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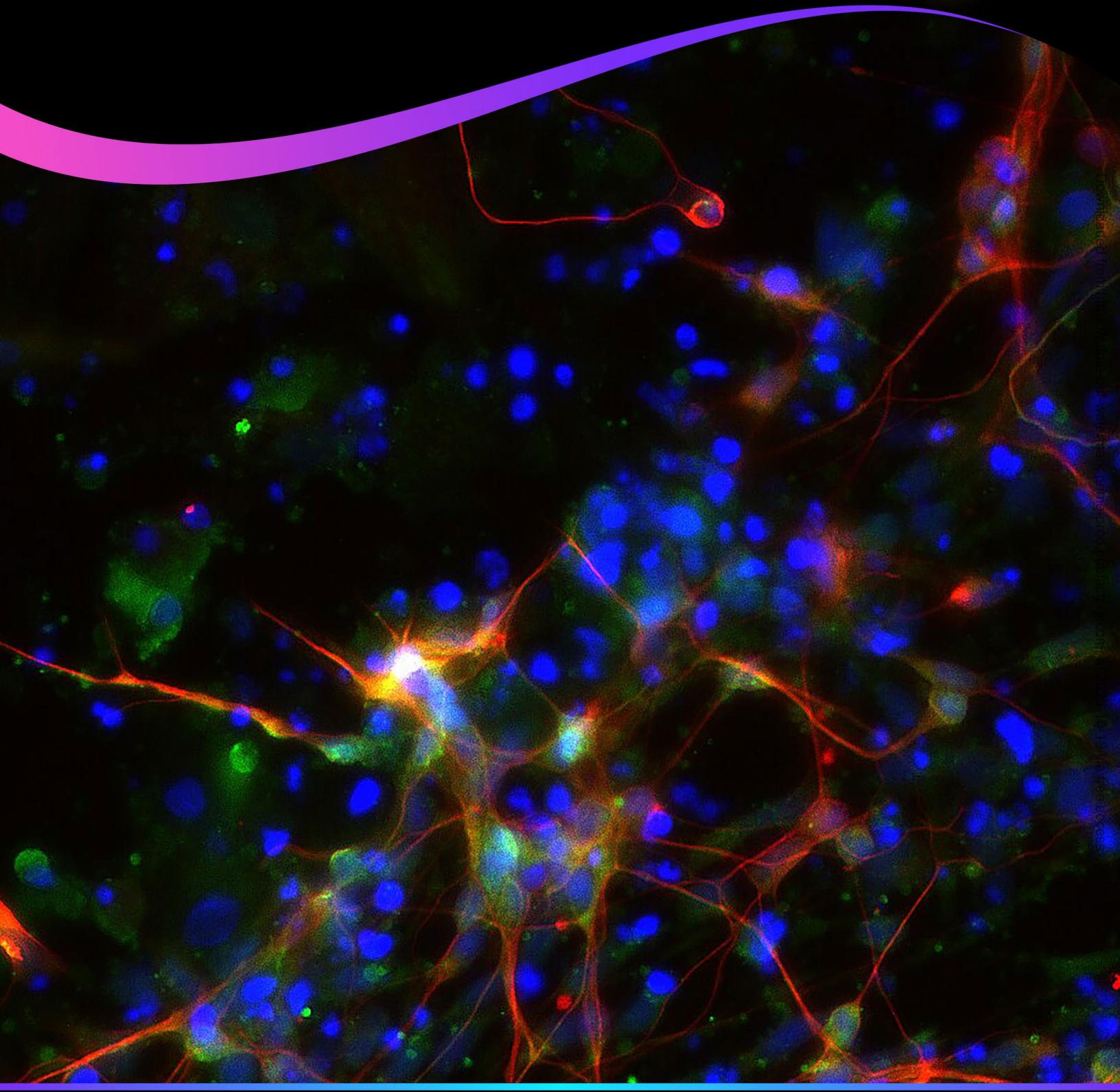
Genomic Psychiatry

Advancing science from genes to society



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Featured Rising Star Sophia Shi: https://www.doi.org/10.61373/bm025k.0074

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Genomic Psychiatry

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Cover Art

Cover Image: Human neurons differentiated from induced pluripotent stem cells (iPSC) reveal the complex cellular architecture achievable in modern disease modeling platforms. This fluorescence micrograph captures mature neurons (red, β3-tubulin) and GABAergic interneurons (green, GABA receptor) derived from a patient with familial Alzheimer's disease, illustrating the sophisticated cell types now routinely generated for neuropsychiatric research. Such advances underscore the importance of establishing rigorous validity standards for iPSC models, as discussed by Kolsters et al. in this issue (pages 27–33).

Image credit: Morozless/Wikimedia Commons, CC BY-SA 4.0. Originally submitted to Science Photo Competition 2023 in Ukraine.

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Genomic Psychiatry

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EDITORIAL

Genomic Press and the moon are Flicts: A tale of belonging and innovation

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Finding Our Place: The *Flicts* Story and the Heart of Genomic Press

When Genomic Press started, I never imagined our defining moment would come from a children's book. Yet here we are, finding our soul in the story of a lonely color that could not fit in.

I remember the first time I encountered *Flicts*. I was an adult and a good friend gave me the book with a knowing smile. "Trust me," she said, "this will make sense." She was right, though not in any way I could have anticipated.

Ziraldo: The Godfather of *Flicts*

Ziraldo Alves Pinto (24 October 1932–6 April 2024), known by his first name, Ziraldo, passed away earlier this year at age 91. A giant of Brazilian literature and visual arts, Ziraldo was born in the Brazilian state of Minas Gerais, my own home state, and got his start as a cartoonist, earning wide notice for his satirical drawings before moving on to write and illustrate children's books. Ziraldo's books, such as the beloved *O Menino Maluquinho* (The Nutty Boy), often combined humor with social commentary.

But *Flicts* was a departure into deeper, more allegorical territory. Penned in the same year that humans landed on the moon for the first time (Apollo 11 in July), *Flicts* evoked a world in which rigidity collided with the fluidity of possibility, in which change was not a welcome visitor but an unavoidable guest. It has since become a cornerstone of Brazilian literature, a story that continues to inspire readers of all ages.

Flicts: A Color That did not Belong

Flicts is the story of a dull, nondescript color that does not fit in. The color Flicts shows none of the fiery bravado of Red, the chirpy cheer of Yellow, or the cool depth of Blue. It is an indeterminable color, an undazzling and unremarkable hue, a thing of quiet presence that no one can see, or wants to.

The story tracks Flicts's odyssey through a world where it belongs nowhere. The rainbow, the universal sign of harmony and inclusion, explicitly excludes Flicts from its arc of seven perfect bands. Paintboxes, full of infinite colors for painting lively fields and radiant skies, do not contain Flicts. Not even nature, with its endless palette, can account for this peculiar hue.

What strikes me most about the story is how painfully specific the rejections are. The rainbow's colors do not just ignore Flicts – they actively exclude it. Red smirks that "seven is such a beautiful number," making it clear that there is no room for an eighth color. Blue stands aloof, muttering about having "a name to protect." Even gentle Green talks about being part of a "big family" in a way that makes it clear Flicts is not invited.

And yet, Flicts persists, but the rejections keep mounting: after checking with all the countries in our vast world, each one made it exceptionally clear to Flicts that there would never be anything Flicts in their flags—neither in the traditional flags of old countries nor in the flags of emerging countries. Similarly, no maker of colored pencils, crayons, or paint wanted to add Flicts to their repertoires.

At some point, Flicts starts to realize that nothing on Earth wants to be Flicts, and as it does not find a place in this world, Flicts departs, rising higher and higher. Flicts's ascent takes it above the limits of Earth, above the boxes of paint, flags, and rainbows, until it arrives at its natural home — not on Earth but on the moon, where it imparts its unwanted color to that entire cosmic body. Here, Flicts changes its function from that of a symbol of exclusion to a symbol of belonging, spreading light on the celestial sea with its quiet glow.

The Color That was not There

Flicts occupies a special place in color theory, beyond the story itself. It is formally categorized as a "fictional color"—a mutant shade that does not actually exist in human perception. A fictional color is defined as a color described in a work of fiction that does not exist in real life and would be impossible to create or obtain. Flicts is listed in nearly all inventories of fictional colors worldwide. Many fictional colors are created for alien planets or conceived in speculative biology. Nevertheless, Flicts is different: its uniqueness is due not to its impossibility but to its displacement — a color that exists yet will find no place in the ordinary spectrum.

However, Brazilian artist Zukoski was brave enough to transform Flicts's metaphysical identity into pixels, and she gave Flicts precise and unprecedented numerical values: Hex D49126 or RGB 212,145,38. Zukoski's muted gold-orange-red shade, quiet but distinctive, captures the ambiguity and singularity that characterize Flicts's identity. For Genomic Press, it was the ideal symbol of our commitment to the unconventional and making space for that which does not exist yet.

Neil Armstrong's Cosmic Connection

Flicts had an incredible twist to its story–the encounter of Neil Armstrong, the first man to walk the moon, with the book in Brazil. Entranced by its message, Armstrong famously proclaimed, "The Moon is Flicts." His handwritten note, reproduced in our Figure 1, is now always printed as the last page of the book, and it became part of *Flicts*'s eternal legacy — a poetic metaphor that made its way into people's homes and hearts as something much more universal.

Armstrong's words validated what Ziraldo had imagined—the rise of Flicts to the moon was part of humanity's broader quest to explore and find meaning beyond the known world.

I get goosebumps every time I think about that moment. Armstrong's handwritten note, which now appears in every edition of the book, did more than validate a fictional color. It validated the idea that sometimes not fitting in is not a flaw – it is a sign you are meant for something bigger.

More Than Just a Color

What fascinates me most about *Flicts* is that it is not just a story. Among color theorists, *Flicts* poses a beautiful puzzle – it is what they call a "fictional color," but not in the usual way. Most fictional colors are pure fantasy, like trying to imagine a new primary color. However, Flicts is different. It exists in that strange space between what we can name and what we can see.

When Brazilian artist Zukoski finally gave Flicts a digital identity, Hex D49126 or RGB 212,145,38, she was not just picking a color code. She

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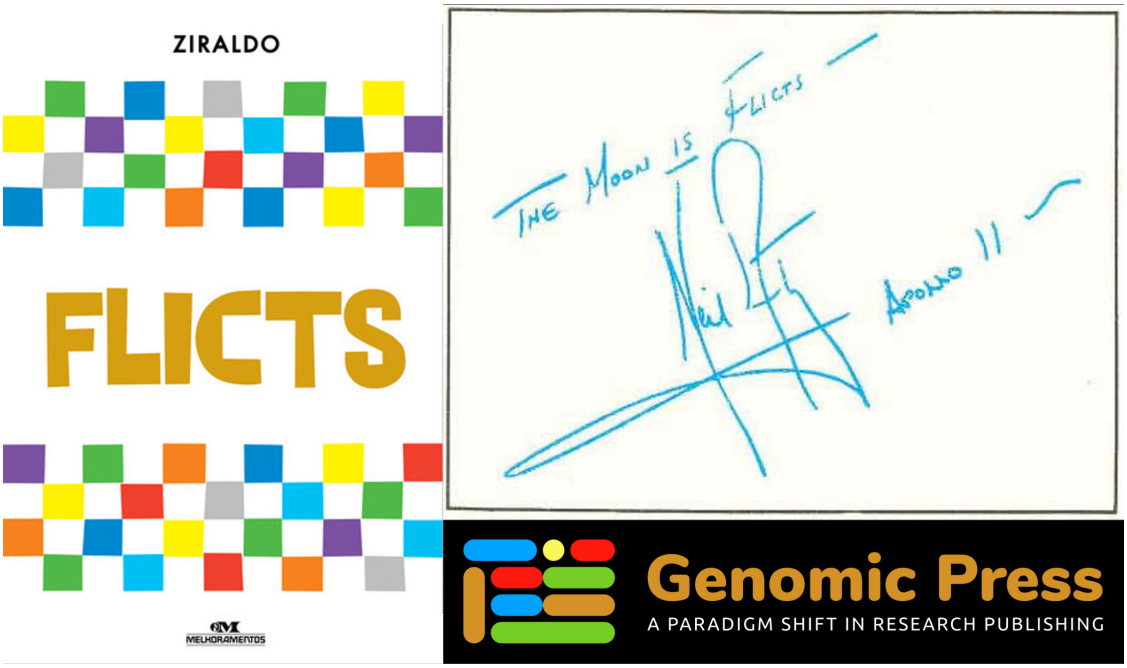

Figure 1. The journey of *Flicts*: A visual representation of Flicts's story and legacy. (Left) The original cover of Ziraldo's *Flicts* (1969), depicting its unplaceable identity with a metallic golden shade that, paradoxically, could not be the true Flicts – a color deemed impossible to reproduce when the book was published. (Top Right) Neil Armstrong's handwritten note, affirming "The Moon is Flicts." (Bottom Right) The Genomic Press logo, utilizing Zukoski's compelling digital interpretation of Flicts (Hex #D49126), which has become one of the most resonant modern visualizations of this historically elusive color.

captured something between categories and made you question how you classify things.

The Heart of Genomic Press

This is why Flicts became more than just our brand color at Genomic Press (see Figure 1). It became our philosophy. In our journals—*Brain Medicine*, *Genomic Psychiatry*, and *Psychedelics*—we are looking for research that, like Flicts, might not neatly fit into traditional categories.

We have seen it happen over and over: the most groundbreaking papers are often the ones that make reviewers at traditional journals uncomfortable. Not because they are flawed but because they exist between established fields, asking questions that do not fit neatly into our current boxes.

Think about it: what color is consciousness? What color spectrum contains the intersection of genetics and mental health? Where in the traditional rainbow do you file research that bridges neuroscience and psychedelic therapy?

Beyond Traditional Boundaries

Every time I look at that quirky Flicts shade in our logo, I think about the researchers who come to us with work that has been rejected elsewhere. Like Flicts being told there is no room in the rainbow, they have often been told their work is "too cross-disciplinary" or "does not fit our scope."

But just as Flicts found its true home in an unexpected place, these researchers often discover that their work is not too strange or too different – it is just reaching for something beyond our current horizons. Sometimes, like Flicts, you must leave Earth entirely to find where you belong.

Conclusion: A Philosophy of Belonging

The story of Flicts reminds us that innovation often comes from the margins, from the spaces between established categories. When I share this story with others in scientific publishing, they sometimes smile at the idea of taking such inspiration from a children's book. But then I show them Armstrong's note, and something shifts in their expression.

Because that is the thing about truth – it does not matter where it comes from. A Brazilian artist writing for children identified something about the moon that resonated with the first human to walk on its surface. That is not just a coincidence; it is a reminder that insight can come from anywhere, and breakthroughs often happen when we look beyond our usual categories.

Fundamentally, *Flicts* teaches us that displacement is not a failure—it is often the precursor to ascension. Just as Flicts found its home on the moon, the ideas that seem out of place in conventional frameworks often hold the key to innovation and progress. At Genomic Press, we are inspired by this lesson. Our mission is to create a space for the innovative, the groundbreaking, and the transformative.

When you see the distinctive shade of Flicts across our journals and platforms, know that it represents more than a branding choice. It is a declaration of purpose: to rise above limitations, to embrace the overlooked, and to illuminate the extraordinary potential that exists beyond the boundaries of the ordinary. Like Flicts, we believe that true belonging is not about fitting in—it is about finding or creating the place where you truly shine.

As we continue building Genomic Press, we carry this lesson from *Flicts*: sometimes, the ideas that do not fit anywhere else are precisely the ones that help us reach the stars.

San Bernardino, California, USA
30 November 2024

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INNOVATORS & IDEAS: RISING STAR

Xuyu Qian: Understanding human brain development and diseases using human-based approaches

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Keywords: Brain, cerebral cortex, stem cell, organoid, neurodevelopment

In this illuminating Genomic Press Interview, Dr. Xuyu Qian, a visionary neuroscientist whose groundbreaking spatial transcriptomics research promises to revolutionize our understanding of human brain development at unprecedented single-cell resolution, shares his remarkable path from an art-infused childhood in Nanjing to becoming a Forbes 30 Under 30 laureate and pioneering force in brain organoid technology. As the newly minted Assistant Professor at the University of Pennsylvania and Children's Hospital of Philadelphia, Dr. Qian has transformed our understanding of human cerebral cortex formation through his innovative fusion of spatial transcriptomics and organoid models. His landmark work, recently published in *Nature* (2025), leveraged state-of-the-art MERFISH technology to analyze over 18 million single cells, thereby redefining our understanding of the emergence of cortical layers and specialized brain regions during fetal development. This breakthrough builds upon his earlier revolutionary development of brain organoid protocols, now cited over 2,000 times and instrumental in shaping CDC guidelines for the prevention of Zika virus. Throughout this candid conversation, Dr. Qian reveals how the anime series Evangelion sparked his passion for biotechnology, explores his generous collaborative philosophy that has led to numerous discoveries, and articulates his commitment to human-centric approaches for decoding neurodevelopmental disorders. His distinctive combination of scientific excellence, creative vision, and infectious enthusiasm establishes him as one of neuroscience's most promising emerging leaders, destined to unravel the fundamental mysteries of human brain development and disease.

Figure 1. Xuyu Qian, PhD, University of Pennsylvania Perelman School of Medicine, USA.

Part 1: Xuyu Qian – Life and Career

Where were you born and where do you live now?

I was born in Nanjing, Jiangsu, China. I live in Boston, Massachusetts, USA, but I will be moving to Philadelphia.

Could you give us a glimpse into your personal history, emphasizing the pivotal moments that first kindled your passion for science?

I grew up in Nanjing, China, in a family that was more artistic than scientific—my dad is a high school art teacher, and my mom is a skilled artisan of brocade crafts. Both of them have always appreciated science, and that quiet enthusiasm rubbed off on me. In their eyes, studying science or engineering was the default path for boys with good school grades. For kids of my generation born in the early '90s China, being a scientist was hands down the most admirable dream job. If you had surveyed a classroom of first graders back then, 95% of us probably would have said we wanted to be scientists when we grew up. To the best of my knowledge, I am the only one from my class who has become a basic scientist. It is hard to pinpoint a single moment that ignited my passion: it was a natural process. I was always drawn to science shows like Beakman's World and later to documentaries on Discovery Channel and National Geographic. I feel lucky to have grown up during a time when those American shows were broadcast on Chinese television—that is no longer the case today.

Please share with us what initially piqued your interest in your favorite research or professional focus area.

My interest in the biological sciences was directly sparked in high school after watching the Japanese anime series *Evangelion*. As dramatic as it sounds, it was the most life-defining moment for me. Set in a post-apocalyptic world, the series centers on humanity's last hope: the "Eva" units-giant, humanoid beings created through advanced bioengineering. Unlike typical sci-fi mechas like Gundam, the Evas are not machines; they are fully living, cloned organisms. That concept completely transformed how I saw biology. It was the first time I realized that biology and

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biotechnology could reshape the world. Inspired by that vision, I chose biomedical engineering as my college major without considering future career paths or job market prospects. It was only after my first year in college that I realized I needed to pursue a PhD to make a difference in the biological sciences.

We would like to know more about your career trajectory leading up to your current role. What defining moments channeled you toward this opportunity?

I attended Worcester Polytechnic Institute (WPI) in Massachusetts, one of the few U.S. universities that offered scholarships to international students. That opportunity enabled me to attend college in the U.S., and I am deeply grateful for it. I graduated in three years, from 2010 to 2013. I started volunteering in a research lab during my first summer. While labs at WPI did not always conduct the kind of cutting-edge science I would later immerse myself in; I quickly realized how much I enjoyed the research process. I spent about a year and a half in Dr. Qi Wen's lab, and I am very thankful for his mentorship. Although I did not produce any publications, the experience taught me how to think like a scientist and confirmed that this was the path I wanted to pursue. Fortunately, PhD applications in 2013 were not as publication-driven as they are today, and I was accepted into Johns Hopkins.

At Hopkins, I joined Dr. Hongjun Song's lab for my third rotation. Within a month, I knew it was the right place for me. I am grateful that Hongjun and Guo-li took me in, even though I had no background in neuroscience or stem cell biology. Under their mentorship, I developed pioneering methods to generate human brain organoids from stem cells—a foundation that shaped the direction of my research.

I continued to pursue my interest in human brain development during my postdoc with Dr. Chris Walsh at Boston Children's Hospital. Chris gives postdocs a great deal of freedom to chart their course. I utilized spatial transcriptomics to analyze preserved human brain samples, thereby constructing a developmental atlas of the human cerebral cortex that was published in Nature (2025) (see Figure 2).

In the fall of 2024, I entered the job market for faculty positions. After numerous interviews, I am thrilled to receive my dream offer and launch my lab at the University of Pennsylvania and the Children's Hospital of Philadelphia. My lab will start this fall, and I could not be more excited for the next chapter.

Figure 2. Dr. Xuyu Qian in his current laboratory, standing beside a collection of external hard drives containing raw spatial transcriptomics data from his groundbreaking human brain development research. The drives, ranging from 5TB to 20TB in capacity, represent the massive scale of single-cell resolution data generated in his recent *Nature* (2025) study analyzing over 18 million cells to map human cerebral cortex development.

What is a decision or choice that seemed like a mistake at the time but ended up being valuable or transformative for your career or life?

Looking back, I have been fortunate in my career. Concrete evidence or detailed reasoning did not drive many of the choices I made- often, they were based on gut instinct. At the time, that felt risky or naive. For example, choosing biomedical engineering as a college major or joining a neuroscience lab without a background in the field could have easily backfired. However, somehow, those intuitive decisions turned out to be exactly right. What initially felt uncertain at the moment ultimately proved to be transformative, and it has taught me to trust my instincts.

What habits and values did you develop during your academic studies or subsequent postdoctoral experiences that you uphold within your research environment?

One of the core values I developed during my training is a strong commitment to collaboration. I genuinely enjoy working with others and believe in leaning on the expertise of collaborators rather than trying to learn and master everything myself, especially in a fast-moving field where techniques quickly become outdated. When I collaborate, I make it a priority to ensure that my collaborators receive full and fair credit for their contributions. I always ask early on what they hope to gain from the collaboration and work to align our goals. If I cannot offer enough benefit in return, I do not ask for significant effort.

This approach has been shaped by both of my mentors, Hongjun and Chris, who lead highly collaborative labs and are generous with credit and authorship. I learned from them that strong science comes from strong partnerships. Like them, I try to be generous with co-authorship—anyone who contributes to a project is included, regardless of the size and depth of their role. I do not believe in using credit as a carrot to motivate more work. Instead, I want people to feel that working with me is enjoyable and rewarding, so they will want to collaborate again or recommend others to do so.

Please tell us more about your current scholarly focal points within your chosen field of science.

My research focuses on understanding how the human cerebral cortex develops and how this process goes awry in neurodevelopmental disorders. The cortex is the most evolutionarily advanced part of the brain and underlies many aspects of cognition, yet it is also particularly vulnerable to developmental disruption. Conventional animal and cell culture models often fail to capture the unique features of human cortical development, which has limited our understanding of human-specific diseases.

To address this, my research program integrates three key human-based strategies: brain organoid models, spatial omics technologies, and human genetics. In the short term, my lab will focus on uncovering the mechanisms that drive the specification of cortical area-specific neuronal subtypes. By combining advanced organoid models with spatial omics, we aim to decipher how cortical regions acquire their unique identities and why some are more susceptible to conditions such as malformations of cortical development and autism spectrum disorder. My long-term goal is to identify the developmental programs disrupted in these disorders and build a foundation for future therapeutic strategies.

What impact do you hope to achieve in your field by focusing on specific research topics?

I want to contribute to driving a paradigm shift in the field, moving from a reliance on animal models to directly studying the human brain. This is especially critical for the cerebral cortex, the most uniquely human part of the brain and the key to what makes us so special. My approach combines two complementary systems: direct analysis of human brain specimens and brain organoid models. Human tissue provides the ground truth, offering real data that allows us to build accurate models and form meaningful hypotheses. Organoids, on the other hand, provide a living, manipulable system in which we can test those hypotheses experimentally. While organoids are not the real brain, benchmarking them against human tissue helps us define what they can or cannot replicate and how we might engineer improved models.

By integrating these platforms, I aim to identify cellular and molecular disruptions that are directly relevant to human disease. One of my core interests is neuronal subtype specification. This is a key point of vulnerability in brain development, where the disruption of even a single gene can dramatically alter the identity and function of neurons, leading to the profound consequences we see in many neurodevelopmental disorders. Through this work, I aim to advance both our mechanistic understanding and our ability to develop targeted therapeutic strategies.

What do you most enjoy in your capacity as an academic or research rising star?

What I enjoy most is the freedom to ask questions that genuinely excite me and the sense of possibility that comes with building a new research direction. As I prepare to launch my lab, it is gratifying to define the kind of science I want to pursue and the questions I want to tackle. It is also significant to connect with others in the scientific community, whether it is learning from senior scientists, exchanging ideas with peers, or encouraging the next generation of researchers. That sense of shared passion and intellectual growth across career stages is one of the most fulfilling parts of academic life. For me, the journey from an initial question to a new insight into the human brain is what makes this work so energizing.

At Genomic Press, we prioritize fostering research endeavors based solely on their inherent merit, uninfluenced by geography or the researchers' personal or demographic traits. Are there particular cultural facets within the scientific community that warrant transformative scrutiny, or is there a cause within science that you feel strongly devoted to?

One cause I feel strongly about is open access to scientific research. The current publishing model in many prominent journals is deeply flawed. Authors often pay high fees to publish, readers or institutions must pay to access the content, and peer reviewers, who are essential to maintaining quality, are not compensated at all. Meanwhile, the profit margins for major publishers exceed those of companies like Google or Amazon. I fully understand that it costs money to run a high-quality journal: editorial staff, infrastructure, and production are all essential. However, the scale of the profits makes it clear that these fees go far beyond simply "keeping the lights on." Moving toward more equitable and open publishing models is essential for making science more impactful.

Outside professional confines, how do you prefer to allocate your leisure moments, or conversely, in what manner would you envision spending these moments given a choice?

As I mentioned earlier, my passion for biology was first sparked by *Evangelion*, so it is no surprise that I am a huge fan of Japanese anime. I also play many video games. I want to proudly highlight these hobbies because they are often seen as "unhealthy" or unproductive, but I would argue that such a perception is misguided. They energize me and often spark creativity. Anime and games tell incredibly inspiring stories, often with complex and thoughtful narratives that provide motivation and perspective. I have also known many other rising scientists who share these same passions.

To stay balanced, I also work out regularly. I am a history enthusiast and love watching YouTube channels on world history while exercising or doing chores. I do not read many books—I am a slow reader and tend to retain visual information more vividly.

I also create science-themed videos on Bilibili, a leading video-sharing platform in China, where I have built a channel of over 370,000 subscribers. I use this platform to make science accessible and exciting, especially for younger audiences, through videos that explain topics ranging from neuroscience breakthroughs to the science behind science fiction.

Part 2: Xuyu Qian – Selected questions from the Proust Questionnaire[1]

What is your most marked characteristic?

My most marked characteristic is focus. I am very clear about my objectives and work with strong intent toward achieving them. Throughout my career, I have set ambitious but realistic goals at each stage and have consistently met them within the planned timeframe. When working on a project or manuscript, I plan the process carefully from the outset. I often sketch a draft version of the figures, complete with panel layouts and the types of analyses I expect, almost like a movie director's storyboard. That focus helps guide the direction, but I am not rigid. I am quick to adapt when data take an unexpected turn, and I am always ready to act when an unanticipated opportunity arises. Focus, for me, is not just about sticking to a plan but about staying attuned to the bigger picture while navigating the unexpected.

Among your talents, which one(s) give(s) you a competitive edge?

One talent that gives me a competitive edge is trusting my intuition and making decisions quickly. I do not waste time hesitating or overanalyzing, which helps me avoid missing opportunities. Especially in science, timing matters, whether it is jumping on a new idea, starting a collaboration, or pursuing a surprising result. I am fortunate that I rarely regret the decisions I make in research. Sometimes, I can almost close my eyes and see the following steps unfold. I am not sure if that qualifies as foresight, but I like to think it is a kind of instinct that guides me in the right direction.

If you could change one thing about yourself, what would it be?

If I could change one thing about myself, I would want to be more thoughtful in my communication with others. I consider myself a kind person—I never intentionally hurt others who treat me fairly. However, in conversation, I can sometimes unintentionally say things that come across as hurtful or offend someone's feelings. I have been working on better understanding how my words might be interpreted from another person's perspective and how to express myself more clearly and sensitively. I have grown a lot in this area over the years—I was much more awkward in college and early grad school—but I think this is something many of us spend a lifetime trying to improve.

What is your current state of mind?

Right now, my mind is juggling a thousand things as I prepare to set up my lab: budgeting, submitting grants, ordering equipment, hiring staff, and managing paperwork. Everything is new, and each task comes with

[1] In the late nineteenth century, various questionnaires were a popular diversion designed to discover new things about old friends. What is now known as the 35-question Proust Questionnaire became famous after Marcel Proust's answers to these questions were found and published posthumously. Proust answered the questions twice, at ages 14 and 20. In 2003 Proust's handwritten answers were auctioned off for $130,000. Multiple other historical and contemporary figures have answered the Proust Questionnaire, including among others Karl Marx, Oscar Wilde, Arthur Conan Doyle, Fernando Pessoa, Stéphane Mallarmé, Paul Cézanne, Vladimir Nabokov, Kazuo Ishiguro, Catherine Deneuve, Sophia Loren, Gina Lollobrigida, Gloria Steinem, Pelé, Valentino, Yoko Ono, Elton John, Martin Scorsese, Pedro Almodóvar, Richard Branson, Jimmy Carter, David Chang, Spike Lee, Hugh Jackman, and Zendaya. The Proust Questionnaire is often used to interview celebrities: the idea is that by answering these questions, an individual will reveal his or her true nature. We have condensed the Proust Questionnaire by reducing the number of questions and slightly rewording some. These curated questions provide insights into the individual's inner world, ranging from notions of happiness and fear to aspirations and inspirations.

its learning curve. It has made me realize what a privilege it was as a PhD student and postdoc to be able to focus entirely on science. I am excited about this next chapter, but I also look forward to the day when my lab is running smoothly, and I can return my full focus to discovery and experimentation, the part I love most.

What is your idea of perfect happiness?
My idea of perfect happiness is the conviction that I am not just one of many human beings who have lived but a truly unique being: a singularity. I want to know that I am special in a way that everyone can recognize and that there is something I can achieve that sets me apart.

When and where were you happiest? And why were so happy then?
I feel happy now because I am making steady progress toward achieving my goals. There is satisfaction in the momentum, in seeing things move forward. However, at this stage, I am still one of many junior scientists working hard to establish myself. My happiest moment will come when I truly accomplish something one-of-a-kind.

What is your greatest fear?
My greatest fear is death. Human life is far too short to unravel the universe's mysteries. There is so much to discover, but not enough time to see it all. We need to advance life science to a point where each year of progress is enough to extend human life by at least one more year. We do not have to achieve immortality overnight—but if we can stay ahead of the clock, we can keep going. That is not asking too much.

What is your greatest regret?
I have been fortunate not to have any deep or essential regrets. If anything, my regrets are lighthearted ones—like not buying Bitcoin in 2013.

What are you most proud of?
I am most proud of my research achievements and the tangible impact they have had in the field. From developing early brain organoid models to mapping human cortical development, I have contributed tools and insights that others have found valuable. My cortical organoid method was among the earliest in its domain- and the paper has now been cited over 2,000 times. Many labs have adopted the protocol, and it is gratifying to meet researchers at conferences who tell me they are getting great results with my protocols.

Using organoids, we were also among the first to provide experimental evidence for the causal link between the Zika virus and congenital brain malformations. The CDC referenced our study to support biological plausibility in their declaration that Zika causes congenital disabilities, and it informed public health guidelines advising pregnant women to avoid affected regions. Although the impact was indirect, knowing that my research helped prevent Zika-related harm—and may even have saved lives—is something I take immense pride in.

What do you consider your greatest achievement?
My most outstanding achievement is earning the opportunity to open my own lab at the University of Pennsylvania. For years, this has been my dream. It represents both a personal milestone and the beginning of a new chapter that I am incredibly excited about.

What or who is your greatest passion?
My greatest passion is scientific discovery—uncovering how the human brain works. Outside of science, I have also had a lifelong passion for anime.

What is your favorite occupation (or activity)?
Rewatching *Evangelion* and analyzing it frame-by-frame.

What is your greatest extravagance?
Traveling to Midgard and Asgard ... in the video game God of War.

What is your most treasured possession?
Cards from former lab members and trainees.

Where would you most like to live?
Italy—perhaps in the Tuscany countryside. The weather is beautiful, the food is incredible, the people are friendly, and the landscape is rich in history and culture.

What is the quality you most admire in people?
Resilience. I am inspired by those who can face setbacks, uncertainty, or failure and still keep moving forward with purpose.

What is the trait you most dislike in people?
Dishonesty.

What do you consider the most overrated virtue?
Perfectionism.

What do you most value in your friends?
What I value most in my friends is their ability not to take offense easily. I appreciate people who understand that honesty and bluntness are not the same as bad intentions. I speak very directly, and I'm grateful for friends who know it comes from sincerity, not criticism. That kind of mutual trust makes for strong, lasting friendships.

Which living person do you most admire?
Anno Hideaki, the director of *Evangelion* (see Figure 3). He is a true genius and a legend in the creative industry. I admire how he poured himself into his creation, often at a significant personal cost. Now, having earned worldwide acclaim and creative and financial independence, he is free to follow his teenage dreams without worrying about audience expectations, critics, or investors. That kind of authenticity—staying true to one's vision—is incredibly inspiring to me.

Who are your heroes in real life?
My real-life heroes are my mentors, Chris and Hongjun as well as many other leaders in science whom I deeply admire. They have not only made extraordinary contributions to the field, but they have also done so with generosity, integrity, and kindness.

If you could have dinner with any historical figure, who would it be and why?
Alexander the Great. At the dinner, I would tell him the food is poisoned. If he survived because of it and goes on to conquer the rest of the world, I would find a strange satisfaction in knowing that my action altered the course of history forever.

Who are your favorite writers?
I am a Lord of the Rings nerd, so J.R.R. Tolkien is my favorite writer. I love how his story draws a clear line between good and evil without the political ambiguity or moral grayness that often dominates modern narratives. However, within that clarity, Tolkien still creates incredible depth, complexity, and resonance with the real world.

More contemporarily, I admire Hanada Jukki, one of the most prolific anime screenwriters in recent years. His work captures emotions that are deeply resonant yet difficult to articulate- those unspoken feelings that many writers shy away from. Nevertheless, he brings them to light with remarkable subtlety and honesty.

Who are your heroes of fiction?
As someone who watches a lot of anime, I find new fictional heroes to admire almost every season. But the greatest, of course, has to be Ikari Shinji, the protagonist of *Evangelion*. He is often misunderstood and criticized for his fear and hesitation, but that is precisely what makes him so compelling. Beneath the surface, Shinji is a profoundly human character—flawed, vulnerable, and constantly struggling with self-worth and purpose. His growth comes full circle in the 2021 series finale movie *Evangelion 3.0 + 1.0*, where he learns to take responsibility, make decisions, and face their consequences. That transformation felt profoundly earned, and it is why he remains my greatest fictional hero.

Figure 3. Sitting at Ube-Shinkawa Station, Ube, Japan. This unassuming station served as the real-world backdrop for Evangelion 3.0 + 1.0's final scene, a place where fiction and reality converge. As the series came full circle in this quiet setting, I found myself at my own threshold: visiting this endpoint of one story just as I embarked on a new chapter felt like a moment of perfect synchronicity.

What aphorism or motto best encapsulates your life philosophy?
"Anywhere can be paradise as long as you have the will to live" – from *Evangelion.*

Boston, Massachusetts, USA
13 June 2025

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INNOVATORS & IDEAS: RESEARCH LEADER

C. Robert Cloninger: Mechanisms and conditions by which temperament, character, and personality development can regulate health and well-being

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Keywords: temperament, character, personality, epigenetics, well-being

Dr. C. Robert Cloninger's contributions have been foundational in understanding temperament, personality, and their biological and genetic underpinnings. Cloninger explored how temperament, character, and personality traits are influenced by genetic factors and how they predict various psychological disorders, such as alcoholism and personality disorders. His prospective studies involving adoptees reared apart from their biological parents provided crucial insights into the heritability and development of personality traits independent of environmental influences. Moreover, Cloninger's pioneering work in conducting the first genome-wide association and linkage studies of normal personality traits laid the groundwork for subsequent research in psychiatry and behavioral genetics, linking specific genetic profiles to patterns of temperament and personality. He is also the creator of two extensively employed personality assessment instruments: the Tridimensional Personality Questionnaire (TPQ) and the Temperament and Character Inventory (TCI). Dr. Cloninger serves as Director of the Anthropedia Institute and Professor Emeritus at Washington University in St. Louis, where he studies the biopsychosocial foundations of personality that influence health and illness. The Anthropedia Institute is the research branch of the Anthropedia Foundation, a non-profit organization dedicated to the promotion of human well-being through initiatives in health care and education. He served as Wallace Renard Professor of Psychiatry & Genetics, Professor of Psychological and Brain Sciences, and founding Director of the Sansone Center for Well-being at Washington University until July 2019. Dr. Cloninger is a member of the National Academy of Medicine USA, Fellow of the American Academy for Advancement of Science, and an editor of various journals in psychiatry, psychology, and genetics. We are pleased to share Dr. Cloninger's answers to the Genomic Press Interview.

Figure 1. C. Robert Cloninger, Anthropedia Institute and Washington University in St. Louis, USA.

Part 1: C. Robert Cloninger – Life and Career

Could you give us a glimpse into your personal history, emphasizing the pivotal moments that first kindled your passion for science?

Learning about nature and living things has always fascinated me. Among my fondest childhood memories are exploring forests and streams, where I observed wildlife, collected insects, and enjoyed immersion in nature. I loved gardening with my grandfather and discussing character development with my mother, an actress who directed the local community theater, and my father, who taught literature. I arranged to enroll in schools with the best available science curriculum because by age 10, I planned to become a medical doctor. In high school, I loved all math and science, especially biology and chemistry. My first taste of research came while in high school during a National Science Foundation college program in biological sciences. At the University of Texas (UT) in Austin, I was in the Plan II Honors Program, studying philosophy, anthropology, and psychology in addition to pre-medical courses. For my honors thesis at UT with the anthropologist and science fiction writer Chad Oliver, I examined the worldview of William Golding to ask questions about the nature of a good and healthy life. I also worked as a research assistant doing operant learning experiments, but I found these theories needed to be revised to explain human capacities for awareness, as considered in my studies of phenomenology with philosophers Irwin Lieb and John Silber. Decades later, I was able to test these various views of human learning capacities in research on the science of well-being.

I picked the research-intensive Washington University (WU) School of Medicine (WUSM), where I began research with psychiatrists Samuel Guze and Eli Robins, doing both laboratory and clinical research that was influential in the development of DSM-III and IV. At each step there were mentors and colleagues who helped me develop and deepen my insights.

We would like to know more about your career trajectory leading up to your most relevant leadership role. What defining moments channelled you toward that leadership responsibility?

Where I trained, the Department of Psychiatry at WUSM emphasized direct measurement of categories with little use of psychodynamic or motivational hypotheses. Nevertheless, Sam Guze was receptive when I suggested that we could begin hypothetico-deductive research to understand why antisocial personality disorder and somatization disorder often occur in the same individual and the same families. By studying female criminals and their family members, we found that both disorders shared familial predispositions, but for women to become antisocial required a stronger predisposition than men. After finishing my residency, I continued that approach in longitudinal studies of psychiatric outpatients and

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their families, developing expertise in clinical assessment and differential diagnosis.

Then, I applied new quantitative genetic techniques to the family data I had collected with Guze and Ted Reich. Ted's new analytic methods allowed us to systematically test hypotheses about alternative inheritance models, which I found satisfying. From 1975 to 1978, I developed models of combined genetic and cultural inheritance using path analysis at WU. In 1978-79, at the University of Hawaii, I extended those models with geneticists Newton Morton and DC Rao in consultation with Sewall Wright. We were awarded a succession of NIH grants at WU that allowed us to put together a creative multidisciplinary group, eventually resulting in my leading a Clinical Research Center in Psychiatric Genetics.

I continued to try to understand the role of personality in the patterns of comorbidity in psychiatric disorders. However, I found that the assessment techniques in psychiatry for personality disorders needed improvement, particularly those restricted to directly observed behavior without considering motivational processes within the person. I studied the psychometric methods of leading personality theorists whom I met through sociologist-epidemiologist Lee Robins, including Ray Cattell, Hans Eysenck, Jeffrey Gray, Robert Hare, and Daisy Schallings. However, I had no formal theory about personality structure until 1986, when two events converged. Beginning in 1980, I conducted adoption studies of alcoholism and related psychiatric disorders in collaboration with Michael Bohman in Sweden. We found personality variables to be associated with risk for alcohol abuse and related behaviors. Then, in 1986, I was invited to describe the factors differentiating susceptibility to generalized anxiety and somatization. To explain this, I developed my theory of temperament dimensions (Cloninger, 1987), drawing on operant learning theory as the underlying basis for behavioral conditioning of temperament in a model that accounted well for personality disorder subtypes.

Soon, I found that a behavioral conditioning model of temperament alone could not account for whether an individual was mature or not. I vividly remember the moment when I was faced with the temperament profiles of two men with nearly identical temperament scores: one was a violent criminal, whereas the other was a responsible and civilized executive. This forced me to develop my model of character as a moderator of temperament in 1993, reawakening my early interests in the humanities and phenomenology.

The combined model of temperament and character provided a comprehensive description of personality needed to diagnose whether someone had a personality disorder (based on character) and what subtype they had (based on temperament). It also predicted associations with physical and mental health disorders and levels of well-being (physical, mental, and social). Together with psychiatrist Dragan Svravic and his twin brother, physicist Nenad Svrakic, I investigated the dynamics of personality change in longitudinal studies, confirming in 1997 that, if there was a change, we could predict what changes would occur in a self-organizing adaptive system. However, it remained unclear what conditions were needed to elicit personality change.

Please share with us what initially piqued your interest in your favorite research or professional focus area.

The dearth of empirical information about the mechanisms and conditions by which personality development can regulate health and well-being indicated a need for leadership to establish an integrative, multidisciplinary center to understand the underlying mechanisms of personality development as a complex adaptive system or, more fully, as a multi-modular network of complex adaptive systems. A Center for Well-being was endowed for this purpose under my leadership at Washington University in 2001. Shortly after that, in 2004, a large group of scientists, educators, and physicians formed the non-profit Anthropedia Foundation to develop educational programs for training coaches and therapists with a strong understanding of the role of personality in the biopsychosocial and spiritual aspects of health and well-being.

My book Feeling Good: The Science of Well-being described the initial foundation for a transdisciplinary approach in 2004. I described the broad roots of a science of well-being in scientific fields from physics, chemistry, and genetics to psychology, sociology, and philosophy. This allowed for the consideration of biopsychosocial processes, from molecules to cells, organs, and organ systems in the body, and on to society, the planet, and the cosmos. Fortunately, interest in the use of the TCI was strong around the world, and the Centers for Well-being at WU and the Anthropedia Foundation have been able to organize international collaborations to study the science of well-being with a highly multidisciplinary team using innovative methods in well-characterized data sets. The team includes psychiatrists, psychologists, anthropologists, neuroscientists, geneticists, and experts in bioinformatics and artificial intelligence, with data from many cultures around the world, as summarized in recent articles on genomics (Cloninger & Zwir, 2022), transcriptomics (del Val et al., 2024), and related studies of all aspects of health (physical, emotional, cognitive, social, and spiritual).

What impact do you hope to achieve in your field by focusing on specific research topics?

Just as the quantum revolution led to a paradigm shift in physics in the early 20th century, the epigenetic revolution has spurred a paradigm shift in the 21st century (Cloninger, 2004; del Val et al., 2024). There is growing evidence that all life forms involve complex information-processing networks that are self-organized as specialized functional modules that interact collaboratively to turn one another on and off to adapt to changing external and internal conditions. Our collaborative team just completed the first study of the transcription of the whole genome in relation to human personality. We found that human personality orchestrates gene expression networks for neuronal plasticity, epigenesis, and adaptive functioning that influence all aspects of physical, mental, social, and spiritual health. We are pioneering ways to extend methods that have previously been restricted to experimental animals to humans in ways that are non-intrusive and beneficial, such as identifying the fundamental changes at a deep molecular level that underlie changes in gene expression and brain connectivity in response to increased awareness from interventions that promote health and well-being.

Please tell us more about your current scholarly focal points within your chosen field of science

We aim to investigate epigenetic change during personality development further by studying chromatin modification and by longitudinal studies of transcriptomics and brain connectivity before and after therapeutic interventions for a range of clinical syndromes and disease spectra.

What habits and values did you develop during your academic studies or subsequent postdoctoral experiences that you uphold within your research environment?

In my experience, the most effective habits and values for academic studies are the same temperament and character traits that promote health and well-being in general: complex systems for reliability, resilience, and creative self-awareness.

At Genomic Press, we prioritize fostering research endeavors based solely on their inherent merit, uninfluenced by geography or the researchers' personal or demographic traits. Are there particular cultural facets within the scientific community that warrant transformative scrutiny, or is there a cause within science that deeply stirs your passions?

The well-being of scientists influences their productivity, integrity, and creativity. Well-being is impaired when people strive to publish from extrinsic pressures (such as fear or to profit personally) rather than from intrinsic drives to uncover what is accurate and beneficial for society. Science has not been immune to the increasing pressure to compete for money for profit like a business rather than to function as a community of mutually respectful scholars seeking greater awareness of truth to benefit individual and collective well-being.

What do you most enjoy in your capacity as an academic or research leader?

I enjoy developing new methods to test hypotheses that previously could not be investigated by bringing together experts and approaches from

multiple disciplines. The satisfaction comes from the experience of helping all members of the team to develop their skills and share in the generation of increased awareness of what is accurate and beneficial for all.

Outside professional confines, how do you prefer to allocate your leisure moments, or conversely, in what manner would you envision spending these moments given a choice?
For leisure around home, I enjoy gardening, hiking in nature, reading, and discussions with friends. I like to travel to other countries and cultures with their diverse rituals, traditions, and cuisines (which is a benefit of international research!).

Part 2: C. Robert Cloninger – Selected questions from the Proust Questionnaire[1]

What is your idea of perfect happiness?
Happiness is the enduring satisfaction that comes from service to others, letting go of all struggles and complaints, and growing in awareness. It is not seeking transient pleasures, which stimulate insatiable and self-defeating desires.

What is your greatest fear?
I fear that humanity is responding inadequately to the existential threats to civilized life as we know it. We are postponing the kind of creative response that has allowed us to survive prior urgent threats over the past 100,000 years.

Which living person do you most admire?
Sherry Lee Cloninger, an artist and my wife, confidante, and best friend.

What is your greatest extravagance?
My passion for Mediterranean and Maghrebian cuisines.

What are you most proud of?
My family and friends.

What is your greatest regret?
I appreciate music and regret never learning to play a musical instrument. I had the opportunity as a child but refused because I preferred to play outdoors. I could have managed to do both.

What is the quality you most admire in people?
Wisdom, which comprises all the virtues.

What is the trait you most dislike in people?
Cruelty.

What do you consider the most overrated virtue?
Pride (because the lack of humility is not self-respect or a sense of intrinsic dignity).

What is your favorite occupation?
What I am doing now is what I most love to do.

Where would you most like to live?
Along the Mediterranean coast of Europe because of the culture, cuisine, and climate.

What is your most treasured possession?
Mementos of my family, friends, and our activities together.

When and where were you happiest? And why were so happy then?
Here and now because I have learned to enjoy life as a journey of constant learning in which there are always more wonders and mysteries to discover.

What is your current state of mind?
I am happy and thankful for what I have experienced and learned. I am enthusiastic about the amazing scientific tools we now have to better understand the world. I am hopeful for the future for myself and others despite many serious situations in the world that may cause many people to endure suffering. I view such suffering with compassion rather than fear because I have faith in the unconditional resilience of life itself.

What is your most marked characteristic?
My generous friends say it is creativity and intellectual curiosity. To me, it is just a willingness to be open-minded about what is possible and to put those ideas to the test.

Among your talents, which one do you think gives you a competitive edge?
The combination of interpersonal insight with analogical reasoning (to see commonalities between familiar and novel sets of relationships) and synthetic reasoning (to integrate elements of information into a new whole). This combination generates new ideas from available information to be critically examined and refined by further experience. Resilience and persistence also help.

What is a personality/characteristic trait you wish you had?
I wish I were more patient, particularly when interrupted while working.

What do you consider your greatest achievement?
Development of the Temperament and Character Inventory (TCI).

What do you most value in your friends?
Good humor, kindness, and candor so that we can balance serious reflection with playfulness.

Who are your favorite writers?
Plato, Immanuel Kant, Mahatma Gandhi.

Who are your heroes of fiction?
The resourceful and clever leader *Odysseus* and the practical and wise mentor *Athena* (both in Homer's *Odyssey*).

Who are your heroes in real life?
Joseph and Genoveffa Mazzagatti (my kind and intelligent grandparents, Italian immigrants to the USA. They taught their extended family to love learning and discovery of the wonders of nature)*; and Sewall Wright* (a founder of population genetics who developed path analysis and the analysis of complex adaptive systems in genetics and evolution. At age 7, he wrote his first book, *The Wonders of Nature,* and then continued to publish scientific works until he died in 1988 at age 98. Wright was a Unitarian who recognized the irreducibility of consciousness to matter, as did

[1]In the late nineteenth century, various questionnaires were a popular diversion designed to discover new things about old friends. What is now known as the 35-question Proust Questionnaire became famous after Marcel Proust's answers to these questions were found and published posthumously. Proust answered the questions twice, at ages 14 and 20. In 2003, Proust's handwritten answers were auctioned off for $130,000. Multiple other historical and contemporary figures have answered the Proust Questionnaire, including among others Karl Marx, Oscar Wilde, Arthur Conan Doyle, Fernando Pessoa, Stéphane Mallarmé, Paul Cézanne, Vladimir Nabokov, Kazuo Ishiguro, Catherine Deneuve, Sophia Loren, Gina Lollobrigida, Gloria Steinem, Pelé, Valentino, Yoko Ono, Elton John, Martin Scorsese, Pedro Almodóvar, Richard Branson, Jimmy Carter, David Chang, Spike Lee, Hugh Jackman, and Zendaya. The Proust Questionnaire is often used to interview celebrities: the idea is that by answering these questions, an individual will reveal his or her true nature. We have condensed the Proust Questionnaire by reducing the number of questions and slightly rewording some. These curated questions provide insights into the individual's inner world, ranging from notions of happiness and fear to aspirations and inspirations.

Spinoza and philosopher Charles Hartshorne, a close friend of both Wright and my other mentor at UT, Irwin C. Lieb).

What aphorism or motto best encapsulates your life philosophy?
Be the change you wish to see in the world.

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INNOVATORS & IDEAS: RESEARCH LEADER

Robin Dunbar: The neurobiology of human sociality

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Keywords: Brain evolution, primates, social networks, endorphins, mentalising

Robin Dunbar is an eminent evolutionary psychologist and anthropologist whose pioneering work has permanently redefined how we think about human social relationships. Dunbar, who is an emeritus Professor of Evolutionary Psychology at the University of Oxford, became world-renowned for, among other contributions, his formulation of "Dunbar's number," a theoretical limit to the number of stable social relationships an individual can maintain, typically cited as about 150. That idea, which he developed as he studied the relationship between brain size and social group size in various primate species, has since had broad influences across areas from social media design to organizational management. Over time, Dunbar's number has become more widely known and discussed, particularly with the advent of social media and online social networks. This has led to renewed interest in its implications for digital social interactions. Dunbar's illustrious career spans multiple prestigious institutions, including, prior to joining Oxford in 2007, the University of Cambridge, the University of Stockholm (Sweden), University College London and the University of Liverpool. He is a fellow of the British Academy and the Royal Anthropological Institute, a Foreign Member of the Finnish Academy of Science and Letters, and an elected Honorary Member of the Hungarian Academy of Sciences. His interdisciplinary expertise is reflected in his professorships in psychology, evolutionary biology, and anthropology at institutions such as Liverpool and Oxford Universities. He has held a visiting chair in statistical physics and computer science at Aalto University, Finland, which has also awarded him an honorary doctorate. Dunbar studies the evolution of social processes in primates and humans, with work that blends neurobiology, cognitive science, and the social dynamics of how we communicate via friendship networks. His insights into friendships and community cohesion continue to shape our understanding of human social behaviour in the digital age. It is an absolute pleasure that Professor Dunbar answered the Genomic Press Interview as we celebrate the extraordinary story of his scientific odyssey with our readers.

Part 1: Robin Dunbar – Life and Career

Could you give us a glimpse into your personal history, emphasizing the pivotal moments that first kindled your passion for science?

Although I went to university to study philosophy, I was quickly attracted to psychology and animal behaviour, which converted me from a humanities person to an enthusiastic scientist. The opportunity to carry out fieldwork on monkeys in Africa as an undergraduate led to a PhD in primate behavioural ecology in Ethiopia. Puzzling about the factors that determined group size in primates and other mammals led to the discovery of the social brain hypothesis (the quantitative relationship between group size and brain size) and later to Dunbar's Number (the natural size of human social groups predicted by this relationship) and the fractal structure of social networks (Dunbar Graphs) in both the animal and human social worlds.

Figure 1. Robin Dunbar MA PhD DSc(Hon), University of Oxford, UK.

We would like to know more about your career trajectory leading up to your most relevant leadership role. What defining moments channelled you toward that leadership responsibility?

Although my academic career started in Psychology, my subsequent postdocs were in Zoology Departments, where I acquired new skills in ecology and energetics, genetics, and evolution. An early interest in human evolution led to a faculty post in biological anthropology and then a swing back through the same disciplines (this time with Full Professorships), culminating in a return to my roots in Psychology at Oxford University in 2012.

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This last move back into psychology was motivated by an increasing interest in neuroimaging and what was happening beneath the brain's surface. At the same time, my work on social groups started to attract attention in computer science (and the social media industry) and in network science, leading to the parallel development of several collaborations with mathematicians and network scientists – a genuine coming together of top-down research on structural patterns in networks with bottom-up studies of the neurobiological mechanisms that create these patterns.

Please share with us what initially piqued your interest in your favourite research or professional focus area

Undertaking field studies of monkeys and ungulates (small antelopes and wild goats) in Africa and elsewhere piqued my interest in the social world. It made me think about the differences and similarities between species. Spending hours each day observing wild animals going about their daily lives, day after day, month after month, gives deep insights into what animals are doing and why.

What impact do you hope to achieve in your field by focusing on specific research topics?

The psychological and behavioural sciences typically view the social world as purely dyadic (you do something, and I respond somehow). However, in social species like primates (and, of course, humans), this is a multi-individual web of relationships – how I respond to your action depends not just on who you are but also on who your friends are and how they might see my actions. Most sciences (and medicine) ignore the most important features of our world. I aim to introduce a better understanding of this extraordinarily complex, multi-dimensional world into the psychological and life sciences. Epidemiological evidence from the last decade consistently points to the medical benefits of this multi-individual world.

Please tell us more about your current scholarly focal points within your chosen field of science

My principal focus now lies in two directions: building a better understanding of the structural constraints that limit the size of our social world (your personal social network) and achieving a better understanding of its neurobiological underpinnings.

What habits and values did you develop during your academic studies or subsequent postdoctoral experiences that you uphold within your research environment?

First, never be satisfied with an answer: the answers to all questions always raise more questions at deeper and higher levels, and pursuing these often challenges our initial assumptions and can lead to unexpected discoveries. Additionally, I have learned that it is fundamental to rely on an instinctive understanding of how the organism sees the world (based on deep ethological observation of the organism behaving in its natural environment) when framing questions and hypotheses.

At Genomic Press, we prioritize fostering research endeavours based solely on their inherent merit, uninfluenced by geography or the researchers' personal or demographic traits. Are there particular cultural facets within the scientific community that warrant transformative scrutiny, or is there a cause within science that deeply stirs your passions?

Science rises above the particularities of culture because it focuses on the fundamental principles of nature. Disciplines that have lost sight of that (as many social sciences did in the 1980s) invariably end up in various degrees of unproductive chaos – usually because they devote their time to worrying about definitions (an invention of our minds) rather than trying to understand the natural world for its own sake.

What do you most enjoy in your capacity as an academic or research leader?

Trying to unpack the puzzle that is the world. We sit amid a giant jigsaw puzzle: we have all the pieces, but we do not know where they fit. Most of us spend our time playing with the pieces in one small corner of the picture. However, if we try to spend time in other corners as well, that often helps us see the big picture faster. The magical moment when suddenly everything starts to fit, and the picture begins to emerge is exhilarating.

Figure 2. Robin Dunbar defending the future of science at the famous Speakers' Corner in Hyde Park, London, where any member of the public is, by tradition, allowed to say anything they like, no matter how controversial or treasonous, without fear of intervention by the police or the state.

Outside professional confines, how do you prefer to allocate your leisure moments, or conversely, in what manner would you envision spending these moments given a choice?

Music, hill walking (though I am getting too old to do that....), local history, reading novels, and poetry when I can find time.

Part 2: Robin Dunbar – Selected questions from the Proust Questionnaire[1]

What is your idea of perfect happiness?

Listening to music.

[1] In the late nineteenth century, various questionnaires were a popular diversion designed to discover new things about old friends. What is now known as the 35-question Proust Questionnaire became famous after Marcel Proust's answers to these questions were found and published posthumously. Proust answered the questions twice, at ages 14 and 20. In 2003 Proust's handwritten answers were auctioned off for $130,000. Multiple other historical and contemporary figures have answered the Proust Questionnaire, including among others Karl Marx, Oscar Wilde, Arthur Conan Doyle, Fernando Pessoa, Stéphane Mallarmé, Paul Cézanne, Vladimir Nabokov, Kazuo Ishiguro, Catherine Deneuve, Sophia Loren, Gina Lollobrigida, Gloria Steinem, Pelé, Valentino, Yoko Ono, Elton John, Martin Scorsese, Pedro Almodóvar, Richard Branson, Jimmy Carter, David Chang, Spike Lee, Hugh Jackman, and Zendaya. The Proust Questionnaire is often used to interview celebrities: the idea is that by answering these questions, an individual will reveal his or her true nature. We have condensed the Proust Questionnaire by reducing the number of questions and slightly rewording some. These curated questions provide insights into the individual's inner world, ranging from notions of happiness and fear to aspirations and inspirations.

What is your greatest fear?
Dying before I have had time to write all the books I want to write.

Which living person do you most admire?

What is your greatest extravagance?
Good whisky.

What are you most proud of?
Dunbar's Number.

What is your greatest regret?
No longer being able to run effortlessly.

What is the quality you most admire in people?
Honesty.

What is the trait you most dislike in people?
Not reading what I have actually written.

What do you consider the most overrated virtue?
Faith.

What is your favourite occupation (or activity)?
Writing.

Where would you most like to live?
In a small community on an island.

What is your most treasured possession?
Photographs and belongings of my ancestors.

When and where were you happiest? And why were so happy then?
Every day brings new reasons to be happy.

What is your current state of mind?
Better than it was 50 years ago.

Among your talents, which one(s) give(s) you a competitive edge?
Persistence in asking questions and seeing the link between different phenomena.

What do you consider your greatest achievement?
Dunbar's Number. It was completely unexpected, and so, at the time, it seemed just mildly interesting. Its significance became increasingly apparent later on – mainly thanks to other people's perceptiveness.

What do you most value in your friends?
Loyalty and a sense of humour.

Who are your favourite writers?
Dylan Thomas, T.S. Eliot, Robert Burns

What aphorism or motto best encapsulates your life philosophy?
"Per ardua ad astra." This Latin phrase means "through adversity to the stars." These few words do a great job representing the scientific endeavour and personal development in general. Throughout my career, as in life, the projects that have been most rewarding are often those that require the surmounting of considerable obstacles. It reminds me that the road to our highest hopes is not a smooth one, but those bumps and hedges bring value as we get there.

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INNOVATORS & IDEAS: RESEARCH LEADER

Philippe Courtet: "Hell is other people." How social pressure shapes suicidal thoughts

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Keywords: Suicidal behaviour, psychological pain, decision-making, social relationship

Philippe Courtet is a distinguished figure in psychiatry and suicidology who has significantly contributed to the field. As an influential PU-PH (Professeur des Universités-Praticien Hospitalier), he is a Professor of Psychiatry at the University of Montpellier, France, and head of Emergency Psychiatry at the University Hospital of Montpellier. Leading the Chair of Excellence in suicide prevention at the Fundamental Foundation and the 'biomarkers, environment and neuropsychiatry' research group at the National Institute of Health and Medical Research (Institut National de la Santé et de la Recherche Médicale, INSERM), Professor Courtet has focused on understanding the vulnerability to suicidal behaviour in mood disorders. His innovative work combines genomics, brain imaging, and social research. With over 500 peer-reviewed articles, an H-index of 82, and 27,000 citations, he stands as one of France's most productive psychiatrists and an international leader in suicidology. His role as Chair of the Suicidology and Suicide Prevention Section of the European Psychiatric Association further cements his influence in the field. Professor Courtet's groundbreaking research and dedication to improving care through the development of connected health tools for suicide risk assessment underscore his commitment to advancing treatment. We are pleased that Professor Courtet has participated in an exclusive Genomic Press Interview, which he entitled "*Hell is other people.*[1]" *How social pressure shapes suicidal thoughts*, offering our readers unique insights into his life and vision for the future of psychiatry and suicide prevention.

Figure 1. Philippe Courtet, MD, PhD, University of Montpellier, France.

Part 1: Philippe Courtet – Life and Career

Could you give us a glimpse into your personal history, emphasizing the pivotal moments that first kindled your passion for science?

During my medical studies, I was passionate about Surrealism, its painters and writers, which steered my choice towards psychiatry because it was the most 'cultural' of the medical disciplines. At the end of my medical studies, as soon as I started my residency in psychiatry, I combined the desire to understand these illnesses better and not be satisfied with just learning the clinical side of the discipline. The time was ripe for the advent of molecular genetics in psychiatry and the possibility of conducting association studies, with which I embarked on learning neuroscientific research into bipolar disorder and suicidal behaviour. This first encounter

[1] In the title of his interview, Professor Courtet uses a line from the French philosopher Jean-Paul Sartre (1905–1980): "Hell is other people," which goes as follows in the original French – "L'enfer, c'est les autres" or "Hell is [the] other(s)." This comes from Sartre's famous one-act play *No Exit*. According to Kirk Woodward, the best-known English translation of the play, by Paul Bowles, actually renders the line "Hell is just – other people"). We get a little more of the flavor of the line in English if we read it as "Hell is the Other." That's closer to the point, I believe. Sartre says that the Other – that which is not ourselves – is, or can be, a source of our distress. Sartre himself spelled out this meaning in a talk that preceded a recording of the play issued in 1965: *"hell is other people" has always been misunderstood. It has been thought that what I meant by that was that our relations with other people are always poisoned, that they are invariably hellish relations. But what I really mean is something totally different. I mean that if relations with someone else are twisted, vitiated, then that other person can only be hell. Why? Because. . . when we think about ourselves, when we try to know ourselves, . . . we use the knowledge of us which other people already have. We judge ourselves with the means other people have and have given us for judging ourselves. Into whatever I say about myself someone else's judgment always enters. Into whatever I feel within myself someone else's judgment enters. . . . But that does not at all mean that one cannot have relations with other people. It simply brings out the capital importance of all other people for each one of us.* Source: Rick on Theater's blog.

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with science, to see, was decisive and gave birth to a passion for clinical research. I was also lucky enough to count on excellent mentors from a very early stage who played a decisive role in my professional development, instilling in me the necessary rigor in my clinical, scientific, and managerial activities. It soon became clear to me that rigor, curiosity (and a dose of rebelliousness necessary to challenge the obvious) were essential values in my career in academic medicine.

We would like to know more about your career trajectory leading up to your most relevant leadership role. What defining moments channelled you toward that leadership responsibility?

The decisive moment was the one described above when I enrolled in a master's program to do research during my residency. Then, my passion for research was born, and the rest was a straight line. Combining clinical and scientific work has been my constant priority for the last 30 years, and in France, the only way out if you want to do both at the same time is to embark on a career in a university hospital. So, publications, projects, competitions, publications, and related academic activities led me to become a Professor of Psychiatry and rapidly the Head of a hospital department where every member of staff knows that research is as much a priority as patient care. More than decisive moments, a decisive person led me to this responsibility. My mentor, a former boss who became a friend, involved me early in managing the clinical department and strongly supported me in developing research there. We then built up a genuine research culture in this hospital department, which ultimately gave me the most legitimacy and authority with the various nursing staff. Everyone is sharing in the same adventure: care and research.

Please share with us what initially piqued your interest in your favourite research or professional focus area

Mystery! What is it that in *ictu oculi*, an individual can commit an act that will change the course of his or her life, and that of others? Why do some people act so impulsively? What happens in their mind? Why some people and not others? Where does this behaviour come from? This is how I became interested in the vulnerability to psychiatric disorders in a general sense, in the mechanisms that intervene upstream of pathologies. In a kind of coincidence that probably was not a real one, I was seduced by my training period in a department for suicide attempters and by its team and by new types of care that are far removed from traditional psychiatry (see Figure 2 for a symbolic view on teamwork). This led me to my profound interest in two aspects: (*i*) scientifically, the vulnerability to suicidal behaviour, initially from a genetic point of view and then from a cognitive, biological, or other point of view; (*ii*) clinically, reversing the way psychiatry provides care: not waiting for patients to seek care, but reaching out to them proactively.

What impact do you hope to achieve in your field by focusing on specific research topics?

My most outstanding achievement would be to contribute to suicide being more widely recognised as a medical issue and not just the consequence of a social problem, to have been able to help highlight some of the psychobiological mechanisms that are important for understanding suicide and that could perhaps lead to the development of effective interventions. My main concern remains that of the necessary evolution of psychiatric care, in a country that still has a strong tradition of psychiatric hospitalization. For example, demonstrating that suicidal patients have decision-making abnormalities that inherently prevent them from acting as psychiatrists would wish (asking for help, being compliant with care, doing as they are told…) should lead us to reverse the care proposals and go toward them where they are.

Please tell us more about your current scholarly focal points within your chosen field of science

Social and relational stress factors, which often precipitate suicidal acts, lead to social and psychological pain and cognitive impairments, and they are also potent inducers of inflammatory activity. More specifically, the aim is to understand this pathophysiology, which includes the question of the links between what is observed peripherally and centrally and the role of the blood-brain barrier. This research, which has the merit of combining physiology, brain imaging, neuropsychology, immunopsychiatry, and genomics, is all the more exciting.

Figure 2. Team life, according to the Zen teachings of harmony in Japan. This image captures the essence of teamwork and harmony as understood in Japanese Zen philosophy. The stone basin, a tsukubai, represents the team's foundation. Just as the basin holds water for purification, a team should be a source of renewal and clarity. The surrounding forest showcases the interconnectedness of all elements, mirroring how team members rely on each other. The single purple flower on the basin's rim symbolizes the unique contribution of each individual to the team's success. Together, these elements illustrate how a well-functioning team, like a balanced ecosystem, thrives on diversity, mutual support, and a shared purpose. The weathered appearance of the basin reminds us that true harmony develops over time through shared experiences and challenges overcome together.

Because physiology is at the heart of medicine, the mechanisms of interoception are fascinating for understanding how psychological pain and emotional signals are transformed into the destruction of the body that is suicide, even if cognitive interpretations blur our understanding. This field, combined with measurements of the cardiovascular stress response, now represents a significant development for us. Because care methods need to support patients as closely as possible to their lives, we are also betting heavily on the possibilities offered by connected health. All this leads us to integrate our work into the model of precision suicidology, which we hope will finally lead to real medical progress in suicide prevention.

What habits and values did you develop during your academic studies or subsequent postdoctoral experiences that you uphold within your research environment?

Collaboration. Multidisciplinary approaches. Humility. To transform suffering into pleasure, like a marathon runner.

At Genomic Press, we prioritize fostering research endeavors based solely on their inherent merit, uninfluenced by geography or the researchers' personal or demographic traits. Are there particular cultural facets within the scientific community that warrant transformative scrutiny, or is there a cause within science that deeply stirs your passions?
It is dangerous to be trying to introduce the cognitive biases of the social sciences everywhere.

What do you most enjoy in your capacity as an academic or research leader?
To have the opportunity to meet and exchange ideas with international colleagues, to arouse the interest of young psychiatrists or researchers in these subjects, and to promote them at an academic level.

Outside professional confines, how do you prefer to allocate your leisure moments, or conversely, in what manner would you envision spending these moments given a choice?
People do not change; they evolve. My passions remain those of my youth: psychiatry and art. I dedicate most of my leisure time to art, and more specifically, contemporary art, which I am lucky to share with my partner. This involves traveling, visiting museums and galleries, and meeting artists, to finally ask ourselves a lot of questions about how each of us perceives art and beauty and how art heals us.

Part 2: Philippe Courtet – Selected questions from the Proust Questionnaire[2]

What is your idea of perfect happiness?
Love and health.

What is your greatest fear?
Not achieving perfect happiness.

Which living person do you most admire?
Bullfighters who aim to create art by risking their lives.

What is your greatest extravagance?
Go to see a bullfight in Jerez de la Frontera on a whim.

What are you most proud of?
Putting psychiatry at the forefront of my institution's research teams and creating a collection of contemporary art.

What is your greatest regret?
Not being nice enough.

What is the quality you most admire in people?
Humility.

What is the trait you most dislike in people?
Narcissism.

What do you consider the most overrated virtue?
Benevolence, because the more we talk about it, the less we have it.

What is your favourite occupation (or activity)?
Visiting art fairs.

Where would you most like to live?
Latin European countries.

What is your most treasured possession?
My art collection.

When and where were you happiest? And why were so happy then?
With my partner and some very good friends sharing good wines.

What is your current state of mind?
Optimistic.

What is your most marked characteristic?
Demanding both of myself and of others.

Among your talents, which one(s) give(s) you a competitive edge?
Energy and curiosity.

What do you consider your greatest achievement?
I am proud to have succeeded in combining a career in psychiatry with my passion for collecting art, which I dreamed of doing when I was young.

If you could change one thing about yourself, what would it be?
Kinder.

What do you most value in your friends?
Freedom.

Who are your favourite writers?
Louis Ferdinand Celine, Charles Buckowski, Gabriele D'Annunzio, and Allen Ginsberg.

Who are your heroes of fiction?
Don Quixote.

Who are your heroes in real life?
Marcel Duchamp, bullfighters.

What aphorism or motto best encapsulates your life philosophy?
"Carpe Diem" – a Latin phrase meaning "Seize the day." It reminds us to make the most of the present moment rather than worrying about the future, encouraging us to embrace life's opportunities as they come and enjoy each day to the fullest.

[2]In the late nineteenth century, various questionnaires were a popular diversion designed to discover new things about old friends. What is now known as the 35-question Proust Questionnaire became famous after Marcel Proust's answers to these questions were found and published posthumously. Proust answered the questions twice, at ages 14 and 20. In 2003 Proust's handwritten answers were auctioned off for $130,000. Multiple other historical and contemporary figures have answered the Proust Questionnaire, including among others Karl Marx, Oscar Wilde, Arthur Conan Doyle, Fernando Pessoa, Stéphane Mallarmé, Paul Cézanne, Vladimir Nabokov, Kazuo Ishiguro, Catherine Deneuve, Sophia Loren, Gina Lollobrigida, Gloria Steinem, Pelé, Valentino, Yoko Ono, Elton John, Martin Scorsese, Pedro Almodóvar, Richard Branson, Jimmy Carter, David Chang, Spike Lee, Hugh Jackman, and Zendaya. The Proust Questionnaire is often used to interview celebrities: the idea is that by answering these questions, an individual will reveal his or her true nature. We have condensed the Proust Questionnaire by reducing the number of questions and slightly rewording some. These curated questions provide insights into the individual's inner world, ranging from notions of happiness and fear to aspirations and inspirations.

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INNOVATORS & IDEAS: RESEARCH LEADER

Carrie Bearden: What causes the onset of psychosis in adolescence, and how can we predict (and ultimately prevent) it?

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Keywords: Neurodevelopment, developmental psychopathology, psychosis spectrum, neurogenetic disorders, brain mechanisms

Dr. Carrie E. Bearden is a Professor of Psychiatry and Biobehavioral Sciences and Psychology at the University of California, Los Angeles (UCLA). Dr. Bearden received her Ph.D. in Clinical Psychology from the University of Pennsylvania and completed her clinical training at UC San Diego. She joined the UCLA faculty in 2003. Her work aims to understand neurobiological risk factors for the development of severe mental illness in youth, both in clinically defined high-risk cohorts and in highly penetrant genetic conditions. She is particularly known for her research taking a 'genetics first' approach to studying brain mechanisms underlying the development of severe mental illness. Dr. Bearden is the Director of the UCLA Center for Assessment and Prevention of Prodromal States (CAPPS), a clinical research program for youth at high risk for psychosis and Co-director of UCLA's Neurogenetics Training Program. She has over 350 peer-reviewed publications and is among the world's most highly cited scientists, according to Clarivate, Web of Science. Currently, she serves as Deputy (Reviews) Editor for the journal *Biological Psychiatry*, as Chair of the DSM-V Serious Mental Disorders Committee and is President-Elect of the Society of Biological Psychiatry. She has received numerous awards and honors, both for her research achievements and for teaching and mentorship, including the Joel Elkes Research Award (ACNP), the A.E. Bennett Neuropsychiatric Research Award (Society of Biological Psychiatry), and an NIH Method to Extend Research in Time (MERIT) award. Professor Bearden is delighted to engage in the Genomic Press Interview, sharing insights about her personal and professional journey with our readers.

Figure 1. Carrie E. Bearden, PhD, University of California, Los Angeles, USA.

Part 1: Carrie E Bearden – Life and Career

Could you give us a glimpse into your personal history, emphasizing the pivotal moments that first kindled your passion for science?

I mostly grew up in Hawaii, on the island of O'ahu. It was a great place to grow up to truly appreciate nature, particularly the power of the ocean and the incredible range of marine life it supports. I also loved mysteries as a child- first the Nancy Drew series, then Agatha Christie. I loved solving puzzles and envisioned a career as either a marine biologist or a glamorous private detective. In high school, my love of literature took over, and I started college at the University of California (UC) Berkeley as an English and Theater major. However, the light bulb went on when I took a Biological Psychology course in my sophomore year. What could be a better mystery to focus on than the human brain?

We would like to know more about your career trajectory leading up to your most relevant leadership role. What defining moments channeled you toward that leadership responsibility?

As a graduate student at the University of Pennsylvania in Ty Cannon's lab, I started working on very large epidemiological datasets, linking prenatal and early childhood history information to psychiatric hospital records to investigate the earliest precursors of schizophrenia. This led to some quite interesting discoveries. As early as you look, there are indicators of subtle developmental delay or differences. This was when we as a field began re-conceptualizing schizophrenia as a neurodevelopmental disorder, recognizing that the onset of full-blown psychotic symptoms represents a late stage of the illness. So this was incredibly interesting, but I also yearned to have more direct contact with patients and involvement in hands-on data collection rather than only working with large databases. Serendipitously, I began a neuropsychology placement at the Children's Hospital of Philadelphia, where through their multidisciplinary Center, I started seeing a large number of children with 22q11.2 deletions.

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I noticed that the vast majority had a characteristic cognitive and neurobehavioral phenotype. At CHOP, cutting-edge research was ongoing on the genetics and other medical aspects of the disorder, but there was no active research program at the time focused on the brain and behavioral phenotype. So I was given pretty free rein to start collecting data; retrospectively, it was an incredible opportunity for a graduate student. This was how my research interests were born and raised; with this background, when the opportunity arose to get involved in a new research program at UCLA focused on early intervention for psychosis risk, I jumped at the chance. Building this program from the ground up was quite challenging, but I am really proud of the program we have built, which is now well known for providing free access to high quality assessment and early intervention for severe mental illness.

However, regarding leadership, I did not seek it out. The process was more analogous to a twig getting caught up in a fast-flowing current. I took on leadership roles at UCLA when more senior people stepped down from those positions, and initially, it was incredibly stressful. Nevertheless, I have become much more comfortable in these roles over time. From a scientific perspective, I have enjoyed having leadership roles in major international multisite projects, where you can collaborate with people worldwide. I began to appreciate how important it is for more junior folks in an organization to see kind, compassionate leadership. And from that vantage point, I realized that if I really care about changing structures I need to forge the path. I cannot just keep my head down and focus on my own work, no matter how attractive that often seems.

Please share with us what initially piqued your interest in your favorite research or professional focus area.

Two courses at UC Berkeley – Biological Psychology and Developmental Psychopathology, taught by Steve Hinshaw- really brought things into focus for me. I wanted to understand the developing brain and what might cause that development to go awry; I was able to do a senior Honors project at UC Berkeley in the context of an advanced seminar focused on sleep. Therefore, I decided to focus my paper on nightmares and psychopathology. This got me diving into the stacks at the Biomedical Library, furiously digging up research articles and scribbling notes everywhere. Marrying my interests in detective work and science was a dream come true. And then, in graduate school, it was pure serendipity that I had the opportunity to combine my clinical and research interests, working with a youth population with a rare genetic disorder at high risk for psychosis.

What impact do you hope to achieve in your field by focusing on specific research topics?

I was very naïve when I started in this field, thinking that by focusing on a highly penetrant genetic variant with a well-understood genetic etiology, we would be able to 'solve' schizophrenia in short order. Of course, nothing is that simple, but it is astonishing when we look at how far we have come in psychiatric genetics in the past 20 years or so. That progress, combined with considerable advances in neuroimaging technology, stem cell biology, big data, and artificial intelligence (AI)– my work has become increasingly interdisciplinary. I enjoy a highly translational approach, working with big teams with diverse expertise. I firmly believe that the investigation of pre-onset or prodromal clinical risk syndromes to psychosis offers hope of overcoming reliance on a post-hoc perspective of disease causation. Further, our two-pronged strategy – that is, taking both a behaviorally defined and genetically defined approach to the problem – holds promise for understanding points of convergence along the risk pathway(s). For example, I am excited by the opportunity that we now have to connect cellular and molecular phenotypes in neurons derived from 22q11.2 deletion carriers to neurobehavioral phenotypes from the same individuals. My vision is to continue to expand this work into the development of specific molecular targets for novel preventive treatments.

Please tell us more about your current scholarly focal points within your chosen field of science.

I find the dynamic changes in the brain and accompanying behavioral shifts that occur in adolescence- and the corresponding risk for neuropsychiatric disorders – to present both an incredibly scientifically interesting and clinically important area. As a period of rapid development of social competence and increased plasticity in social-affective neural networks, adolescence provides a unique opportunity to understand how abnormalities arise in the structure and function of these brain networks, as well as an opportunity to define biomarkers for the development of treatments to improve outcomes. Interestingly, I have recently returned to my interest in sleep, sparked as an undergraduate. Sleep is still poorly understood, but it is essential for health and well-being, changing dramatically in adolescence. So, I think it holds much promise as a modifiable treatment target. A graduate student in the lab convinced me that we should venture into it in the next phase of our prospective longitudinal study; new wearable technology has made this feasible to do in kids with neurodevelopmental disorders, which was not possible before. I am now excited to see these results.

What habits and values did you develop during your academic studies or subsequent postdoctoral experiences that you uphold within your research environment?

Stubborn determination has been valuable, as has learning how to work as part of a multidisciplinary team with people who may be very different from me. The importance of kindness, compassion, and empathy cannot be overstated. I am proud of the inclusive environment we have built in our lab, aimed at making people feel supported and welcome.

At Genomic Press, we prioritize fostering research endeavors based solely on their inherent merit, uninfluenced by geography or the researchers' personal or demographic traits. Are there particular cultural facets within the scientific community that warrant transformative scrutiny, or is there a cause within science that deeply stirs your passions?

I am really concerned about academic silos and 'ivory towers', and the lack of trust in science and medicine in many communities. It is critical that we are able to make science accessible to everyone.

What do you most enjoy in your capacity as an academic or research leader?

I absolutely love mentoring trainees and seeing them get excited about a discovery, or something they are working on.

Outside professional confines, how do you prefer to allocate your leisure moments, or conversely, in what manner would you envision spending these moments given a choice?

On a steep rocky hiking trail with beautiful views. And after that, a delicious plant-based dinner.

Part 2: Carrie E Bearden – Selected questions from the Proust Questionnaire[1]

What is your idea of perfect happiness?

Sitting on a beach watching the sunset with my family. Including our dogs! And eating ice cream.

[1]In the late nineteenth century, various questionnaires were a popular diversion designed to discover new things about old friends. What is now known as the 35-question Proust Questionnaire became famous after Marcel Proust's answers to these questions were found and published posthumously. Proust answered the questions twice, at ages 14 and 20. In 2003 Proust's handwritten answers were auctioned off for $130,000. Multiple other historical and contemporary figures have answered the Proust Questionnaire, including among others Karl Marx, Oscar Wilde, Arthur Conan Doyle, Fernando Pessoa, Stéphane Mallarmé, Paul Cézanne, Vladimir Nabokov, Kazuo Ishiguro, Catherine Deneuve, Sophia Loren, Gina Lollobrigida, Gloria Steinem, Pelé, Valentino, Yoko Ono, Elton John, Martin Scorsese, Pedro Almodóvar, Richard Branson, Jimmy Carter, David Chang, Spike Lee, Hugh Jackman, and Zendaya. The Proust Questionnaire is often used to interview celebrities: the idea is that by answering these questions, an individual will reveal his or her true nature. We have condensed the Proust Questionnaire by reducing the number of questions and slightly rewording some. These curated questions provide insights into the individual's inner world, ranging from notions of happiness and fear to aspirations and inspirations.

Figure 2. Carrie Bearden hiking in the Santa Monica mountains (Westridge Trail) with her two rescue dogs Loona (female lab/bulldog mix, age 8) and Otis (male jindo mix, age 5) against the backdrop of the Los Angeles cityscape.

What is your greatest fear?
For me personally, cognitive decline. More broadly, an unlivable planet.

Which living person do you most admire?
Jane Goodall; also Greta Thunberg. They are just incredibly brave, fearless women.

What is your greatest extravagance?
Korean spa days.

What are you most proud of?
My two kids. They are both incredible human beings.

What is your greatest regret?
Not wearing sunscreen as a kid.

What is the quality you most admire in people?
Standing up for what you believe in and not giving up.

What is the trait you most dislike in people?
Hypocrisy and being a bully.

What do you consider the most overrated virtue?
Patience.

What is your favorite occupation (or activity)?
The one that I've got!

Where would you most like to live?
Right here in Los Angeles. I do occasionally fantasize about moving to the mountains, though.

What is your most treasured possession?
Photo albums of my family

When and where were you happiest? And why were so happy then?
I am pretty happy right now. But I am happiest when my whole family is together.

What is your current state of mind?
Unsettled. There is a lot to do.

What is your most marked characteristic?
My family would say that I do not compromise. I don't know if that's good or bad from their perspective!

Among your talents, which one(s) give(s) you a competitive edge?
Grit.

What do you consider your greatest achievement?
In addition to my children, all of the trainees who have gone on to flourish in their own careers. It brings me joy to see that.

If you could change one thing about yourself, what would it be?
I am an absolutely awful singer. It would be really cool to have a beautiful singing voice.

What do you most value in your friends?
Love and support. They are there for me for the good, the bad, and the ugly!

Who are your favorite writers?
Haruki Murakami, Elena Ferrante, and Annie Proulx. I am drawn to Murakami's blend of the everyday with the surreal, particularly in novels

like *The Wind-Up Bird Chronicle*. Ferrante's Neapolitan novels captivated me with their raw honesty about female friendship, and Proulx's ability to create such vivid characters and landscapes, especially in works like *Brokeback Mountain*, is remarkable.

Who are your heroes of fiction?
When I was a kid, probably Jane Marple (Agatha Christie). Also, Uncle Iroh from Avatar: The Last Airbender.

Who are your heroes in real life?
Jane Goodall, Michelle Obama.

What aphorism or motto best encapsulates your life philosophy?
Keep calm and carry on (at least I try).

Los Angeles, California, USA
23 December 2024

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INNOVATORS & IDEAS: ACADEMIC LEADER

Takeo Yoshikawa: Exploring the biological underpinnings of psychiatric disorders, such as schizophrenia, through genetics and metabolic insights

Genomic Psychiatry July 2025;1(4):24–26; doi: https://doi.org/10.61373/gp024k.0022

Keywords: Schizophrenia, genetics, energy metabolism, reductive stress, hydrogen sulfide

Dr. Yoshikawa currently serves as the Administrative Director of the RIKEN Center for Brain Science in Japan. His journey with the institution began in 1999 when he assumed the Principal Investigator (Team Leader) role and established the Laboratory of Molecular Psychiatry. Before this, he gained experience at various esteemed institutes, including the Department of Psychiatry at Tokyo Medical and Dental University, the National Institute of Physiology in Japan, and the National Institute of Mental Health (NIMH) in the USA. For 22 years, Dr Yoshikawa dedicated himself to unraveling the molecular intricacies of psychiatric diseases within his laboratory. Transitioning to his current position, he now lends his expertise to the operational endeavors of the Center. Beyond his administrative responsibilities, Dr. Yoshikawa actively engages with the academic communities, serving as a grant reviewer, scientific advisor, and editorial board member for scientific journals. Remarkably, he maintains his clinical practice, caring for patients as a psychiatrist. Dr Yoshikawa graciously participated in the Genomic Press interview, sharing insights into his life and career and providing valuable reflections for our readers.

Part 1: Takeo Yoshikawa – Life and Career

Could you give us a glimpse into your personal history, emphasizing the pivotal moments that first kindled your passion for science?

As a young child, my insatiable curiosity led me to incessantly pepper my late father with questions about the world around me. Remarkably, he always had an answer ready. While my father harbored the aspiration of becoming a researcher, the uproar of WWII steered him toward a career in teaching instead. Undoubtedly, his influence played a notable role in shaping my professional journey. Initially drawn to the complexities of physics and chemistry, I pursued my studies at the University of Tokyo. However, the growth of molecular biology in the 1970s stimulated my interest, prompting me to delve deeper into the field of biology at Osaka University Medical School. During my undergraduate tenure, I seized the opportunity to immerse myself in immunology, dedicating my spare hours to research under the guidance of Professor Tadamitsu Kishimoto, whose groundbreaking work included the discovery of IL-6 and its receptor. Despite my immersion in the intricacies of immunology, I gravitated toward psychiatry as graduation approached. Its enigmatic nature at the time intrigued me deeply, and I sensed untapped potential within the field. Additionally, perhaps subconsciously, I was drawn to psychiatry for reasons that will become clearer as I delve further into my narrative.

We would like to know more about your career trajectory leading up to your most relevant leadership role. What defining moments channeled you toward that leadership responsibility?

I completed my psychiatry training at the Tokyo Medical and Dental University. During that period, there persisted a prevailing sentiment of antipsychiatry, and research into the biological underpinning of mental illness was often dismissed. However, I embarked on psychopharmacological research, focusing on the CCK peptide, a neurotransmitter known to coexist with dopamine. My endeavors centered on elucidating how antipsychotics and psychotomimetics regulated CCK levels.

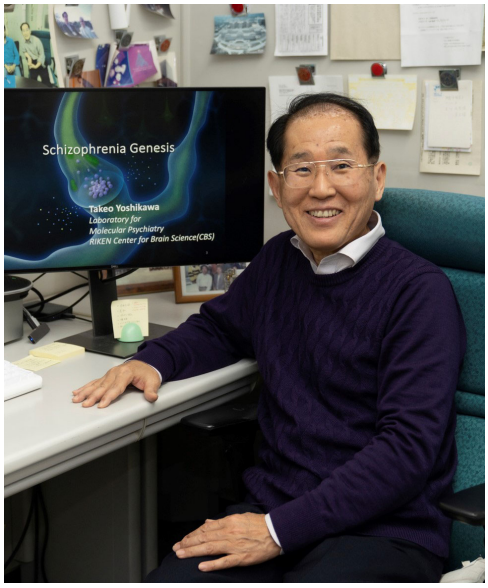

Figure 1. Takeo Yoshikawa, MD, PhD, RIKEN Center for Brain Science, Japan.

I was driven by an unwavering quest to understand the fundamental origins of mental disorders and recognized the necessity for a comprehensive understanding of neuroscience. Consequently, I joined the esteemed laboratory of Professor Kunihiko Obata at the National Institute of Physiology in Japan. Professor Obata had made groundbreaking discoveries regarding GABA as an inhibitory neurotransmitter in the central nervous system. In this new environment, I delved into investigating the molecular mechanism underlying the phenomenon of amphetamine-induced behavioral sensitization in rodents, an established animal model for schizophrenia.

Please share with us what initially piqued your interest in your preferred area of research or professional focus.

While in animal research, I sensed a notable divergence between animal models and human psychiatric disorders. By the late 1980s, some researchers began performing linkage analysis to approach the causes of psychiatric disorders more closely, sparking my interest in the human genetics approach. In 1993, I seized the opportunity to join the Neurogenetics Branch of NIMH under Dr Elliot Gershon's leadership, where intensive linkage analysis of bipolar disorder was conducted. Working under

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Dr Sevilla Detera-Wadleigh, a thoughtful mentor and exceptional researcher, I delved into genetic analysis. I consider myself fortunate to have crossed paths with her.

What were the key impact areas of your research topics?
Upon my return to Japan, I was able to establish my laboratory at RIKEN in 1999. There, I initiated a nationwide consortium aimed at gathering DNA samples for schizophrenia and other psychiatric disorders, making the commencement of our genetic studies. From the late 1990s to today, we have found ourselves amidst an exhilarating era characterized by remarkable advancement in human genetics, spurred by numerous technical and conceptual breakthroughs. While acknowledging the potency of genetics as a formidable tool, it has become increasingly evident that mental disorders exhibit extreme polygenicity. They manifest as disruption in gene expression precipitated by an amalgamation of multiple risk variants and epigenetic alternations. My laboratory has undertaken various approaches using human-derived materials to elucidate potential therapeutic interventions and yield actionable insights. These include conducting postmortem brain studies and engaging in research involving induced pluripotent stem cells (iPS).

Could you tell us more about your most relevant focal points within your chosen field of science?
Although I did not initially realize it when I embarked on my journey into psychiatry, my research journey may have been influenced by a notable experience I had as a teenager: an intense episode of depersonalization. This ordeal proved to be formidable and challenging, exacerbated by the lack of effective medication available at the time and even now. Driven by a fervent desire to comprehend the mechanisms underlying mental disorders, I have recently developed a novel hypothesis. I propose that energy metabolism within the brain plays an essential role in mental illness. Specifically, I hypothesize that excessive hydrogen sulfide production may impair energy metabolism, leading to what I term "reductive stress." Consequently, I am inclined to shift the focus of my research toward exploring the anti-inflammatory mechanisms triggered by "sulfide stress" rather than solely adhering to the conventional inflammatory theory of psychiatric illness. This approach holds particular significance for disorders such as schizophrenia, which I view as a progressive and fundamentally complex disease process.

What habits and values did you develop during your academic studies or subsequent postdoctoral experiences that you have maintained throughout your life?
If I struggle to navigate challenges and experience distress, it may signify that my approach is still tentative. However, by delving deeper into my thoughts and contemplating more extensively, I can uncover clues that will guide me toward a solution. This principle holds not only in the realm of science but also in personal life.

At Genomic Press, we prioritize fostering research endeavors based solely on their inherent merit, uninfluenced by geography or the researchers' personal or demographic traits. Are there particular cultural facets within the scientific community that you think warrant transformative scrutiny, or is there a cause within science that deeply stirs your passions?
The Internet has enabled the free sharing of resources, yet it has recently underscored English speakers' advantage in spreading scientific results. In numerous countries, particularly developing regions, it would be best for individuals to pursue scientific studies in their preferred language. The evolution of artificial intelligence (AI) presents a partial solution to this issue. I am hopeful that future progress will lead to improvements in this regard.

What have you most enjoyed in your capacity as academic or research leader?
I have found immense joy in my academic journey, navigating the challenges of psychiatric research with a foundation in clinical psychiatry and basic biology. Equally gratifying has been the opportunity to nurture a spirit of teamwork among my laboratory staff, united in our pursuit of a common goal. Witnessing their career growth has been a source of great satisfaction.

Outside professional confines, how do you prefer to allocate your leisure moments, or conversely, in what manner would you envision spending these moments given a choice?
I cherish the opportunity to travel alongside my wife, indulging in leisurely drives and unwinding in soothing hot springs known as "Onsen." Additionally, I derive immense pleasure from loading my bicycle into the car and venturing to the outskirts for cycling excursions on my days of respite.

Part 2: Takeo Yoshikawa – Selected questions from the Proust Questionnaire[1]

What is your idea of perfect happiness?
I find true fulfillment when I become completely engrossed in my thoughts, allowing myself to forget everything else around me.

What is your greatest fear?
I am accustomed not to dwelling on things too deeply. I believe that everything unfolding in our world, even in the vast expanse of the universe, is intricately intertwined with destiny.

Which living person do you most admire?
I deeply admire and am grateful to my laboratory staff, who worked tirelessly together toward our common goal.

What is your greatest extravagance?
My wife and I own a second house located 600 km away from our residence, which my wife's parents previously inhabited. One of my indulgences is embarking on journeys up the highway to visit them occasionally. We enjoy staying there, immersing ourselves in the tranquility of country life and reveling in the beauty of the surrounding nature.

What are you most proud of?
I have been fortunate and proud to pursue what I truly desire, largely due to my wife's unwavering support and dedication.

What is your greatest regret?
I refrain from dwelling on thoughts of the past.

What is the quality you most admire in people?
Compassion and empathy toward others.

What do you consider the most overrated virtues?
Every individual possesses unique virtues and strengths, each of which holds immeasurable value and significance.

[1]In the late nineteenth century, various questionnaires were a popular diversion designed to discover new things about old friends. What is now known as the 35-question Proust Questionnaire became famous after Marcel Proust's answers to these questions were found and published posthumously. Proust answered the questions twice, at ages 14 and 20. In 2003, Proust's handwritten answers were auctioned off for $130,000. Multiple other historical and contemporary figures have answered the Proust Questionnaire, including among others Karl Marx, Oscar Wilde, Arthur Conan Doyle, Fernando Pessoa, Stéphane Mallarmé, Paul Cézanne, Vladimir Nabokov, Kazuo Ishiguro, Catherine Deneuve, Sophia Loren, Pelé, Valentino, Yoko Ono, Elton John, Martin Scorsese, Pedro Almodóvar, Richard Branson, Spike Lee, Hugh Jackman, David Bowie, and Zendaya. The Proust Questionnaire is often used to interview celebrities: the idea is that by answering these questions, an individual will reveal his or her true nature. We have condensed the Proust Questionnaire by reducing the number of questions and slightly rewording some. These curated questions provide insights into the individual's inner world, ranging from notions of happiness and fear to aspirations and inspirations.

What is your favorite occupation?
Engaging in research endeavors and associated tasks, alongside providing consultation to patients.

Where would you most like to live?
I currently reside in Japan and immensely enjoy it. I relish the beauty of four distinct seasons and abundant natural landscapes. However, the region is prone to frequent natural disasters.

What is your most treasured possession?
What holds the utmost value for me are the friendships I have built.

When and where were you happiest? And why were so happy then?
In Japan, there is a saying that "misfortune and fortune are like the strands of a rope, intertwined." It is important to remain cautious during times of prosperity to preserve hope during adversity.

What is your most marked characteristic?
While I may tend to prioritize my desires, potentially inconveniencing others, I make a concerted effort to collaborate and cooperate with them whenever possible.

Among your talents, which one do you think gives you a competitive edge?
A personality that endeavors to approach things slightly differently from others.

What is a personality/characteristic trait you wish you had?
I cannot change my inherent nature, so I am content with who I am, but I acknowledge that I could benefit from cultivating a much sharper sense of humor.

What do you consider your greatest achievement?
The public stigma surrounding mental illness in Japan was notably potent. It would bring me immense satisfaction if I could have played a minor role in reducing this stigma by advocating for research into the biological aspects of mental disorders.

What do you most value in your friends?
Trust and integrity.

Who are your favorite writers?
Yasushi Inoue, the renowned Japanese writer, resonates with my mindset.

Who are your heroes of fiction?
There are many heroes to admire in Japanese manga. However, I am particularly drawn to individuals who, despite not being overly intelligent, possess a genuine and flawed humanity.

Who are your heroes in real life?
My parents and my wife.

What aphorism or motto best encapsulates your life philosophy?
If one dies after being enlightened about the true path, one has not lived in vain.

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PERSPECTIVE

Establishing validity standards for iPSC modeling of neuropsychiatric disorders

Nikki Kolsters[1], Anthony C. Vernon[2,3], Nael Nadif Kasri[1], and Brooke L. Latour[1]

Neuropsychiatric disorders impact over 3 billion individuals globally, posing significant challenges due to their molecular complexity, phenotypic diversity, and limited clinical translation of genetic insights. Advances in induced pluripotent stem cell (iPSC) technology offer unprecedented opportunities to model these disorders in human-relevant contexts. Human iPSC-derived two-dimensional neurons and glia, and three-dimensional organoids recapitulate key aspects of brain development and cellular functions, enabling the study of disease mechanisms and therapeutic responses on the relevant genetic background. Pioneering studies have begun to demonstrate the potential of iPSC models for precision medicine. However, translating these findings to clinical applications at scale requires robust validity assessments. Building on established frameworks of construct, face, and predictive validity derived from animal models, this perspective examines their application within an iPSC context. These approaches offer valuable insights to refine iPSC-based modeling systems and enhance their translational relevance as well as address the complexities of modeling neuropsychiatric disorders.

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Introduction

Neuropsychiatric disorders are a molecularly complex group of disorders that impact over 3 billion individuals worldwide and profoundly shape the social, economic, and personal well-being of those affected (1). Over the past decade, significant strides have been made in uncovering the genetic underpinnings of both polygenic and monogenic neuropsychiatric disorders through genome-wide association studies, as well as exon and genome sequencing efforts. These advances have enhanced our understanding of the mechanisms underlying certain conditions, particularly those caused by monogenic factors. Despite this progress, a considerable gap persists between these genetic discoveries and their clinical application. Insights into disease mechanisms and potential therapeutic strategies have yet to be fully translated into effective and routine clinical practice, for example, prediction of drug response, outcome, and new therapeutic targets. Moreover, the substantial phenotypic diversity and varied treatment responses seen in these disorders underscore the urgent need for precision medicine approaches—not only to design targeted therapies but also to develop robust models for understanding disease mechanisms at the individual level.

The diverse clinical manifestations, complex etiology, and limited access to patient brain tissue have curtailed an effective understanding of the molecular framework of many of these disorders. Although animal models of relevance for neuropsychiatric disorders provide valuable insights into multiple aspects of these conditions, they are limited by inherent interspecies differences, including variations in the timing and trajectory of brain development, tissue architecture, and cell-type specificity (2). Human-derived induced pluripotent stem cell (iPSC) disease modeling offers an unprecedented opportunity to study neuropsychiatric disease within the appropriate genetic context and tissue or cell types of interest. Accumulating evidence suggests that iPSC-derived models have the potential to recapitulate various molecular and cellular features of neuropsychiatric disease (3–5). Thus far, protocols for reliably generating specific cell types have been established including glial cells such as astrocytes, oligodendrocytes, and microglia and neuronal subtypes such as glutamatergic, GABAergic, dopaminergic, serotonergic, cholinergic, and motor neurons (6–14). These iPSC-derived neural cell types can be cultured alone or in combination, in two-dimensional (2D) or three-dimensional (3D) organoids, to give rise to more complex systems to study various parameters such as excitatory-inhibitory balance or model brain regions of interest. Assessment of cellular morphology, functional electrophysiological parameters, protein expression, organelle structure, and transcriptional profile can be used to characterize iPSC-derived models of neural cell types.

Protocols pioneered by the Sasai group enabling the development of 3D optic cup (15) and cortical structures (16), laid the foundation for modeling embryonic development in 3D using embryonic and iPSC cells. Human iPSC-derived 3D cultures of neural development recapitulate key aspects of human brain development including self-organizing neural architecture, cell type formation, some electrophysiological parameters, and precise spatiotemporal signaling to establish regional identity. Transcriptomic and proteomic studies of iPSC-derived brain models indicate that these models display expression profiles akin to human fetal brain (17–20) between 8 and 16 weeks postconception (21) with neuronal classes from diverse developmental stages with heterogeneous cell intrinsic maturation states (22, 23). Disease modeling with organoids is complex but holds the potential to bridge the gap between humans and animal models, offering valuable insights into disease mechanisms and treatment strategies. Brain organoids can be used to assess known genetic risk factors for structure brain defections such as macrocephaly and microcephaly and screen potential therapeutic agents, as was demonstrated for Angelman syndrome where pharmaceutical attenuation of potassium channel activity with Paxillin normalized neuronal excitability (24).

iPSC technology circumvents many obstacles currently impeding progress toward developing effective therapies for psychiatric illness. For the first time, we have the opportunity to track the developmental trajectory of neuropsychiatric disorders, investigate the role of genetic background, and study disease material throughout disease progression—rather than relying on postmortem samples taken at the end stage of disease. This approach allows us to examine disease dynamics in real time

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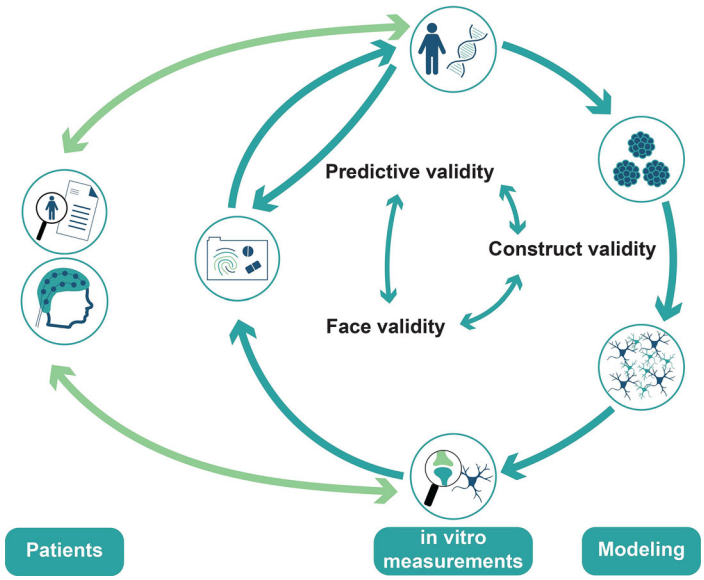

Figure 1. Interdependency of construct, face, and predictive validity. Construct, face, and predictive validity are highly interdependent. Depending on the available information, a model system can be built starting at any of the three validities. For example, if a patient's response to medication (predictive validity) and the appropriate in vitro measurement (face validity) are known, this can be used to define what cell types are needed (construct validity) to accurately model a neuropsychiatric disorder. By working closely together with clinicians, relevant patient information, like EEGs or questionnaires, can be informative to generate a patient iPSC-derived model system that has high construct, face, and predictive validity.

for fetal neurodevelopment (23, 25). However, while these advancements are promising, they also present challenges—many of which are shared with animal models. Of particular concern, how can we determine the validity of the chosen models and readouts to enable effective translation? This question is particularly pressing in the iPSC field, where bridging the translational gap remains a primary goal. To address this, we can draw valuable insights from the validity frameworks already established for animal models and by adapting such a framework, we may uncover solutions to ensure more reliable and impactful translational outcomes for iPSC models.

Classically animal models have been held to a multidimensional set of criteria of validities to be considered a relevant interface for human pathology, namely construct, face and predictive validity. The definition of construct validity in animal research is complex and the views on what it exactly entails are dependent on the author as has been extensively summarized in Lemoine and Belzung (2011). For iPCS-derived model systems, we define construct validity as follows: (1) The model system has the correct genetic etiology, for example, a relevant mutation in the causative gene for a specific disease, or a high or low polygenic risk score; (2) The biological processes underlying the disease in the relevant cell types are present (26–28). Face validity refers to the similarities between the model and the condition being modeled or essentially the extent to which a model measures the concept it is intended to measure and is therefore linked to assay validating readouts of cell-based assays. Predictive validity in animal science has traditionally been defined in one of two ways. Most commonly, predictive validity emphasizes the similarity in treatment responses between patients and the model system. However, it is sometimes defined as the model's ability to predict specific markers of the disease, referring to biomarkers used to monitor the disorder's progression (26). To achieve high predictive validity, both high construct validity and high face validity are essential. In this Perspectives, we assess the potential of this framework to be applied in the context of iPSC studies of neuropsychiatric disorders and explore how these validities pertain to such research. Below, we examine the three types of validity in detail (Figure 1).

Construct Validity

Construct validity defines the extent to which an assessment accurately measures the concept it was designed to evaluate. For iPSC-based modeling of psychiatric diseases, two critical aspects underpin construct validity: the genetic framework and cell-type specificity. iPSCs can be generated from healthy individuals where a relevant mutation can be introduced using CRISPR-Cas9, or directly from patient material. The primary advantage of using patient-derived iPSCs is that the patient's genetic background is retained during reprogramming. However, it is important to acknowledge that genomic instability and genetic alterations may occur during or after the reprogramming process, where iPSCs generated via genome integrating methods have higher incidences of genomic aberrations compared to those generated by nonintegrating methods (29, 30). These changes include chromosomal aneuploidy, copy-number variants, or point mutations and may provide mutated iPSCs with a growth advantage during extended culture, thus introducing passage-dependent effects (31). In addition, iPSCs derived from fibroblasts accumulate more mutations and chromosomal abnormalities due to repeated exposure to ultraviolet light (32). iPSCs would therefore benefit from regular assessment of genomic integrity, to ensure they remain effective for disease modeling. This consideration is particularly pertinent for polygenic neuropsychiatric disorders, whose complexity is challenging to replicate in alternative models, underscoring the unique relevance of patient-derived iPSCs (33).

Experimental designs in iPSC studies generally employ either case-control approaches or gene-editing methods using isogenic controls. A key advantage of case-control studies is that the groups can be defined solely on a patient's clinical features or their polygenic risk score, eliminating the need to identify the exact causative mutations. However, a notable limitation of case-control studies is that they typically have limited cohort sizes, resulting in low statistical power. The use of multiple isogenic iPSC-lines can help mitigate this limitation for rare cases with monogenic contributions (34). While isogenic iPSC-based study designs are widely accepted as the gold standard, a major disadvantage is that there is no patient associated with an isogenic iPSC, limiting their translational applicability.

iPSCs can be differentiated into nearly any brain cell type, providing extensive versatility in neuropsychiatric disease modeling. However, this flexibility introduces challenges in selecting the appropriate cell types to include. Decisions about the model system are often driven by pragmatic considerations, such as speed, homogeneity, and reproducibility. iPSC-derived models range from simple 2D systems involving a single cell type

to complex 3D systems comprising diverse cell types. Simpler 2D models offer advantages in terms of rapid generation, uniformity, and reproducibility, making them ideal for high-throughput applications. However, they may lack the cellular complexity required to capture the full pathophysiology of neuropsychiatric disorders. Conversely, 3D models, while more time-intensive and variable, provide a more comprehensive representation of cellular interactions and the microenvironment, which are critical for understanding multifaceted disease mechanisms. Selecting the appropriate model system for a study requires careful consideration of the specific research question and available data. One valuable source of information is postmortem analysis. For example, a recent study using postmortem cortical brain samples from patients with autism spectrum disorder (ASD) and controls found that most alterations in neuronal gene expression were localized to glutamatergic neurons in the superficial layers of the cortex (35). Alternatively, insights can also be derived from the behavioral symptoms associated with a disorder and the regions of the brain implicated in these symptoms, or from the known mechanisms of action of medications used in treatment. For instance, in attention-deficit hyperactivity disorder, dysfunction is observed in areas such as the superior longitudinal fasciculus and cortico-limbic structures, and medications like methylphenidate are known to work by blocking presynaptic dopamine and norepinephrine transporters (36, 37). Additionally, single-cell data can provide further insights on the developmental trajectory of a specific disease gene (38).

In the case of monogenic neuropsychiatric disorders, selecting the appropriate model system often depends on identifying which cell types express the gene of interest. For example, Timothy syndrome, caused by mutations in *CACNA1C* and associated with autism, bipolar disorder, and schizophrenia (SCZ) (39), exhibits the highest expression of *CACNA1C* in both excitatory and inhibitory neurons (40). Consequently, research using both mouse and iPSC-derived models has primarily focused on neuronal function. These iPSC-derived models are typically generated through an intermediate neural progenitor cell (NPC) stage, resulting in a system that includes a variety of cell types, including progenitors and multiple neuronal subtypes (41–43). However, these models often lack sufficient glial cells, which are crucial for neuronal development and function. This limitation could hinder the model's ability to fully represent the biological processes impacted by *CACNA1C* mutations. Future models incorporating glial cell populations could offer a more comprehensive understanding of *CACNA1C* deficiency. While patient iPSC-derived glial cells would be ideal to fully understand the full pathophysiology, healthy rodent glial cells have also been shown to support human neuronal function, as described by Frega *et al*., and can aid in characterizing the neuronal phenotype (14). One potential strategy is creating a chimeric model by transplanting human iPSC-derived organoids into rodent brains, as demonstrated by Chen *et al*. (2024). In this study, *CACNA1C*-deficient cortical organoids were transplanted into the somatosensory cortex of newborn rats to integrate into sensory and motivation-related circuits and evaluate an Antisense oligonucleotide (ASO)-based treatment strategy (44). This approach highlights the potential of combining in vitro patient-derived models with in vivo systems to study neuropsychiatric diseases, particularly when animal models fail to fully capture human genetics and pathophysiology. However, using animal-derived glial cells cannot exclude human-specific roles of a gene, as even low gene expression may still contribute to development of a disease.

Another example can be found in *SETD1A*, a gene with a significant, genome-wide association to SCZ (45, 46). All preclinical models, including both mouse and iPSC-derived models, for *SETD1A* have focused the role of SETD1A dysfunction in neurons (47). While it is true that, in the mouse brain, *Setd1a* is most expressed by neurons followed closely by astrocytes, in the human brain astrocytes have higher *SETD1A* expression followed closely by neurons (40, 48). For this reason, it follows logically that iPSC-derived model systems with good construct validity should contain both neurons and astrocytes with a *SETD1A* deficiency. To date, there are two studies using different iPSC-derived neuronal models, however, neither study has included *SETD1A*-deficient astrocytes (49, 50). While these studies have provided valuable insights into *SETD1A* deficiency in neurons, a key advantage of iPSC-based models is the capacity to incorporate diverse cell types. For *SETD1A* research, this means that a future model could integrate iPSC-derived astrocytes, allowing for a more comprehensive investigation into *SETD1A*-related pathology.

Although the concept of construct validity may seem straightforward when a disorder has a clear genetic cause, many neuropsychiatric disorders are understood to be highly polygenic, involving thousands of common and rare genetic variants (51). These genetic factors, combined with environmental risk factors, increase the likelihood for an individual to develop a neuropsychiatric condition. While the use of patient-derived iPSCs addresses the genetic etiology aspect of construct validity, identifying the relevant cell types and biological processes remains challenging when the specific genes involved in the disorder are unknown. In recent years, the Ziller lab has addressed this issue using a large cohort ($n = 104$) of iPSCs from healthy controls and individuals with SCZ, bipolar disorder, and major depressive disorder. They found differences in alternative polyadenylation (APA) in the 3′ untranslated region of many transcripts related to synapse biology between iPSC-derived neurons from patients with SCZ and healthy donors. These differences were associated with a reduction in synaptic density on the cellular level. In addition, they showed that 3′APA was highly correlated with SCZ polygenic risk and concluded that the cumulative effects of polygenic risk converge on 3′APA as a common molecular mechanism underlying synaptic impairment in SCZ (52). An alternative strategy could be to use models that encompass most cell types, like cerebral organoids, which may provide a more comprehensive understanding of polygenic risk in diverse cellular populations. In addition, it is important to calculate the polygenic risk score for each individual, not just for the disorder of interest.

Face Validity

Face validity is essentially the degree of descriptive similarity between a model and an individual affected by a neurobehavioral disorder. This concept was initially defined within the context of depression by Wilner to encompass both treatment and symptomatic features, specifically the response to pharmacological intervention and the experiential profile (27). Geyer and Markou, and Sarter and Bruno, expounded upon face validity to mean "the degree of phenomenological similarity between the model and the disorder to be modeled" (53, 54). This suggests that face validity corresponds to the ability of a model system to mimic (generally behavioral or cognitive) diagnostic criteria of psychiatric conditions, yet it remains largely uncharacterized at the molecular level. As psychiatric disorders are defined primarily based on behavior, something iPSCs are inherently unable to model, the focus of translational models must shift to the molecular and cellular level. Despite this necessity for physiological and molecular profiling, we currently lack clear biomarkers and cellular profiles of neuropsychiatric disorders. Additionally, for most studies it is difficult to assess face validity since there is often no golden standard data, such as fetal brain tissue from the case of interest.

Despite challenges, there are credible examples of face validity within iPSC-derived models that demonstrate dimensions of neuropsychiatric disorders in large part due to the inclusion of patient data. de Vrij *et al*. adopted a family-based patient assessment approach for genetic discovery in SCZ coupled with functional analysis using patient-derived iPSCs to define variants in chondroitin sulfate proteoglycan 4 (*CSPG4*), an oligodendrocyte progenitor specific marker, as a potential cause of familial SCZ (55). This approach allowed researchers to characterize genetic and functional evidence of oligodendrocyte progenitor cell dysfunction in SCZ. In some cases, in vivo measurements in patients can be translated into molecular insights. 22q11.2 Deletion syndrome is associated with increased SCZ risk, in a pilot study, dopamine synthesis capacity was assessed via ^{18}F-DOPA PET imaging in patients with 22q11.2 deletions. By generating iPSC-derived dopaminergic neurons from these patients, they observed alterations in gene expression related to dopamine metabolism and signaling, with differences noted between 22q11.2 hiPSC lines corresponding to distinct clinical presentations (56) suggesting that dopamine metabolism dysfunction may contribute to SCZ.

Another potential strategy to circumvent the absence of behavior modeling is to focus on the underlying neuronal patterns that drive the behavior. For example, Romero-González *et al*. recently demonstrated

that in a cohort of children with ASD, those with greater impairment in executive functioning also exhibited abnormal epileptiform electroencephalography (EEG) activity (57). EEG abnormalities in patients are significantly elevated in patients with neuropsychiatric disorders, and current research is evaluating the use of EEG as a diagnostic tool (58, 59). Similar to EEGs, which measure the summation of synchronous activity in the brain (60), the synchronous activity of iPSC-derived neuronal models can be assessed using microelectrode arrays (MEAs). MEAs can be used to study neuronal activity patterns relevant to human neurological conditions in both 2D networks and brain organoids (61–63). For instance, Trujillo *et al*. showed that cortical organoids have similar developmental trajectories, specifically pertaining to frequency of oscillations and duration of events (63), in their functional neural network activity as those observed in neonatal human EEGs (64). These results indicate that interrogating developmental oscillatory patterns in neuropsychiatric and developmental disorders may offer valuable perspectives into both normal and abnormal brain development and function. These insights, coupled with the findings that administration of the benzodiazepine drug diazepam—known to facilitate GABAergic signaling—to organoids decreased spiking complexity within neural circuits, suggest that both neural circuitry abnormalities and neuropsychotropic drugs can be assessed via this platform (65).

Molecular insights into disease can be gleaned with the translation of patient data into in vitro studies which can then be translated back into the clinical setting. In ASD, Bruining *et al*. developed an algorithm to estimate the excitation-inhibition (E/I) ratio using EEG to assess neural oscillations, the functional E/I ratio was capable of detecting E/I shifts associated with pharmacological intervention in human EEG. E/I ratio profoundly affect optimal processing of stimuli (66) and aberrancies therein interrupt neuronal network dynamics and impair their function (67). This approach was validated in nonmedicated children with ASD and in healthy controls under pharmacological enhancement of GABAergic synaptic inhibition (68). Measuring the E/I ratio at the cellular level may provide a translational link between patient data informed in vitro models and the clinic. Additionally, using disease signatures of neural activity can offer insights into assessments of neural circuitry in patient-derived organoids. Using calcium imaging and extracellular recording to assess local field potentials, Samarasinghe *et al.* demonstrated that brain organoids derived from individuals with Rett syndrome (RTT), displayed complex circuitry dynamics akin to intact brain preparations and demonstrated a deficit in low frequency oscillations and frequent epileptiform-like activity (69). Additionally, they discovered that the antiapoptotic, p53 inhibitor pifithrin-α rescued many of these physiological parameters within the organoid model.

Additional examples of face validity within iPSC-derived neural systems include the ability to recapitulate macrocephaly and microcephaly in neural organoids, which is validated by their clear structural readout. Urresti *et al*. demonstrated that cortical organoid model the macrocephalic and microcephalic effects of the reciprocal deletion and duplication, respectively, of the 16p11.2 region associated with ASD (70). Morphological measures of the brain could also serve this purpose. For Williams syndrome, Chailangkarn *et al*. generated patient iPSC-derived NPCs and cortical neurons, demonstrating that increased NPC proliferation and apoptosis could be traced to a single gene, FZD9, within the Williams-Beuren Syndrome Critical Region. These findings were further supported by morphological abnormalities observed in postmortem brain tissue, particularly in neurons from cortical layers V and VI (71). Non-neuropsychiatric disorders, including epilepsy and neurodegenerative diseases, offer a potentially more straightforward approach for modelling diseases using iPSCs, owing to their distinct cellular phenotypes and potential for electrophysiological readouts. However, these models face shared challenges with neuropsychiatric disorders that need to be fully realized for accurate disease modeling, such as reflecting developmental timepoints that may precede disease onset by decades.

Predictive Validity

To assess the validity of a model system, the inclusion of positive and negative controls is essential. Considering that there are currently no known biomarkers for neuropsychiatric disorders, we will define predictive validity as the ability of the model system to replicate treatment responses observed in patients using relevant measurements. For example, lithium has been widely used to treat mania in bipolar disorder. Mertens *et al*. generated iPSC-derived hippocampal dentate gyrus granule-like neuronal networks from both lithium responders and nonresponders. Their study showed that lithium treatment induced significant changes in the neuronal networks of lithium responders, while it had no apparent effect on the neuronal networks of nonresponders (72). Interestingly, this suggests that a model containing only iPSC-derived dentate gyrus cells could suffice for building a predictive model for bipolar disorder, as it effectively differentiates between lithium responders and nonresponders. This study highlights the value of incorporating clinical insights at the individual level to guide experimental design. An extension of this study showed this could also be replicated in cortical organoids derived from lithium responders and nonresponders (73). Instead of prioritizing construct and face validity first, identifying known medication responders and nonresponders can help determine the most suitable iPSC-derived model for a disorder. It is worth noting that it may be difficult to accurately define treatment-resistant groups and therefore, close collaboration with clinicians and use of established guidelines or standards for diagnosing treatment resistance is advantageous.

Another study that utilized this concept used iPSCs from clozapine responders and nonresponders. Here, they showed that clozapine increased activity in neuronal networks from both control and clozapine responders, while there was no effect for clozapine nonresponders (74). Unfortunately, the available clinical information is not always applied effectively. In one study, researchers used iPSC-derived neuronal cultures from a patient that clinically has no response to clozapine, yet they did not include clozapine as a positive control (75). In contrast, there are instances where clinical information is unavailable. For example, a study aiming to model disease predisposition used iPSCs lines from multiple patients attempted to investigate whether antipsychotic manipulation could rescue deficits in NPC migration during in vitro neurodevelopment. However, the absence of detailed medication histories made it unclear whether the tested medications had been clinically effective for those patients (76). That being said, the readouts used in this study did not reflect aspects of adult treatment efficacy, suggesting limitations in the model's face validity and highlighting how all three validities contribute to a valid stem cell model.

Recently, the US Food and Drug Administration (FDA) approved a new therapy for RTT, consisting of a peptide fragment of insulin-like growth factor 1 (IGF-1) which was shown to restore multiple aspects of RTT pathology in a mouse model (77, 78). This information was later used to develop a predictive iPSC-derived model for RTT by Marchetto *et al*. (2015), who generated both NPCs and neurons from clinically affected female patients with RTT. Here, they showed that RTT patient-derived neurons have reduced number of synapses and dendritic spines, as was previously shown in the mouse model. In addition, IGF-1 treatment was able to recover the synapse number back to control levels (79), suggesting that this iPSC-derived model can be used for translational purposes and discovery of additional novel treatments for RTT.

To assess the potential for precision therapy in early infantile epileptic encephalopathy type 13, also known as SCN8A-related epilepsy, Tidball *et al.* treated iPSC-derived excitatory neuron from three patients with missense variants in *SCN8A* (80). They were able to show within their model system that iPSC-derived neurons displayed altered sodium currents and treatment with riluzole, a drug used to treat amyotrophic lateral sclerosis, reduced spontaneous firing and heightened the action potential firing threshold. As a result of this study, riluzole was prescribed off-label to 2 patients whose iPSC-derived neurons demonstrated responsiveness.

Many neuropsychiatric disorders are managed through antipsychotics, antidepressants, mood stabilizers, and stimulants. Despite significant efforts, the current psychiatric medications show little improvement in effectiveness or functional outcomes compared to the original treatments introduced over 50 years (81). The development of patient-derived iPSC-based model systems offer the potential for testing future novel medications in a more personalized manner. For example, the FDA has

recently approved a new therapy for schizophrenia (82). Inclusion of known responders and nonresponders from these clinical trials in iPSC studies could validate these models, resulting in a model that is more suited for forward translation and discovery of new therapeutic approaches. To achieve this, it is essential to establish a model system with high predictive validity. However, before a model can provide meaningful insights into the efficacy of new medications, its predictive validity must be established using positive and negative controls based on a patient's medical history at the individual level.

Discussion

One limitation of iPSC-derived model systems is their restriction to early prenatal neurodevelopmental stages. For instance, on a gene expression level, brain organoids replicate cell states normally observed in the first and second trimester but generally fail to capture later developmental stages (23, 83). Similarly, 2D neuronal cultures most closely resemble fetal brain tissue (17, 84, 85). Considering that neuropsychiatric disorders can emerge anywhere from childhood through adulthood, iPSC-derived models are therefore best suited to study disease predisposition rather than fully modeling the disease itself. A critical challenge in iPSC-based research is therefore identifying measurable features that are related to clinical manifestations of the disorder. For example, neuropsychiatric disorders are primarily defined by behavioral characteristics, a trait that iPSC-derived model systems inherently cannot replicate. This limitation not only affects the ability to model these disorders but also complicates the validation of these models and requires alternative approaches to capture this aspect of the disorder. One potential strategy could be to compare electrophysiological MEA recordings from patient iPSC-derived neuronal models to a control cohort to reveal neuronal mechanisms underlying the behavioral symptoms, functioning as an effort to capture a phenomenon that iPSCs are inherently unable to model and providing a basis for model validation. Another possible approach is to analyze the transcriptional signature underlying brain activity. Although establishing a direct correlation between brain activity and transcriptomics in the human brain is ethically and clinically challenging, Bahl *et al*. recently developed a deep learning toolbox designed to predict neuronal activation based on transcriptomic signals (86). Likewise, transcriptional signatures of iPSC-derived neuronal networks can be directly integrated with electrophysiological profiling using MEA recordings (87). This integration enables the identification of disorder-related pathways and opens opportunities for future therapeutic strategies.

The development of iPSC-derived models in recent years has revolutionized the study of human brain development, providing opportunities to model complex disorders *in vitro*. However, these advancements have also introduced new challenges, particularly in selecting the most appropriate model to address specific research questions. Drawing on examples from animal models of neuropsychiatric disorders, the well-established validity framework can serve as a foundation for selecting the most suited iPSC model. This framework is built upon three key types of validity, specifically construct, face, and predictive validity, each of which plays a critical role in model development and is interdependent. Conceptually, any of the three validities could serve as a starting point. For instance, in the case of monogenic disorders, it would be logical to start with construct validity. Alternatively, clinical data could be informative for the predictive validity, particularly when information about known responders and non-responders to a specific medication is available. With proper rigor and validity standards, iPSC modeling of neuropsychiatric disorders stands to provide insights that contribute to elucidating disease mechanisms as well as prognostic and preventative indicators of disease. Predictive iPSC modeling of disease would benefit from the synthesis of data from patients and data gleaned from current cellular models to generate prediction models of biological patterns and mechanisms and how they relate to disease.

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Author Contributions

N.K., A.C.V., N.N.K., and B.L.L. conceived the ideas and wrote the manuscript.

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Author Disclosures

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PERSPECTIVE

Illuminating synucleinopathies: Advances in α-synuclein PET tracer development for in vivo neuroimaging

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Abnormal α-synuclein aggregation is a pathological hallmark of Parkinson's disease, multiple system atrophy, and dementia with Lewy bodies. A suitable radiotracer that can noninvasively map synucleinopathies through positron emission tomography (PET) will lead to breakthroughs in early diagnosis, monitoring disease progression, and evaluating treatment responses. However, the development of PET tracers for α-synuclein is lagging due to several challenges. In this perspective, we provide a brief review of the advancements in PET tracers targeting α-synuclein and summarize recent clinical studies aimed at mapping synucleinopathies in neurodegenerative patients using these PET tracers.

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Aggregation of α-synuclein is found in Lewy bodies (LBs) and Lewy neurites (LNs), which are the pathological hallmarks of Parkinson's disease (PD) and related disorders known as synucleinopathies (1, 2). However, the regional distribution, conformation, and seeding capacity of synucleinopathies are heterogeneous among the diseases. In PD, neuronal inclusions of LBs and LNs first emerge in the brainstem, spreading through the midbrain/substantia nigra to the medial temporal cortex, and through the mesocortex to the neocortex (3). In comparison, the cellular pathology of dementia with Lewy bodies (DLB) is characterized by glial cytoplasmic inclusions found in oligodendrocytes, which are particularly prominent in the white matter of the brainstem and cerebellum (4). SNCA gene located in chromosome region 4q21-4q23 is responsible for encoding the α-synuclein protein in humans. The protein consists of three distinct regions: (1) an amphipathic domain (residues 1–60) that contains apolipoprotein lipid-binding motifs, which are predicted to form amphiphilic helices, thereby conferring a propensity to adopt α-helical structures upon membrane binding, (2) a non-amyloid β-component (NAC) (residues 61–95), which is crucial for potential β-sheet aggregation, and (3) an acidic domain that is highly negatively charged and prone to being unstructured (Figure 1) (5–7). In 1997, Polymeropoulos *et al.* identified the first missense variant in SNCA, leading to an A53T amino acid change and, as a result, a prolonged β structure that is prone to aggregation (8). Since then, several other point mutations in SNCA have been reported, including E46K (9), G51D (10), E83Q (11), and V15A (12) depicted in Figure 1. Nevertheless, a common mechanism by which SNCA point mutations might result in synucleinopathies has yet to be discovered, indicating that the diverse genetic architecture may influence clinical and pathological presentations through distinct signaling pathways (13). In addition, a diverse set of variants contributing to mitochondrial and mitophagy function, lysosomal and trafficking pathways, and so on, are inversely or directly correlated with the level of synucleinopathies, such as LRRK2, PARK7 (also known as DJ-1), PRKN (also known as PARK2), and PINK1 (14).

Molecular probes with an appropriate affinity, high selectivity, and specificity in vivo for α-synuclein will be useful in the understanding and monitoring of synucleinopathy-related diseases using positron emission tomography (PET). This could be exemplified by radioligands targeting tauopathies, which provide PET-based Braak staging as an effective method to differentiate between phases of the AD continuum (15). Our recent studies have shown that synaptic loss in the brain measured by [^{18}F]SynVesT-1, a ^{18}F-radiolabeling radiotracer that targets synaptic vesicle glycoprotein 2A, correlates with clinical, fluid, and imaging biomarkers of neurodegeneration (16, 17). This finding supports the use of PET imaging as a valuable tool for assessing interconnected pathologic processes, including pathologic structures, neuroinflammation, and synaptic dysfunction. Furthermore, *in vivo* PET quantification can expedite drug discovery and development by providing valuable information on accessing target occupancy and monitoring treatment feedback. Aβ-targeting monoclonal antibodies such as aducanumab and lecanemab gained accelerated FDA approval based on the reductions of amyloid-β (Aβ) PET signals in clinical trials. Undoubtedly, there is much interest to develop radiotracer for mapping synucleinopathies in the brain regions. In this perspective, we aimed to briefly review the development of PET tracers targeting α-synuclein (Figures 2 and 3) and highlight recent advances that may illuminate the path for future development.

Thioflavin-T derivative [^{11}C]Pittsburgh compound-B ([^{11}C]PIB) and benzoxazole [^{18}F]BF227 were first investigated as non-selective probes for β-sheet structures of α-synuclein aggregates. Using α-synuclein or Aβ_{1-42} fibrils, [^{18}F]BF227 showed high binding affinity to β-sheet structures of both species with two classes of binding sites on Aβ_{1-42} fibrils (dissociation constants $K_{d1} = 1.31$ and $K_{d2} = 80$ nM, respectively) and one class of binding sites on α-synuclein fibrils ($K_d = 9.63$ nM) (18). Fluorescent BF227 staining of the substantia nigra from patients with PD showed also colocalization with immunohistochemistry staining using an α-synuclein–targeting antibody. However, no binding of [^{18}F]BF227 was detected in pure DLBs homogenates in the absence of Aβ plaques. This observation suggests that the structure of recombinant fibrils may not accurately reflect the structure of fibrils existed in brain homogenates, particularly due to the potential impact of posttranslational modifications and protein interactions on the conformation of β-sheet structures and the accessibility of binding sites *in vivo*. Bagchi and Yu *et al.* subsequently demonstrated that the phenothiazine analog [^{125}I]SIL23 bound to a site identified on recombinant α-synuclein fibrils, as well as to fibrillar α-synuclein in LBs and LNs found in the brains of patients with PD (19). The density of corresponding binding site was proven to be sufficiently high to be detected by PET imaging using high affinity

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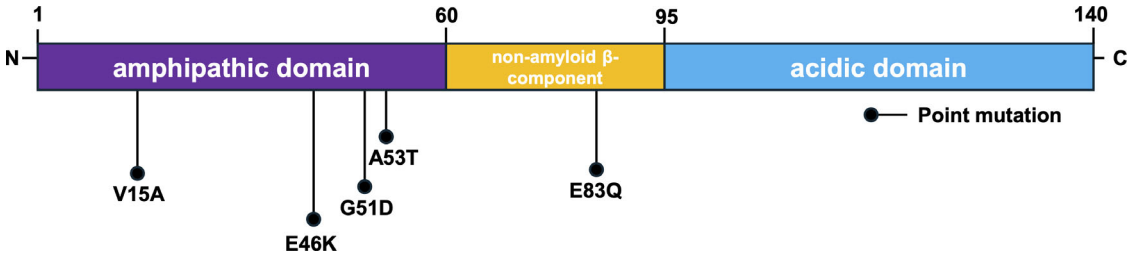


Figure 1. Schematic of α-synuclein domains.

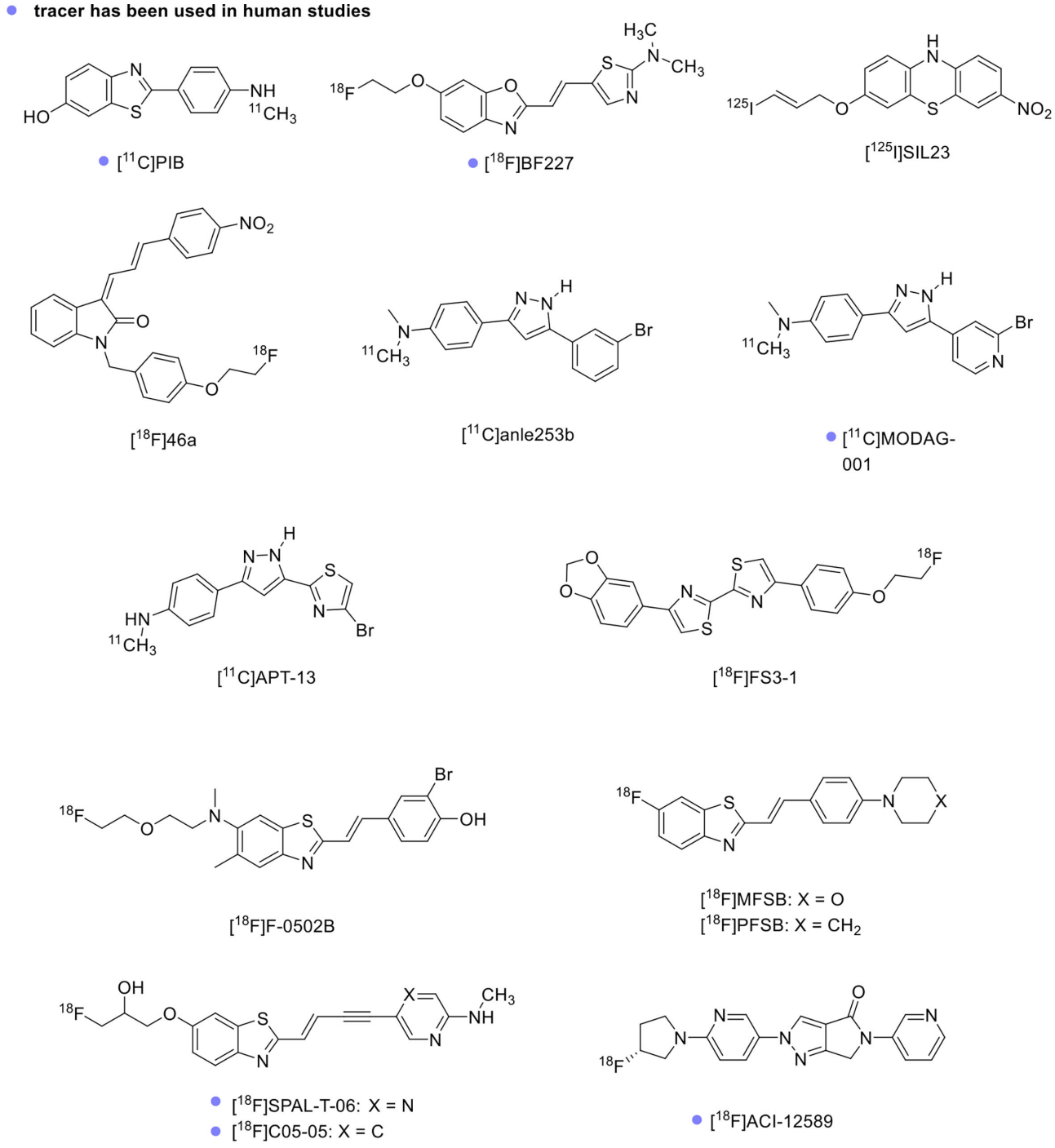
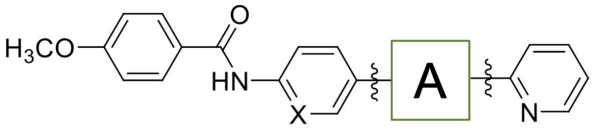
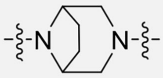
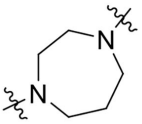
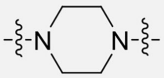
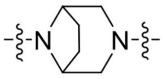


Figure 2. Chemical structures of α-synuclein PET tracers.

A



Cpd.	X	A	a-syn K_i (nM)	Aβ K_i (nM)
4f	H		21.9 ± 9.7	≥ 760
4i[a]	H		6.1 ± 2.1	N. A[b]
4m	H		8.7 ± 0.6	>1000
4o	N		4.9 ± 0.4	43.1 ± 3.8
4p	N		44.1 ± 32.0	N. A

B

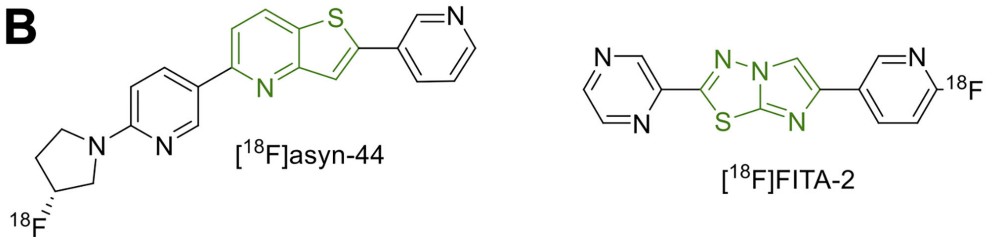


Figure 3. (A) Structure-affinity relationship of **4i** and its derivatives (32). [a]The compound was labeled with carbon-11 and evaluated as a PET tracer targeting α-synuclein. [b]N.A stands for not active (no displacement at 1000 nM). (B) Chemical structures of [^{18}F]asyn-44 and [^{18}F]FITA-2.

radioligands. However, not yet a tricyclic structure based on SIL23 has been reported for imaging synucleinopathies in patients. [^{18}F]46a reported by Chu *et al.* was optimized from selective fluorescent dyes and displayed good selectivity on α-synuclein fibrils (20). The high lipophilicity and potential reduction of the nitro group in the structure were believed to contribute to a high level of nonspecific binding in vivo, hindering its application as a suitable PET probe for neuroimaging. Inhibition pathological aggregation of prion protein and α-synuclein using diphenylpyrazole led to the discovery of [^{11}C]anle253b, from which [^{11}C]MODAG-001 was developed (21, 22). In dynamic PET imaging of normal mice, [^{11}C]MODAG-001 demonstrated good brain penetration; however, several radiometabolites were identified in the brain homogenates at 5 min postinjection. Further *in vitro* autoradiography studies showed no significant binding on brain sections from patients with Lewy body dementia using [^{3}H]MODAG-001. Like [^{18}F]46a, its high log*D* value was proposed as the reason for the low signal-to-noise ratio in human brain tissue with synuclein pathology. Arylpyrazolethiazole derivative [^{11}C]APT-13 was recently reported to have a K_i value of 27.8 ± 9.7 nM and a 3.3-fold selectivity over Aβ. In preliminary studies, it exhibited high initial brain penetration in healthy mouse brains and emerged as a lead for further development (23). Diarybisthiazole derivative [^{18}F]FS3-1 showed promising sensitivity in a rat model overexpressing human E46K-mutated α-synuclein (24).

Meanwhile, numerous structures containing polyphenols such as gallic acid and flavonoids such as quercetin have been found to have good inhibitory effect on α-synuclein aggregation (25, 26). It's hypothesized

that phenol or catechol group promotes the interaction with α-synuclein–containing species. Accordingly, the dimethylamino group on BF227 may be accountable for its affinity toward Aβ fibrils, sabotaging its specificity. A novel PET tracer targeting α-synuclein ([^{18}F]F-0502B, Figure 2) has recently been reported (27). It is structurally similar to [^{18}F]BF227 but features a free phenol group, with molar activities ranging from 18.5 to 37 GBq/μmol. Using saturation binding assays, [^{18}F]F-0502B exhibited K_d values of 3.68, 107.8, and 151.2 nM in brain homogenates from PD, Alzheimer's disease (AD) with Aβ fibrils and AD with tau fibrils, respectively, demonstrating its specific interactions with α-synuclein fibrils. In healthy non-human primates, [^{18}F]F-0502B showed an initial brain uptake with a standardized uptake value (SUV) below 1.5 and fast washout within 5 min postinjection. The tracer was further investigated in non-human primate models of PD with nigrostriatal degeneration induced by intracranial injection of adeno-associated virus encoding A53T mutant human α-synuclein (AAV-A53T-α-Syn) or preformed fibrils of α-synuclein. PET images averaged from 30 to 60 min postinjection of [^{18}F]F-0502B showed a higher radioactivity accumulation in the striatal regions of PD models compared with the control group. Further investigations, including radiometabolite analysis, kinetic modeling, and human translation, are warranted to see whether [^{18}F]F-0502B could serve as a useful tracer for imaging α-synuclein aggregates in living patients. Notably, Maurer *et al.* disclosed the development of a library of 2-styrylbenzothiazoles in 2023 (28). Using human recombinant α-synuclein fibrils and [^{3}H]PIB, less lipophilic analog MFSB also exhibited enhanced affinity to α-synuclein aggregates ($K_i = 10.3 \pm 4.7$ nM) compared with that of PFSB. PET imaging demonstrated significant brain penetration of [^{18}F]MFSB, with a SUV of 1.79 in healthy wild-type mice. However, the slow washout of [^{18}F]MFSB from the brain, along with increased radioactivity accumulation in white matter–rich areas such as the midbrain and brainstem, indicates a high degree of nonspecific binding in vivo.

[^{18}F]SPAL-T06 with an (*E*)-hex-2-en-4-yne linker in the backbone structure was reported by Higuchi *et al.* (29). The affinity of [^{18}F]SPAL-T06 was determined to be 2.49 nM using putamen homogenates of patients with multiple system atrophy (MSA) with predominant parkinsonism. It successfully visualized the synucleinopathies in patients with MSA without Aβ deposition. However, its rapid metabolism and, consequently, insufficient intact radioligand in the bloodstream preclude its ability to capture α-synucleinopathies in cases of PD and DLB with low target abundance. The same group further developed [^{18}F]C05-05 to overcome the rapid clearance issue of [^{18}F]SPAL-T06 (30). In in vitro autoradiography, [^{18}F]C05-05 showed specific accumulation in the amygdala from a patient with DLB and substantia nigra from a patient with PD with dementia. The concentrations of this tracer to induce 50% homologous inhibition were determined to be 1.5 and 1.7 nM in DLB and MSA homogenates, respectively. Ten patients meeting clinical diagnostic criteria for PD or DLB, and eight healthy controls were included for an exploratory clinical PET study of [^{18}F]C05-05. The study focused on the synucleinopathies in the midbrain, as the midbrain substantia nigra is a common area affected by Lewy pathologies in "body-first" and "brain-first" subtypes of PD and DLB at a clinical stage. Using the deep white matter as the reference region, subjects in PD/DLB group showed significantly higher ratios of $SUV_{midbrain}/SUV_{reference\ region}$, which is well correlated with the degree of motor impairments assessed by Movement Disorder Society revised Unified Parkinson's Disease Rating Scale part III scores. Although patients with AD pathologies were pre-excluded, this study provided the first essential evidence on the capability of PET probe for imaging α-synuclein pathologies in humans. However, it is important to note that both [^{18}F]SPAL-T06 and [^{18}F]C05-05 interact with the groove-like binding pocket in the β-sheet structure of α-synuclein fibril cores. This binding characteristic complicates the achievement of high selectivity over tau pathology, due to the significant resemblance of cross-β structures. Consequently, the accumulation of radioactivity from these tracers in vivo will be confounded by the presence of tau aggregations. Meanwhile, the increased nonspecific binding of [^{18}F]C05-05 to myelin components may potentially elevate background noise in other regions with high white matter fractions. Further studies in a relatively large cohort are warranted to investigate whether the aforementioned factors have a profound impact on its clinical value regarding disease progression and companion diagnosis.

[^{18}F]ACI-12589, developed by the biotech company AC Immune, was recently published and demonstrated promising results in distinguishing MSA from other neurodegenerative diseases (31). By *in vitro* autoradiography, the K_d values of [^{3}H]ACI-12589 was estimated to be 17 nM using brain tissues from a familiar PD and 28 nM using brain tissues from a MSA case. High-resolution autoradiography revealed radioactive accumulation on individual α-synuclein inclusions, aligning with the pathologies identified through immunohistochemical staining. Using AD tissues with Aβ and tau aggregates, [^{3}H]ACI-12589 showed a K_d value of 300 nM and a low maximal binding capacity, indicating its excellent specificity towards α-synuclein pathologies. In the preliminary clinical studies, compared to patients with PD, DLB, and healthy controls, participants diagnosed with MSA exhibited a greater retention of [^{18}F]ACI-12589 in the cerebellar white matter, particularly in the phenotype dominated by parkinsonism (MSA-P) rather than in the phenotype dominated by cerebellar ataxia (MSA-C). The low density of α-synuclein pathologies, along with the variations in conformation and posttranslational modifications across different synucleinopathies, may explain the lack of specific accumulation in PD and DLB. Impressively, the study further included participants with progressive supranuclear palsy (PSP, three cases), hereditary ataxias (two cases), and AD (five cases). The radioactivity accumulation of [^{18}F]ACI-12589 exhibited overlaps with pathologies identified by the tau tracer [^{18}F]RO948, but showed a weak correlation with the positive regions observed by the Aβ PET tracer [^{18}F]flutemetamol. Similarly, the retention of [^{18}F]ACI-12589 in PSP matched the expected tau pathology. This may be clarified by the co-pathologies of α-synuclein in AD and PSP. Further characterizations of the tracer's binding sites in various neurodegenerative diseases, along with an in-depth clinical investigation involving larger patient cohorts, would provide an answer.

Immense efforts have been devoted to developing α-synuclein PET tracers with enhanced selectivity. Mach *et al.* introduced heterocyclic moieties, such as diazaspirocyclic or bridged amino cores, to replace the piperazine in the nonselective lead compound (32). The key structure-affinity relationship is illustrated in Figure 3A. In comparison to compound **4o**, linking the 4-methoxy-N-phenylbenzamide and pyridine with either 2,8-diazaspiro[4.5]decane (**4f**), 1,4-diazepane (**4m**), or 3,8-diazabicyclo[3.2.1]octane (**4i** and **4p**) enhanced the selectivity of the structures toward α-synuclein. [^{11}C]**4i** was subsequently obtained with high molar activity (106 ± 56 GBq/μmol) and peaked brain uptake in non-human primates with SUV values of 1.68 ± 0.54 at 4 min postinjection. *In vitro* binding assays using [^{3}H]**4i** suggest an off-target to 4R tau, which may limit the application of [^{11}C]**4i** in certain circumstances. [^{3}H]asyn-44, featuring a pyridothiophene core structure, was reported by Neil *et al.* to have a potent K_i value of 1.85 nM using PD homogenates (Figure 3B, left) (33). In in vitro autoradiography studies, it generated a distinct radioactive signal in brain sections from MSA and PD, aligning with neuropathology visualized through anti-pS129 α-synuclein immunohistochemistry. The corresponding PET tracer [^{18}F]asyn-44 was hindered by the penetrance of radiometabolite in the brain, preventing further evaluations. Imidazo[2,1-*b*][1,3,4]thiadiazole derivatives were proposed by Cui *et al.* as a novel scaffold, and [^{18}F]FITA-2 was screened out with moderate affinity ($IC_{50,\ \alpha\text{-synuclein}} = 245$ nM, Figure 3B, right) (34). It possessed suitable brain uptake with sufficient clearance and good stability in healthy SD rats and is currently being evaluated in patients. Other chemical structures that may fulfill α-synuclein neuroimaging are discussed in recent reviews (35, 36).

In summary, extensive posttranslational modifications in vivo, including phosphorylation, truncation, and acetylation, may lead to changes in aggregation characteristics, such as structure and properties within the binding pocket. This results in varying binding potency of the structures to synthetic α-synuclein aggregates and human tissues. It seems to be an unavoidable trend to include brain sections or homogenates from donors with neurodegenerative conditions for compound screening. Binding assays of this kind include also the inherently low density of synucleinopathies. Molecular docking and photoaffinity labeling, alongside cryo-electron microscopy techniques, may assist in identifying

potential binding sites and optimizing structure (32, 37). Second, α-synucleinopathies are often accompanied by Aβ and tau aggregations that share a similar β-sheet structure, making it important and somewhat challenging to achieve selectivity. Recently, immunomagnetic cell sorting following in vivo radiotracer injection dissected the cellular allocation of 18-kDa translocator protein (TSPO)–PET signals in human glioma samples (38). We speculate that this approach may serve as a valuable tool to untangle the sources of radioactive signals in vivo from newly established α-synuclein PET tracers. In addition to the points mentioned above, the development of α-synuclein PET tracers encounters the typical challenges faced by molecular probes for central nervous system. It is essential to ensure sufficient molar activity, adequate brain penetration and to avoid confounding signals from radiometabolites in the brain (39). Nonetheless, the first promising clinical results have been disclosed. We believe that continuous scientific contributions from multiple disciplines will eventually pave the way for the development of α-synuclein PET tracers to illuminate synucleinopathies during disease progression.

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Author Contributions

Y.H. and L.Q. performed the literature review and wrote the original draft. Q.Y. and X.F. participated in reviewing and editing the manuscript. The manuscript has been read and approved by all authors. No related work is under consideration elsewhere.

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Author Disclosures

The authors have confirmed that no conflict of interest exists.

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THOUGHT LEADERS INVITED REVIEW

Mood disorders polygenic scores influence clinical outcomes of major psychiatric disorders

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Polygenic scores (PGS), summarizing the cumulative contribution of common genetic variants to psychiatric phenotypes, are increasingly investigated as putative predictors of treatment response and illness course. In major depressive disorder (MDD), several studies have associated higher MDD PGS with a modestly increased risk of nonresponse, lower remission rates, and treatment resistance. Conversely, bipolar disorder (BD) PGS have yielded more heterogeneous findings, with largely null or weak associations in unipolar depression but a possible on lithium response in BD cohorts, while lower MDD PGS showed a more consistent beneficial effect on lithium response in BD. MDD PGS may also have a modulating effect on clinical features of schizophrenia and a range of other psychiatric disorders. Nonetheless, the variance explained remains limited and predictive power improves only marginally when PGS are used in isolation. Integrative approaches that combine clinical predictors, environmental measures, and biomarker data appear to enhance prediction over genetics alone, which is increasing due to the most recent large genomewide studies. However, ancestral diversity remains limited, with most research conducted in Caucasian samples. Taken together, current evidence supports the incremental value of MDD and BD PGS in informing prognosis and treatment response, though clinical implementation remains premature. Replication in ancestrally diverse samples, integration with dimensional phenotypes, and improved modeling strategies will be essential to translate genetic liability into clinically actionable insights in precision psychiatry.

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Introduction

Major depressive disorder (MDD) and bipolar disorder (BD) are two of the most prevalent and disabling psychiatric conditions worldwide, contributing significantly to the global disease burden through chronic distress, functional impairment, and elevated mortality risk (1, 2). Despite advances in clinical assessment and psychopharmacology, predicting disease onset, course, and treatment response remains a central challenge for mental health practitioners. Traditional clinical features alone often fail to capture the broad heterogeneity of mood disorders, highlighting the need for more precise, biologically informed markers (3). In the past decade, genome-wide association studies (GWAS) have substantially expanded our understanding of the genetic architecture of psychiatric disorders, culminating in the development of polygenic scores (PGS) as potential tools for disentangling genetic contributions to complex psychiatric phenotypes (4).

PGS aggregate the effects of hundreds to thousands of common genetic variants, each exerting a small effect, into a single quantitative index of genetic liability. This approach is particularly relevant for mood disorders: MDD and BD, like most psychiatric conditions, are highly polygenic, with hundreds of variants collectively accounting for a proportion of disease risk (5, 6). The same polygenic influences may also shape symptom severity, comorbidities, and response to pharmacological or psychosocial interventions in other psychiatric disorders. Over the last few years, an increasing number of studies have leveraged PGS to investigate whether individuals carrying a higher genetic burden for MDD or BD exhibit specific clinical features (e.g., remission, treatment resistance, or different patterns of psychiatric comorbidity). Consequently, an evidence base is emerging that seeks to integrate these genetic features with traditional clinical factors, hoping to refine prognostic models and personalize treatment choices.

Given the rapidly evolving landscape of genetic research, there is a need to synthesize findings on how MDD and BD PGS relate to psychiatric outcomes.

Recent reviews provide a useful overview about the potential impact of PGS on treatment outcome (7, 8); however, the broad approach in these previous reviews does not focus on mood PGS alone and it does not include a substantial number of very recent studies across all major psychoses.

In this review, we present a narrative synthesis of the literature investigating MDD and BD PGS in relation to treatment outcomes (response, remission, and treatment resistance) and other clinically relevant phenotypes (comorbidity, illness course, and environmental exposures) in major psychoses.

Results

Treatment Outcome

Major Depression The first application of PGS in treatment outcome was reported in 2013 (9). The study reported a meta-analysis of three genome-wide pharmacogenetic studies—GENDEP ($N = 672$), MARS ($N = 604$), and STARD ($N = 980$)—in individuals with MDD treated with various antidepressants for up to 12 weeks, and derived PGS from the GENDEP and MARS cohorts which, when applied to the STARD sample, modestly predicted antidepressant response by explaining between 0.5% and 1.2% of the variance in percentage improvement and remission rates, the very small sample size of the original samples from which PGS have been calculated may explain the low predictive power in this early study. A subsequent study (10) examined two large cohorts, NEWMEDS ($N = 1791$) and again STARD ($N = 1107$), specifically testing whether BD PGS, derived from the largest Psychiatric Genomics Consortium (PGC) BD GWAS of that time (7481 cases and 9250 controls), could predict antidepressant response in MDD; however, the analyses revealed no significant association, with BD PGS explaining less than 0.01% of variance overall for selective serotonin reuptake inhibitors (p values ranging from 0.829 to 0.934) and only slightly higher yet nonsignificant variance (0.15%–0.34%, p ranging from 0.184 to 0.999) in the norepinephrine reuptake inhibitor subgroup. Also in this case, the GWAS sample where PGS have been calculated was

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relatively small and much smaller than the following ones that have been used later, which led to PGS with much less predictive power (11).

A few years later, García-González *et al*. (12) investigated MDD PGS in antidepressant treatment response across seven pharmacogenetic studies, with primary analyses in GENDEP ($n = 736$) and STARD ($n = 1409$) and validation in five additional independent samples totaling 3756 subjects, but found that MDD PGS did not significantly predicted symptom improvement or remission, as p values remained greater than 0.1 across nine significance thresholds, though rarer variants, $p < 0.0001$, showed a modest trend. Other than the relatively small original GWAS sample size (13), in this and many older studies PGS were calculated without more recently optimized Bayesian tools (14) and multiple thresholds were used, moreover the target samples heterogeneity may have influenced results. Indeed, MDD is recognized to be a more heterogeneous disorder when compared to other major psychoses, as evidenced by the lower genetic heritability, and this constitutes a challenge in biological studies. In fact, in a more homogeneous study some more significant results were reported. Ward *et al*. (15) analyzed 760 patients with MDD from three cohorts (GENDEP, AMPS–1, and AMPS–2) treated with escitalopram, nortriptyline, or citalopram/escitalopram over 8–12 weeks, computing MDD PGS and for neuroticism (NEU PGS) using GWAS data from the largest studies at the time (16, 17); in this study meta-analyses revealed for the first time some nominal associations, such as MDD PGS at $p < 5 \times 10^{-5}$ showing a β of -0.019 ($p = 0.009$) for 4–week response and NEU PGS at $p < 0.1$ showing a β of -0.017 ($p = 0.03$) for 8–week response; however, the variance explained remained very low ($\leq$1.2%), and the associations did not survive stringent correction for multiple testing.

The first community sample, including a larger and more powered sample, was studied in 2020, it examined antidepressant treatment resistance using prescription data from the Generation Scotland: Scottish Family Health Study (GS:SFHS) and the GENDEP cohort, with a meta-analysis of 4213 individuals (358 cases and 3855 controls) and a separate GWAS on stages of resistance (n = 3452) (18). PGS using summary statistics for MDD and BD revealed again nominal associations between treatment resistance and MDD PGS at thresholds of $<$0.1, $<$0.5, and $<$1, whereas BD PGS showed no significant relationship. This study performed in a relatively large sample therefore confirmed previous MDD PGS results.

Treatment-resistant depression (TRD) was then analyzed in the context of esketamine response by analyzing 527 European-ancestry individuals from two phase III trials (SUSTAIN–2 and TRANSFORM–3, the latter restricted to those with age of onset $<$55 years), where the primary outcome was the percentage change in Montgomery–Åsberg Depression Rating Scale (MADRS) score at 4 weeks (19); the GWAS identified a significant Single Nucleotide Polymorphism (SNP) in IRAK3 (rs11465988, $p = 3.57 \times 10^{-8}$, $\beta = -51.6$, SE = 9.2) and a gene-level association for NME7 ($p = 1.73 \times 10^{-6}$), and PGS based on depressive symptoms (20) were nominally associated with esketamine response ($p = 0.001$, $\beta = -3.1$, SE = 0.9), whereas BD PGS (21) was not significantly associated ($p = 0.076$ for MADRS change, $p = 0.141$ for remission), expanding the potential role for depressive symptom genetic loading in antidepressant outcome also to esketamine efficacy, but not for bipolar liability. This finding is of interest given that it was the first focusing on the narrow phenotype of TRD and it could reduce the heterogeneity of antidepressant response by focusing on esketamine treatment outcome (22–25).

Shortly after, the focus shifted to late-life antidepressant response in 335 older adults ($\geq$60 years) with MDD treated with venlafaxine XR over 12 weeks in the IRL–GREY (26) clinical trial (27); although the GWAS did not reveal genome-wide significant SNPs for remission or symptom improvement, and PGS constructed for depression and Alzheimer's disease were not significantly associated with treatment response, a PGS for cardioembolic stroke was significantly linked to nonremission [OR = 0.63, 95% confidence interval (CI) = 0.48–0.83, $p = 0.001$, permutation p = 0.006], suggesting that vascular factors, frequently associated with depression, might play a role in antidepressant resistance among older individuals, as suggested by recent genetic correlation studies between MDD and cardiometabolic factors (28). However a later reanalysis of the same sample investigated antidepressant response in late–life depression in 342 adults aged $\geq$ 60 from the same IRL–GREY study with the same treatment, and observed that while the BD PGS was nominally associated with better remission (OR = 0.75, 95% CI = 0.58–0.97, $p = 0.031$) and symptom improvement ($\beta = 4.27$, SE = 2.17, $p = 0.049$), the MDD was not associated with treatment outcomes, though with a trend in the same direction of the other papers here reviewed ($p = 0.086$) and intriguingly, the ADHD PGS was nominally associated with higher odds of remission (OR = 1.36, 95% CI = 1.07–1.73, $p = 0.011$), contrary to a previous finding (29). Though none of these associations survived Bonferroni correction, suggesting that in late-life depression genetic predictors of treatment response may partially differ from those in younger populations (30), trends were mostly in line with other studies. The BD and MDD PGS results difference across the two studies on the same sample could be explained by the use in the second sample of PGS calculated excluding 23andme data, that may be less powerful in explaining phenotypic variance given the self-report bias (31).

In 2021, the European Group for the Study of Resistant Depression (GSRD) sample of 1148 patients with MDD was used to assess the relationship between PGS for BD, MDD, and neuroticism (NEU) with treatment nonresponse and treatment resistance (TRD defined as failure of two or more antidepressants) (32); we found that that MDD PGS was nominally associated with non–response ($p = 0.032$) in the same direction of previous studies, while BD PGS and NEU PGS did not show significant effects. In a following meta-analysis, we examined PGS for MDD, BD, and NEU in relation to antidepressant nonresponse and nonremission in a larger sample of 3637 and 3184 patients respectively from six European clinical samples, and confirmed that the MDD PGS was nominally associated with nonresponse (OR = 1.10, 95% CI = 1.02–1.19, $p = 0.013$, pseudo–$R^2 = 0.24$%) and nonremission (OR = 1.14, 95% CI = 1.04–1.24, $p = 0.004$, pseudo–$R^2 = 0.57$%) at specific p–value thresholds, while BD PGS showed no significant association; however, though none of these associations survived correction for multiple testing, the observed trends of MDD PGS effects remained in a relatively large and heterogeneous target sample (33).

A smaller, but with a more complex design study, investigated predictors of clinical outcome in 174 patients with TRD admitted to a specialist inpatient unit where patients received a multimodal treatment regimen including pharmacotherapy, cognitive behavioral therapy (CBT), behavioral activation, and, if indicated, electroconvulsive therapy (34); while clinical predictors such as later age of onset, a higher number of previous depressive episodes, and lower treatment resistance (as measured by the Maudsley Staging Method) were significantly associated with a favorable response, PGS for MDD, BD, and schizophrenia (SCZ) did not predict treatment outcome, as none of the genetic variables reached significance in either univariate or multivariate analyses, suggesting that in the context of intensive inpatient treatment for TRD, clinical factors may overshadow the modest effects of genetic liability, the relatively small target sample may also have influenced results. Indeed, variance explained in the range of 1%–2% need larger samples to be detected.

In a large population study, we analyzed TRD using primary care records from UK Biobank ($n = 230{,}096$, with MDD cases numbering 19,979 and TRD cases 2430) and the EXCEED cohort ($n = 8926$, with 1271 MDD cases and 159 TRD cases) (29), finding that while MDD PGS robustly predicted MDD diagnosis ($p = 1.89 \times 10^{-71}$ in UKB and $p = 6.05 \times 10^{-6}$ in EXCEED), PGS for BD were only weakly associated with MDD in UKB and not in EXCEED, and crucially, when comparing TRD with non–TRD MDD, these PGS did not show significant differences after correction for multiple testing, although MDD PGS showed a nominal positive correlation ($p = 0.028$). This study underlines the importance of investigating large target samples that may overcome the outcome heterogeneity observed in MDD, though populations samples such as this one may add other stratification factors when compared to clinical samples.

Placebo response was examined for the first time, versus antidepressant treatment, in 1364 patients with MDD from seven randomized, double–blind, placebo–controlled vortioxetine trials, with an additional self–reported validation sample from 23andMe ($N = 642$), constructing PGS for antidepressant response as well as for MDD and BD, NEU, subjective well–being, and cognition (35); although no PGS reached significance

after Bonferroni correction, analyses showed that higher MDD PGS was nominally linked to a better placebo response on measures of somatic anxiety ($\beta = 0.54$, $p = 0.011$), suggesting that genetic predisposition may influence not only drug response but also placebo effects, albeit with small effect sizes and limited clinical utility on their own. It remains to be investigated whether MDD PGS effects are limited to antidepressant treatment or have a disease course modulatory effect.

PGS for antidepressant response (PGS–AR), computed from GWAS summary statistics (36), was then investigated with electroencephalogram (EEG) biomarker data in a sample of 1123 participants (including 1061 psychiatric patients and 62 healthy controls) to determine whether a specific EEG component (component 4) could predict treatment response in MDD (37); in men, PGS–AR was significantly associated with EEG component 4 ($\beta = 0.172$, $R^2 = 2.91\%$, $p = 0.000567$), and this EEG component significantly predicted symptom improvement in an independent iSPOT–D sample ($\beta = -0.153$, $R^2 = 2.3\%$, $p = 0.019$) as well as in a dataset of rTMS plus psychotherapy patients ($\beta = -0.230$, $R^2 = 5.3\%$, $p = 0.022$), although these associations were not observed in women. This study investigated PGS-AR, which in theory may be more powerful than MDD PGS, but it should be considered that the origin samples to calculate PGS-AR are usually much smaller that MDD GWAS samples, therefore the power is much reduced. However, the value of this study is to be a proof–of–concept that combining genetic and neurophysiological markers may enhance the prediction of antidepressant outcomes.

PGS for antidepressant and lithium response were then investigated in a large sample of 4572 patients with MDD from three Swedish cohorts (PREFECT, iCBT, and STAGE) using three distinct definitions of TRD (38), and found that while the PGS-AR did not significantly differ between TRD and non–TRD groups (e.g., broad definition: mean difference $= -0.015$, $p = 0.631$), the PGS for lithium response was significantly higher in TRD cases (e.g., broad definition: mean difference $= 0.094$, $p = 0.003$; logistic regression showed an OR of 1.12 per SD increase, 95% CI $= 1.04$–1.20, $p = 0.003$), with a dose–response effect evident in the top PGS quartile ($P_{trend} < 0.005$), suggesting that TRD may be characterized by a higher genetic predisposition to respond to lithium and emphasizing the pleiotropic effects of PGS in both MDD and BD and, from a clinical point, supporting the potential utility of a targeted lithium use in TRD.

We recently examined the interaction between PGS for mood disorders, including MDD and BD, and environmental factors in the UK Biobank (with sample sizes ranging from 33,000 to 380,000) (39), finding that while both PGS and environmental variables had additive effects on well-being, significant interactions emerged such that higher MDD PGS intensified the negative impact of recent stress on loneliness ($\beta = 0.0156$, SE $= 0.0025$, $p = 3.20 \times 10^{-10}$) and BD PGS interacted with stress to predict lower household income ($p = 1.17 \times 10^{-4}$), even though these interactions explained only an additional 0.01%–0.02% of variance, supporting the differential susceptibility hypothesis and suggesting that genetic liability for mood disorders can modulate the effects of environmental adversity, as it will be discussed in the conclusion section.

A secondary analysis of the Early Medication Change (EMC) trial was recently performed involving 481 patients with MDD (compared with 3215 controls from the Heinz Nixdorf Recall study) undergoing an 8–week treatment algorithm starting with escitalopram and switching to venlafaxine or lithium (40), and although the MDD PGS was significantly associated with disorder status (Nagelkerke's $R^2 = 2.48\%$, $p < 1 \times 10^{-12}$), it did not predict treatment outcomes such as early improvement, response, or remission (with Nagelkerke's R^2 values ranging from 0.007% to 0.256%); however, the relatively small sample size of the target sample may be considered as a limitation also of this study.

Following with the large series of very recent studies, Monistrol–Mula *et al*. (41) explored the impact of polygenic liability to various mental disorders on COVID–19 outcomes in 4405 individuals with a history of depression from the Australian Genetics of Depression Study (AGDS), and found that the MDD PGS was significantly associated with higher COVID–19 burnout ($\beta = 0.36$, SE $= 0.12$, $p = 0.003$, adjusted $R^2 = 0.089$), with individuals in the top 10% having 4.17–fold higher odds of burnout (95% CI $= 1.47$–11.86), while the BD PGS showed a trend toward a protective effect against burnout that did not survive multiple testing, suggesting that genetic liability for depression may predispose individuals to greater psychological distress during the pandemic through its influence on anxiety, which fully mediated the observed association.

Pregnancy and postpartum are relevant periods for depression (42) and they were the focus of another study that investigated whether PGS for MDD and BD predicted antidepressant treatment trajectories in a Danish cohort of 2316 women with mood disorders (43), but found no significant associations between these PGS and treatment trajectories (categorized as continuers, early discontinuers, late discontinuers, or interrupters), with clinical factors such as higher prepregnancy antidepressant dose, longer treatment duration, and prescription of multiple antidepressant classes being the primary predictors of continued antidepressant use, thus suggesting that, at least in the perinatal period, treatment trajectories are largely driven by clinical severity and possibly by other environmental factors rather than genetic liability.

Positive results have also been reported in another recent study aimed at validating an antidepressant response algorithm across multiple electronic health record (EHR) systems from Vanderbilt University Medical Center, the All of Us Research Program and the Mass General Brigham Healthcare System (44). It demonstrated that higher polygenic risk scores for MDD (OR $= 1.07$, $p = 2.84 \times 10^{-8}$), and BD (OR $= 1.04$, $p = 1.99 \times 10^{-3}$) were significantly associated with poorer antidepressant response, with an estimated heritability of antidepressant response of 3.84% (SE $=$ 0.007) and significant genetic correlations with these psychiatric traits (rg $= 0.23$ for MDD, rg $= 0.15$ for BD), thereby supporting previous findings on MDD PGS though the overall predictive power of PGS remains modest.

Genetic analyses are mainly performed in Caucasian populations, but information on Asians is also needed, given the known differences in the genetic background (45, 46). Shao *et al*. (47) examined the association between MDD PGS and early antidepressant efficacy in 912 Han Chinese patients with nonpsychotic MDD (aged 18–65, with baseline HAM–D17 $\geq$ 18 and medication–free for at least 2 weeks), and reported that a higher MDD PGS was significantly associated with a lower percentage reduction in HAM–D17 scores after 2 weeks ($p = 0.009$; Spearman $r = -0.075$, $p =$ 0.024; in multivariate regression, $\beta = -4.086$, $p = 0.039$, adjusted $R^2 =$ 0.086), no significant interaction with negative life events was observed, suggesting that the direction of the effect may be the same when compared to Caucasians and that the effect is quite high, this is quite encouraging for generalizability of the MDD PGS effect.

A larger longitudinal population study utilized data from the iPSYCH 2015 sample in Denmark to investigate polygenic liabilities in early–onset MDD (diagnosed between ages 10 and 25, $N = 10{,}577$) and identified four treatment trajectories over 7 years using latent class growth analysis, brief contact, prolonged initial contact, later re–entry, and persistent contact, and found that the MDD PGS was nominally associated with the later re–entry trajectory (OR $= 1.09$, 95% CI $= 1.02$–1.17, $p = 0.01$) and significantly associated with continued antidepressant treatment in primary care (OR $= 1.11$, 95% CI $= 1.05$–1.17, $p = 0.0003$), while BD PGS did not show significant associations, suggesting that the MDD PGS effect may be detected also in early–onset depression and during longitudinal clinical course. This study also supports the usefulness of large population sample, that, despite the limitations inherent to registries, may well contribute to the definition of the genetic modulating effects (48).

Bipolar Disorder MDD PGS were also studied on lithium response in patients with BD using data from the Consortium on Lithium Genetics (ConLi + Gen), comprising 2586 patients (with analyses stratified by ethnicity: multiethnic, European, and Asian subsamples) (49); employing weighted PGSs constructed from a large PGC GWAS (135,458 MDD cases and 344,901 controls), the authors found that higher MDD PGS were significantly associated with poorer lithium response, with continuous outcomes showing significant R^2 values of approximately 0.8% in the multiethnic sample (and similar findings in the European subsample). Stratified analyses showed that patients in the lowest quartile of MDD PGS had significantly better outcomes (e.g., OR $= 1.54$ in the multiethnic sample and OR $= 1.75$ in Europeans). This study suggested for the first time that patients with BD with lower polygenic liability for depression may

represent a distinct lithium-responsive biotype; sensitivity analyses using unrelated trait PGS (bone mineral density) confirmed the specificity of the effect.

Lithium response was again the focus in patients with BD in the same largest sample collected so far described before ($N = 2283$ from ConLi + Gen) and the study examined MDD PGS associated with treatment outcomes as measured by the Alda scale (50); the study found higher MDD PGS (OR = 1.61, $p = 0.04$) significantly associated with poorer lithium response, and that a meta–analytic approach combining SCZ and MDD PGS into a MET2–PGS improved prediction (OR = 2.54, $p = 0.002$, with Nagelkerke's R^2 of 0.91%), whereas BD PGS alone did not significantly predict response; functional pathway analyses of MET2–PGS implicated histone modification and glucose metabolism pathways, suggesting epigenetic and metabolic mechanisms may underlie lithium efficacy. This result is in line with the previously reported detrimental effect of SCZ PGS (51) and suggests a synergic effect of both SCZ and MDD PGS on BD maintenance outcome.

The same ConLi + Gen sample was again analyzed with a machine–learning approach in a subsample of 1034 patients with BD to predict lithium response using both clinical predictors and PGS for SCZ and MDD (52); the study demonstrated that while PGS alone explained only modest variance (1.2% in linear models and 2.0% in nonlinear models), combining PGS with clinical variables improved prediction to 4.7% (and up to 13.7% in PGS–stratified models), with patients in the lowest quartile of MDD PGS being 67.7% more likely to respond to lithium than those in the highest quartile (OR = 1.68, 95% CI = 1.14–2.47, $p = 0.009$). This study clearly underlines the benefit of a combined model with the perspective of a potential clinical utility of integrating clinical and genetic risk information for personalized treatment stratification.

Lithium pharmacogenetics in BD was also investigated by developing a lithium response polygenic score (Li + PGS) in the mentioned large ConLi + Gen cohort ($N = 2367$) and replicating the findings in PsyCourse ($N = 89$) and BipoLife ($N = 102$) cohorts (53); lithium response, measured via the ALDA scale (both continuously and categorically with a cutoff of 7), was significantly predicted by Li + PGS (categorical outcome: $p = 9.8 \times 10^{-12}$, $R^2 = 1.9\%$; continuous outcome: $p = 6.4 \times 10^{-9}$, $R^2 = 2.6\%$), with patients in the highest Li + PGS decile having 3.47–fold higher odds of a favorable response (95% CI = 2.22–5.47), and gene–based pathway analysis implicated cholinergic and glutamatergic systems, thereby reinforcing the notion that lithium responders may have a distinct genetic profile in addition to the previously reported lower polygenic liability for depression.

Collectively, these studies (Table 1), spanning from 2013 to 2025 and incorporating diverse samples, from large-scale meta-analyses and clinical trials to population-based and EHR studies, strongly suggest that MDD PGS show modest but consistent associations with both disorder severity susceptibility and treatment outcomes (with higher MDD polygenic load generally predicting poorer antidepressant or lithium response). BD PGS have shown a less robust predictive value for antidepressant response in MDD, but they may play a role in influencing lithium response in BD when considered in conjunction with other PGS. In any case the overall variance explained by these genetic predictors remains low, underscoring the complexity of treatment response phenotypes and the need for integrating genetic information with clinical, environmental, and other biological markers to enhance predictive accuracy.

Outcome-Related Traits A series of papers investigated clinical aspects that, though not directly measuring short-term treatment outcome, may inform about the possible influence of PGS. Kowalec *et al.* (54) analyzed 24,706 individuals with SCZ from the Swedish national registers, with a genomic subset of 4936 cases, to investigate clinical, demographic, and genetic factors associated with treatment-resistant schizophrenia (TRS), defined both by clozapine prescription ($N = 4813$) and by clozapine prescription or antipsychotic polypharmacy for $\geq$ 90 days ($N = 13,779$); although they found that a higher SCZ family history burden [highest quartile vs. lowest quartile: OR = 1.31, 95% CI = (1.19–1.42), $p = 4.8 \times 10^{-8}$] and lower premorbid IQ in males (per 1 SD decrease: OR = 0.94, 95% CI = [0.90–0.98], $p = 0.002$) were robust predictors of TRS, none of the PGS, MDD or BD reached significance ($p > 0.1$ for both), though both PGS showed a nonsignificant trend in the direction of increasing TRS.

A smaller but well-designed study conducted an integrative genomic–epigenomic analysis in 44 patients with refractory psychosis treated with clozapine [31 with SCZ (70.45%), 9 with schizoaffective disorder (20.45%), and 4 with BD (9.09%)], computing PGS for BD and MDD, and found that BD PGS was significantly associated with clozapine metabolic ratio (pseudo-$R^2 = 0.2080$, $p = 0.0008$, adjusted $p = 0.0189$), while MDD PGS was only nominally associated with clozapine dose (pseudo-$R^2 = 0.386$, $p = 0.0035$, adjusted $p = 0.0759$), suggesting that bipolar genetic liability could influence clozapine metabolism, and, probably more interesting, that MDD genetic risk may lead treating clinicians to raise the dose possibly for an observed poor response (55).

A more recent study focused more directly on the interplay of MD and BD PGS with clinical and environmental factors (56). The study examined data from the AGDS ($N = 14,146$; 75% female, mean age = 44.0 years) to assess associations between PGSs for multiple mental disorders, MDD and BD, and exposure to 32 stressful life events (SLEs) categorized by childhood, past-year, lifetime, and cumulative events. Using logistic and linear regression models adjusted for age and sex with false discovery rate (FDR) correction, they found that higher MDD PGS was significantly associated with increased odds of exposure to all childhood SLEs (ORs = 1.07–1.12, p's < 0.013, FDR-corrected), as well as with specific adverse events such as physical assault [OR = 1.06, 95% CI = (1.02–1.11), $p = 0.006$], unwanted or uncomfortable sexual experiences, sexual assault [OR = 1.10, 95% CI = (1.05–1.16), $p < 0.001$], severe human suffering [OR = 1.17, 95% CI = (1.05–1.30), $p = 0.003$], life-threatening illness or injury [OR = 1.09, 95% CI = (1.03–1.15), $p = 0.003$], and assault with a weapon [OR = 1.12, 95% CI = (1.04–1.21), $p = 0.003$]; additionally, higher MDD PGS was associated with increased cumulative SLEs (ORs = 1.05–1.24, FDR-corrected p's < 0.05), whereas higher BD PGS was associated with lower odds of experiencing physical assault [OR = 0.95, 95% CI = (0.91–0.99), $p = 0.014$], major financial troubles [OR = 0.93, 95% CI = (0.88–0.98), $p = 0.004$], and living in unpleasant surroundings [OR = 0.92, 95% CI = (0.87–0.98), $p = 0.008$], as well as with fewer reported childhood SLEs [OR = 0.97, 95% CI = (0.95–0.99), $p = 0.01$]. Results from this complex study may suggest that, while genetic liability for depression may predispose individuals to greater exposure to stress, BD genetic risk appears inversely related to such exposure. The large sample and the evaluation of stressful life events are positive aspects of the study that add to the overall outcome domain and are in line with previous evidence therefore starting to identify specific modulating effects of MDD versus BD PGS.

A converging evidence comes from another study (57), that used data from two population-based cohorts, the Avon Longitudinal Study of Parents and Children (ALSPAC; $N = 5521$, mean age = 11.8 years, SD = 0.14, 50.3% female) and the Twins Early Development Study (TEDS; $N = 4625$, mean age = 11.27 years, SD = 0.69, 53.2% female), to compute MDD PGS among other traits and to examine their associations with psychopathology symptoms measured by the Short Mood and Feelings Questionnaire (SMFQ) and the Strength and Difficulties Questionnaire (SDQ); the study found that the depression PGS was significantly associated with the symptom "not enjoying anything" ($r = 0.04$) and with "being bullied" ($r = 0.06$) on the peer problems subscale, supporting the evidence that genetic risk for depression may be broadly influencing the complex interplay with environmental stressors and possibly reducing the heterogeneity of treatment outcome (58).

Another interesting possible effect, diagnostic transition, was recently investigated (59). The study included 10,565 individuals from a Danish registry with eating disorders (Anorexia nervosa [AN], $n = 6901$; Bulimia nervosa [BN], $n = 1417$; Eating Disorder Not Otherwise Specified [EDNOS], $n = 2247$) and calculated PGS for 422 traits including MDD and BD using LDpred2 and meta-PGS approaches; the study found that a higher PGS for MDD was significantly associated with a 15% greater hazard of transitioning from anorexia nervosa to either bulimia nervosa or EDNOS (HR = 1.15 per SD increase, $p < 1.57 \times 10^{-4}$), whereas the BD PGS was not significantly associated with diagnostic transitions. Though not directly investigating outcome, results from this study are interesting because

Table 1. Summary of studies investigating MDD and BD PGS and outcome

Study	Objective	Design	Treatment	Subjects	Findings	Implications
Amare *et al.*, 2021	MDD PGS and lithium response in BD	Analysis within ConLi + Gen; multiethnic sample with subgroup analyses	Lithium treatment; response measured by the Alda scale (continuous and categorical)	BD patients: Multiethnic $N = 2586$ (European: $N = 2366$; Asian: $N = 220$)	Higher MDD PGS associated with poorer lithium response (multiethnic: continuous $R^2 = 0.8\%$, categorical $R^2 = 0.7\%$; European quartile OR = 1.75, decile OR = 1.74; Asian: nominal, $p = 0.034$)	Lower polygenic load for MDD in patients with BD predicts better lithium response
Amare *et al.*, 2023	Lithium response PGS in BD	Cohort study in ConLi + Gen with replication in PsyCourse ($N = 89$) and BipoLife ($N = 102$)	Lithium treatment; response measured by the ALDA scale (categorical and continuous outcomes)	ConLi + Gen: $N = 2367$; Replication cohorts: PsyCourse $N = 89$, BipoLife $N = 102$	Li + PGS associated with lithium response in ConLi + Gen (categorical: $p = 9.8 \times 10^{-12}$, $R^2 = 1.9\%$; continuous: $p = 6.4 \times 10^{-9}$, $R^2 = 2.6\%$); patients in the 10th decile had 3.47-fold higher odds (95% CI: 2.22–5.47); replication $p = 3.9 \times 10^{-4}$, $R^2 = 0.9\%$	Lithium response in BD is partly genetically determined, with evidence implicating cholinergic and glutamatergic pathways
Cearns *et al.*, 2022	PGS-guided stratification for lithium response in BD	Retrospective analysis with machine-learning; training set $n = 692$, test set $n = 342$	Lithium treatment; response via Alda scale	Patients with BD from ConLi + Gen: $N = 1034$	Combining PGS (PGS-SCZ and PGS-MDD) with clinical predictors improved variance explained to 5.1% (linear models) and up to 13.7% in stratified models; lower MDD PGS associated with better response (OR = 1.677, 95% CI = 1.14–2.47, $p = 0.009$)	Integrating PGS with clinical data enhances lithium response prediction in BD
Elsheikh *et al.*, 2024	BD and MDD PGS effects on late-life antidepressant response	12-week trial analysis (IRL-GRey study) in adults aged ≥60 years	Venlafaxine XR titrated from 37.5 mg/day up to 300 mg/day for 12 weeks	Late-life depression: $N = 342$ adults	BD PGS was nominally associated with remission (OR = 0.75, 95% CI = 0.58–0.97, $p = 0.031$) and with symptom improvement (β=4.27, SE = 2.17, $p = 0.049$); MDD were not significant; ADHD PGS nominally (OR = 1.36, $p = 0.011$)	In late-life depression, BD genetic liability may modestly influence treatment response
Fabbri *et al.*, 2021	MDD/BD PGS associations with treatment-resistant depression (TRD)	Retrospective cohort analysis using primary care records (UKB and EXCEED)	TRD defined as ≥2 antidepressant switches (each ≥6 weeks)	UKB: MDD $n = 19979$ (TRD $n = 2,430$); EXCEED: MDD $n = 1271$ (TRD $n = 159$); UKB total = 230,096, EXCEED = 8926	MDD PGS nominally associated with TRD vs. non-TRD (p = 0.028); BD PGS not significant (p = 0.07)	TRD in MDD may involve genetic liabilities beyond MDD and BD (e.g. ADHD
Fabbri *et al.*, 2024	MDD/BP PGS effects on wellbeing	Cross-sectional analysis; sample size varied from 33,000 to 380,000 (UK Biobank)	Observational study	UK Biobank participants (using mood disorder PGS among others)	Higher MDD and BP PGSs interacted with environmental stress (e.g., BP PGS increased odds of lower income, $p = 1.17 \times 10^{-4}$); PGS × E interactions added ~0.01%–0.02% variance	Genetic liability for mood disorders modulates the impact of adverse/protective environments on well-being
Fanelli *et al.*, 2021	MDD PGS in predicting antidepressant nonresponse in MDD	Cross-sectional analysis in the European Group for the Study of Resistant Depression (GSRD)	Antidepressant treatment; patients classified as responders, nonresponders (failure of 1), or TRD (failure of ≥2)	Patients with MDD from GSRD: $N = 1148$	MDD PGS was nominally associated ($p = 0.032$); BD PGS was not significant	Increased MDD genetic liability may indicate an MDD subtype less responsive to treatment
Fanelli *et al.*, 2022	Mood disorder PGS impact on antidepressant nonresponse/ nonremission	Meta-analysis across six European clinical samples; PGS computed at eight thresholds	Antidepressant treatment; outcomes: nonresponse and nonremission	Nonresponse sample: $n = 3637$; Nonremission sample: $n = 3184$	MDD PGS was nominally associated with nonresponse (OR = 1.10, 95% CI = 1.02–1.19, $p = 0.013$) and nonremission (OR = 1.14, 95% CI = 1.04–1.24, $p = 0.004$); BD PGS not significant	A higher genetic burden for depression may increase the risk of poor antidepressant outcomes
García-González *et al.*, 2017	MDD PGS prediction of antidepressant response	Analysis in GENDEP ($n = 736$) and STAR*D ($n = 1409$) with validation in five independent studies	Antidepressants administered over 12 weeks	Combined discovery sample: $N \approx 2145$; Validation: total $n = 3756$	MDD PGS not associated with antidepressant response ($p > 0.1$ across nine thresholds). rarer variants, $p < 0.0001$, showed a modest trend	MDD PGS modest effect in line with other reports

(*continued*)

Table 1—*Continued*

Study	Objective	Design	Treatment	Subjects	Findings	Implications
GENDEP Investigators *et al.*, 2013	Antidepressant response polygenic prediction	Meta-analysis of three GWAS pharmacogenetic studies; 12-week treatment duration	GENDEP: escitalopram (10–30 mg/day) or nortriptyline (50–150 mg/day); MARS: various antidepressants (naturalistic inpatient setting); STAR*D: citalopram (20–60 mg/day)	Total $N = 2256$ individuals with MDD; GENDEP: $N = 672$; MARS: $N = 604$; STAR*D: $N = 980$	PGS derived from GENDEP/MARS significantly predicted STAR*D improvement (PGS explained 0.5%–1.2% of variance, $p = 0.005$–0.048) and remission (PGS explained 0.8%–1.2% of variance, $p = 0.017$–0.041)	In this early study, the small origin sample size may limit informativeness
Li *et al.*, 2020	Depressive PGS influence on esketamine response in TRD	GWAS and PGS analysis in two phase III trials (SUSTAIN-2 and TRANSFORM-3); analysis in European ancestry (TRANSFORM-3 limited to onset <55 years)	Esketamine treatment; primary outcome: % change in MADRS at 4 weeks; also responder and remission status	Total sample: $N = 527$ (from SUSTAIN-2 [$n \approx 598$] and TRANSFORM-3 [$n = 95$], with inclusion criteria applied)	Depressive symptom PGS was nominally associated with MADRS change ($p = 0.001$, $\beta = -3.1$) and with remission ($p = 0.002$); BD PGS showed a trend of association ($p = 0.076$)	Genetic loading for depressive symptoms may modestly affect esketamine efficacy in TRD
Liu *et al.*, 2024	PGS impact on perinatal antidepressant treatment trajectories	Retrospective cohort study from Danish registers	Antidepressants prescribed prepregnancy; trajectories: continuers, early/late discontinuers, interrupters	Women with affective disorders: $N = 2316$; Trajectory distribution: continuers 38.2%, early discontinuers 22.7%, late discontinuers 23.8%, interrupters 15.3%	PGS for MDD and BD were not associated with treatment trajectories (e.g., for continuers vs. early discontinuers: MDD PGS RRR = 0.93, 95% CI = 0.81–1.06)	Antidepressant use during the perinatal period appears driven by clinical factors rather than by genetic liability for mood disorders
Marshe *et al.*, 2021	Depression PGS impact on late-life antidepressant response	GWAS and PGS analysis in a 12-week trial in older adults (≥60 years)	Venlafaxine XR, titrated from 37.5 mg/day up to 300 mg/day for 12 weeks	Older adults with MDD: $N = 335$ (IRL-GREY trial)	MDD PGS was not significantly associated with remission; PGS for cardioembolic stroke was associated with nonremission (OR = 0.63, $p = 0.001$)	Vascular and neuroinflammatory genetic factors may be more influential than depression PGS in late-life antidepressant response
Monistrol-Mula *et al.*, 2024	MDD/BD PGS effects on COVID-related burnout	Cross-sectional analysis with mediation (and moderation) in AGDS	Observational study	Individuals with depression from AGDS: $N = 4405$	MDD PGS associated with higher COVID-19 burnout ($\beta = 0.36$, SE = 0.12, $p = 0.003$; top 10% vs lowest: OR = 4.17, 95% CI = 1.47–11.86); BD PGS showed a trend (highest 10% OR = 0.27, 95% CI = 0.09–0.76)	Genetic liability for depression may predispose to COVID-related burnout, an effect fully mediated by anxiety
Müller *et al.*, 2024	MDD PGS in disorder risk vs. treatment response	Secondary analysis of an 8-week treatment trial (EMC)	Initial escitalopram; switch to venlafaxine or lithium per algorithm	EMC: enrolled $N = 889$, genetic data available for 560, final analysis $N = 481$; Controls from HNR: $N = 3215$	MDD PGS associated with disorder status (Nagelkerke's $R^2 = 2.48\%$, $p < 1 \times 10^{-12}$) but ADR-PGS did not predict early improvement ($R^2 = 0.007\%$, $p = 0.879$) or remission ($R^2 = 0.194\%$, $p = 0.464$)	Common polygenic variation may influence MDD risk
Mundy *et al.*, 2024	PGS influence on treatment trajectories in early-onset MDD	Danish register-based study using latent class growth analysis over 7 years	Secondary psychiatric care for MDD (no active treatment trial)	Early-onset MDD individuals (diagnosed age 10–25): $N = 10,577$	MDD PGS was nominally associated with the later re-entry trajectory (OR = 1.09, 95% CI = 1.02–1.17, $p = 0.01$) and with continued antidepressant use (OR = 1.11, 95% CI = 1.05–1.17, $p = 0.0003$); BD PGS not significant	Genetic liability for depression may predict recurrent treatment needs in early-onset MDD
Nøhr *et al.*, 2022	PGS associations with vortioxetine/placebo response in MDD	Randomized, double-blind trials (vortioxetine $N = 907$, placebo $N = 455$) plus a 23andMe self-report sample ($N = 642$)	Vortioxetine vs. placebo	Clinical trials: $N = 1364$; additional self-reported sample: $N = 642$	No PGS reached significance after correction; nominally, PGS MDD was nominally linked to better placebo response ($\beta = 0.54$, $p = 0.011$)	Genetic predictors may differentially affect drug versus placebo response

(*continued*)

Table 1—*Continued*

Study	Objective	Design	Treatment	Subjects	Findings	Implications
Schubert *et al.*, 2021	Combined SCZ and MDD PGS predict lithium response in BD	Cross-sectional analysis using logistic and Tobit regression	Lithium treatment; response measured by the Alda scale	Patients with BD from ConLi + Gen: $N = 2283$	PGS MDD (OR = 1.61, $p = 0.04$) predicted poorer response; combined MET2-PGS improved prediction (OR = 2.54, $p = 0.002$; Nagelkerke $R^2 = 0.91\%$)	Patients with BD with higher genetic liability for MDD are less likely to respond favorably to lithium
Sealock *et al.*, 2024	Psychiatric PGS association with antidepressant response via an EHR algorithm	Multisite EHR validation study using ordinal regression models	First antidepressant trial; response categorized as responder, intermediate, nonresponder	Data pooled from VUMC, All of Us, and MGB (sample size not specified)	Higher PGS for MDD (OR = 1.07, $p = 2.84 \times 10^{-8}$) and BD (OR = 1.04, $p = 1.99 \times 10^{-3}$) were associated with poorer response	Genetic risk for mood disorders is linked to diminished antidepressant response
Shao *et al.*, 2025	MDD PGS and clinical factors and early antidepressant efficacy	Observational study with multiple stepwise linear regression; outcome measured after 2 weeks	Antidepressant treatment; outcome: % reduction in HAM-D17 scores after 2 weeks	Patients with nonpsychotic MDD: initial $N = 999$, final $N = 912$; Han Chinese, age 18–65, baseline HAM-D17 ≥ 18	Higher MDD PGS was linked to a lower HAM-D17 reduction ($r = -0.075$, $p = 0.024$; $\beta = -4.086$, $p = 0.039$, adjusted $R^2 = 0.086$)	A higher genetic burden for depression modestly predicts reduced early antidepressant efficacy
Tansey *et al.*, 2014	BD PGS influence on antidepressant response in MDD	Meta-analysis of two cohorts (NEWMEDS: $N = 1{,}791$; STAR*D: $N = 1107$) over 12 weeks	Antidepressants: SSRIs (e.g., escitalopram, citalopram) and NRIs (e.g., nortriptyline, reboxetine)	NEWMEDS: $N = 1791$; STAR*D: $N = 1107$; patients with MDD	BD PGS explained <0.01% to 0.34% of variance in response (p values ranging from 0.829 to 0.999); no significant association was observed	Negative results in the early study may be linked to the reduced power of the origin samples
Taylor *et al.*, 2021	PGS influence on intensive inpatient TRD outcome	Observational study in a specialist inpatient service	Individualized pharmacotherapy, CBT, occupational and couples therapy, ECT if indicated	TRD patients: $N = 174$; responders = 82 (47%)	No significant associations were found between MDD PGS, BD PGS, and treatment response; clinical predictors (e.g., later age of onset) were modest (AUC < 0.6)	Genetic liability for mood disorders does not strongly influence short-term outcome in intensive inpatient treatment for TRD
Ward *et al.*, 2018	MDD and neuroticism PGS in antidepressant response	Meta-analysis across three cohorts (GENDEP $N = 267$; AMPS-1 $N = 357$; AMPS-2 $N = 138$) over 8–12 weeks	Antidepressants: escitalopram, nortriptyline, citalopram/ escitalopram	Total $N = 760$ patients with MDD	MDD PGS nominally associated with lower 4-week response ($\beta = -0.019$, $p = 0.009$) and PGS NEU nominally with lower 8-week response ($\beta = -0.017$, $p = 0.03$); variance explained was ≤1.2%	Higher genetic liability for depression and neuroticism may modestly predict poorer short-term antidepressant outcomes
Wigmore *et al.*, 2020	PGS associations with antidepressant treatment resistance in MDD	GWAS and PGS analysis; meta-analysis of GS:SFHS and GENDEP; additional GWAS on stages of resistance	Based on health service prescription data (antidepressant resistance defined via treatment stages)	Meta-analysis: $N = 4213$ (cases = 358, controls = 3855); GS:SFHS subanalysis: $n = 3452$	Antidepressant resistance was nominally associated with MDD PGS (at PT < 0.1, <0.5, <1); BD PGS was not significant	Genetic liability for MDD may contribute to antidepressant resistance
Xiong *et al.*, 2023	Lithium and antidepressant PGS in TRD MDD	Cross-sectional analysis across three Swedish cohorts (no explicit trial duration)	PREFECT: severe MDD received ECT; iCBT: mild–moderate MDD treated with internet CBT; STAGE: population MDD	Total $N = 4572$; PREFECT $N = 1{,}922$ (ECT), iCBT $N = 964$, STAGE $N = 1686$; TRD defined: broad (1778 vs 2264), narrow$_1$ (1487 vs 1483), narrow$_2$ (1081 vs. 1483)	Antidepressant response PGS showed no difference (broad diff = −0.015, $p = 0.631$); lithium response PGS was higher in TRD (broad diff = 0.094, $P = 0.003$; narrow$_1$ OR = 1.12 per SD, 95% CI = 1.04–1.20, $p = 0.003$)	TRD in MDD may be genetically predisposed to lithium responsiveness

Abbreviations: ADR, antidepressant response; BD, bipolar disorder; ECT, electroconvulsive therapy; EMC, Early Medication Change; GWAS, genome-wide association study; iCBT, internet-based cognitive behavioral therapy; MDD, major depression; NRI, norepinephrine reuptake inhibitor; PGS, polygenic score; TRD, treatment-resistant depression; R^2, coefficient of determination; RCT, randomized controlled trial; SSRIs, selective serotonin reuptake inhibitors.

report for the first time that that depression genetic liability may play a role also in the clinical evolution of eating disorders.

Nguyen *et al*. (60) then used Swedish and Danish national registries to study psychotic MDD, defined using ICD-10 subcodes F32.2/F32.3 and analyzing approximately 30,000 genotyped MDD cases from the UK Biobank and a Swedish clinical cohort. It reported that the heritability of psychotic MDD was estimated at 30.17% (95% CI = 23.53–36.80), with individuals with psychotic MDD having higher mean BD PGS [OR = 1.28, 95% CI = (1.20–1.36)], while the MDD PGS was associated with lower odds of psychotic MDD [OR = 0.93, 95% CI = (0.88–0.99)], this study is of particular interest given that it may suggest that, as expected, BD PGS may influence the risk of psychotic behavior, given its higher correlation with SCZ PGS (61, 62), while PGS MDD could define a less severe depressive subtype (31).

On the other hand, in patients with SCZ MDD PGS may have a similar effect. In fact, a study of first-episode psychosis cases examined 583 individuals from the EU-GEI study and derived transdiagnostic symptom dimensions via a bifactor model from measures such as the Positive and Negative Syndrome Scale (PANSS), finding that MDD PGS was significantly associated with lower positive [$\beta = -0.48$, 95% CI = (-0.765, -0.200), $p = 0.002$] and negative symptom scores [$\beta = -0.48$, 95% CI = (-0.754, -0.199), $p = 0.002$] but that it interacted with childhood trauma [as measured by the CTQ (Childhood Trauma Questionnaire)] to amplify positive symptoms [interaction $\beta = 0.42$, 95% CI = (0.155–0.682), $p = 0.004$], while BD PGS showed a trend toward association with lower positive symptoms [$\beta = -0.49$, 95% CI = (-0.875, -0.102), $p = 0.021$] and a significant interaction with childhood trauma on positive symptoms [$\beta = 0.45$, 95% CI = (0.106–0.798), $p = 0.010$], suggesting that genetic liability for mood disorders can influence SCZ symptomatology in a similar direction to the one observed in MDD and possibly also modulate the impact of adverse early-life experiences on psychosis symptomatology (63).

A converging evidence comes from a population study. In the Norwegian MoBa cohort, Bakken *et al*. (64) assessed 54,839 children at ages 1.5, 3, 5, and 8 years using latent growth models and latent profile analysis to characterize trajectories of emotional and behavioral difficulties, finding that the PGS for depression was significantly associated with higher baseline emotional difficulties [$\beta = 0.029$, 95% CI = (0.018–0.041), $p < 0.001$] and with a steeper increase in behavioral difficulties [$\beta = 0.041$, 95% CI = (0.024–0.058), $p < 0.001$], whereas the BD PGS was not significantly associated with overall trajectories but was specifically associated with a latent profile characterized by severe behavioral dysregulation [OR = 1.52, 95% CI = (1.21–1.90), $p = 0.001$]. This large and well powered study adds to the potentially broad effect of MDD PGS that may apply also to subjects not affected by MDD or other major psychoses.

The complex interplay with genetic liability and trauma was very recently investigated in a large study including 96,002 individuals from hospital-linked biobanks at VUMC and MGB to investigate the interaction between sexual trauma and PGSs (65). The results suggest that in individuals without sexual trauma, BD PGS was significantly associated with BD diagnosis [OR = 1.36, 95% CI = (1.31–1.42), $p < 0.002$] and MDD PGS with MDD diagnosis [OR = 1.20, 95% CI = (1.17–1.22), $p < 0.002$], while in those with documented sexual trauma, the association for BD PGS was attenuated [OR = 1.11, 95% CI = (0.99–1.23), $p = 0.072$] yet the MDD PGS association remained robust [OR = 1.21, 95% CI = (1.08–1.37), $p < 0.002$], suggesting that severe trauma may diminish the predictive power of bipolar genetic liability but not the one of depressive genetic liability, which may have a synergic contribution with trauma.

In Taiwan, Wu *et al*. (66) used data from 106,806 participants to examine associations between BD PGS with educational attainment and cognitive aging (assessed via the mini–mental state examination (MMSE) in 27,005 individuals aged ≥ 60 with longitudinal data from 6194 participants over a mean follow-up of 3.9 years), and found that BD PGS was significantly associated with higher educational attainment (OR = 1.021 per SD increase, $p = 0.001$) and that its concordant variants explained 0.48% of variance (vs. 0.39% overall), and in terms of cognitive aging, BD PGS was associated with better cognitive performance ($\beta = 0.054$, $p = 0.020$). Indeed, this study supports the complex effect of BD PGS, an effect that is not unequivocally detrimental, possibly depending on other clinical and environmental factors.

Similarly, Jiang *et al.* (67) investigated cardiovascular disease risk in 345,169 European-ancestry individuals from the UK Biobank and found that each 1-SD increase in MDD PGS was significantly associated with increased risk of atrial fibrillation [HR = 1.04, 95% CI = (1.02–1.06), $p = 1.5 \times 10^{-4}$], coronary artery disease [HR = 1.07, 95% CI = (1.04–1.11), $p = 2.6 \times 10^{-6}$], and heart failure [HR = 1.09, 95% CI = (1.06–1.13), $p = 9.7 \times 10^{-10}$] in females, whereas BD PGS showed no significant associations. This finding supports the previously discussed correlation between MDD and cardiovascular diseases, that is an area of relevant clinical and research interest.

Another piece of evidence comes from the study by Scott *et al*. (68) which examined 1473 individuals aged 15–25 from the Brisbane Longitudinal Twin Study and, through principal component analysis of four PGSs (for MDD, BD, SCZ, and NEU), derived a BD-SCZ dimension (explaining 35.7% of variance) and an MDD-NEU dimension (34.2% variance), finding that the BD-SCZ dimension was significantly higher in individuals meeting Composite International Diagnostic Interview (CIDI) criteria for a full-threshold mood or psychotic disorder ($p = 0.005$) and was significantly associated with help-seeking behavior ($p = 0.02$), while the MDD-NEU dimension was only associated with help-seeking ($p = 0.003$). One interesting aspect of this study is the further evidence of a similarity between BD and SCZ liability as well as between MDD and NEU liability while they are quite independent from each other.

An onset focused study compared 207 older adults with BD from the PsyCourse Study, distinguishing 144 early-onset BD cases (onset <50 years) from 63 late-onset cases (onset $\geq$ 50 years) (69), and found that BD PGS was significantly higher in early-onset BD ($p = 0.005$), explaining a quite relevant 6.0% of the variance (Nagelkerke's pseudo-R^2 = 6.0%), whereas MDD PGS ($p = 0.66$) were not associated with age of onset. Also in this case, the small sample size may not have been powered for the low effect sizes observed in other studies.

A similar lack of effect was reported by another study, this time on neuropsychological measures (70). The study included a network analysis in 132 first-episode psychosis patients, assessing cognitive functioning and psychopathology at 2 months and 2 years, and found that no mood PGS were significantly associated with cognitive domains, but, again, the small sample size and the complex network analysis could suggest power issues.

A much larger study reported interesting findings on symptomatology (71). The study analyzed UK Biobank data from 409,630 participants for chronotype and 239,918 for insomnia, reporting that BD PGS ($p = 4.8 \times 10^{-3}$) and MDD PGS ($p = 8.07 \times 10^{-4}$) were both significantly associated with an evening chronotype, and that both BD PGS ($p = 2.9 \times 10^{-7}$) and MDD PGS ($p < 2.2 \times 10^{-16}$) were significantly associated with insomnia, suggesting that genetic liability for mood disorders contributes to symptomatology heterogeneity and circadian dysregulation. This finding is of relevant clinical interest given the known impact of circadian dysregulation in mood disorders outcome (72–75).

Harrington *et al*. (76) investigated peripartum depression in 178 parous female inpatients from an Italian sample, dividing them into MDD ($n = 72$) and BD ($n = 106$) subgroups and applying a multipolygenic risk framework with 341 PGSs, finding that both MDD and BD PGS were negatively associated with peripartum depression, though not consistently in the two subgroups. However, the relatively small sample size and the many comparisons in the study suggest caution in interpreting findings of this study.

Suicidality is another potentially interesting phenotype and it was the focus of another very recent study in 232 youth (mean age 16.7 years) with BD ($n = 125$) or at high risk for BD ($n = 107$) in Canada (77). Results suggest that MDD PGS was nominally associated with suicidal ideation ($\beta = 0.36$, SE = 0.16, $p = 0.017$; remaining significant when controlling for family history ($\beta = 0.37$, SE = 0.15, $p = 0.016$), whereas BD PGS did not significantly predict any suicidality outcomes, again suggesting a different effect of MDD genetic liability versus BD liability also on suicidal behaviors, an area that should be further investigated for its potential clinical benefit.

The different effect may reflect on the potential diagnostic power of the two PGS. In a very large study Panagiotaropoulou *et al*. (78) analyzed 51,149 individuals (15,532 BD cases, 12,920 MDD cases, and 22,697 controls) from the Psychiatric Genomics Consortium with replication in an independent iPSYCH cohort ($n = 25{,}966$, including 2524 BD and 23,442 MDD cases) to differentiate BD from MDD using genome-wide association analyses and PGS calculated with SBayesR, finding that BD PGS significantly differentiated BD from MDD (AUC = 0.62, Nagelkerke $R^2 = 2.29\%$) and that combining BD PGS, MDD PGS, and a BD versus MDD GWAS-based PGS improved classification (AUC = 0.64, $R^2 = 4.56\%$), with MDD PGS alone contributing little, thereby reinforcing that BD and MDD PGS, though correlated, have different effects. Though focusing only on diagnostic status and not on outcome, the study is interesting in further underlining the specific effects of MDD versus BD PGS.

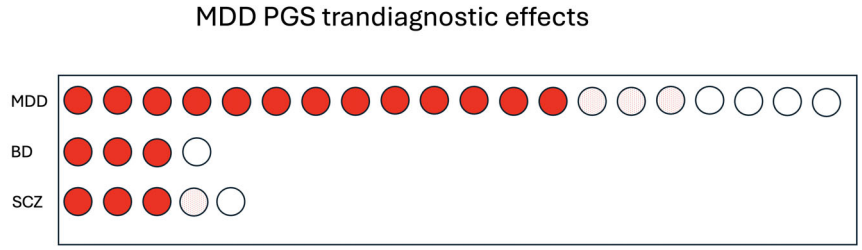

Figure 1. Visual summary of results, circles indicate independent studies on outcome or related features. Full circle: positive or nominal association, dotted circle: consistent trend, empty circle: no association. See text and tables for details.

In a similar study, Chen *et al*. (79) applied deep learning algorithms to genetic data from multiple large datasets (including MGS, SCCSS, CATIE, PGC, WTCCC, among others) to classify SCZ, BD, and MDD based on PGSs for 42 comorbid traits, and reported that for BD classification, the target BD PGS achieved an accuracy of 0.895 ± 0.020 and an AUC of 0.965 ± 0.003, while for MDD classification, the target MDD PGS achieved an AUC of 0.854 ± 0.010, with performance improving when PGSs for comorbid traits were added, suggesting that although disorder-specific polygenic risk is informative, genetic overlap with comorbid traits can further enhance diagnostic classification and possibly outcome.

Following on the possible pleiotropic effects of PGS, Segura *et al*. (80) examined the impact of PGSs on antipsychotic-induced metabolic dysregulation in a longitudinal study of 231 first-episode psychosis (FEP) patients over 6 months and found that MDD PGS, but not BD PGS were associated with total cholesterol levels (FDR = 0.006) and also at month 2 (FDR = 0.030). Though interesting, and in line with previous evidence of a correlation between metabolic and depressive backgrounds (81), also in this study the relatively small sample size suggests caution.

The complex interplay with the environment was further investigated in a study involving 573 FEP cases and 1005 controls from the EU-GEI study to investigate PGSs and environmental risk interactions (82). Results suggest that for affective psychosis, BD PGS was the strongest genetic predictor [OR = 1.50, 95% CI = (1.18–1.91), $p = 0.001$] and MDD PGS was also significant but with a smaller effect [OR = 1.34, 95% CI = (1.10–1.63), $p = 0.004$], though not interacting with environment. Therefore, whereas SCZ-spectrum disorder was primarily driven by SCZ PGS, affective psychosis may be influenced from a combination of mood disorder genetic liability and environmental factors.

Finally, Song *et al*. (83) examined 5180 BD cases from Sweden and 2577 BD cases from the UK to assess associations between BD PGS and MDD PGS with BD subphenotypes, finding that BD PGS was positively associated with full interepisode remission [OR = 1.16, 95% CI = (1.10–1.23), $p = 1.05 \times 10^{-7}$] and with higher Global Assessment of Functioning (GAF)-function scores [$\beta = 0.78$, 95% CI = (0.38–1.17), $p = 1.06 \times 10^{-4}$], and was negatively associated with comorbid anxiety disorders [OR = 0.88, 95% CI = (0.83–0.93), $p = 1.60 \times 10^{-5}$], whereas MDD PGS was negatively associated with remission [OR = 0.84, 95% CI = (0.80–0.89), $p = 2.78 \times 10^{-11}$] and GAF-function [$\beta = -0.70$, 95% CI = (−1.00 to −0.40), $p = 3.76 \times 10^{-6}$] and positively associated with comorbid anxiety [OR = 1.15, 95% CI = (1.09–1.21), $p = 8.73 \times 10^{-7}$], thus providing further evidence that MDD and BD are quite distinct polygenic liabilities that underpin different aspects of BD heterogeneity. This study is interesting also because it focuses on more detailed outcome and severity phenotypes, a much needed line of investigation, and that results further confirm the detrimental effect of MDD PGS while a mixed effect of BD PGS.

Collectively, these studies (Table 2) suggest a comprehensive picture of how PGSs for MDD and BD may influence a wide range of phenotypes that may relate to outcome: environmental exposures, symptom expression, diagnostic transitions, developmental trajectories, cognitive and educational outcomes, help-seeking behavior, treatment response itself, and even pharmacokinetic parameters, suggesting that, while higher MDD PGS is frequently associated with increased exposure to adverse events and poorer functional outcomes, BD polygenic risk exhibits a more complex pattern that can sometimes be linked to better clinical outcomes (such as higher remission rates and functioning in BD subphenotypes) and cognitive advantages, yet also modulates other aspects in a detrimental direction such as psychosis; however, the overall predictive power of these PGSs remains modest, and their clinical utility would benefit from further refinement through larger, multiancestry, longitudinal studies that integrate genetic data with environmental, clinical, and biomarker information.

Discussion

The evidence reviewed underscores both the promise and the current limitations of mood disorder PGS use in understanding the complex clinical features and treatment outcomes in major psychoses. Also, thanks to the rapid advancements in genomic discovery, due to ever-larger GWAS and more refined statistical approaches, several consistent themes emerge that clarify the clinical significance of these genetic score markers.

First, the majority of studies point to a modest but consistent relationship between MDD PGS and antidepressant treatment outcomes (Figure 1). In numerous samples, higher polygenic load for depression correlates with a greater likelihood of nonresponse, nonremission, or resistance to conventional antidepressant therapies. MDD PGS showed also a detrimental effect on BD and SCZ outcomes, this is of interest because of the transdiagnostic effect of MDD genetic liability that applies also to other diagnoses. When significant effects do arise, however, they generally explain less than 1% of variance in treatment outcomes. This small effect size highlights the persistent "missing heritability" challenge in psychiatric genomics: though large consortia have identified hundreds of common risk variants for MDD (5), they still exerts only a small influence on complex traits like symptom improvement or remission (36, 84).

By contrast, BD PGS have more variably predicted treatment outcomes (Figure 2). Some studies show little to no association with antidepressant

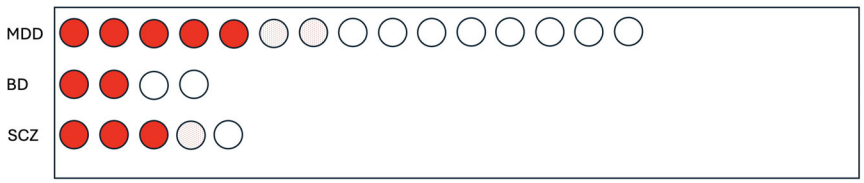

Figure 2. Visual summary of results, circles indicate independent studies on outcome or related features. Full circle: positive or nominal association, dotted circle: consistent trend, empty circle: no association. See text and tables for details.

Table 2. Summary of studies investigating MD and BD PGS and outcome-related aspects

Study	Objective	Design	Treatment	Subjects	Findings	Implications
Abdulkadir *et al.*, 2025	ED transitions and MD/BD PGS	Registry-based analysis of Danish hospital records (1995–2018)	None	$N = 10,565$ individuals with eating disorders (AN = 6901; BN = 1417; EDNOS = 2247)	Higher MDD PGS associated with a 15% greater hazard of transitioning from anorexia nervosa to bulimia nervosa or EDNOS (HR = 1.15 per SD increase, $p < 1.57 \times 10^{-4}$); BD PGS was not significantly associated with diagnostic transitions.	Genetic liability for depression may influence diagnostic shifts in eating disorders
Alameda *et al.*, 2024	MD/BD PGS and childhood adversity in FEP	Cross-sectional analysis in FEP cases from the EU-GEI study	None	$N = 583$ FEP cases	MDD PGS was inversely associated with both positive and negative symptom scores ($\beta = -0.48$, $p = 0.002$) and interacted with childhood trauma (CTQ) on positive symptoms ($\beta = 0.42$, $p = 0.004$). BD PGS showed a trend for lower positive symptoms ($\beta = -0.49$, $p = 0.021$) with a significant CTQ interaction ($\beta = 0.45$, $p = 0.010$).	Genetic liability for MD and BD modulates the impact of childhood adversity on psychosis symptoms
Bakken *et al.*, 2024	Childhood trajectories and MD/BD PGS	Longitudinal analysis using latent growth models and latent profile analysis in the MoBa cohort	None	$N = 54,839$ children (assessed at 1.5, 3, 5, and 8 years)	PGS for depression (PGSDEP) associated with higher baseline emotional difficulties [$\beta = 0.029$, 95% CI = (0.018–0.041), $p < 0.001$] and with a steeper increase in behavioral difficulties [$\beta = 0.041$, 95% CI = (0.024–0.058), $p < 0.001$]; BD PGS was not significantly associated with trajectories.	Depression genetic liability influences early emotional and behavioral development, whereas bipolar genetic risk does not manifest in early childhood trajectories.
Chen *et al.*, 2025	Deep learning classification using MD/BD PGS	Multidataset classification using elastic net regression and deep neural networks; cross-sectional	None	Cases of schizophrenia, BD, MDD, and controls from datasets including MGS, SCCSS, CATIE, PGC, WTCCC, etc.	BD classification using BD PGS achieved accuracy = 0.895 ± 0.020 and AUC = 0.965 ± 0.003; MDD classification using MDD PGS achieved accuracy = 0.782 ± 0.015 and AUC = 0.854 ± 0.010; adding comorbid trait PGSs further improved performance.	Integrating disorder-specific and comorbid PGSs with deep learning substantially improves diagnostic classification of BD and MDD
Crouse *et al.*, 2024	Stress exposure and MD/BD PGS	Cross-sectional analysis using data from the Australian Genetics of Depression Study	None	$N = 14,146$ (75% female; mean age = 44.0 years, SD = 15.3); adults with depression	Higher MDD PGS associated with increased odds of childhood stressful life events (SLEs) (ORs = 1.07–1.12, p's < 0.013, FDR-corrected) and specific events [physical assault: OR = 1.06 (1.02–1.11), $p = 0.006$; sexual assault: OR = 1.10 (1.05–1.16), $p < 0.001$; severe human suffering: OR = 1.17 (1.05–1.30), $p = 0.003$]. In contrast, higher BD PGS was associated with lower odds of physical assault [OR = 0.95 (0.91–0.99), $p = 0.014$], major financial troubles [OR = 0.93 (0.88–0.98), $p = 0.004$], unpleasant surroundings [OR = 0.92 (0.87–0.98), $p = 0.008$], and fewer childhood SLEs [OR = 0.97 (0.95–0.99), $p = 0.01$].	MD genetic liability may increase exposure to stress, whereas BD genetic risk appears linked to fewer reported SLEs, challenging traditional subtype distinctions.
Fahey *et al.*, 2024	Sleep traits & MD/BD PGS	Cross-sectional analysis using UK Biobank data	None	For chronotype: N = 409,630; for insomnia: N = 239,918	BD PGS associated with an evening chronotype ($p = 4.8 \times 10^{-3}$) and with insomnia ($p = 2.9 \times 10^{-7}$); MDD PGS associated with evening chronotype ($p = 8.07 \times 10^{-4}$) and with insomnia ($p < 2.2 \times 10^{-16}$).	Shared genetic liability for mood disorders influences sleep patterns, implicating circadian dysregulation as an intermediate phenotype in psychiatric disorders.
Gil-Berrozpe *et al.*, 2025	BD/MDD PGS and cognition in FEP	Longitudinal network analysis in first-episode psychosis with assessments at 2 months and 2 years	None	$N = 132$ first-episode psychosis patients	Neither BD PGS nor MDD PGS showed associations with cognitive functioning	The small sample size and the complex network analysis could suggest power issues.
Harrington *et al.*, 2024	PPD risk and MD/BD PGS in peripartum	Cross-sectional machine-learning analysis (PLS regression) in an Italian inpatient sample	None	$N = 178$ parous female inpatients (MDD = 72; BD = 106; PPD present = 62, absent = 116)	MDD and BD PGS were negatively associated with peripartum depression	The relatively small sample size and the many comparisons in the study suggest caution

(*continued*)

Table 2—*Continued*

Study	Objective	Design	Treatment	Subjects	Findings	Implications
Jiang *et al.*, 2024	MD/BD PGS and cardiovascular risk	Observational cohort study using UK Biobank data with replication in BioVU	None	UK Biobank: $N = 345{,}169$ (European ancestry)	In females, each 1-SD increase in MDD PGS was associated with increased risk of atrial fibrillation [HR = 1.04, 95% CI = (1.02–1.06), $p = 1.5 \times 10^{-4}$], coronary artery disease [HR = 1.07, 95% CI = (1.04–1.11), $p = 2.6 \times 10^{-6}$], and heart failure [HR = 1.09, 95% CI = (1.06–1.13), $p = 9.7 \times 10^{-10}$]; BD PGS showed no significant associations.	Genetic liability for depression may elevate cardiovascular risk in females even in the absence of a clinical diagnosis
Kowalec *et al.*, 2021	TRS predictors: MD/BD PGS	Registry and genomic study in Swedish national registers	None	$N = 24{,}706$ SCZ cases (genomic subset $N = 4936$)	BD PGS, and MDD PGS were not significant, though both PGS showed a non significant trend in the direction of TRS	In treatment-resistant SCZ, familial and cognitive factors are more predictive than common MD or BD polygenic risk
Lake *et al.*, 2025	Sexual trauma and MD/BD PGS interactions	Cross-sectional analysis from hospital-linked biobanks (VUMC and MGB) with retrospective trauma data	None	$N = 96{,}002$ individuals	In individuals without sexual trauma, BD PGS was associated with BD diagnosis [OR = 1.36, 95% CI = (1.31–1.42), $p < 0.002$] and MDD PGS with MDD diagnosis [OR = 1.20, 95% CI = (1.17–1.22), $p < 0.002$]. Among those with sexual trauma, the BD PGS association was attenuated [OR = 1.11, 95% CI = (0.99–1.23), $p = 0.072$] while MDD PGS remained significant [OR = 1.21, 95% CI = (1.08–1.37), $p < 0.002$]	Sexual trauma moderates the effect of bipolar genetic risk on diagnosis, suggesting that severe environmental stress may diminish the predictive power of BD PGS, whereas MDD PGS remains robust.
Mayén-Lobo *et al.*, 2021	BD/MDD PGS and clozapine metabolism	Cross-sectional integrative genomic-epigenomic analysis in clozapine-treated refractory psychosis	Clozapine treatment	$N = 44$ patients (SCZ = 31; Schizoaffective disorder = 9; BD = 4)	BD PGS was significantly associated with clozapine metabolic ratio (pseudo-$R^2 = 0.2080$, $p = 0.0008$, adjusted $p = 0.0189$); MDD PGS was nominally associated with clozapine dose ($p = 0.0035$, adjusted $p = 0.0759$).	Bipolar genetic liability may affect clozapine metabolism and MDD PGS the prescribed dose
Montejo *et al.*, 2025	BD PGS and age of onset in BD	Cross-sectional comparison in older adult bipolar disorder from the PsyCourse Study	None	$N = 207$ older adults with BD (early-onset BD = 144; late-onset BD = 63)	BD PGS was significantly higher in early-onset BD compared to late-onset BD ($p = 0.005$), explaining 6.0% of the variance (Nagelkerke's pseudo-R^2 = 6.0%); PGS-SCZ ($p = 0.27$) and MDD PGS ($p = 0.66$) were not significantly associated with age of onset.	Higher bipolar genetic liability characterizes early-onset BD
Nguyen *et al.*, 2025	Genetic profile in psychotic depression	Population-based registry analysis using Swedish and Danish national data; PGS analysis	None	>5.1 million individuals; PGS analyses performed on ~30,000 genotyped MDD cases (from UK Biobank and a Swedish clinical cohort)	Psychotic MDD cases showed higher BD PGS (OR = 1.28, 95% CI = 1.20–1.36) but lower MDD PGS (OR = 0.93, 95% CI = 0.88–0.99) compared to nonpsychotic MDD.	Psychotic depression exhibits a distinct genetic profile, with greater overlap with bipolar liability, suggesting it is genetically less similar to typical MDD.
Panagiotaropoulou *et al.*, 2025	Differentiate BD from MDD via PGS	GWAS and PGS analysis using PGC data with replication in the iPSYCH cohort	None	PGC: $N = 51{,}149$ (BD = 15,532; MDD = 12,920; controls = 22,697); iPSYCH: $N = 25{,}966$ (BD = 2524; MDD = 23,442)	BD PGS significantly differentiated BD from MDD (AUC = 0.62, Nagelkerke's $R^2 = 2.29\%$); combining BD PGS, MDD PGS, and BD vs. MDD PGS improved classification (AUC = 0.64, R^2 = 4.56%); MDD PGS alone contributed little.	BD and MDD are genetically distinct; BD-specific polygenic risk may aid in differential diagnosis
Piazza *et al.*, 2024	Depression PGS and symptom networks	Cross-sectional network analysis in two population-based cohorts (ALSPAC and TEDS)	None	ALSPAC: $N = 5521$ (mean age = 11.8 years, SD = 0.14; 50.3% female); TEDS: $N = 4625$ (mean age = 11.27 years, SD = 0.69; 53.2% female)	The depression PGS was significantly associated with the symptom "not enjoying anything" ($r = 0.04$) and with "being bullied" ($r = 0.06$) in the peer problems subscale.	Genetic risk for depression appears concentrated on specific core symptoms and environmental stressors
Rodriguez *et al.*, 2024	Genetic and environmental risk in affective psychosis	Multisite case-control study (EU-GEI) with cross-sectional PGS and environmental risk (MERS) analysis	None	$N = 573$ FEP cases and 1005 controls (European ancestry)	For affective psychosis, BD PGS [OR = 1.50, 95% CI = (1.18–1.91), $p = 0.001$] and MDD PGS [OR = 1.34, 95% CI = (1.10–1.63), $p = 0.004$] were significantly associated; no significant interaction with MERS was observed, indicating additive effects.	Affective psychosis appears to arise from a combination of bipolar and depressive genetic liability plus environmental risk

(*continued*)

Table 2—*Continued*

Study	Objective	Design	Treatment	Subjects	Findings	Implications
Scott *et al.*, 2025	PGS & help-seeking in youth mood disorders	Cross-sectional analysis from the Brisbane Longitudinal Twin Study with principal component analysis of PGS	None	N = 1473 individuals aged 15–25; 29% (n = 409) met CIDI criteria for mood/psychotic disorders; 26% (n = 388) sought professional help	A BD-SCZ dimension (explaining 35.7% variance) was significantly higher in individuals with a CIDI diagnosis (p = 0.005) and was significantly associated with help-seeking (p = 0.02); an MDD-NEU dimension (34.2% variance) was also associated with help-seeking (p = 0.003).	Genetic liability for mood disorders influences help-seeking behavior in youth
Segura *et al.*, 2022	Metabolic effects and MD/BD PGS in FEP	Longitudinal study (6-month follow-up) in first-episode psychosis patients	Antipsychotic treatment (including exposure to olanzapine/clozapine)	*N* = 231 FEP patients (baseline: 192–220; 6-month follow-up: 118–179)	Higher MDD PGS associated with increased total cholesterol over time (FDR = 0.006 overall; FDR = 0.030 at month 2); PGS-BD was not significantly associated with metabolic progression.	Depression polygenic risk may modestly influence antipsychotic-induced metabolic dysregulation, whereas bipolar genetic risk appears uninvolved.
Song *et al.*, 2024	BD subphenotypes and MD/BD PGS	Multicohort analysis in BD cases from Sweden and the UK	None	Sweden: *N* = 5180; UK: *N* = 2577 BD cases	BD PGS was positively associated with full interepisode remission [OR = 1.16, 95% CI = (1.10–1.23), $p = 1.05 \times 10^{-7}$] and higher GAF-function [β = 0.78, 95% CI = (0.38–1.17), $p = 1.06 \times 10^{-4}$], and negatively with comorbid anxiety [OR = 0.88, 95% CI = (0.83–0.93), $p = 1.60 \times 10^{-5}$]; conversely, MDD PGS was negatively associated with remission [OR = 0.84, 95% CI = (0.80–0.89), $p = 2.78 \times 10^{-11}$] and GAF-function [$\beta = -0.70$, 95% CI = (−1.00 to –0.40), $p = 3.76 \times 10^{-6}$] and positively with anxiety [OR = 1.15, 95% CI = (1.09–1.21), $p = 8.73 \times 10^{-7}$].	Different polygenic liabilities shape BD heterogeneity: BD PGS is linked to better clinical outcomes, whereas MDD PGS correlates with poorer functioning and greater anxiety
Wu *et al.*, 2024	BD PGS and education/cognition	Cross-sectional and longitudinal analysis using data from the Taiwan Biobank	None	For education: *N* = 106,806; for cognitive aging: *N* = 27,005 aged ≥ 60 (longitudinal follow-up: *n* = 6194; mean follow-up = 3.9 years)	BD PGS associated with higher educational attainment (OR = 1.021 per SD increase, *p* = 0.001) and with better cognitive performance (β = 0.054, *p* = 0.020)	Bipolar genetic liability may confer advantages in education and cognition, reflecting a complex pleiotropic influence of BD risk alleles.
Zai *et al.*, 2025	PGS and suicidality in youth BD	Cross-sectional analysis from the Centre for Youth Bipolar Disorder, Canada	None	*N* = 232 youth (mean age = 16.7; BD = 125; high risk for BD = 107)	MDD PGS was nominally associated with suicidal ideation (β = 0.36, SE = 0.16, *p* = 0.017), while BD PGS not significantly associated with any suicidality outcomes.	Depression genetic liability may contribute to suicidal ideation in youth at risk for bipolar disorder

Abbreviations: ALSPAC, Avon Longitudinal Study of Parents and Children; AN, anorexia nervosa; AUC, area under the curve; BD, bipolar disorder; BLTS, Brisbane Longitudinal Twin Study; BN, bulimia nervosa; CI, confidence interval; CIDI, Composite International Diagnostic Interview; CNV, copy-number variant; CTQ, Childhood Trauma Questionnaire; DNN, deep neural network; ED, eating disorder; EDNOS, eating disorder not otherwise specified; FDR, false discovery rate; FEP, first-episode psychosis; GAF, global assessment of functioning; HR, hazard ratio; iPSYCH, Integrative Psychiatric Research; MD, major depressive disorder; MERS, Maudsley Environmental Risk Score; MoBa, Norwegian Mother, Father, and Child Cohort Study; OR, odds ratio; PGS, polygenic score; PPD, peripartum depression; SCZ, schizophrenia; SD, standard deviation; TEDS, Twins Early Development Study; TRS, treatment-resistant schizophrenia; URV, ultra-rare variant.

response in unipolar depression, while others suggest that BD PGS contributes to the likelihood of a favorable response to lithium in BD. Notably, multiple investigations of lithium pharmacogenetics converge on the finding that bipolar patients with lower MDD PGS are more likely to respond well to lithium. Conversely, a higher MDD PGS tends to predict less favorable outcomes under lithium treatment. These complementary patterns raise the possibility that BD is a heterogeneous construct composed of partially distinct subsamples wherein one subgroup has less polygenic liability for depression and better lithium responsiveness. This could be explained by the hypothesis that MDD PGS are indeed an indicator of higher NEU (31), therefore high MDD PGS could select a subsample of patients that are less responsive to treatment because of personality traits (85, 86). Recent approaches in detecting biotypes could further inform on those aspects (87).

A second prominent theme in the reviewed literature is the difficulty in translating these genetic markers into robust clinical tools. Even when PGS reach nominal significance, their additional explanatory power in predicting outcomes beyond conventional clinical predictors, such as baseline severity, comorbidities, or duration of illness, often remains marginal (88). Similarly, the ability of PGS to distinguish clinical phenotypes (e.g., early- vs. late-onset BD, or those with vs. without rapid cycling) is often modest, typically explaining well under 5% of variance. While these small effect sizes do not negate the scientific value of PGS, they underscore the need for polygenic information to be further improved in their predictive value and integrated into multifactorial models that also capture environmental, demographic, and biomarker data.

Third, a recurring observation is that higher MDD polygenic liability correlates with increased exposure to stressors and poorer psychosocial outcomes in both individuals with and without depression. Several studies find that participants with higher MDD PGS report more stressful life events, greater feelings of loneliness under adversity, higher incidence of cardiometabolic dysfunction, and overall poorer functional trajectories. While it may seem counterintuitive that genetic features correlate with environment, it has been clearly suggested that this may indeed be possible via specific at-risk behaviors (89). By contrast, the BD PGS often exhibits more complex or even seemingly paradoxical patterns. On

one hand, it can associate with better educational outcomes or higher cognitive functioning; on the other, it may predispose to certain affective or psychotic dimensions in specific contexts. This duality likely reflects the polygenic overlap between BD and creativity/cognition, as well as the broader pleiotropy observed for psychiatric and cognitive traits. Such evidence highlights how BD liability does not uniformly confer negative outcomes and may, in some contexts, be advantageous (90, 91). The PGS effects on stressors further complicates multivariable analyses, given the reciprocal effects, this should be taken into account when modeling the analysis.

Fourth, ancestry and sample size constraints remain major concerns. Most GWAS to date have been based on Caucasian populations, limiting the generalizability of polygenic findings. Although recent studies in Asian populations, especially Han Chinese samples, have shown broadly consistent directions of effect for MDD PGS, differences in linkage disequilibrium structure and allele frequencies may lead to attenuation of predictive power if PGS are primarily derived from European samples (46). Likewise, studies with small or heterogeneous target samples reduce statistical power and can inflate the risk of spurious findings. As the field moves forward, replication in large, multiancestry cohorts with harmonized phenotyping will be essential to refine the clinical validity of PGS. Otherwise results will be much limited in terms of model portability and prediction reliability. Recent GWAS are increasingly including other ancestries and will lead to more widely generalizable results (5).

Beyond these core themes, the reviewed studies point toward promising new directions. Efforts to integrate PGS with neurophysiological measures (e.g., EEG biomarkers) or to combine multiple PGS into meta-analytic risk profiles are providing incremental gains in predictive accuracy. Machine-learning approaches that incorporate both genetic and clinical data can, in some circumstances, yield more substantial improvements in outcome prediction compared to linear models (88, 92–94). Moreover, the discovery of gene-by-environment interactions, though so far modest, suggests that specific PGS may modulate the impact of stressors, trauma, or other risk exposures on disease severity or symptom manifestation.

Taken together, the most consistent finding is that MDD PGS may modulate both risk and outcome: it correlates with susceptibility to depression, can subtly shape treatment response and clinical features also transdiagnostically, and it seems to confer broad liability for symptomatology profile in population studies. Meanwhile, BD PGS exerts a distinctly different, and more variegated, set of influences, sometimes correlating with positive functional traits and other times associating with bipolar-specific clinical features and psychotic features. Yet in no instance has the predictive power of either MDD or BD PGS reached a threshold that would recommend immediate translation into routine psychiatric practice, though in some cases the use of extreme deciles could in a near future offer a clinically relevant prediction. Rather, these scores should be viewed as incremental predictive markers, useful in large-scale risk stratification or as part of research aimed at dissecting the heterogeneity of mood disorders and psychiatric disorders in general. Hopefully, in a near future, PGS could support clinicians in the choice of treatment, as an example with a more intensive treatment at baseline in case of negative outcome PGS prediction, at least in extreme deciles. Indeed protocols with this aim are underway (95).

Results presented in this review should be interpreted according to some limitations, the selection of the studies, though broad and performed according to convergent methods, was not following common guidelines, in order to offer a broad view of the topic. The sample size of the studies varied from small samples to large population ones, with issues on one side of adequate power and on the other of heterogeneity and poor phenotyping. Most relevant for the aim of this review is the fact that in many studies the PGS was calculated in relatively small origin GWAS samples, particularly in older studies, and this may have reduced the power of the analyses. In fact, most of the reviewed studies rely on relatively old GWAS summary statistics that are less informative in terms of explained variance when compared to the most recent studies. However in the coming years, ongoing GWAS expansions, coupled with better computational and statistical methodologies, will increase the accuracy of MDD and BD PGS thanks to the very recent large studies with public summary statistics (5, 6).

Broader ancestry representation and deeper phenotyping, incorporating longitudinal treatment response data, real-time symptom monitoring, and biological markers like inflammatory or neuroimaging signatures, could lead to a more predictive framework. Ultimately, the goal is precision psychiatry, wherein PGS could be integrated with clinical profiling to tailor interventions for each patient. While the studies synthesized here indicate that the field has taken meaningful steps toward that goal (Table 3), they also reveal how far we have to go. The modest effect sizes of current PGS demand caution, but, together with results of SCZ PGS (51), they also highlight an evolving scientific frontier that, with continued investment and methodological refinement, holds significant promise for improving care in mood and psychotic disorders.

Materials and Methods

This review synthesized evidence on the relationship between MD and BD PGS and major psychoses outcomes using a nonsystematic approach. A nonsystematic review was chosen to allow for a broad and flexible exploration of the available literature, given the heterogeneous methodologies, diverse study populations, and varying definitions of treatment response, remission, and resistance across studies. Systematic reviews require predefined inclusion criteria and structured data synthesis, which may not be suitable for topics with rapidly evolving research, methodological diversity, and studies using different polygenic scoring techniques (96). A nonsystematic approach may enable a more inclusive examination of the findings while integrating insights from various study sampling, designs and relevant phenotypes.

Study Selection

Studies were selected based on their relevance to PGS and treatment outcomes, including treatment response, remission, resistance, and disease severity. Inclusion criteria focused on original research that involved primarily adult populations diagnosed with MDD, BP, or SCZ. Nonoriginal articles, such as reviews, meta-analyses, commentaries, and editorials, were excluded, along with studies that did not explicitly assess disease outcomes in relation to PGS.

Search Strategy

A targeted literature search was conducted using PubMed and Google Scholar, employing a range of relevant keywords and search terms related to PGSs and psychiatric disorders. These included:

> "polygenic score", "PGS", "risk profile score", "genetic risk score", "genetic score", "polygenic", "depression", "mood", "schizophrenia", "antidepress", "treatment resistance", "bipolar", "BP", "BD", "treatment outcome", "antipsycho*", "stabiliz*", and "remission"*, in various combinations.

To ensure comprehensive coverage, additional studies were identified through citation tracking, including forward citation searches (examining studies that have cited key papers) and backward citation searches (reviewing references cited within relevant articles). Studies known to the authors or cited in prior literature reviews were also considered when relevant. Given the pleiotropy of the genetic factors and the complex interplay of clinical and environmental factors, relevance of the selected papers was based on the previously defined outcomes and possible outcome-related phenotypes.

Data Extraction and Synthesis

Extracted data included: Sample size, population characteristics (e.g., diagnosis, demographic details), definitions of treatment outcomes (response, remission, resistance, and severity), PGS calculation methods, statistical results (associations between PGS and psychiatric outcomes). Given the heterogeneity in study methodologies, including differences in PGS computation, sample populations, outcome definitions, and statistical approaches, a meta-analytic approach was not feasible. Instead, findings were synthesized narratively, summarizing trends and highlighting key associations between PGS and psychiatric treatment outcomes and relevant phenotypes.

Table 3. Results summary

Focus/Phenotype	MDD PGS	BD PGS
Antidepressant response	• Consistently shows modest but significant correlations with poorer outcomes (lower remission, higher risk of nonresponse or TRD).	• No strong link to MDD antidepressant response.
Treatment-resistant depression (TRD)	• Frequently associated with TRD, though not always surviving strict multiple testing corrections.	• No consistent or notable association with TRD.
Lithium response in bipolar disorder	• In BD cohorts, higher MDD PGS → poorer lithium response.	• BD PGS alone sometimes shows a positive (or neutral) link to lithium response, but is less consistent than MDD or SCZ PGS.
Bipolar course and subtypes	• Within BD, higher MDD PGS often predicts more depressive episodes, poorer remission, and increased anxiety.	• BD PGS can be linked to better overall functioning or remission in BD, but also sometimes to psychotic/affective features.
SCZ/psychosis dimensions	• In first-episode psychosis, higher MDD PGS can correspond to lower "core psychosis" severity but might exacerbate affective or stress-related symptoms.	• BD PGS shows a somewhat similar pattern of reducing core psychosis but interacting with adversity to worsen positive symptoms.
Environmental and stress interactions	• Higher MDD PGS correlates with increased exposure to life stress and heightened susceptibility to negative emotional outcomes in stressful settings.	• BD PGS has shown weaker or inconsistent G × E interactions compared to MDD PGS.
Comorbidities and functional traits	• Linked to higher rates of cardiometabolic risk, suicidality, or anxiety. • Can heighten the likelihood of a "switch" to more severe conditions (e.g., from anorexia to bulimia).	• Often associated with higher educational attainment or better cognition in some populations, but also with risk of mania or psychotic features in others. • High BD PGS in BD populations linked to better remission and functioning.
Overall effect sizes and clinical utility	• MDD PGS consistently shows a subtle negative impact on depression outcomes but rarely exceeds 1% in variance explained.	• BD PGS alone does not strongly predict MDD outcomes, but in BD it can contribute to lithium response, remission, and subtypes.

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RESEARCH ARTICLE

5′LysTTT tRNA fragments support survival of botulinum-intoxicated neurons by blocking ferroptosis

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Botulinum neurotoxins (BoNTs) block cholinergic signaling at neuromuscular junctions, inducing transient muscle paralysis while avoiding neuronal death. However, the mechanism(s) underlying these dual features are yet unknown. Here, we report accumulation of 5′Lys transfer RNA fragments (tRFs) in both BoNT/A-intoxicated cultured human neuroblastoma cells and submandibular glands from BoNT/A-intoxicated rodents. Importantly, we show that 5′LysTTT tRFs balance ferroptosis by cointeracting with the RNA-binding ferroptosis-inducing protein *HNRNPM* and the 3′ untranslated region of the ferroptosis-inhibiting *CHAC1* mRNA. Moreover, approximately 20% of the BoNT/A-induced tRFs shared an 11-nucleotide-long LysTTT and LysCTT tRFs-included motif, CCGGATAGCTC, which may target transcripts containing complementary sequences, including the *UNC5B* transcript that can regulate cell survival. Collectively, the multiple regulatory roles of tRF-5′LysTTT and the shared repetitive motif reveal mechanism(s) supporting the survival of cholinergic neurons under BoNT/A exposure. This understanding may predict the development of novel BoNT/A therapeutic avenues for treating diverse neuromuscular disorders and BoNT/A cosmetic procedures.

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Introduction

Botulinum neurotoxins (BoNTs) are the most potent biological toxins known to humans, with an estimated lethal dose of approximately 1 ng/kg (1). These toxins are the causative agents of botulism, a severe and potentially fatal neuroparalytic illness affecting both humans and animals (2). Paradoxically, BoNTs also form the basis for a variety of cosmetic and therapeutic applications (3). Produced by anaerobic *Clostridium* bacteria, BoNTs are initially synthesized as single-chain precursor proteins, which are subsequently processed into a 100 kDa heavy chain and a 50 kDa light chain. Different *Clostridium* strains produce seven BoNT serotypes (A to G) (4), of which serotypes A, B, E, and rarely F, are associated with human botulism. Annually, a few hundred cases of infant or adult intestinal botulism are reported worldwide (5). Mechanistically, BoNTs act by entering peripheral cholinergic neurons through a multistep cellular intoxication process that culminates in the cleavage of core SNARE proteins. This interrupts acetylcholine (ACh) trafficking and release at the neuromuscular junction (NMJ), leading to paralysis (6, 7). Infants are particularly vulnerable to botulism, and since 1979, infant botulism has been the most commonly diagnosed form of this poisoning in the United States (8). Neurological symptoms in infants are similar to those in adults, but are often overlooked because infants cannot verbalize their discomfort. The most common sources of infant botulism are contaminated honey and environmental exposure (9, 10). From 2000 to 2019, infant botulism represented 71% of the 3241 human botulism cases detected in the United States (11).

While BoNT/C and BoNT/E serotypes have been shown to cause neurodegeneration in both cultured neurons and live mice (12, 13), BoNT/A does not induce neurodegeneration despite its similar ability to block synaptic vesicle exocytosis and cleave the SNAP25 protein (albeit at different positions from BoNT/E) (13). The sustained neuronal survival observed under BoNT/A intoxication has enabled its therapeutic application at picomolar or higher concentrations to induce prolonged flaccid paralysis (14). This effect is utilized in treating disorders such as dystonia, hyperhidrosis, and essential tremors (15). However, the underlying mechanism of action remains largely unexplained. Subsequent studies identified certain proteasome enzymes as key regulators of the duration of post-BoNT/A effects (16, 17) with some analyses focusing on mRNA transcripts. Additionally, BoNT/A injections have become the most popular cosmetic procedure worldwide, used to reduce wrinkles with effects lasting several months and impacting over 7 million people annually (18). Despite these advancements, neither basic research studies nor investigations into cosmetic applications have elucidated the downstream processes triggered by BoNT intoxication or the molecular mechanisms supporting neuronal survival. To address this gap, we aimed to elucidate the cellular responses to BoNT/A intoxication. Given recent insights into the regulatory roles of microRNAs (miRNAs) (19) we studied the involvement of small noncoding RNAs (sncRNAs) in these processes. Specifically, we profiled sncRNA transcripts in cultured human neuroblastoma cells following BoNT/A intoxication to identify miRNA, small-interfering RNAs, and the recently re-discovered transfer RNA fragments (tRFs) (20–22) that may contribute to postintoxication cellular responses.

tRFs are derived from the cleavage of both mature transfer RNA (tRNA) and tRNA precursors. Their biogenesis is highly regulated, depending on tissue, physiological state, and developmental stages of the cells or tissues in which they accumulate. This process reflects conserved and specific tRNA cleavage mechanisms, rather than random tRNA degradation (22, 23). tRFs typically range from 16 to 50 nucleotides (nt) in length and are endonucleolytic cleavage products of specific ribonucleases to generate shorter, functional fragments (24–26). Recent studies have revealed that tRFs play diverse roles across various nervous system cell types and organisms. Notably, tRFs targeting cholinergic transcripts have been

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identified to predominantly originate from the mitochondrial genome (21) and exhibit a decline in abundance with age (23).

Multiple nucleases mediate the cleavage of tRNA and tRNA precursors to generate tRFs (27, 28). Importantly, tRFs can originate from either the nuclear or mitochondrial genome (29), and their functional roles are diverse. They may regulate protein translation in a manner akin to miRNAs (30) silence genes through base pairing with target mRNAs (20, 22, 31), or interact with RNA-binding proteins (32). The cleavage of tRNA molecules produces tRFs that can mediate neuroprotective effects by interacting with specific mRNA targets (33, 34). However, the potential role of tRFs in supporting the survival of human-originated neurons under botulinum intoxication, as well as the underlying mechanisms involved, remained unknown.

The immediate stress-induced accumulation of specific subtypes of tRFs facilitates the intracellular formation of stress granules, where these fragments localize to protect cells from acute stress (35). However, the processes occurring after the acute exposure phase remain poorly understood. In this respect, neuronal death in numerous neurodegenerative and neurological disorders has recently been linked to ferroptosis, a nonapoptotic cell death mechanism characterized by increased membrane lipid peroxidation, accumulation of lipid peroxides, and an inadequate capacity to counteract these processes (36–38). Neurons, being enriched in phospholipids containing polyunsaturated fatty acids (PUFAs) and iron (39, 40), rely on protective mechanisms to prevent lipid peroxidation under normal conditions. Interestingly, BoNT/A has been shown to alleviate cartilage degradation and inhibit osteoarthritis progression by preventing ferroptosis in chondrocytes (41). However, under pathological conditions, these defense mechanisms are compromised, promoting the recently identified ferroptosis cell death pathway (42). Despite this, the role of BoNT/A in modulating neuronal ferroptosis remains largely unexplored.

To study the molecular mechanisms underlying botulinum intoxication in neurons, we utilized the human-derived LAN5 neuroblastoma cell line (21). Small RNA profiling of BoNT/A-intoxicated LAN5 cells revealed profound changes in the neuronal transcripts landscape, suggesting a link between these changes and the mechanisms by which cholinergic neurotransmission is blocked while neuronal survival is maintained. Unexpectedly, our analysis uncovered a previously unrecognized accumulation of tRFs that initiate multiple protective pathways to both counteract neuronal death and inhibit ferroptosis. Furthermore, we identified a BoNT/A-induced accumulation of a repetitive tRNA-derived motif among the elevated postintoxication tRFs, which together appear to ensure the survival of intoxicated neurons by blocking ferroptosis. Deciphering these simultaneous protective pathways offers valuable insights into the mechanisms of BoNT/A-induced neuronal survival and may pave the way for novel BoNT/A-based therapeutic applications.

Results

Neuronal Survival Under BoNT/A Intoxication Involves Massive Transcriptomic Changes

The lethal dose of BoNT/A per person is estimated to range between 0.03 and 1 μg depending on the route of administration (5). To explore the cellular response to BoNT/A intoxication, LAN5 human neuroblastoma cells were exposed to a potentially lethal dose of BoNT/A (10,000 $MsLD_{50}$/mL, equivalent to ∼ 50 ng; Figure 1A), a concentration significantly higher than doses used in cosmetics applications (18). ELISA-based quantification of cleaved-SNAP25 levels (43) was used to establish a time-course assay, enabling the assessment of BoNT/A effects on LAN5 cells (Figure 1B). A dose–response curve spanning a wide range of BoNT/A concentrations was generated, yielding an EC_{50} of approximately 4000 $MsLD_{50}$/mL (∼120 pM toxin concentration) and a detection limit of 2–3 pM BoNT/A (Figure 1C).

An in vitro cell-based assay demonstrated neuronal survival following BoNT/A intoxication (13) (Figure 1D), providing a platform to explore molecular mechanisms regulating survival under such conditions. Long-read RNA sequencing (RNA-seq) of BoNT/A-intoxicated LAN5 cells identified differentially expressed (DE) transcriptomic changes, with 217 transcripts significantly downregulated and 135 transcripts upregulated under BoNT/A exposure (Figure 1E and F and Supplementary Table S1), using a false discovery rate (FDR) of 0.05. Gene Ontology (GO) enrichment analysis of the downregulated transcripts revealed significant enrichment in biological processes such as *tRNA aminoacylation for protein translation* (Figure 1G). These findings suggested an altered repertoire in intoxicated cells of noncoding tRNAs, critical for protein synthesis and involved in diverse cellular processes and regulatory pathways.

BoNT/A-intoxicated LAN5 Cells Display Massive tRFs Accumulation

Small RNA-seq of total RNA from BoNT/A-intoxicated LAN5 cells and nontreated (NT) cells (Figure 2A, >12 million single reads per sample) was analyzed using miRExpress 2.1.4 (44) for miRNA levels and the MINTmap pipeline (45) with default parameters for tRF levels (using only reads mapping exclusively to the tRNA space). This analysis showed modest changes in miRNA levels (Figure 2B and Supplementary Table S2), which contrasted with massive increases and decreases in tRFs levels of both nuclear and mitochondrial genome origins (Figure 2C and D and Supplementary Table S3). Specifically, only 2 DE miRNAs were identified, whereas 335 DE tRFs were detected, with 63% upregulated under BoNT/A exposure. Among these, tRF-5′LysTTT (also known as tDR-1:31-Lys-TTT-3-M2 or tRF-31-PS5P4PW3FJHPB) emerged as the most significantly elevated tRF in BoNT/A-intoxicated LAN5 cells (Figure 2E) compared to BoNT/E and TeNT toxin (Supplementary Figure S2). Classifying the DE tRFs by fragment type (Figure 2C and F) revealed that 189 of the 335 DE tRFs were internal tRFs, with 38 (all upregulated) originating from lysine tRNA and 51 from glutamate tRNA (most of them upregulated) (Figure 2G). Subdividing the DE tRFs by length showed that all of the upregulated tRFs were relatively long (25 nt and above), whereas the downregulated tRFs were short (less than 25 nt) and therefore more lilkely to function like miRNAs (Figure 2H). Together, these findings indicate a nonarbitrary fragment generation of regulatory sncRNAs during BoNT/A intoxication. Furthermore, the massive tRNA fragmentation observed likely corresponds to cell viability, as tRFs are known to be regulated under cell proliferation and are necessary for ensuring cell survival (46). To examine whether the cleavage we observed under BoNT/A exposure is unique to this condition or is shared by other types of stress, we utilized the GSE113751 dataset (47) of wildtype HEK293T cells under amino acid starvation (either Arginine (Arg) or Leucine (Leu)) or under no treatment for 3 or 6 h ($n = 12$, see *Materials and Methods*). Out of the 20,274 tRFs that were expressed in these cells, only 134 tRFs were shared with the 1654 tRFs found in the BONT DE analysis. Moreover, none of those was significantly DE under amino acid starvation. Further, when comparing the log (FoldChange) of these 134 tRFs under BoNT/A intoxication and under amino acid starvation, only 14 showed the same trend of change (namely, over 1 or under −1 log (FoldChange) in both cases). The rest of the tRFs were either changed in one case only, or altered in opposite directions under the two stressors (Supplementary Figure S1). Thus, we show that the tRFs whose levels are changed under BoNT exposure reflect a "BoNT/A specific" signature.

BoNT/A-exposed LAN5 Cells and Rat Submandibular Glands Undergo Correlated Intoxication-induced tRFs Changes

To evaluate the relevance of our cell culture findings to *in-vivo* intoxication, we analyzed the small-read RNA-seq dataset from the submandibular glands of BoNT/A-injected rats versus control animals [GSE 141815 (48)], using the human MINTmap pipeline. This dataset revealed three DE tRFs out of the total of 92 detected tRFs (Figure 3A and Supplementary Table S4) ($P < 0.05$). Two of those (66%) corresponded to DE tRFs from BoNT/A-intoxicated human-originated LAN5 neurons (Figure 3B). Subdividing the entire tRFs repertoire based on the originating amino acid type families showed that most tRFs originated from lysine, glutamate, or histidine tRNAs ($Log_2FC > 1$), Furthermore, of the 52 total upregulated tRFs (DE and non-DE) in the BoNT/A-exposed rat dataset, 14 tRFs (27%) corresponded to DE tRFs from BoNT/A-intoxicated LAN5 neurons (Figure 3C). Specifically, among the 17 and 18 tRFs originating from lysine and glutamate tRNAs in BoNT/A-exposed rat glands (all nuclear-genome-originated), eight tRFs corresponded to lysine (47%) and 10 to glutamate (55%) DE tRFs identified in BoNT/A-intoxicated LAN5 cells (Figure 3D and E). Therefore, similarities between the DE tRFs identified in BoNT/A-exposed LAN5 cells were identical to tRFs with altered levels in poisoned

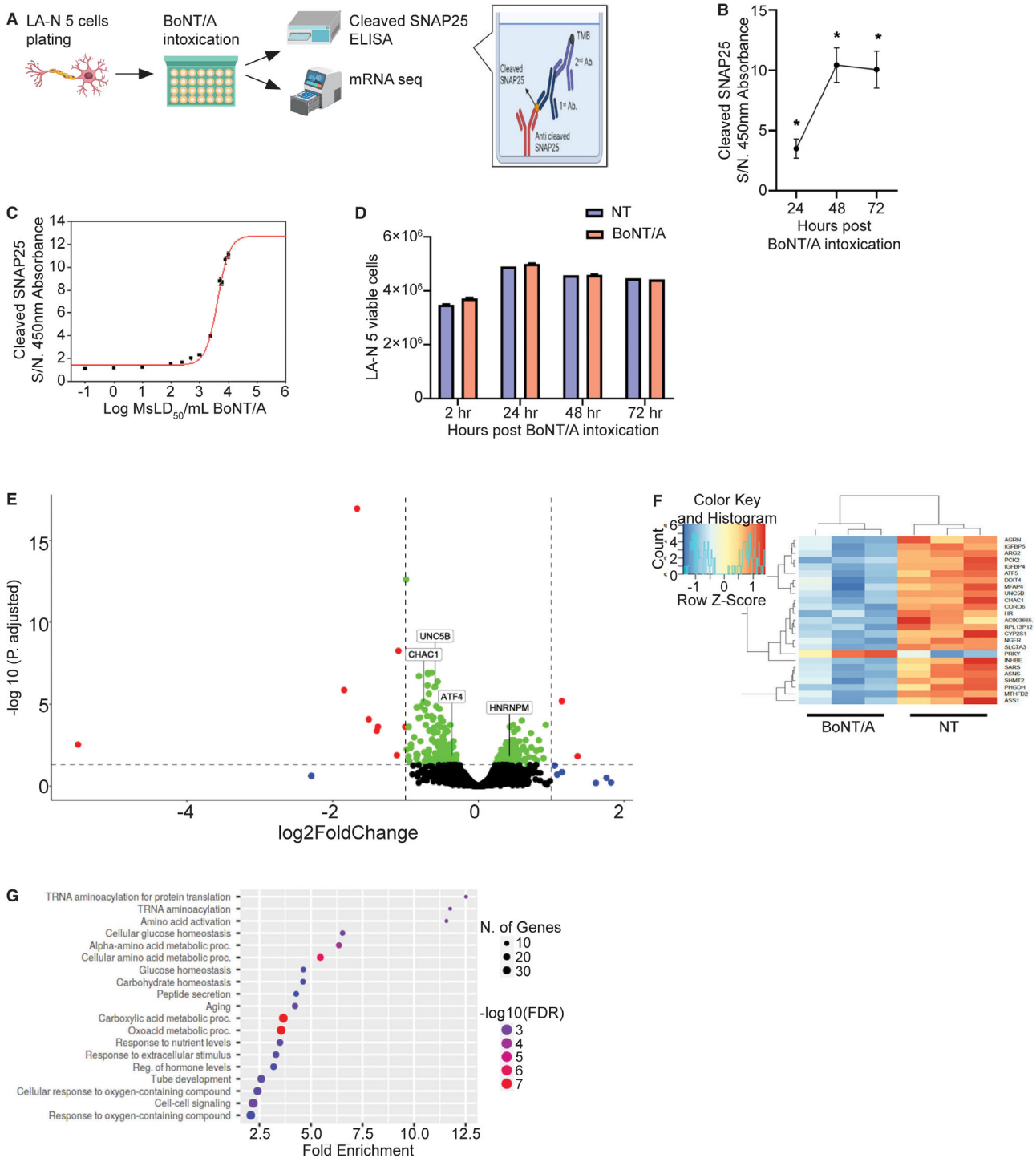


Figure 1. Assessing BoNT/A potency and molecular characterization of intoxicated LAN5 neuroblastoma cells. (A) Experimental setup: LAN5 cells (2×10^6 cells/well) were intoxicated with 10,000 $MsLD_{50}$/mL BoNT/A. Cleavage of SNAP25 was assessed using a specific sandwich ELISA assay. Long RNA-seq was performed for mRNA and lncRNA profiling. (B) Time-dependent signal-to-noise ratio (S/N) of cell lysates during BoNT/A incubation. S/N was calculated as the absorbance of BoNT/A-exposed cells divided by the absorbance of nontreated (NT) cells for all samples (duplicates). Data represent $N = 3$ biological replicates, analyzed by one-way ANOVA ($^*P < 0.05$). (C) Dose–response curve for LAN5 cells exposed to BoNT/A concentrations ranging from 0.1 to 10,000 $MsLD_{50}$/mL, showing S/N ratios of cell lysates. (D) Time-course viability assay comparing NT cells to those exposed to 10,000 $MsLD_{50}$/mL BoNT/A. Viability was assessed using Alamar Blue fluorescence intensity (Ex: 530 nm, Em: 580 nm) at 2, 24, 48, and 72 h postpoisoning. Fluorescence signals were normalized to cell counts across three technical replicates. (E) Volcano plot displaying DE transcripts from long RNA-seq at 48 h postintoxication with 10,000 $MsLD_{50}$/mL BoNT/A. Red and green dots represent DE genes (FDR < 0.05), while blue and black dots indicate non-DE genes (FDR >0.05). Differential expression analysis was performed by using the DESeq2 tool and Wald statistical analysis. Data reflect three biological replicates per treatment. (F) Heat map of the top 25 DE transcripts identified, visualized after variance-stabilizing transformation. (G) GO enrichment analysis of DE biological processes. Shown are processes with a fold enrichment >1.5.

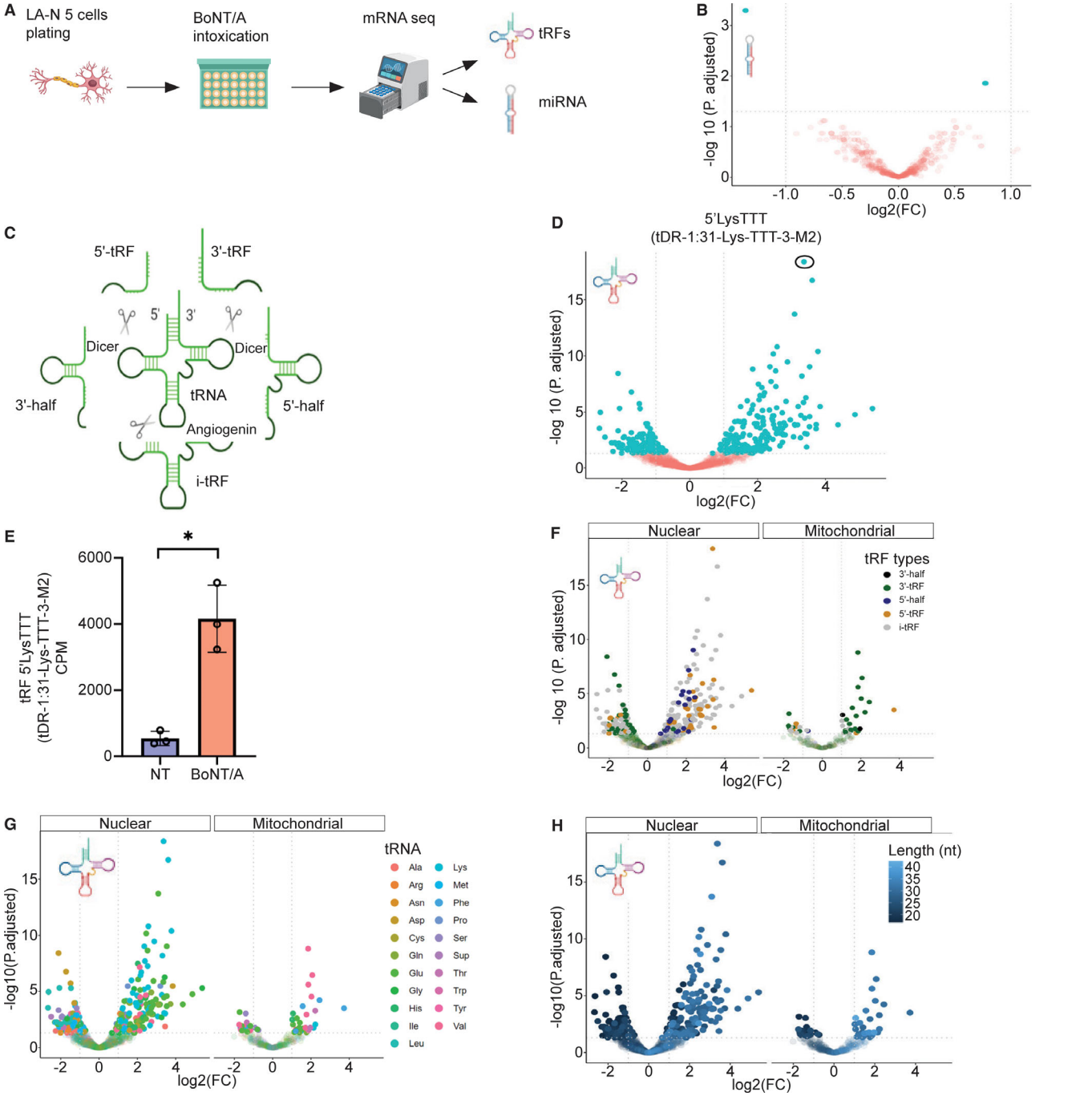


Figure 2. BoNT/A intoxication of LAN5 cells induces massive tRF changes. (A) Experimental design: 2×10^6 LAN5 cells/well were intoxicated by 10,000 $MsLD_{50}$/mL BoNT/A, and small RNA-seq profiles from these cells were compared to nontreated (NT) cells, revealing differences in miRNAs and tRFs. Volcano plots of DE transcriptomes (FDR < 0.05) were generated from three biological triplicates per treatment. Differential expression analysis was performed using the EdgeR tool. (B) DE miRNAs. (C) Schematic representation of tRNA cleavage. (D) DE tRFs. (E) Levels of tRF-5′LysTTT were drastically elevated in BoNT/A-intoxicated LAN5 cells. Data represent $N = 3$ biological replicates; $^*P < 0.05$. (F, G, H) Classification of DE tRFs by fragment type, corresponding amino acid, and length.

rat submandibular glands. These findings suggest an evolutionary conservation of the BoNT/A-intoxication response and its dose-dependent effects across mammalian tissues and neurons.

BoNT/A-upregulated Cholino-tRFs Predictably Target Numerous Cholinergic Transcripts

The observed changes in the transcriptomic landscapes of BoNT/A-exposed rat glands and human neuronal cells may reflect interrelated alterations in small RNA regulators, such as miRNAs (19) and tRFs (20). This further predicted corresponding changes in the mRNA targets of those small RNAs whose levels were changed. To challenge this prediction and study the association between the analyzed small- and long-read RNA-seq datasets, we used the MR-microT DIANA prediction tool to identify predicted targets of the DE tRFs based on sequence motifs (49). This analysis was grounded in the conceptual framework that tRFs and miRNAs share the ability to interact complementarity with the 3′-untranslated region (UTR) of mRNA coding sequences (50). Using a combined prediction score that incorporates site conversion across species (51), we

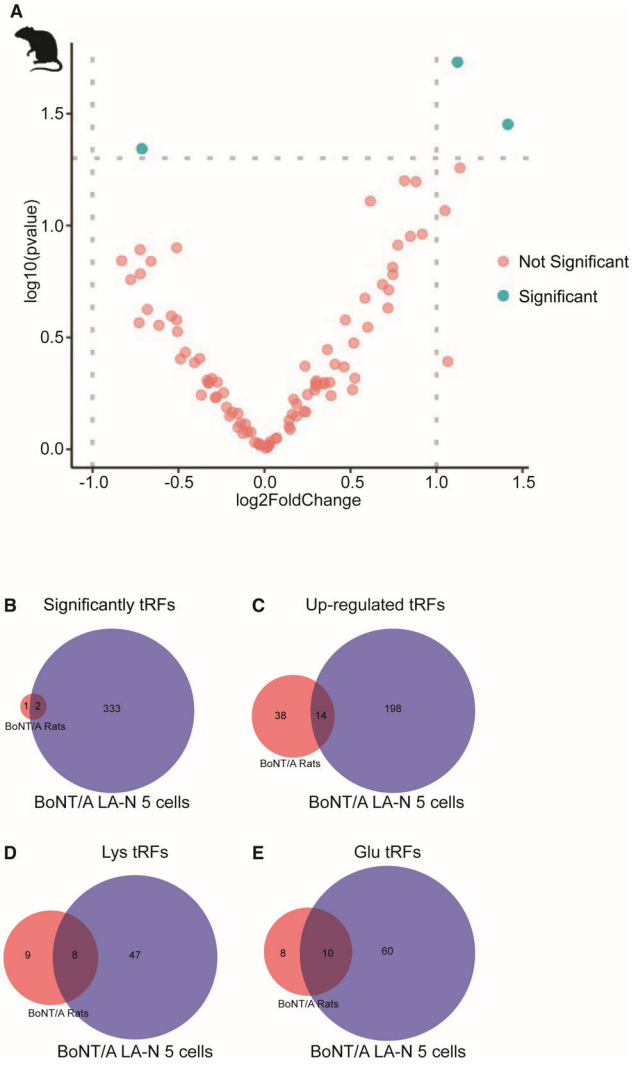


Figure 3. BoNT/A-intoxicated rat submandibular glands (GSE 141815) and human-derived LAN5 cells show closely related tRFs profiles. Small RNA-seq of rat submandibular glands following intoxication with six $MsLD_{50}$/0.1 mL BoNT/A compared to a control group. $N = 4$ biological replicates. (A) Volcano plot depicting DE tRFs (P-value <0.05), with four biological triplicates per treatment. Differential expression analysis was performed by using the DESeq2 tool and Wald statistical analysis. (B) Venn diagram illustrating the correlative significance of tRFs between LAN5 and rat datasets. (C) Venn diagram showing correlative upregulated tRFs between LAN5 cells and rat datasets. (D, E) Venn diagrams presenting correlative lysine tRFs levels between LAN5 cells and rat datasets and correlative glutamate tRFs levels between LAN5 cells and rat datasets.

extracted targets with a score below 0.8 for reliability (Figure 4A). Given that BoNT/A acts on cholinergic neurons forming cholinergic synapses at the NMJ, we further examined the potential of tRFs and miRNAs as regulators of synaptically expressed cholinergic genes. We defined "Cholino-tRFs" and "Cholino-miRs" as tRFs or miRs predicted to target either one core cholinergic gene [responsible for acetylcholine synthesis or breakdown, based on a cholinergic gene list acquired from (52)] or at least five genes associated with cholinergic activity. Sequence complementarity predictions formed the basis of this analysis (see *Materials and Methods*). Using this approach, our cell culture–derived small RNA-seq dataset included 11 DE Cholino-tRFs (Figure 4B and Supplementary Table S5) but no DE Cholino-miRs (Figure 4C). Among these, nine Cholino-tRFs were upregulated, while two were downregulated, all nuclear-originated, with 36% derived from glutamate tRNA fragments. These findings suggested that Cholino-tRFs, rather than Cholino-miRs, may impact the cholinergic balance in BoNT/A-intoxicated neurons by regulating the expression of cholinergic genes.

Further analysis of the top 14 upregulated and 5 downregulated DE tRFs (originating from both mitochondrial and nuclear genomes) identified biological processes linked to their predicted target genes (Figure 4D and E). Processes such as "*Neurogenesis*," "*Generation of neurons*," and "*Nervous system development*" were enriched among the predicted targets of both upregulated and downregulated tRFs. These findings highlight the significant role of tRFs, compared to miRNAs, in modulating the neuronal response to BoNT/A exposure.

5′LysTTT tRFs Inhibit Ferroptosis in BoNT/A-intoxicated LAN5 Cells

5′LysTTT tRFs are known for their role in responding to stress by delaying cell death (53). Ferroptosis, a regulated nonapoptotic cell death mechanism dependent on iron and characterized by peroxidation of membrane lipids, is modifiable by cellular pathway modulation (54). This process involves the accumulation of lipid reactive oxygen species (ROS) or lipid peroxides derived from PUFAs, including arachidonic acid (AA), which serve as inducers of ferroptosis (55) (Figure 5A). BoNT/A intoxication triggered an endoplasmic reticulum (ER)-stress response that involves glutathione (GSH)-specific gamma-glutamylcyclotransferase 1 (*CHAC1*) (56) a regulator of ferroptosis that functions via GSH depletion (57). In BoNT/A-intoxicated LAN5 cells, we observed an accumulation of AA (Figure 5B), alongside other fatty acids (Figure 5C). This accumulation might stem from BoNT/A's inhibition of AA release, previously reported in neurons (58). Correspondingly, we found increased levels of fluorescent ROS compounds (Figure 5D), indicating oxidative stress. However, we also found reduced transcription of cystine transporter solute carrier family 7 member 11 (*SLC7A11*) (Figure 5E), a cystine transporter critical for ferroptosis balancing as well as reduced transcription of Arginase 2 (*ARG2*) in both the long RNA-seq (Supplementary Table S1) and qRT-PCR data (Supplementary Figure S3), recent studies which have elucidated the role of *ARG2* as a key regulator in ferroptosis. Correspondingly, knockdown of *ARG2* increases lipid peroxidation, a hallmark of ferroptosis (59). Ferroptosis is governed by a complex network of genes and pathways, key regulators found in BoNT/A-intoxicated LAN5 long RNA-seq data that include those involved in iron metabolism (*FTH1, TFRC*, *TSC1, TSC2, EIF4EBP1*; Supplementary Table S1), lipid metabolism (*DGAT1, ACADVL, CPT1B*; Supplementary Table S1), antioxidant defense (*GCH1*; Supplementary Table S1) and metabolic enzymes (*PHGDH, SHMT2, MTHFD2;* Supplementary Table S1) which support NADPH production for GSH recycling, all regulating ferroptosis sensitivity (60, 61). Despite these changes, no ferroptosis was observed, potentially due to compensatory mechanisms. Supporting this prediction, long RNA-seq (Figure 1E) and qRT-PCR data (Figure 5F–H) revealed suppressed ferroptosis-related transcripts, including *CHAC1*, and those declined transcripts were exclusively decreased under BoNT/A intoxication, compared to BoNT/E or Tetanus toxin (Figure 5H).

Notably, *CHAC1* reduction may lead to GSH elevation (Figure 5I) and glutathione peroxidase 4 (*GPX4*) upregulation (Figure 5J), a potent ferroptosis antagonist (57). Using the tRFtarget 2.0 algorithm, we have further identified potential interactions between 5′LysTTT tRF and the 3′UTR of *CHAC1* mRNA (Figure 7K), indicating that tRF-5′LysTTT may exert a miR-like function to silence the *CHAC1* mRNA transcript. Experimental validation through a dual-luciferase reporter assay (62) showed a 20% reduction in Firefly/*Renilla* luciferase activity when 5′LysTTT mimic was cotransfected, confirming its miR-like function in silencing *CHAC1* mRNA (Figure 5L). Together, these findings highlight the role of 5′LysTTT tRFs in suppressing ferroptosis by targeting *CHAC1* and regulating neuronal ferroptosis-related genes, including *ATF4*, *DDIT3*, *CHAC1*, and *SLC7A11*. Furthermore, CHAC1 transcript levels were not changed under overexpression of 5′LysTTT in LAN5 cells compared to control, indicating active contribution of BoNT/A to the reduction in CHAC1 transcripts (Supplementary Figure S4). These molecular mechanisms likely contribute to neuronal survival after BoNT/A intoxication via ferroptosis inhibition and underscore the functional significance of tRFs in support of neuronal viability (Figure 5A).

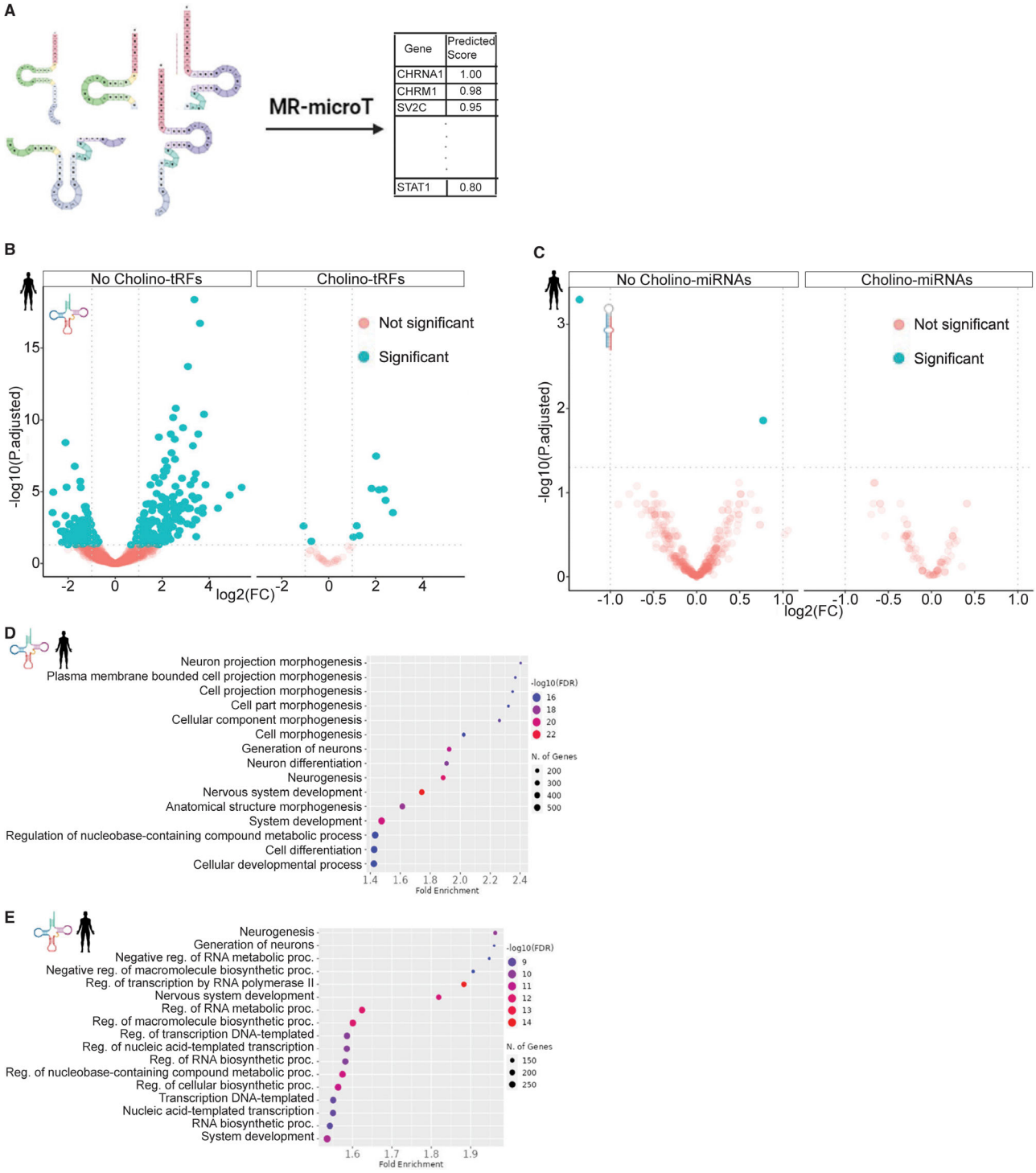


Figure 4. BoNT/A intoxication selectively upregulates nuclear genome-originated "Cholino-tRFs" affecting their predicted target genes. (A) A scheme representation of the predicted target gene analysis from small RNA-seq profiling of miRNAs and tRFs. LAN5 cells (2×10^6 cells/well) were intoxicated for 48 h by 10,000 $MsLD_{50}$/mL BoNT/A and compared to nontreated (NT) cells. DE transcriptomes (FDR < 0.05) were identified using three biological triplicates per treatment. (B) Proportion of DE tRFs and the fraction attributed to "Cholino-tRFs." (C) Proportion of DE miRNAs and the fraction attributed to "Cholino-miRNAs." (D) GO enrichment analysis of the top biological processes associated with the 14 most enriched upregulated DE tRFs (originating from mitochondrial and nuclear gemones). Analysis was conducted using www.ShinyGO.com with a fold enrichment cutoff >1.4. (E) GO enrichment analysis of the top biological processes associated with the five most enriched downregulated DE tRFs (originating from mitochondrial and nuclear genomes). Analysis was conducted using www.ShinyGO.com with a fold enrichment cutoff >1.5.

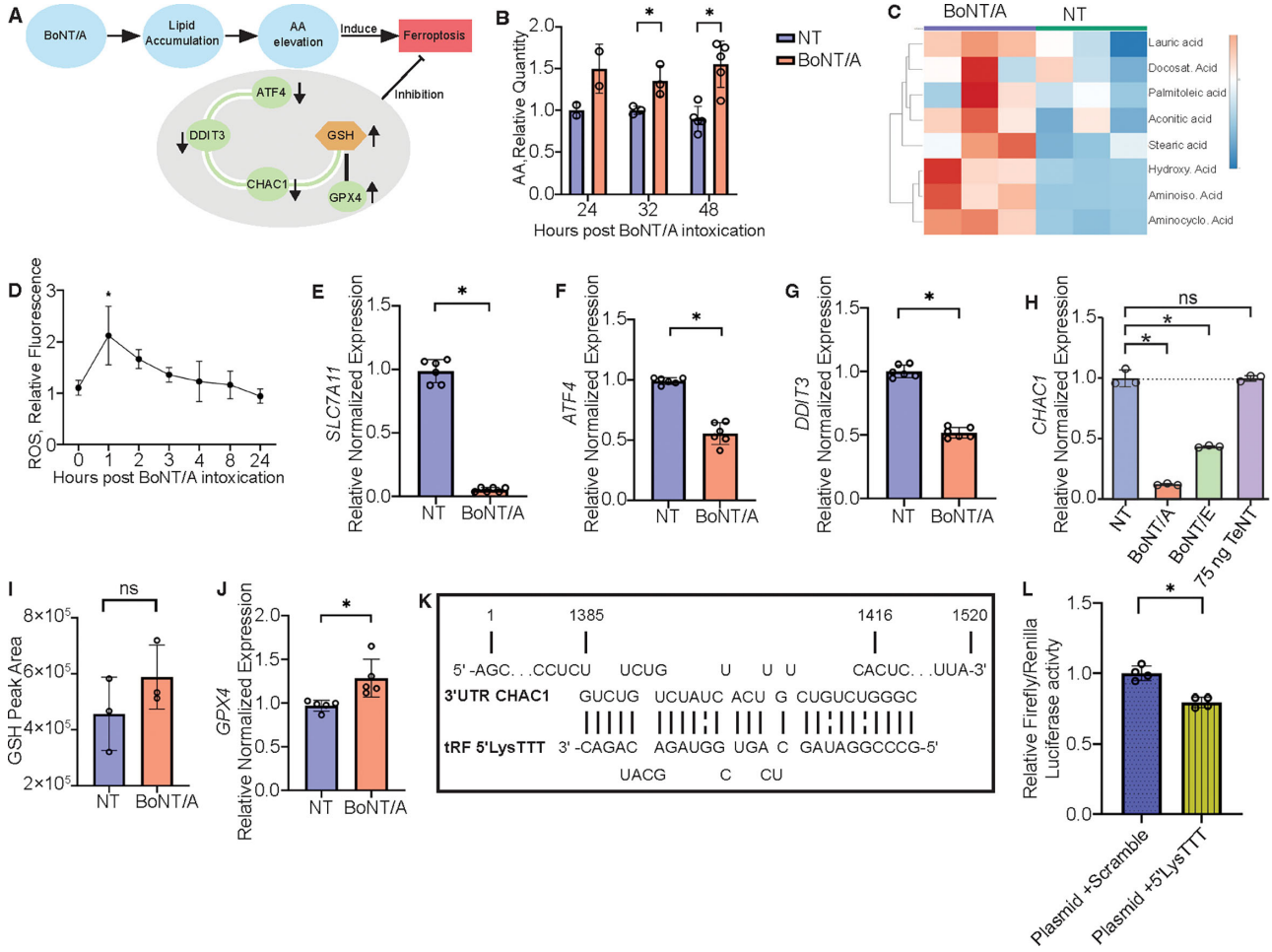
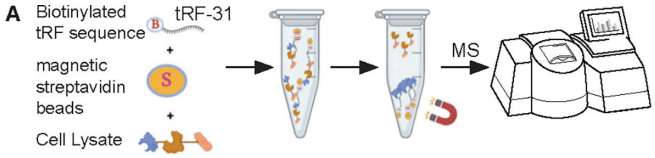


Figure 5. Declined ferroptosis-hub genes may support neuronal viability under BoNT/A intoxication. (A) Proposed mechanism by which ferroptosis is regulated after BoNT/A intoxication to maintain neuronal viability. (B) Arachidonic acid (AA) levels were measured under BoNT/A intoxication. AA was extracted from cells exposed to 10,000 $MsLD_{50}$/mL BoNT/A over 24–48 h and analyzed using LC-MS/MS. Levels were normalized to nontreated (NT) control cells. (C) Heat map of peak areas from fatty acids, analyzed by semitargeted metabolomics profiling. Polar extracts from BoNT/A-intoxicated LAN5 cells (10,000 $MsLD_{50}$/mL) were compared to NT samples and normalized to protein concentration. $N = 3$ biological replicates. (D) Relative ROS levels measured in BoNT/A-intoxicated cells (10,000 $MsLD_{50}$/mL) over 0–24 h. ROS were fluorescently detected, and data presented as mean ± SE. $N = 3$ biological replicates. (E) qPCR analysis of the *SLC7A11* transcript in LAN5 cells exposed to 10,000 $MsLD_{50}$/mL BoNT/A for 48 h. *GAPDH* served as a housekeeping gene. $N = 6$ biological replicates. $^*P < 0.05$. (F, G) qPCR of ferroptosis-related transcripts (*ATF4, DDIT3*) in LAN5 cells exposed to 10,000 MsLD50/mL after 48 h of BoNT/A intoxication compared to NT cells. *GAPDH* served as a housekeeping gene. $N = 6$ biological replicates. $^*P < 0.05$. (H) qPCR of the *CHAC1* transcript in LAN5 cells exposed to 10,000 $MsLD_{50}$/mL after 48 h of BoNT/A/E and 75ng TeNT intoxication compared to NT cells. GAPDH served as a housekeeping gene. $N = 3$ biological replicates. $^*P < 0.05$. (I) Glutathione (GSH) levels were analyzed using semitargeted metabolomics profiling of polar extracts from BoNT/A-intoxicated cells (10,000 $MsLD_{50}$/mL). Data normalized to protein concentrations. $N = 3$ biological replicates. (J) qPCR of *GPX4* transcripts in LAN5 cells exposed to 10,000 $MsLD_{50}$/mL BoNT/A for 48 h. *GAPDH* served as a housekeeping gene. $N = 5$ biological replicates. $^*P < 0.05$. (K) Sequence alignment of tRF-5′LysTTT with the 3′UTR of *CHAC1* mRNA, predicted using the tRFtarget.net 2.0 algorithm, suggesting potential sense–antisense interactions. (L) Dual-luciferase reporter assay demonstrating interaction of tRF-5′LysTTT with the 3′UTR of *CHAC1* mRNA. Cotransfection of the 3′UTR of *CHAC1* (psiCHECK-2 plasmid) with tRF-5′LysTTT mimic resulted in a significant 20% reduction in Firefly/Renilla luciferase activity compared to a scrambled sequence. $N = 4$ biological replicates. $^*P < 0.05$.

BoNT/A Suppresses the Formation of 5′LysTTT-HNRNPM Protein Complex, Reflecting the Outcome of Ferroptosis

To further explore the role of the modulated tRFs in BoNT/A-intoxicated cells, we next examined whether the DE tRFs could reflect the sustained viability of these cells. The most significantly modulated tRF in BoNT/A-intoxicated cells was tRF-5′LysTTT (as shown in Figure 2D and E). Due to its length and sequence features, we hypothesized that 5′LysTTT could interact with RNA-binding proteins. To test this hypothesis, biotinylated 5′LysTTT tRF oligonucleotides were conjugated to streptavidin magnetic beads, exposed to lysates from BoNT/A-intoxicated LAN5 cells, and the proteins selectively bound to the 5′LysTTT were analyzed (Figure 6A). Proteomic analysis using tandem mass spectrometry identified several proteins interacting with the biotinylated 5′LysTTT (Figure 6B and Supplementary Table S6), one of which was heterogeneous nuclear ribonucleoprotein M (*HNRNPM*). HNRNPM is a well-known RNA-binding protein that regulates alternative splicing (63) and plays a role in neuronal ferroptosis (64) In our proteomic profiling, HNRNPM was found to be significantly enriched in BoNT/A-intoxicated cell lysates, as reflected by 15 unique HNRNPM-derived peptide fragments (Supplementary Table S6). Correspondingly, the *HNRNPM* mRNA transcript was significantly upregulated in the mRNA sequencing (mRNA-seq) dataset from BoNT/A-exposed cells (Figure 1E and Supplementary Table S1), and this was confirmed experimentally by qRT-PCR (Figure 6C). However, contrasting the upregulation of *HNRNPM* at the mRNA level, protein analysis revealed significantly reduced levels of HNRNPM in BoNT/A-treated cell lysates (Figure 6D), while no such reduction was observed in BoNT/E-treated cells (BoNT/E



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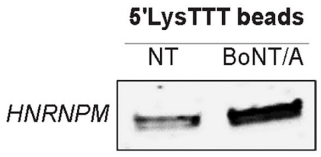
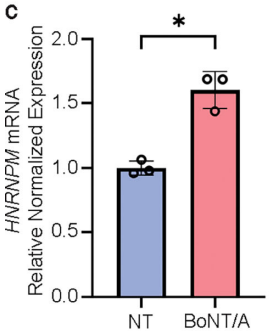
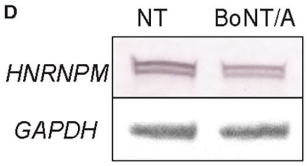
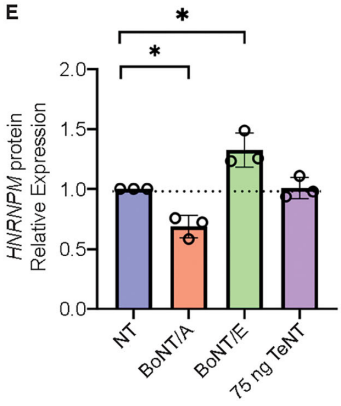
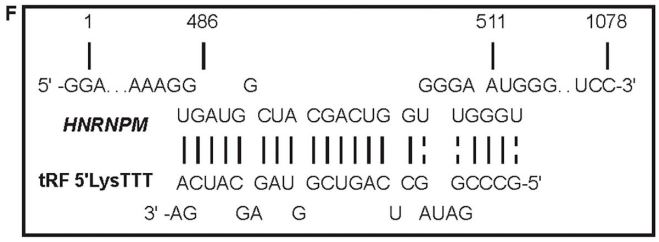
Protein	Gene Symbol	Enriched FC (treated/NT)	Unique peptides	mRNA sequencing
Heterogeneous nuclear ribonucleoprotein M	HNRNPM	32.4	15	Up 34%
Microtubule-associated protein 4	MAP4	25.4	5	–
Nucleolysin	TIAL1	24.2	4	–
Secreted frizzled-related protein 1	SFRP1	21.5	3	–











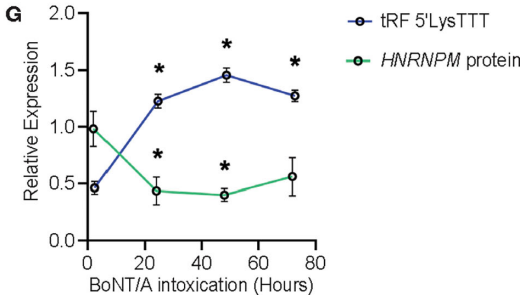


Figure 6. 5′LysTTT tRFs elevate *HNRNPM* regulation under BoNT/A intoxication. (A) A scheme illustrating the RNA pull-down assay conducted using biotinylated 31 nt 5′LysTTT tRF. (B) Proteins interacting with biotinylated 5′LysTTT tRF were analyzed by LC-MS/MS. The four highest scoring proteins that interacted with the tRF are shown, with high confidence peptides passing the 1% FDR threshold (left panel). The expression of *HNRNPM* was validated using immunoblot (right panel). $N = 2$ biological replicates. (C) qPCR analysis of *HNRNPM* mRNA expression was conducted on RNA extracts from BoNT/A-intoxicated LAN5 cells after 48 h of exposure to 10,000 $MsLD_{50}$/mL BoNT/A. Data normalized to *GAPDH*. $N = 3$ biological replicates. $^*P < 0.05$. (D) Representative immunoblots of the *HNRNPM* protein in cell lysates from BoNT/A-intoxicated LAN5 cells compared to nontreated (NT) cells, after 48 h of exposure to 10,000 $MsLD_{50}$/mL BoNT/A. The blots were normalized to *GAPDH*. $N = 3$ biological replicates. (E) Graphical analysis of HNRNPM relative expression in cell lysates (48 h, 10,000 $MsLD_{50}$/ml or equal) calculated $N = 3$ biological replicates. $^*P < 0.05$. (F) A sequence alignment of tRF-5′LysTTT (tDR-1:31-Lys-TTT-3-M2) and the *HNRNPM* mRNA was conducted using the tRFtarget.net 2.0 algorithm. The alignment showed potential sense–antisense interactions between this tRF and *HNRNPM* mRNA. (G) A time-course experiment analyzing the relative expression of *HNRNPM* by Western blot and quantifying 5′LysTTT tRF expression by qPCR after exposure to BoNT/A for 2–72 h (10,000 $MsLD_{50}$/mL). $N = 3$ biological replicates.

also cleaves SNAP25, but at a different position) or cells exposed to Tetanus neurotoxin (Figure 6E and Supplementary Figure S5). Using the tRFtarget 2.0 algorithm revealed predicted targeting by tRF-5′LysTTT of the *HNRNPM* transcript within its coding region (Figure 6F). This suggested that the elevation of 5′LysTTT and the parallel reduction in HNRNPM protein levels over time (Figure 6G) might indicate a direct impact of this sncRNA on HNRNPM protein levels following BoNT/A intoxication. HNRNPM is functionally involved in a variety of cellular processes including alternative splicing (63), synaptic activity (64), immune response regulation (65), and the modulation of neuronal ferroptosis hub genes (66). Specifically, knockdown of HNRNPM has been shown to significantly enhance ferroptosis (67), highlighting its protective role. The inverse relationship between HNRNPM knockdown and ferroptosis aligns with the observed pattern of tRFs-5′LysTTT elevation and HNRNPM protein reduction, indicating a complex regulatory network balancing ferroptosis-related processes under BoNT/A intoxication. This reflects a delicate balance between upregulation and downregulation of ferroptosis pathways, ultimately influencing neuronal survival.

BoNT/A Upregulated tRFs Share an Amplified Sequence Motif

The intricate balance between regulators of ferroptosis following NoNT/A poisoning promoted a search into the controllers of this balanced response. In this respect, all tRNAs share significant portions of their sequences (20, 25). Furthermore, our findings of similar tRF responses to BoNT/A intoxication in human-derived cultured neuorns and rat submandibular glands suggest an evolutionary conservation of this response. This raised the question of whether DE tRFs in humans and rodents share internal motifs (Figure 7A). Supporting this hypothesis, we identified a highly conserved 11-nt internal motif, "CCGGATAGCTC" (Figure 7B), to be present in approximetly 20% of the upregulated tRFs in BoNT/A-intoxicated human cells and rat tissues. This shared motif was also detected in the datatset from BoNT/A-intoxicated rat submandibular glands (Figure 3), with a minor variation at position 5 (adenine or cytosine in humans and only cytosine in rats, Figure 7B). Surprisingly, this motif was found in 14% of the tRFs in the rat gland dataset, despite the heterogeneous cell composition of these glands, which likely includes a lower abundance of neurons compared to human cell line cultures. This suggests that the observed elevation of motif-containing tRFs reflects the intoxication event rather than cell-type composition. Further analysis of sequences containing shared motifs in BoNT/A-intoxicated LAN5 cell datasets revealed a clear trend: longer motifs were progressively less represented among the total tRF numbers (Figure 7C). The 11-nt motif, derived from one of two lysine tRNAs (codons TTT and CTT), was detected in 20% of the upregulated tRFs but only 3% of the total tRFs in BoNT/A-intoxicated LAN5 cells (Figure 7D). This motif was absent in downregulated tRFs and exclusively originated from the 5′ side of nuclear genome-derived tRNAs (Figure 7E). Moreover, this motif's composition significantly differred from that of the 7-nt motif previously identified in Parkinson's disease tissues and body fluids (68), suggesting its specificity to BoNT/A intoxication. Analysis using the MEME tool confirmed the overpresentation of this motif in the analysed systems. This outcome predicted that RNA sequences carrying complementary regions to these motifs may be efficiently blocked during BoNT/A intoxication. Further, this also implies that the regulatory impact of this repetitive tRF motif (e.g., by targeting complementary mRNA transcripts) would be considrably more pronounced than that of single-copy tRF sequences.

Predicting that BoNT/A-induced tRFs may function similarly to miRNAs by interacting with complementary mRNA sequences to inhibit translation and promote degradation, (69) we searched for mRNAs with complementary sequences to the motif. Using the tRFtarget 2.0 algorithm, we identified the *UNC5B* transcript as a predicted target (Figure 7F). This mRNA, encoded by the cholinergic *UNC5B* gene, was first discovered in *Caenorhabditis elegans* flatworms exposed to anti-cholinesterases, resulting in movement impairments. (70) *UNC5B*, a netrin receptor, plays a critical role in axon extension during neural development (71). Therefore, we hypothesized that BoNT/A-induced motif-containing tRFs, acting in a miRNA-like capacity, (20) may silence *UNC5B* or reduce its expression, thereby impairing neurite extension. Indeed, *UNC5B* transcript levels were reduced by ∼50% in BoNT/A-exposed cells (Figures 1E, 7G and Supplementary Table S1). This aligns with reports of BoNT/A-triggered arrest of nerve terminal sprouting, reflecting the toxin's effective duration. (72) Specifically, neuronal sprouting is crucial for restoring muscle contraction and re-establishing motor endplates by accelerating synaptic vesicle recycling (73). Remarkably, *UNC5B* also functions as a dependence receptor, promoting survival in the presence of its ligand, netrin-1. In the absence of netrin-1, *UNC5B* can induce apoptosis (74). However, mRNA-seq data from BoNT/A-intoxicated LAN5 cells showed no detectable netrin-1 (*NTN1)* transcripts (Figure 1E and Supplementary Table S1). This indicates that the downregulation of *UNC5B* in BoNT/A-intoxicated cells impairs cholinergic functions without compromising cell survival.

Together, these findings reveal the multifaceted roles for tRF-5′LysTTT and the intoxication-induced repetitive motif in orchestrating the cosuppression of ferroptosis and cholinergic signaling while maintaining the survival of BoNT/A-intoxicated neurons.

Discussion

The impact of both whole-body BoNT/A intoxication and the locally injected toxin for cosmetic purposes (at far lower doses) may last 3–6 months in humans (75), and the protein changes involved have been well characterized (17, 76). However, the molecular regulators driving these changes and supporting neuronal survival under BoNT/A intoxication remain incompletely understood. To better understand the molecular processes underlying the response to BoNT/A, we profiled both long RNA (coding and long noncoding) and small noncoding RNAs (including miRNAs and tRFs) in BoNT/A-treated human-originated LAN5 cells, and sought relationships between these two datasets. Surprisingly, the short RNA profiles revealed a massive elevation of intoxication-induced tRFs and a contrasting minor miRNA response. Importantly, many of the DE tRFs contained sequences complementary to mRNAs expressed in these cells, and 20% of the intoxication-induced tRFs contained a repetitive 11-nt-long internal motif. Justifying this dual short and long RNA-seq approach, this comparison suggested a potentiated impact through short miRNA-like targeting by both miRNAs and tRFs of long cholinergic and pro-apoptotic transcripts, whose levels indeed declined in BoNT/A-intoxicated cells.

Unlike miRNAs, much is still unknown regarding the role of the recently re-discovered tRFs, their biogenesis, and functional mechanisms. Furthermore, while tRFs elevation under acute stress conditions has been studied by others and us in blood cells from postischemic stroke patients (20) and in degenerating brain neurons (21, 22) steatotic hepatocytes (77), and other human diseases, an in-depth understanding of the molecular mechanisms underlying specific cellular responses of tRFs to acute conditions is still in its infancy. Our findings of significant changes in tRFs profiles versus mild miRNA profile changes under BoNT/A intoxication may reflect the different impact and kinetics of miRNA changes compared to tRFs responses under acute stressors. Briefly, miRNA responses depend on transcription and transport to the site of their activity (in our case, the neuromuscular synapse), whereas tRFs changes are far more immediate, as they only require rapid cleavage by nucleases, including Angiogenin, Dicer, and Argonaut (31), from local tRNAs that are always present in all cells.

Exposing LAN5 human neuroblastoma cells to BoNT/A poisoning enabled us to reveal their BoNT/A sensitivity and EC_{50} values, which closely correlated with findings from other cell lines and different BoNT/A intoxication protocols (43). Our long-read RNA-seq dataset from intoxicated human-originated neuroblastoma cells revealed numerous DE transcripts related to biological processes of "*tRNA aminoacylation*" which is altered by nucleases-driven breakdown of tRNAs (22, 25). This led us to explore the role of tRFs in BoNT/A intoxication, a recently rediscovered class of sncRNAs.

BoNT/A intoxication, whether accidental or therapeutic, leads to blockade of ACh release from NMJs. Accordingly, we identified DE transcripts that are reported to be NMJ-specific (78). Microarray studies in cell lines and human-induced pluripotent stem cells (hiPSC)-derived neurons under BoNT/A intoxication, as well as our current data, showed upregulation of mRNAs related to collagen deposition and apoptotic factors (79, 80). Based on this evidence, and on the surprising massive changes in BoNT/A-induced tRFs, our search into the molecular mechanisms driving these changes, while ensuring sustained viability of the affected cells, has focused on the unexpected upregulation and downregulation of specific tRFs in response to high leveled BoNT/A intoxication.

In addition to their remarkable toxicity, BoNTs may also positively impact neuronal outgrowth. The observed changes in tRFs profiles following BoNT/A intoxication are likely attributed to immediate posttranscriptional processes induced by neuronal intoxication. Correspondingly, the predicted target genes of those tRFs were enriched in processes such as "*neurogenesis*," "*generation of neurons*," and "*nervous system development*." For instance, neurons treated with BoNT/A are known to sprout new axonal branches (81). However, unlike rabies intoxication (82), this response enables long-term neuronal survival. Together, these findings indicate that BoNT/A intoxication induces a unique phenomenon that is distinct from other poisoning events or neurodegenerative states

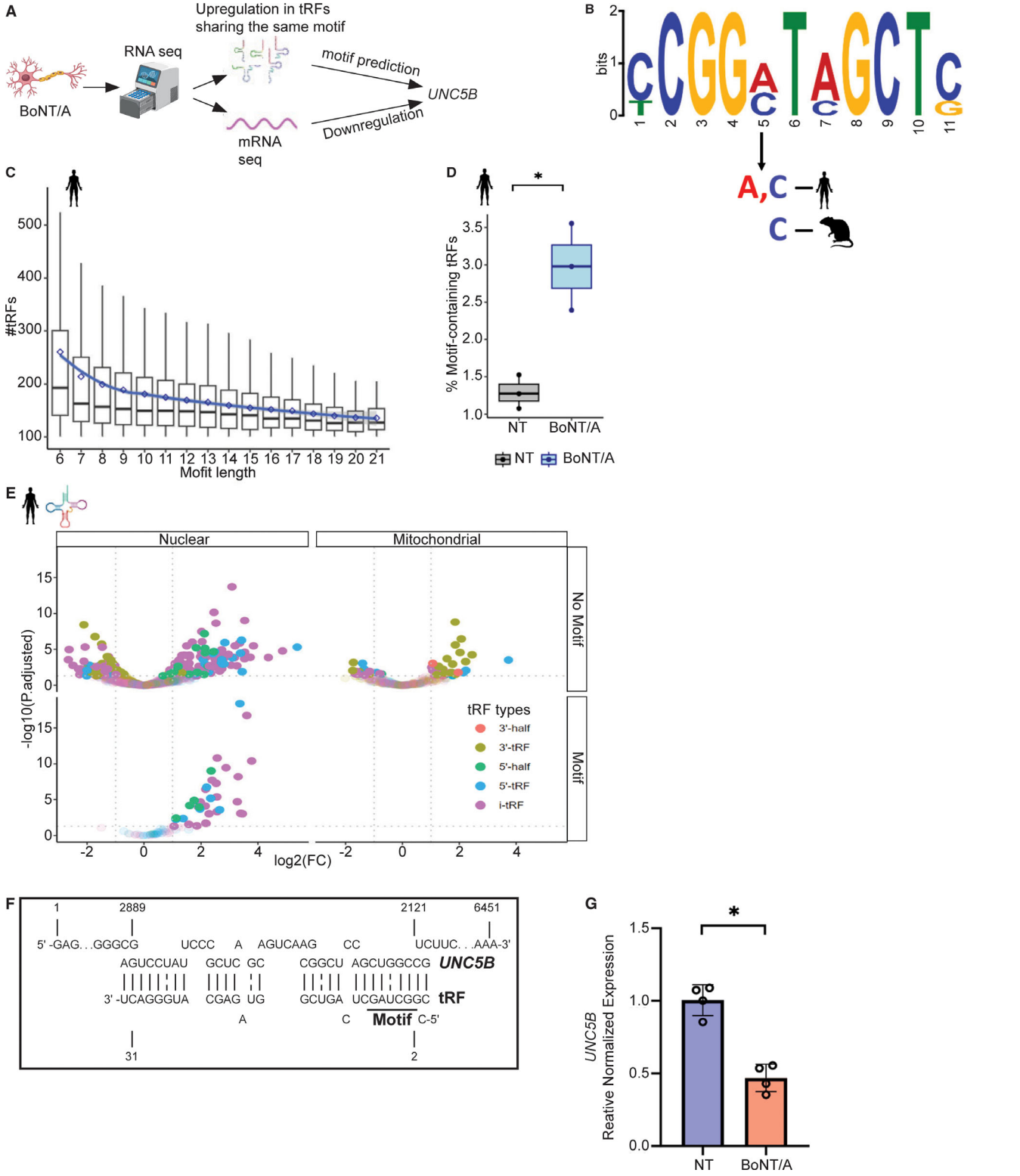


Figure 7. BoNT/A-intoxicated cells overproduce tRFs containing a common motif targeting the *UNC5B* gene. (A) Schematic representation of cholinergic-targeted transcripts identified by both long and short RNA-seq. (B) Visualization of the CCGGATAGCTC motif using the MEME algorithm (https://meme-suite.org/meme/). (C) Box plots showing the number of different tRFs carrying motif sequences of 6–21 nucleotides that appeared in at least 100 tRFs in the BoNT/A-intoxicated LAN5 cells dataset. The graphs display median (box), mean (empty squares), and smoothed data. (D) Comparison of the percentage of tRFs from BoNT/A-intoxicated LAN5 cells (Treated) and nontreated (NT) cells carrying this motif, calculated from total tRFs in the BoNT/A-intoxicated LAN5 cells dataset. The graphs show median (box), mean (empty squares), and smoothed data. (E) Volcano plot of tRFs carrying this motif by fragment type in BoNT/A-intoxicated LAN5 cells. Shown are Volcano plots of DE transcriptomes (FDR < 0.05) with data from 3 biological triplicates per treatment. Differential expression analysis was performed by using the EdgeR tool. (F) Sequence alignment of the CCGGATAGCTC motif and *UNC5B* mRNA using tRFtarget.net 2.0, showing potential sense–antisense interactions. (G) qPCR validation of *UNC5B* transcript levels. *GAPDH* served as a housekeeping gene ($N = 4$ biological replicates). $^*P < 0.05$.

(21, 22). We conclude that tRFs may play a significant role in supporting neuronal survival following BoNT/A intoxication, but our current observations strongly suggest that they are unlikely to be involved in BoNT/A cosmetic uses due to the extremely low toxin concentrations employed.

Our study further established the predictable role for tRFs in forming complexes with RNA-binding proteins. In an in vitro pull-down assay, proteins interacting with biotinylated 5′LysTTT tRF from BoNT/A-cells lysate demonstrated affinity for HNRNPM, one of those RNA-binding proteins whose levels were elevated in BoNT/A-intoxicated neurons. Notably, hnRNP proteins play key roles in mRNA metabolism and are heavily involved in several neurodegenerative disorders (83). Further, *HNRNPM* suppression has been shown to promote ferroptosis in glioma cells (67). Hence, we postulate that the modulation of *HNRNPM* levels following BoNT/A intoxication may similarly arrest ferroptosis while supporting neuronal survival, processes which are inversely modulated by ferroptosis blockers.

Specific cells and structures of living organisms tend to carefully maintain homeostasis, suggesting that cells may deploy common mechanisms to respond to traumatic events. Instead of transcribing and transporting a miRNA to provide such response, cells can rapidly cleave tRNAs that harbor the required "seed" sequence (i.e., a sequence matching the 3′-UTR of a complementary miRNA target transcript) to generate multiple "miRNA-like" oligomers targeting the same transcripts. Given that tRNAs are highly conserved and abundant in all cell types, their cleavage can produce numerous copies sharing specific motif sequences. We have recently identified tRFs carrying a conserved 7-nt-long motif that accumulates in body fluids of Parkinson's disease patients in correlation with the initiation of disease emergence and inversely with their tremor events (68). Here, we report the accumulation of a longer, albeit less abundant 11-nt-long tRF motif derived from Lys tRNA, which can target and suppress the cholinergic *UNC5B* transcript, thus blocking cholinergic neurotransmission by the intoxicated neurons. Notably, this sequence emerges in approximately 20% of the postpoisoning tRFs, suggesting its substantial role in blocking cholinergic transmission in poisoned NMJs at large. Importantly, the tRFtarget.net prediction tool identified the observed internal repetitive motif sequence as capable of targeting several BoNT/A-intoxication downregulated mRNA transcripts, either within their coding regions or at their 3′UTR sites. This reflects a coordinated impact by groups of similar tRFs produced and acting together, generating "tRFs storms" that amplify their ability to silence specific transcript sets in response to BoNT/A intoxication.

The discovery of tRFs carrying repetitive motif sequences emerges from our current study as a reproducible event, reflecting the conserved sequences of mammalian tRNAs preserved in their breakdown products and corresponding to their length. Under random conditions, we calculated that approximately 1% of randomized upregulated and downregulated tRFs would carry shared sequence motifs. However, in the current dataset of BoNT/A-intoxicated LAN5 cells, an 11-nt-long shared motif appeared in 20% of the upregulated tRFs, suggesting a nonrandom event. The presence of this shared repetitive motif suggests that such sequences may vary depending on the stressor that triggered their nuclease-dependent emergence. The biological impact of their accumulation likely varies based on the stressor, the nuclease responsible for cleavage, the level of amplification under the specific conditions, and the mRNAs targeted by these motifs, which are characteristic of the biological process involved. At the observed concentration, BoNT/A intoxication triggers neuronal accumulation of tRFs carrying the internal "CCGGATAGCTC" motif, which is complementary to the coding region of the cholinergic *UNC5B* mRNA, which recently was found to regulate neuronal ferroptosis (84). This was consistent with a reduction in *UNC5B* mRNA levels in intoxicated cells as well as with the predicted impact of BoNT/A intoxication which arrests cholinergic neurotransmission by the intoxicated neurons.

While the motif sequence identified in Parkinson's disease patients' blood is predicted to interact with and block the translation machinery (68), the motif sequence currently identified (present in approximately 20% of the BoNT/A-intoxication-elevated tRFs) may function as a "seed" domain, levering the tRFs' capacity to act like miRNA oligomers. In this respect, such repetitive sequences could be generated in relatively large quantities through tRNA cleavage, potentiating their impact on mRNAs carrying complementary motifs. This amplification underscores the efficacy of tRNA-derived noncoding responses in mammalian cells and tissues.

Ferroptosis is a nonapoptotic cell death pathway characterized by the accumulation of lipid peroxides and mitochondrial dysfunction. Recent studies suggest that ferroptosis contributes to neurodegeneration in Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis (85). In this context, RNA-seq profiling of BoNT/A-intoxicated neurons revealed a decline in transcripts involved in the ER stress pathway ATF4-CHAC1, which may regulate the threshold for ferroptosis-related death (86, 87). The observed coaccumulation of ferroptosis-related fatty acids and ROS in intoxicated cells from our model may provide a functional explanation for the molecular mechanisms underlying this process.

In addition to the miRNA-like functions of the intoxication-induced tRFs, we identified such interaction between the most potently BoNT/A-induced DE tRFs and *HNRNPM* mRNA, whose levels were significantly elevated postintoxication. Importantly, this tRF response efficiently suppressed HNRNPM protein levels, potentially promoting the ferroptosis pathway of programmed cell death (67). Further analysis of tRFs from BoNT/A-injected submandibular rat glands indicated that tRFs altered in LAN5 cells following BoNT/A exposure were also found *in vivo* in BoNT/A-exposed tissues. Classification of tRFs based on the amino acid encoded by their originating tRNA revealed that approximately 50% of the affected tRFs originated from lysine and glutamate tRNAs. Lysine tRNA fragmentation, previously reported to support the sustained viability of steatotic hepatocytes (77), appears to play a similar role across diverse cell types and tissues, as suggested by our findings.

Small- and long-read RNA-seq datasets revealed that a majority of DE tRFs were upregulated, correlating with a decline in many DE mRNA transcripts carrying complementary sequences to these tRFs. This finding suggests a miRNA-like function for some of the shorter tRFs (20), which may interact with mRNA transcripts carrying complementary sequence motifs (28). Notably, we identified a shared motif present in approximately 20% of BoNT/A-upregulated tRFs, which likely targets cholinergic genes by downregulating mRNA transcripts carrying tRF-complementary domains. This motif's significant occurrence among the upregulated tRFs population could amplify its regulatory impact, explaining the direct and substantial repression of *UNC5B* transcripts and the corresponding arrest of cholinergic neurotransmission that is known to appear under BoNT/A intoxication. Such repression might suppress cholinergic signaling, while the arrested ferroptosis preserves the viability of the intoxicated neurons.

The elevation of specific tRFs containing a repetitive motif sequence may stem from diverse biological origins: (1) BoNT/A intoxication may elevate specific tRNA levels, possibly due to translational arrest or a shortage of particular amino acids, leading to the breakdown of these tRNAs and the subsequent accumulation of their corresponding tRFs; (2) Increased levels or activity of specific nucleases could preferentially cleave certain tRNAs, resulting in the accumulation of selected tRFs; (3) Cellular mechanisms governing tRNA breakdown may be altered, promoting the survival of certain tRFs while degrading others; (4) A combination of the above processes could act together to produce the observed accumulation of tRFs carrying a repetitive motif. Interestingly, all of the motif-carrying tRFs whose levels were altered under BoNT/A intoxication in our human-originated cell line were derived from one of two nuclear genome-originated lysine tRNAs (codons TTT and CTT). This suggests that BoNT/A-induced accumulation of lysine-tRNAs may specifically contribute to the elevated levels of repetitive motif-containing tRFs observed in this study and thus determine its ultimate outcome.

Taken together, our findings suggest that the observed accumulation of AA may represent an immediate cellular response to BoNT/A poisoning, while the suppressed cell death reflects a longer-term outcome of this response. In this context, *CHAC1*, a downstream target of the ER stress pathway (88), plays a pivotal role in cell survival following BoNT/A-intoxicated LAN5 neurons, and may be a key factor selectively contributing to BoNT/A, but not to BoNT/E neuronal survival although both serotypes bind to the same receptor and cleave the same substrate. Correspondingly, the decline in *CHAC1* levels could actively promote the accumulation of GSH and

the upregulation of *GPX4*, both of which inhibit ferroptosis (89). Additionally, since tRF-5′LysTTT targets the 3′UTR of *CHAC1* transcripts, and given that *CHAC1* transcription is suppressed under BoNT/A intoxication, we hypothesize that the elevation of tRFs in response to BoNT/A exposure may serve as a trigger for both suppression of ferroptosis and sustained viability of the affected neurons.

New approaches to balance the duration of BoNT effects are poised to impact its clinical and cosmetic applications. Traditionally, clinical or cosmetic BoNT treatments last 3–4 months, but innovations reflecting the underlying molecular mechanisms might enable prolonged efficacy, lasting up to 6 months or more. In clinical settings, these extended-duration formulations could reduce treatment frequency for conditions like spasticity, migraines, and hyperhidrosis, improving patients' convenience and compliance while lowering health care costs (90). Similarly, in cosmetic applications, longer-lasting BoNT could provide sustained wrinkles reduction and muscle relaxation, appealing to patients seeking fewer appointments for maintenance (90). These advancements are likely to redefine treatment protocols across both therapeutic and aesthetic fields, offering more durable and efficient outcomes.

Our study involves certain limitations as well. First, the mechanisms observed here should be further challenged in other experimental models, as they may involve varying levels of BoNT/A intoxication due to differences in exposure time, toxin concentrations, and the use of purified neurotoxin, which could reflect its impact in other applications. Second, this study would benefit from validations using clinical samples from human BoNT/A intoxication cases, which were not available for this research.

In conclusion, our findings highlight distinct roles of sncRNAs, particularly poisoning-induced tRFs, in BoNT/A intoxication, acting as key drivers of the intoxication outcome and molecular pathways it activates, and ultimately supporting neuronal survival. The observed changes in tRFs induced by BoNT/A provide a plausible explanation for the sustained viability of intoxicated neurons in culture and poisoned rat glands, while also shedding light on the molecular mechanisms underlying the effects of poisoning over both short and long timelines. Enriching our knowledge of the central aspects of BoNT intoxication etiology may, therefore, pave the way for novel pharmaceutical strategies aimed at treating BoNT intoxication and/or enhancing its medical applications, ultimately improving human health and well-being.

Materials and Methods

Cell Culture, BoNT Intoxication, and Transfection

LAN5 neuroblastoma cells (DSMZ Catalog no. ACC-673), derived from a male donor, were cultured in RPMI-1640 medium (Biological Industries) supplemented with 10% FBS (Biological Industries), 1% L-Glutamine (200 mM, Biological Industries), and 1% penicillin-streptomycin-amphotericin solution (Biological Industries). The cells were maintained at 37°C in a humidified atmosphere containing 5% CO_2 and were passaged weekly using Trypsin-EDTA Solution A (0.25%) with EDTA (0.02%) (Biological Industries). For experiments, cells were plated in 12-well plates at a density of 2×10^6 cells/well in 1 mL of complete medium. *Clostridium botulinum* serotype A and E strains (A198 and E450, respectively) were sourced from the IIBR collection. Tetanus neurotoxin (TeNT) was procured from Sigma-Aldrich (Catalog no. T3694). Unless otherwise specified, LAN5 cells were intoxicated with 10,000 $MsLD_{50}$/mL (~300 pM) of BoNT/A/E for 48 h diluted in Neuro-Basal Medium (Biological Industries) supplemented with 2% FBS (Biological Industries), 1% L-Glutamine (200 mM, Biological Industries), and 1% penicillin-streptomycin-amphotericin solution (Biological Industries) and were compared to LAN5 cells cultured with Neuro-Basal Medium (Biological Industries) supplemented with 2% FBS (Biological Industries), 1% L-Glutamine (200 mM, Biological Industries), and 1% penicillin-streptomycin-amphotericin solution (Biological Industries) for 48 h without neurotoxin. 5′LysTTT transfection to LAN5 cells was as follows: 100 nM of tRF-5′LysTTT (GCC CGG AUA GCU CAG UCG GUA GAG CAU CAG A) and 100 nM of a control scrambled sequence (CGU UAA CCG CGC AUA AUA CGC GUA CGG GAG) were transfected to LAN5 using Lipofectamine3000 kit (L3000008, Invitrogen) according to the manufacture's instructors.

Sandwich ELISA Assay for Cleaved $SNAP25_{197}$

Synthetic monoclonal antibodies specific to cleaved $SNAP25_{197}$ were generated as described previously (43). These antibodies (100 ng/well) were diluted in 50 mM bicarbonate buffer (pH 9.6) and used to coat polystyrene 96-well microtiter plates (Maxisorp, NUNC). Plates were incubated overnight at 4°C. The wells were washed three times with 300 μL/well of washing buffer (DDW, 0.9% NaCl, 0.05% Tween-20), followed by blocking with 250 μL/well TSTA buffer (DDW, 8.5% NaCl, 1 M Tris, 0.05% Tween-20, 2% BSA, pH 7.6) for 1 h at 37°C. For sample preparation, cells treated with or without neurotoxin were washed once with PBS and lysed in freshly prepared Triton X-100 lysis buffer (50 mM HEPES, 150 mM NaCl, 1.5 mM $MgCl_2$, 1 mM EGTA, 1% Triton X-100, and one EDTA-free protease inhibitor tablet). Lysates were centrifuged at 12,000 rpm for 5 min, and 50 μL of supernatant was added to the wells for 1 h incubation at 37°C. After washing three times, wells were incubated with polyclonal rabbit anti-SNAP25 antibodies (Catalog no. S9684, Sigma-Aldrich) diluted 1:5000 in TSTA buffer containing 1% normal mouse serum (NMS) and 1% normal human serum (NHS) for 1 h at 37°C. Following another wash, 50 μL/well of Horseradish peroxidase (HRP)-conjugated donkey anti-rabbit antibodies (Catalog no. 711-035-152, Jackson) diluted 1:1000 in TSTA buffer with 1% NMS and 1% NHS was added for 30 min at 37°C. The enzymatic reaction was visualized using 50 μL/well of KPL Sureblue (Catalog no. 5120-0081, Seracare) substrate for 10 min at room temperature. The reaction was stopped by adding 100 μL/well 0.5M H_2SO_4, and absorbance was measured at 450 nm. Lysates from neurotoxin-free cells served as controls in all assays. Signal-to-noise (S/N) ratios were calculated for each treatment.

Cell Viability

For calibration curves, 2 mL of 800,000 cells/mL were added to one well of a 24-well plate (COSTAR). A total of 1 mL of fresh medium was placed in the other five wells in the same row. Then, 1 mL of 800,000 cell/mL was moved to the adjacent well and cells were serially diluted across the entire row so that wells contained 800,000, 400,000, 200,000, 100,000, and 50,000 cells/mL. The last well in the row was left with 1 mL fresh medium as a negative control. 1 mL samples of counted cells were added to each well and supplemented with 100 μL Alamar blue (Catalog no. G8081, Promega). Plates were covered by aluminum foil for protection from light and incubated for 1 h at 37°C without CO_2. Then, plates were read in a M200 plate reader (TECAN, NEOTEC BIO), with excitation: 550 nm, emission: 580 nm filters. Remaining cell numbers were calculated based on the calibration curves.

Long-read mRNA-seq

Total RNA was extracted from LAN5 cells intoxicated with 10,000 $MsLD_{50}$/mL BoNT/A for 48 h and from nontreated LAN5 cells (NT) using the RNeasy Mini Kit (Catalog no. 74104, Qiagen) according to the manufacturer's instructions. RNA quantity and quality were assessed using Bioanalyzer (Cat# G2964AA, Agilent) with the RNA High Sensitivity Kit ScreenTape (Catalog no. 5067-5579, Agilent). RNA integrity numbers (RIN) were calculated, and samples with RIN value >8.0 were selected for sequencing at the Columbia Genome Center (NY, USA) and the Genomic Technologies Facility at the Hebrew University of Jerusalem, Israel. Libraries were generated from 1 μg of total RNA using the TruSeq RNA Library Preparation Kit (Catalog no. RS-122-2001, Illumina), and whole transcriptome sequencing (total RNA-seq) was performed using an Illumina HiSeq. Over 20 million single-end 100 nt reads per sample were generated. RTA (Illumina) for base calling and bcl2fastq2 (version 2.19) for converting BCL to fastq format coupled with adaptor trimming was performed. Pseudoalignment to a Kallisto index was created from transcriptomes (Human:GRCh38.p12) using Kallisto (0.44.0) (91). Normalization, visualization and differential transcript analyses were performed using the DESeq2 tool to test differential expression between two experimental groups from RNA-seq counts data, with significantly altered transcripts identified by calculating FDR < 0.05. GO enrichment analysis was conducted using the Gene Ontology Resource (http://geneontology.org/) as per the provided guidelines.

Table 1. Primers used in this study

Transcript	Forward Primer	Reverse Primer
UNC5B	GTCGGACACTGCCAACTATAC	CCGCCATTCACGTAGACGAT
5′Lys TTT	GCCCGGATAGCTCAGTCGGT	TTTTTTTTTTCTGATGCTCT
HNRNPM	CTCTTAATGGACGCTGAAGGAAA	CGCTCAGACTATGCTTGTTTAGG
SLC7A11	GGTCCATTACCAGCTTTTGTACG	GGTCCATTACCAGCTTTTGTACG
ATF4	CCCTTCACCTTCTTACAACCTC	TGCCCAGCTCTAAACTAAAGGA
DDIT3	GGAAACAGAGTGGTCATTCCC	CTGCTTGAGCCGTTCATTCTC
CHAC1	GTGGTGACGCTCCTTGAAGA	GAAGGTGACCTCCTTGGTATCG
GPX4	GAGGCAAGACCGAAGTAAACTAC	CCGAACTGGTTACACGGGAA
GAPDH	AGGGGTCTACATGGCAACTG	CGACCACTTTGTCAAGCTCA
3′UTR CHAC1	TCTACTCGAGGTGCTCATGTGGACATCAGG	TAAGCGGCCGCTAAAGAGATAGTTTTATGGG

Small-read RNA-seq

Total RNA was extracted from LAN5 cells intoxicated with 10,000 $MsLD_{50}$/mL BoNT/A for 48 h and from nontreated LAN5 cells (NT) using the miRNeasy Mini Kit (Catalog no. 1038703, Qiagen) according to manufacturer's instructions. RNA yields were quantified, and RNA quality was assessed using the Bioanalyzer (Catalog no. G2964AA, Agilent), with the RNA High Sensitivity Kit ScreenTape (Catalog no. 5067-5579, Agilent). RIN values were calculated and samples with RIN value >8.0 were sent to LC Sciences (Houston, USA) for small-read RNA-seq of a small RNA library generated from 1 μg of total RNA using the TruSeq Small RNA Sample Prep Kits (Catalog no. RS-200-0012, Illumina). Single-end sequencing of up to 50-nt-long reads was performed on an Illumina Hiseq 2500. Over 12 million single reads per sample were generated. FASTQ files were quality checked with FastQC (92) according to the pipeline recommended definitions, and then adaptors were removed using FLEXBAR (93) according to the pipeline instructions. Adaptor-less reads were aligned to miRs (miRBase v21) using miRExpress 2.1.4 (44) with default parameters, and to tRFs using the MINTmap pipeline (45) with default parameters for tRF levels, and using only reads that mapped exclusively to the tRNA space. After the alignment, differential expression analysis of miRs and tRFs was conducted using EdgeR (94): Lowly expressed features were filtered with filterByExpr. Normalization factors were calculated with calcNormFactors, and dispersion estimates were obtained using estimateDisp. A generalized linear model was fitted (glmQLFit) and calculated, and differential expression was assessed using quasi-likelihood F-tests (glmLRT). Finally, results were extracted with topTags, applying false discovery rate (FDR) correction.

Analysis of the GSE113751 Dataset

To enable consistent and straight forward analysis, we analysed only HEK293T samples from the GSE113751 dataset, and only of wildtype background (i.e., noninfected cells or cells infected with an AAV that carries GFP alone). We regarded both Arg and Leu starvation as "stress" and the DE design matrix consisted of comparing stressed to control cells, accounting for starvation time, and noted if cells were transfected with a GFP-carrying AAV or not transfected at all. This analysis (alignment, normalization and differential expression analysis) was similar to the analysis for small-read RNA-seq (the previous section) (47).

Quantitative PCR and Gene Expression Assessment

Total cellular RNA was extracted from LAN5 cells intoxicated with 10,000 MsLD50/mL BoNT/A for 48 h and from nontreated LAN5 cells (NT) using the TRI reagent (Catalog no. T9424, Sigma-Aldrich) with either RNeasy (Catalog no. 74104, Qiagen) or miRNeasy Mini Kit (Catalog no. 1038703, Qiagen; for enrichment of small RNA fragments) according to manufacturer's instructions. Total RNA concentration was determined using the Qubit RNA HS Assay Kit (Catalog no. Q10211, Invitrogen). RNA was reverse-transcribed to cDNA using the Maxima First Strand cDNA Synthesis Kit (Catalog no. K1671, Thermo Fisher Scientific) which includes a DNase treatment step to eliminate genomic DNA, and diluted 1:10 in double-distilled water before preparing the quantitative PCR (qPCR) plate. For tRF-5′LysTTT quantification, total RNA was reverse-transcribed using the qScript miRNA cDNA Synthesis Kit (Catalog no. 95107-025, QuantaBio). qPCR was performed in 96-well plates on the LightCycler 96 Instrument (Roche) using Universal SYBR Green Supermix (Catalog no. 1725150, Bio-Rad) with a final well volume of 15 μL. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) served as the housekeeping gene. Gene expression was calculated as ΔΔCt values using the LightCycler 96 System Performance Data by Roche. Primer sequences are provided in Table 1.

RNA Pull-down Assay

Biotin-labeled tRF-5′LysTTT and a corresponding scrambled sequence were synthesized by IDT (Coralville, IA, USA). RNA pull-down from BoNT/A-intoxicated and NT cell lysates was performed using The Pierce Magnetic RNA-Protein Pull-Down Kit (Catalog no. 20164, Thermo Fisher Scientific) following the manufacturer's protocol, as described previously (22). tRF-5′LysTTT (GCC CGG AUA GCU CAG UCG GUA GAG CAU CAG A) and a control scrambled sequence (CGU UAA CCG CGC AUA AUA CGC GUA CGG GAG G) were designed with the two penultimate bases protected by 2′-O-methylation to prevent 3′-end degradation. Pull-down proteins were analyzed by mass spectrometry at the Smoler Proteomics Center, Technion, Israel.

Western Blot Analysis

SDS-PAGE was performed using NuPAGE 10% Bis-Tris gels (Catalog no. NP0301BOX, Invitrogen). Cell lysate samples, diluted in a Tris-Glycine SDS Sample Buffer, were subjected to transfer using the Nitrocellulose Western iBlot Gel Transfer Semi-dry system (Invitrogen). Membranes were blocked with 5% skim milk diluted in PBST (PBS containing 0.05% Tween-20) and incubated with monoclonal mouse anti-HNRNPM1-M4 (Catalog no. NB200-314SS, Novus) primary antibody, followed by HRP-conjugated Donkey anti-Mouse (Catalog no. 715-035-152, Jackson) secondary antibody. Immunoreactive bands were visualized using the TMB Liquid Substrate System for Membranes (Catalog no. T0565, Sigma-Aldrich), detected with the FUJIFILM LAS-3000 imaging system, and analysed using ImageJ software.

AA Extraction and Measurement by LC-MS/MS

AA extraction, purification, and quantification were performed using stable isotope dilution liquid chromatography/tandem mass spectrometry (LC-MS/MS) as previously described (95). Briefly, AA from BoNT/A-intoxicated and NT cell lysates was precipitated using ice-cold acetone and 50 mM Tris buffer (pH 8.0). An ice-cold extraction buffer (1:1 MeOH/Tris Buffer) containing the internal standard [$_d$4-AEA] was added to the samples, followed by homogenization. The homogenates were extracted with ice-cold $CHCl_3$: MeOH (2:1, vol/vol), and the extraction was repeated with three washes of ice-cold chloroform. The samples were then dried under a nitrogen stream and reconstituted in MeOH. LC-MS/MS analysis was conducted on an AB Sciex (Framingham, MA, USA) QTRAP 6500 + mass spectrometer coupled with a Shimadzu (Kyoto, Japan) UHPLC system. Liquid chromatographic separation was

performed on a Kinetex 2.6 μm C18 (100 × 2.1 mm) column (Phenomenex, Torrance, CA, USA) with the autosampler temperature set to 4°C, and the column maintained at 40°C throughout the analysis. Gradient elution was carried out using mobile phases of 0.1% formic acid in water (phase A) and 0.1% formic acid in acetonitrile (phase B). AA was detected in a positive ion mode using electron spray ionization and a multiple reaction monitoring (MRM) acquisition mode. AA levels were quantified against standard curves and normalized to total protein concentration in the samples. Relative AA quantities were compared between BoNT/A-exposed and untreated cells.

LC-MS Metabolomics Analysis

For fatty acids and GSH measurements, pellets from BoNT/A-intoxicated and NT cells were harvested with 400 μL of cold (−20°C) metabolite extraction solvent (MeOH:acetonitrile:water, 5:3:2) and kept on ice. Extracts were centrifuged at 18,000 × *g* for 15 min at 4°C, and the supernatants were collected into microcentrifuge tubes. These were re-centrifuged at 18,000 × *g* for 10 min at 4°C, then transferred to glass high-performance liquid chromatography (HPLC) vials and stored at −70°C. LC-MS metabolomic analysis was performed as described previously (96). Briefly, a Dionex Ultimate 3000 high-performance liquid chromatography (UPLC) system coupled to an Orbitrap Q-Exactive Mass Spectrometer (Thermo Fisher Scientific) was used with a resolution of 35,000 at 200 mass/charge ratio (m/z). Electrospray ionization and polarity switching mode enabled transport of both positive and negative ions across a mass range of 67 to 1000 m/z. The UPLC system utilized a ZIC-pHILIC column (SeQuant; 150 mm × 2.1 mm, 5 μm; Merck, Darmstadt, Germany) with a ZIC-pHILIC guard column (SeQuant; 20 mm × 2.1 mm). A 5 μL sample of cell extract was injected, and the compounds were separated using a mobile phase gradient over 15 min, starting at 20% aqueous (20 mM ammonium carbonate, adjusted to pH 9.2 with 0.1% of 25% ammonium hydroxide) and 80% acetonitrile, then terminating with 20% acetonitrile. The flow rate was set to 0.2 mL/min and the column temperature to 45°C, with a total run time of 27 min. Metabolite analysis was performed with a mass accuracy of less than 5 ppm. Data acquisition was done using Thermo Xcalibur, followed by analysis in TraceFinder 4.1 (Thermo Fisher Scientific, Waltham, MA, USA). The exact mass and known retention time of singly charged ions were determined using an in-house MS library created by running commercial standards for all detected metabolites. The intensity of each identified metabolite was normalized to total protein concentration of each sample.

Measuring Oxidative Stress

Total cellular ROS levels in adherent cells were determined using 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA, Catalog no. D6883; Sigma-Aldrich) staining, as described previously (97). DCFH-DA is a widely used probe for total ROS detection, where it is taken up by cells and cleaved by cellular esterases, releasing DCFH. Oxidation of DCFH by ROS produces for 2′-7′dichlorofluorescein (DCF), which emits green fluorescence upon excitation at 485 nm and emission at 530 nm. To assess ROS levels, 20 μM DCFH-DA was added to both BoNT/A-intoxicated and NT cells, which were incubated for 30 min under standard culture conditions, protected from light. After incubation, the medium was replaced with fresh medium, and DCF fluorescence was measured using a TECAN M200 plate reader (NEOTEC BIO). Relative fluorescence intensities were calculated by comparing the signals from BoNT/A-treated cells to untreated controls.

Construction of a psiCHECK-2 Plasmid Containing the 3′UTR of *CHAC1* mRNA

Genomic DNA extracted from LAN5 neuroblastoma cells (DNeasy Blood & Tissue Kit, Catalog no. 69504, Qiagen) was used as the template for PCR amplification of the 3′UTR of the *CHAC1* gene (717 bp). Specific primers, including flanking regions for *PspXI* and *NotI* HF restriction enzymes (Table 1), were used for amplification. The amplified product was gel-purified using the QIAquick Gel Extraction Kit (Catalog no. 28704, Qiagen). The PCR product was then digested with *PspXI* and *NotI* HF restriction enzymes in rCutSmart buffer (10 U; New England Biolabs, Ipswich, MA) and purified using the QIAquick PCR Purification Kit, Catalog no. 28104, Qiagen). The psiCHECK-2 plasmid (1 μg; Promega) was digested sequentially with *PspXI* and *NotI* HF in rCutSmart buffer (10 U; New England Biolabs, Ipswich, MA). The purified insert and vector were ligated with T4 ligase (200 U, 20 μL reaction, 1:3 vector/insert ratio; New England Biolabs). Transformation of ligated constructs into competent *Escherichia coli* cells following by PCR colony screening identified positive clones. The composition of the resulting psiCHECK-3′UTR *CHAC1* plasmid was confirmed by PCR.

Transfection and Dual-luciferase Assay of the psiCHECK-3′UTR *CHAC1* Vector

HEK293 cells (1×10^5 cells/well) were seeded in a 24-well plate. After 24 h, the psiCHECK-3′UTR *CHAC1* vector was cotransfected with 100 nM of tRF-5′LysTTT or a corresponding scrambled sequence using Lipofectamine 3000 (Catalog no. L3000015, Invitrogen) according to the manufacturer's instructions. Following 48 h of incubation, the media were removed, and cells were lysed. Dual luciferase activity was measured using the Dual Luciferase Reporter Assay Kit (Catalog no. K1910, Promega).

Statistics

Data are presented as mean ± SEM. Statistical differences between two groups were assessed using unpaired two-tailed Student's *t* test. For comparisons among multiple groups, one-way ANOVA followed by a one-sided Tukey test was used. All analyses were performed using GraphPad Prism v8 for Windows (San Diego, CA). A significance level of $P < 0.05$ was set for all comparisons.

Computational Tools

Repetitive sequence motifs in the characterized tRFs were identified using the MEME suite tool (https://meme-suite.org/meme/index.html) (98). For target prediction of these sequences, the tRFtarget.net 2.0 and MR-microT DIANA algorithms were employed (49, 99, 100).

Data Availability

The data that support the findings of this study are available upon request from the corresponding author: hermona.soreq@mail.huji.ac.il

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Author Contributions

A.M., N.M., J.T., O.R. and H.S.: Conceptualization; A.M., J.T., O.R. and H.S: Methodology; A.M. and O.R.: Investigation; A.M, N.M, S.V.T, O.I., L.H.: Formal Analysis; A.M., N.M.: Writing – Original Draft; J.T., D.S.G., O.R. and H.S.: Writing – Review & Editing; A.M., N.M., O.R., H.S.: Visualization; J.T., H.S.: Funding Acquisition; O.R., H.S.: Project Administration; J.T., O.R., and H.S.: Supervision.

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Author Disclosures

The authors declare no conflict of interest.

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Varying levels of interleukin 1 receptor antagonist (*Il1rn*) gene expression affect circulating leptin concentrations and fat distribution

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The relationship between leptin and interleukin 1 receptor antagonist (IL1RA) is critical to the understanding of obesity's low-grade chronic inflammatory state. IL1RA antagonizes the proinflammatory effects of IL1, dampens systemic inflammation markers, and in clinical trials improved glycemic control in type 2 diabetes. We show that leptin levels decreased relative to the number of *Il1rn* copies in mice overexpressing IL1RA; these mice have altered white fat distribution with decreased epididymal and retroperitoneal fat tissue masses. They also have increased IL1RA plasma levels and insulin sensitivity. Recombinant IL1RA may be a novel treatment strategy for metabolic syndrome by improving resistance to leptin and insulin.

Low-grade chronic metabolic inflammation is as a pivotal shared pathophysiological feature of neurodegenerative diseases and cognitive deficits as well as obesity, insulin resistance, and progression toward type 2 diabetes (T2D) (1). Increased levels of proinflammatory cytokines, such as interleukin 1B (IL1B), can contribute to pancreatic ß-cell destruction leading to T1D and to insulin resistance (2, 3). IL1 receptor antagonist (IL1RA), a naturally occurring endogenous antagonist for the proinflammatory effects of IL1A and IL1B, is increased severalfold in obesity with concentrations that are correlated with leptin levels (4). IL1RA appears to regulate adipogenesis, energy expenditure, and intake. Its elevated levels in obesity may contribute to obesity-associated outcomes, including leptin resistance (5). The complex interplay between IL1RA and leptin suggests that these molecules engage in bidirectional communication affecting inflammatory and metabolic pathways. Despite the use of recombinant human IL1RA in the treatment of rheumatoid arthritis and in trials for treating T2D, where it improved glycemic control, ß-cell function, and systemic inflammation (6), the net long-term IL1RA effects on metabolism are not well understood.

Using a unique approach of varying exposure to IL1RA levels based on transgenic mouse models with different copy numbers of the secreted form of the *Il1rn* (7), we investigated the effects of IL1RA on adipose tissue function. All experiments used male mice aged 10–14 weeks, acknowledging the sexual dimorphism in fat distribution and leptin biology. As an initial exploratory study, we focused on males, though future studies should examine sex-specific responses to IL1RA overexpression. This transgenic approach allows for investigation of dose-dependent effects that would be difficult to achieve with pharmacological administration, where IL1RA levels fluctuate rapidly after

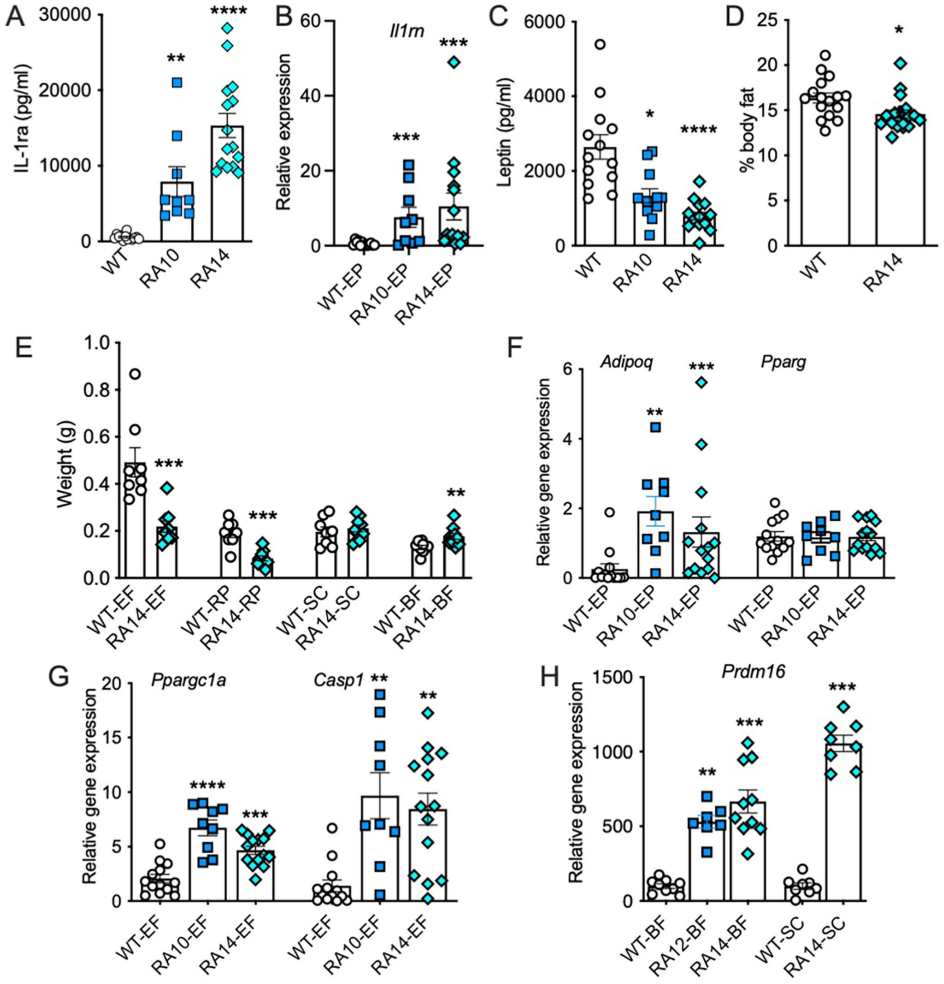

Figure 1. IL1RA overexpression alters fat depot weight and function. Comparing to wild-type (WT) mice, mice expressing a total of 10 (RA10) and 14 (RA14) copies of the *Il1rn* had: (A) higher plasma IL1RA levels and (B) significantly increased *Il1rn* gene expression in the EF (epididymal fat pad); and (C) decreased plasma leptin levels. (D) Dual-energy X-ray absorptiometry (DXA) scan showed decreased body fat percentage in RA14 mice. (E) RA14 mice showed decreased fat tissue mass in the EF and retroperitoneal fat (RF), unchanged subcutaneous fat (SC), and decreased brown fat (BF). (F) IL1RA-overexpressing mice had increased *Adipoq* expression and unchanged *Pparg* expression in the EF. *Ppargc1a* and *Casp1* mRNA expression were increased in the EF of mice overexpressing IL1RA. (G) *Prdm16* expression was increased in BF and SC of IL1RA-overexpressing mice. Columns and error bars = means ± sem; $n > 8$/group; one-way ANOVA or Kruskal–Wallis test and post hoc test (A, B, C, F, G, and BF data in H); Student *t*-test or Mann–Whitney test (D, E, and SC data in G); asterisks depict significant differences compared to WT mice; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{*****}P < 0.0001$.

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dosing. The sustained and genetically regulated elevation of IL1RA provides a model that more closely mimics constitutive overexpression than intermittent therapy. Compared to wild-type (WT) mice, these mice have very high levels of circulating IL1RA (Figure 1A) and significantly increased *Il1rn* gene expression (Figure 1B). Their leptin levels were decreased relative to the copy number of *Il1rn* (Figure 1C), possibly through IL1RA-mediated suppression of NFκB signaling in adipocytes. IL1RA-overexpressing mice had decreased percentage of body fat (Figure 1D) and a decrement in percentage of body fat with decreased epididymal fat (EF) pad and retroperitoneal fat (RF) masses, and increased brown fat (BF) weight (Figure 1E). Their EF adipose tissue showed increased *Adipoq* (adiponectin) gene expression, which is consistent with enhanced insulin sensitivity and glucose uptake (Figure 1F). However, the adipogenic transcription factor *Pparg* expression was unchanged. Adipose tissue *Ppargc1a* [peroxisome proliferator-activated receptor gamma (PPARG) coactivator 1 alpha] expression was also increased, implying increased mitochondrial activity (8). Caspase 1 (*Casp1*) mRNA was increased in the adipose tissue, suggesting adipocyte differentiation (Figure 1F). IL1RA-overexpressing mice also had increased *Pdrm16* (PR domain containing 16) expression in BF and subcutaneous fat (SC) (Figure 1G). PRDM16 is an essential regulator of thermogenesis and induces BF formation (9). Congruent with published work and compared to WT mice, IL1RA-overexpressing mice have normal weight (7), increased insulin sensitivity, reflected in decreased glucose level in the intraperitoneal insulin sensitivity test, and decreased circulating insulin and C-peptide levels. Additionally insulin content in pancreatic ß-cell islets was decreased (Supplemental Figure).

Our findings support the hypothesis that obesity and metabolic syndrome may be autoinflammatory conditions in which endogenous IL1RA levels, though present, are insufficiently high to restore insulin and leptin sensitivity. Administration of human recombinant IL1RA is an approved treatment for autoinflammatory disorders, such as rheumatoid arthritis. Previous studies with these transgenic mouse lines demonstrated that elevated IL1RA expression reduces systemic inflammation markers, including circulating IL1A and IL1B, and dampens IL1 signaling. While we did not measure serum adiponectin levels, the enhanced adiponectin gene expression suggests a potential mechanism for improved metabolic profiles via enhanced glucose uptake and fatty acid oxidation. The inverse relationship between IL1RA copy number and leptin levels observed in our study indicates a dose-dependent effect that could be leveraged therapeutically. The effects on fat depot distribution, with decreased white fat and increased thermogenic capacity in BF, suggest that IL1RA influences not only total adiposity but also the qualitative aspects of fat metabolism. We suggest that pharmacological doses of recombinant IL1RA may be beneficial for treating metabolic syndrome by decreasing leptin and insulin resistances; its administration should take in consideration the nocturnal peak of endogenous leptin (10).

Neuro-immune interactions, including IL1RA-leptin signaling, offer therapeutic targets beyond weight regulation. The neuro-immune axis (11, 12) modulates brain pathways relevant to metabolic and neurodegenerative disorders, suggesting potential applications for treating cognitive decline and depression alongside obesity and diabetes.

Data Availability

All data needed to evaluate the conclusions in the paper are present in the paper and supplemental materials. Original data for gene expression analyses (qPCR), plasma measurements (IL1RA, leptin, insulin, C-peptide), body composition (DXA scans), and tissue weights are available from the corresponding author (juliolicinio@gmail.com) upon reasonable request. The transgenic mouse lines used in this study were obtained from Dr. Emmet Hirsch, currently at University of Chicago.

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Author Contributions

JL and MLW conceived and designed the study; CM and GP performed animal studies; CM performed gene expression and leptin assays; GP performed Dual-energy X-ray absorptiometry scans; CS conceived, designed, and performed pancreatic ß-cell islets studies; CM, GP, and CS analyzed data and plotted figures; JL and MLW wrote, and CM, GP, and CS edited the paper.

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Author Disclosures

The authors declare no competing interests.

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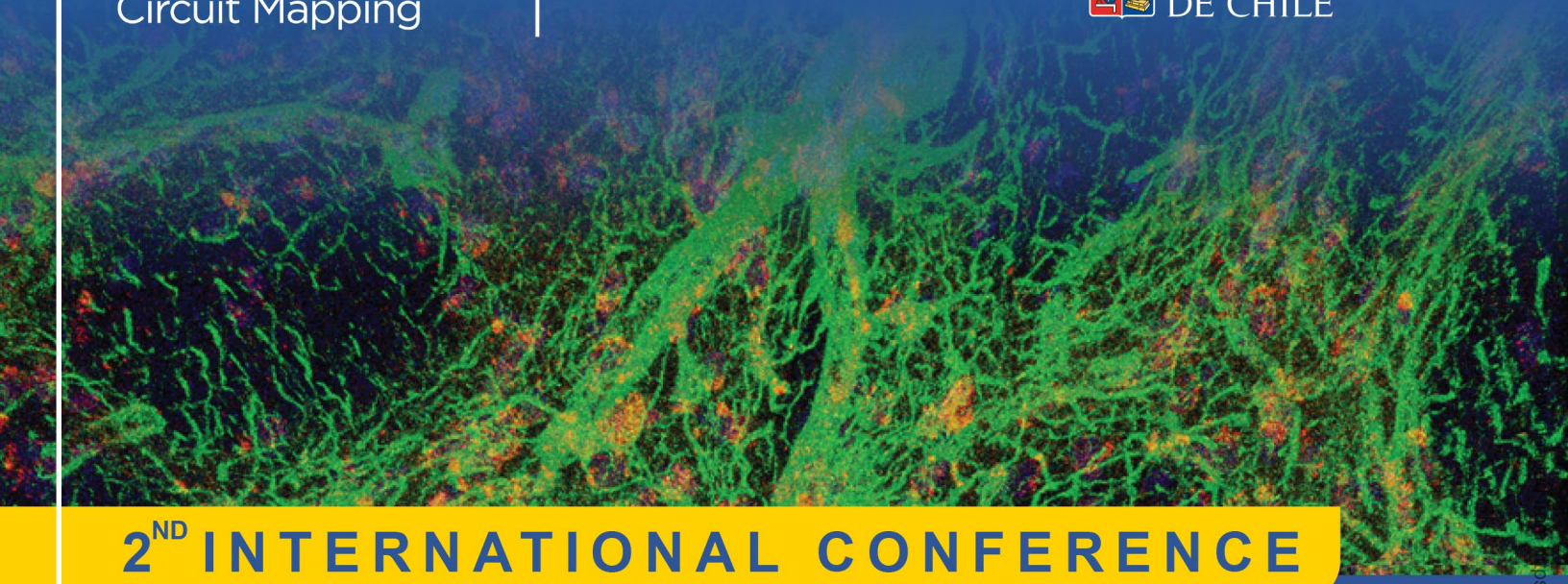

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