Heterogeneity in clinical sequencing tests marketed for autism spectrum disorders

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Autism spectrum disorders (ASD) is now a high profile and common concern in the population. Diagnosis has long been based on clinical findings, but with increased recognition of a strong genetic contribution and a subset of cases with an underlying genetic syndrome, clinical laboratory testing to search for genomic risk variants is now an important component of the diagnostic work-up. Chromosomal microarray is currently the recommended first-tier genetic test for ASD, revealing deleted and duplicated segments of DNA. These relatively large copy number variations (CNVs), often affecting several genes, can account for 5–25% of ASD cases, depending on the cohort examined.

The introduction of next-generation sequencing (NGS) now allows for a more in-depth look at ASD's genetic landscape. Besides CNVs, genomic risk factors for ASD can include single-nucleotide variants, insertion/deletions, and complex structural variations, contained in potentially hundreds of different genes, which may be involved in complex interactions. The NGS technology may be targeted to a selection of genes of interest, or to the coding portions of all genes (exome sequencing), or for entire genome sequencing. Such sequencing approaches are now being marketed for use as second-tier tests for ASD, particularly when chromosomal microarray analysis has not revealed any explanation for the clinical presentation.

We undertook to survey the scope of sequencing tests for ASD (or including ASD) that are primarily being marketed by commercial laboratories as adjuncts or follow-up to chromosomal microarrays. Perhaps not surprisingly, because this is a new territory and guidelines are not yet developed, we found significant heterogeneity among such laboratories with respect to the tests they offer. The most striking finding was the variable number of genes being tested for on panels marketed for ASD (range, 11–2562), with little content overlap, albeit, these encompassed ASD-specific as well as much larger ASD-inclusive panels (Table 1).

Our search began in 2017, using the former GeneTests and current Genetic Testing Registry as resources, supplemented by an internet search (terms: “autism panel”, “autism lab sequencing”, “autism genetic test”). To focus on gene panels, we excluded biochemical assays, chromosomal microarrays, and sequencing tests involving fewer than three genes. To focus on ASD, we excluded tests targeted for general neurodevelopmental disorders, seizure disorders, and intellectual disability, unless they specified autism. That search resulted in 20 DNA testing laboratories offering an ASD gene panel. Updating the survey in June 2018 (adding use of a new resource, Concert Genetics—www.concertgenetics.com), we found that four panels were no longer offered, but five were newly available, for an updated total of 21 laboratories offering an ASD gene panel (Table 1). We then went to the individual laboratory websites for further information.

We compared ASD gene lists from panels of the 21 laboratories. Each entire panel is listed in Supplementary Table S1. Supplementary Table S2 shows the 178 genes included in at least five lab panels ("shared genes") in order of listing frequency. Table 2 summarizes the top 16 most commonly listed genes, along with their associated genetic disorders according to the Online Mendelian Inheritance in Man (OMIM). The latter shows that most (12/16) of these genes are associated with genetic syndromes, where the primary phenotype involves physical/systemic features and not ASD. Almost half of these genes (7/16) were located on the X chromosome. Only one gene was included on all panels offered by the 21 labs: MECP2, which is associated with Rett syndrome (previously considered part of the ASD spectrum, but no longer so under criteria of the Diagnostic and statistical manual of mental disorders (5th edn)). There were 63 genes shared among at least 10 labs, but the vast majority of the cumulative list of 2928 unique genes were included by fewer than five laboratories. There were, nonetheless, some pockets of significant overlap. Two pairs of laboratories each posted identical ASD gene lists. Two other laboratories had lists that encompassed those of separate labs, but with additional genes to create their own collection.

We then compared the gene lists ascertained here to four lists from academic research projects that identify genes with strong association to ASD: Simons Foundation Autism Risk Initiative (SFARI), Simons Foundation Powering Autism Research for Knowledge (SPARK), Autism Speaks – MSSNG, and Autism Sequencing Consortium (ASC) (Supplementary Table S2). Comparing these lists, we found 15 genes to be shared by all, and an additional 24 genes were common to 3 of the 4 lists (Supplementary Table S3). We noted that 39 ASD risk genes identified by at least two of these research sources were not included among the top 178 genes listed by the commercial labs (Supplementary Table S4). Moreover, seven well-studied genes (ADNP, ARID1B, CHD8, POGZ, SCN2A, SLC6A1, and SYNGAP1) identified to be important for ASD by the SFARI, SPARK, MSSNG, and ASC research projects, are not found among the commercial list of the top 47 shared genes (Supplementary Table S2).
| Number | Laboratory company                  | Test name                                      | Number of genes included | Gene list provided<sup>a</sup> | Gene selection criteria/rationale provided<sup>b</sup> | Certification/accreditation notes provided<sup>b</sup> |
|--------|-------------------------------------|-----------------------------------------------|--------------------------|-------------------------------|--------------------------------------------------------|--------------------------------------------------------|
| 1      | Ambry Genetics                      | AutismNext                                    | 48                       | ●                            | ●                                                      | ●                                                      |
| 2      | ApolloGen                           | Autism Spectrum Disorders Panel Test          | 125                      | ●                            | ●                                                      | ●                                                      |
| 3      | Asper Biogene Asper Biogene LLC     | Autism Spectrum Disorders NGS panel           | 52                       | ●                            | ●                                                      | ●                                                      |
| 4      | Ayass BioScience                    | AUTISM - Genetic Testing                      | 98                       | ○                            | ●                                                      | ●                                                      |
| 5      | Blueprint Genetics                  | Autism Spectrum Disorder Panel                | 26                       | ●                            | ●                                                      | ●                                                      |
| 6      | CEN4GEN                             | Autism Spectrum Disorders: Gene Panel         | 63                       | ●                            | ●                                                      | ●                                                      |
| 7      | Center for Human Genetics, Inc      | Autism Spectrum Disorders 53-Gene Panel       | 53                       | ●                            | ●                                                      | ●                                                      |
| 8      | Centogene                           | Syndromic autism panel                        | 50                       | ●                            | ●                                                      | ●                                                      |
| 9      | CGC Genetics                        | Autism                                        | 28                       | ●                            | ●                                                      | ●                                                      |
| 10     | EGL Genetic Diagnostics (Emory)     | Autism Spectrum Disorders: Tier 2 Panel       | 63                       | ●                            | ●                                                      | ●                                                      |
| 11     | Fulgent Genetics                    | Autism NGS Panel                              | 106                      | ●                            | ●                                                      | ●                                                      |
| 12     | GeneDx                              | Autism/ID Xpanded Panel                       | 2562                     | ●                            | ●                                                      | ●                                                      |
| 13     | GENETAQ                             | Autism                                        | 28                       | ●                            | ●                                                      | ●                                                      |
| 14     | Greenwood Genetic Center Diagnostic Laboratories | Syndromic Autism Sequencing Panel       | 83                       | ●                            | ●                                                      | ●                                                      |
| 15     | Munroe-Meyer Institute              | Autism/Intellectual Disability/Multiple Anomalies Panel | 117                   | ●                            | ●                                                      | ●                                                      |
| 16     | Lineagen                            | NextStepDx PLUS                               | 936                      | ○                            | ●                                                      | ●                                                      |
| 17     | Michigan Medical Genetics Laboratories | Autism / Intellectual Disability Panels (Tier 2 and Tier 3) | 11                      | ●                            | ●                                                      | ●                                                      |
| 18     | MNG Laboratories                    | Comprehensive Intellectual Disability/Autism  | 901                      | ●                            | ●                                                      | ●                                                      |
| 19     | PreventionGenetics                  | Autism Spectrum Disorders and Intellectual Disability (ASD-ID) Comprehensive Sequencing Panel with CNV Detection AND Autism Spectrum Disorders Sequencing Panel with CNV Detection | 1908 | ● | ● | ● |
| 20     | Reference Laboratory Genetics       | Autism Spectrum Disorders (Expanded Panel)    | 77                       | ●                            | ●                                                      | ●                                                      |
| 21     | Sema4                               | Autism Spectrum Disorder Sequencing Panel     | 30                       | ●                            | ●                                                      | ●                                                      |

● = provided upon request
<sup>a</sup>See Supplementary Table S1 for full gene list
<sup>b</sup>See Supplementary Table S5 for details
How were these lists derived by the 21 clinical laboratories? We looked for such information from the websites, documented the mention of gene selection criteria or rationale (Table 1) and summarized the comments found (Supplementary Table S5). Some labs indicated nothing, some made general comments, and a few provided notes with or without literature citations. Favorably, one lab provided a dated record of updates to their panel.

From the respective websites, information about sequencing and analysis approaches used by the various laboratories were rarely obvious and sometimes unstated. A few of the labs undertook exome sequencing followed by selective analysis of an ASD gene panel. A few other labs stated that they applied NGS to “selected” or “targeted” genes only, and most did not provide any specification. Some added mention of exons or coding sequences; some also mentioned introns, splice junctions, or non-coding sequence. One lab clearly said they used Sanger sequencing only, and some noted that adjunct Sanger sequencing was applied either for regions poorly covered with NGS or for confirmation of significant findings before reporting.

Our ascertainment was intended to identify clinical tests; therefore, we searched for documentation of laboratory qualification and tabulated key findings (Table 1 and Supplementary Table S5). Additionally, a few websites specified that tests were not direct-to-consumer or could only be ordered by a health professional. Several labs mentioned that the test was intended for individuals with symptoms or clinical diagnosis of ASD, some limiting this to syndromic autism, and a few provided certifications of those preparing the test.

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The diversity unearthed by this survey applied not only to the specific gene tests offered for ASD, but to the extent of supporting information provided by the companies who market these services. Consumers would benefit from greater transparency (including evidence) about items such as gene inclusion criteria, dates of updates, technologies used, interpretation of variants, reporting standards, ownership of data, secondary use policies, etc.

ASD is clearly a heterogeneous disorder, both in clinical presentation and in terms of the underlying etiology. In fact, the genetic predisposition for ASD may be different in almost
every individual. Furthermore, the evidence for association between any given variant and ASD is wide-ranging, and even when an association is strongly supported, there is usually variable expressivity or some degree of non-penetration reflected in the evidence. The NGS tests currently marketed for ASD are mostly being used in a confirmatory manner, in the context of clinical findings. However, expectations are rising, from families and their healthcare providers, to use the same DNA tests to enable early diagnosis, prognosis, and medical management of ASD, and to assess familial risk. This survey highlights the need to develop a clinically validated list of genes appropriate for clinical laboratory analysis for ASD to meet these growing demands.

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AUTHOR CONTRIBUTIONS
All authors conceptualized, researched, and wrote this paper.

ADDITIONAL INFORMATION
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Competing interests: S.W.S. and SickKids Hospital has licensed intellectual property for genetic testing in autism to Athena Diagnostics, Lineagen, Population Bio. S.W.S is also on the Scientific Advisory Committees of Population Bio and Deep Genomics, and directs the Autism Speaks MSSNG Open Science project. The remaining authors declare no competing interests.

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