Validation and Implementation of a 62-Gene Next Generation Sequencing Panel for Syndromic Autism: Experience from a Clinical Diagnostic Laboratory

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Abstract

Autism spectrum disorders (ASD) are complex developmental disabilities affecting as many as one in 150 individuals. Coexisting genetic disorders are found in a subgroup of patients with autism (estimated to be between 11% and 37%). In an effort to provide comprehensive testing for this subgroup, the Molecular Diagnostic Laboratory at the Greenwood Genetic Center (GGC) collaborated with investigators at Emory Genetics Laboratory and RainDance Technologies™ to design a Next Generation Sequencing (NGS) panel for 62 genes associated with ASD (available commercially from RainDance Technologies™ as the ASDseq™ Research Screening Panel). These genes were selected to represent the most common single gene etiologies associated with autism as a significant clinical feature. The panel utilizes droplet-based multiplex PCR technology to enrich for 1164 exons for NGS with greater than 98% coverage of the targeted regions. The coverage of the panel includes all exons for each gene, at least 50 nucleotides upstream and downstream of each exon and 1 kb of both the 5’ promoter region and the 3’ UTRs. The panel was recently validated at GGC as a diagnostic test on both the SOLiD™ 3 Plus and 5500xl systems using 16 positive controls. In addition, 40 patients with autism of unknown etiology have been tested to date. Here we describe our validation process and the implementation of diagnostic testing using the NGS Syndromic Autism 62-Gene Panel. This panel provides comprehensive testing for patients with autism and may also serve as a useful 2nd tier test for patients with features overlapping those of Rett and Angelman syndromes.

Methods

5 µg of each genomic DNA sample was fragmented to 4-6 kb using a Covaris. RainDance enrichment for the 62 genes was performed on the RT15000 instrument (RainDance Technologies, Lexington, MA). Standard fragment libraries for analysis by SOLiD sequencing were prepared for each sample. Library amplification was performed using emulsion PCR, and the products of the emulsion PCR were purified (AMPure) and then deposited onto a glass slide for analysis by either the SOLiD™ 3 Plus or 5500xl system (Applied Biosystems, Foster City, CA). After bioinformatically processing the raw data, a tertiary analysis was performed using the Beast software (SoftGenetics, State College, PA). The data was then reviewed with an initial processing the data, a tertiary analysis was performed using NextGENe software Database (HGMD: http://www.hgmd.cf.ac.uk/ac/index.php). The alterations emphasis on novel alterations and those reported in the Human Gene Mutation Database (HGMD).

Validation of the Syndromic Autism 62-Gene Panel

Gene Alteration Detected by

MECF2 c.1530C>T het (p.Q510X) Yes (3 Plus)
MECP2  c.1178C>T het  (p.T393I) Yes (5500)
PTEN  c.2350G>A het (p.A793T) Yes* (3 Plus)
PTEN  c.3936C>T het (p.T1312X) Yes (5500)
PTEN  c.559C>T het (p.Q187X) Yes (5500)
SHANK3_cds1 c.1643_1644delTG hemi Yes (5500)
SHANK3_cds12 c.116_117delAC hemi Yes (5500)

d.1643_1644delTG hemi Yes (5500)

Average fold coverage of targeted regions: 168X

Average exon span coverage: 97.6%

Average number of dropouts: 18

Summary

For each of the 40 patients tested thus far, an average of 5 to 10 variants of unknown clinical significance were detected. Family studies will be performed to help define the pathogenicity of these changes. In addition to these variants, two pathogenic mutations and one likely pathogenic alteration were detected. These three cases are described below:

Patient 1 is a 10 year old female with intellectual disability, autism, speech delay and seizures. A heterogeneous missense change (c.1178C>T; p.Q393X) was detected in the MECP2 gene by NGS which is predicted by both SIFT and PolyPhen to be damaging. The change was confirmed by Sanger and was not maternally inherited. The paternal sample is not available for analysis.

Patient 2 is a non-verbal, non-ambulatory 2 1/2 year old male with autism, intellectual disability, ataxia, global developmental delay and acquired microcephaly. A heterogeneous missense mutation (c.1368C>T; p.R456X) was detected in the MECP2 gene by Sanger and this change was confirmed by NGS.

Patient 3 is an 11 year old female with acquired microcephaly and other features consistent with Rett syndrome. In 2002, a MECP2 analysis was performed by Sanger sequencing which yielded a normal result. NGS using this panel detected a mosaic heterozygous mutation (c.116_117delAC; p.T1312X) in MECP2. Sanger sequencing was repeated, and the abnormal allele was again not detected by the software but could be visualized at a very low level.

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