Many Apparent False Negatives in Detection of Mutations in Gene Associated With Autism Spectrum Disorders

Mary Hames,[1] Alexander Kolevzon,[2] Elizabeth Berry-Kravis,[3] Jimmy Holder,[4] Larry Glass,[1] Nancy E Jones,[1] Brian Eisinger,[1] Geraldine Bliss,[5] Liza Squires,[1] Tess Levy[2]

[1]Neuren Pharmaceuticals, Camberwell, VIC, AUS; [2]Icahn School of Medicine at Mount Sinai, New York, NY, USA; [3]Rush University Medical Center, Chicago, IL, USA; [4]Baylor College of Medicine, Houston, TX, USA; [5]CureSHANK, Los Angeles, CA, USA

Objective

To evaluate US commercial laboratories' *SHANK3* testing quality, estimate the real-world impact of suboptimal testing on the number of undiagnosed individuals with Phelan-McDermid syndrome (PMS), and disseminate actionable findings to support accurate diagnoses of PMS

Conclusions

False negative genetic testing results are likely being reported for individuals with ASD

Consequently, hundreds of cases of PMS have likely gone undiagnosed in recent years, including up to half of those tested at laboratories with poor *SHANK3* testing

These findings may inform diagnostic workflow development, motivate remedial retesting of negative results, and empower future diagnostic decision-making

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L Glass, NE Jones, and L Squires are executives at Neuren and may hold Neuren stock or stock options.

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Background

- Phelan-McDermid syndrome (PMS) is a neurodevelopmental disorder that presents heterogeneously with intellectual disability (ID), speech impairment/absence, problems with social communication, motor impairments, and features of autism spectrum disorder (ASD)[1]
- PMS results from disruptions to the *SHANK3* gene on chromosome 22q13, which encodes a scaffolding protein crucial for synaptic function and neuronal development[2]
  - While many cases of PMS involve chromosome 22q13 deletions, *SHANK3* sequence variants also commonly lead to PMS[3]
  - Individuals with *SHANK3* variants may have milder phenotypes[4]
- *SHANK3* variants are among the most common genetic findings in ASD, affecting ~1% of individuals[5]
- Guidelines for genetic testing for neurodevelopmental disorders vary across societies, with some not reflecting current knowledge and technology[6]
- Variations in genetic testing practices among healthcare providers and the quality of genetic testing offered by diagnostic laboratories contribute to missed or delayed diagnoses
- To understand how laboratory testing quality may impact healthcare providers' ability to accurately diagnose neurodevelopment disorders, we evaluated *SHANK3* testing quality among US diagnostic laboratories

Methods

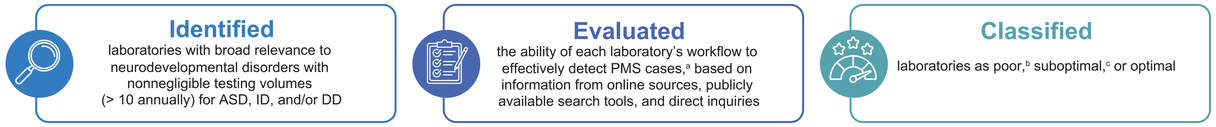
Diagnostic Laboratory Evaluation

- US diagnostic laboratories were identified, evaluated, and classified based on their next-generation sequencing (NGS) workflows (Figure 1)

Impact of Diagnostic Testing Gaps

- We used laboratory-reported testing volumes and published diagnostic yields for chromosomal microarray analysis (CMA; 7.0%) and fragile X testing (0.46%)[7] to estimate the potential impact of the identified testing gaps at 2 laboratories on the ability to identify PMS

Figure 1. Laboratory Identification, Evaluation, and Classification



ASD, autism spectrum disorder; DD, developmental delay; ID, intellectual disability; PMS, Phelan-McDermid syndrome.
[a]Assessed criteria (relative impact on classification): Inclusion of *SHANK3* in whole-genome sequencing, whole-exome sequencing, or panels (critical); inclusion or availability of deletion/duplication analysis (high); quality of sequencing depth and coverage of all *SHANK3* regions (moderate); reflex validation (eg, Sanger sequencing) for challenging regions (moderate); and variant classification and reporting (mild).
[b]*SHANK3* not included in panel-based testing or whole-genome/whole-exome sequencing.
[c]*SHANK3* included in testing but with incomplete gene coverage, a lack of deletion/duplication analysis, or noncomprehensive variant reporting.

Results

Diagnostic Laboratory Evaluation

- Twelve diagnostic laboratories with relevant testing for ASD, ID, or developmental delay were identified and evaluated (Figure 2)
- NGS diagnostic methods and capabilities varied across laboratories, with significant gaps in coverage identified for several laboratories
- Identified gaps included:
  - Incomplete analysis or total omission of *SHANK3* in relevant sequencing panels
  - Lack of *SHANK3* deletion/duplication analysis
  - Reliance on exome sequencing, which may not identify large deletions
- No pattern between testing volume and quality classification was observed

Figure 2. Evaluation of Laboratories' NGS-Based *SHANK3* Diagnostic Testing

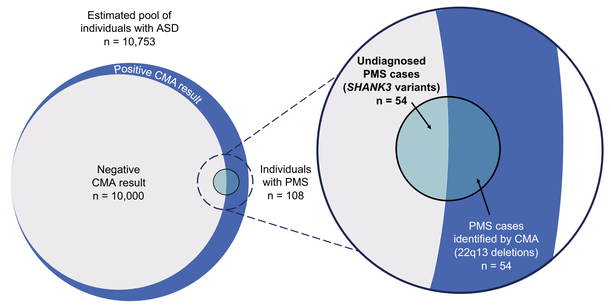
Quality Classification	Laboratory	Areas for Improvement	Annual Testing Volume
Optimal	H	Not applicable	> 10,000
	J	Not applicable	< 100
	C	Not applicable	Not available
Suboptimal	F	Relevant test does not sequence exon 11	1000–10,000
	G	Coverage of coding sequences is not guaranteed > 90%; VUS reporting is not included by default	1000–10,000
	K	Does not include deletion/duplication analysis	1000–10,000
	I	No coverage of exon 1 or portions of exon 12	100–1000
	B	Relevant panels do not include deletion/duplication analysis; exon 11 coverage is not reliable	< 100
	E	Relevant test does not include deletion/duplication analysis	Not available
Poor	D	*SHANK3* is not included in WES or relevant panels	> 10,000
	A	*SHANK3* is not included in relevant panels	< 100
	L	*SHANK3* is not included in relevant panels	Not available

NGS, next-generation sequencing; VUS, variant of uncertain significance; WES, whole-exome sequencing.
Laboratories assessed as having "Optimal" quality classifications display "Not applicable" for areas of improvement.

Example Impact of Diagnostic Testing Gaps

- Laboratory D (poor testing quality) conducts > 10,000 NGS-based tests annually for individuals presenting with ASD or ID
  - Based on a CMA diagnostic yield of 7.0%,[7] NGS tests for 10,000 individuals with ASD who had received negative CMA results would correspond to an original pool of ~10,753 individuals. Of these, 108 (1%) are likely to have PMS[5] (Figure 3)
  - Assuming 50% of PMS cases involve large deletions (based on recent data[8]), 54 of 108 individuals with PMS would have had a 22q13 deletion identified by CMA, leaving approximately 54 individuals who would require testing with NGS methods
  - Because Laboratory D does not include *SHANK3* in NGS testing, all 54 of these individuals would remain undiagnosed

Figure 3. Potential Number of PMS Cases Missed Annually at Laboratory D



ASD, autism spectrum disorder; CMA, chromosomal microarray; PMS, Phelan-McDermid syndrome.

- NGS testing for the same 10,000 individuals with ASD at Laboratory F (suboptimal testing quality), which includes *SHANK3* but excludes exon 11 (9% of the gene's coding sequence), would leave approximately 5 individuals undiagnosed
- Alternatively, if the NGS-based tests were ordered after fragile X testing, which has a diagnostic yield of 0.46%,[7] the original ASD pool would comprise ~10,046 individuals, including 50 and 5 individuals with PMS undiagnosed by Laboratories D and F, respectively

Implications And Future Directions

- Based on our annual estimates of undiagnosed cases, hundreds of individuals with PMS have likely remained undiagnosed after NGS-based testing at diagnostic laboratories across the US
  - Given existing limitations to testing access, individuals receiving false negatives may not be retested
- Identifying common gaps in genetic testing can inform laboratories' diagnostic workflow development, motivate remedial retesting of negative results, and empower decision-making by individuals, families, and healthcare providers
  - NGS-based testing for ASD, ID, and developmental delay should include *SHANK3* and copy number variant analysis to identify pathogenic or likely pathogenic variants
  - If NGS quality control metrics are low, Sanger sequencing should be performed for regions with poor coverage
  - Quality NGS-based testing can also identify PMS cases associated with deletions not detected by CMA. For example, 1 child with PMS received a negative CMA result before a whole-exome sequencing test identified an 84-kb deletion in *SHANK3* (J Holder, personal experience)
- We shared our findings with all 12 assessed laboratories
  - To date, we have received responses from 6 laboratories
  - In follow-up discussions with 3 laboratories, all expressed an interest or intent to improve; 1 laboratory classified as "poor" noted that implementing the necessary improvements could take years
- This analysis was limited to US laboratories; assessment of international diagnostic testing laboratories is needed to understand global impacts