MOLECULAR GENETICS REPORT:
Autism Spectrum Disorders and Intellectual Disability (ASD-ID) Comprehensive Panel
Trio Analysis (Proband and Parents)

SUMMARY OF RESULTS: HETEROZYGOUS FOR DE NOVO VARIANT OF UNCERTAIN SIGNIFICANCE IN MED13

INDICATIONS FOR TESTING: Global developmental delay, coarse facial features, low frustration tolerance

1) Variants in genes known to be associated with phenotype: None detected

2) Variants in genes possibly associated with the phenotype:

<table>
<thead>
<tr>
<th>Gene</th>
<th>Disorder</th>
<th>Mode of Inheritance</th>
<th>Transcript</th>
<th>DNA Variation/Predicted Effect</th>
<th>Reference</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>MED13</td>
<td>Autism Spectrum Disorder</td>
<td>Autosomal Dominant (De Novo)</td>
<td>NM_005121.2</td>
<td>c.6123G&gt;C (p.Gln2041His), Heterozygous</td>
<td>Undocumented</td>
<td>Uncertain</td>
</tr>
</tbody>
</table>

RESULTS AND INTERPRETATIONS: This patient is heterozygous in the MED13 gene for a de novo sequence variant designated c.6123G>C, which is predicted to result in the amino acid substitution p.Gln2041His. To our knowledge, this variant has not been reported in the literature or public databases. The amino acid residue p.Gln2041 of the MED13 protein has been highly conserved during evolution. The amino acid substitution prediction programs PolyPhen-2, SIFT and MutationTaster all predict the p.Gln2041His change to be deleterious. Although we suspect that the c.6123G>C (p.Gln2041His) variant may be pathogenic, its clinical significance is currently uncertain due to the absence of conclusive functional and genetic evidence (see NOTES).

MED13 is a component of the Mediator complex, a multiprotein coactivator that interacts with RNA polymerase II and various transcription factors to carry out gene expression (Sato et al. 2004, PubMed ID: 15175163). A 800 kb de novo deletion encompassing six genes including MED13 has been reported in an individual with moderate intellectual disability, short stature, mild dysmorphism, hearing loss, peripheral cataract, and attention deficit and hyperactivity disorder (ADHD) (Boutry-Kryza et al. 2011, PubMed ID: 22162340). Most recently, putative damaging variants (2 nonsense, 1 missense) in MED13 have been identified in 3 out of 2,620 heterozygous individuals with Autism Spectrum Disorder (ASD) from three different simplex families via whole genome sequencing. However, the clinical features of these individuals were not described. This group also classified MED13 as a new candidate gene for ASD (Supplementary Figure 2, Yuen et al. 2017, PubMed ID: 28263302).
These results should be interpreted in the context of clinical findings, family history and other laboratory data. All genetic tests have limitations. Please see limitations and other information for this test on pages 3 - 5.

**NOTES:**

1) This patient also carries many additional variants not listed here. A list of these variants is available upon request.

2) Genetic counseling is recommended.

**DATA TRANSFER:** PreventionGenetics recommends that DNA sequence information from this test be stored in the patient’s electronic medical record. This will facilitate reinterpretation of the sequence in future, and will best benefit the patient and family members. Upon request, we will be pleased to transfer the sequence results.
Clinical Features: Autism Spectrum Disorders (ASDs) and Intellectual Disability (ID) are a heterogeneous group of neurodevelopmental disorders. ASD is characterized by varying degrees of social impairment, communication ability, propensity for repetitive behavior(s), and restricted interests (Levy et al. 2009); whereas ID refers to significant impairment of cognitive and adaptive development (intelligence quotient, IQ<70) due to abnormalities of brain structure and/or function (American Association of Intellectual and Developmental Disabilities, AAIDD). ID is not a single entity, but rather a general symptom of neurologic dysfunction that is diagnosed before age 18 in ~1%–3% of the population, irrespective of social class and culture (Kaufman et al. 2010; Vissers et al. 2016). In contrast, ASD symptoms usually present by age 3, and diagnosis is based on the degree and severity of symptoms and behaviors (McPartland et al. 2016). ASDs and ID are highly comorbid, suggesting shared etiologies in many forms. For ASD specifically, comorbidities have been observed in more than 70% of cases, and include ID, epilepsy, language deficits, and gastrointestinal problems (Sztainberg and Zoghbi 2016).

Genetics: ASDs and ID are inherited in a multifactorial fashion, with heritability estimates ranging between 50%-90% for ASDs and 15%-50% for ID (Larsen et al. 2016; Karam et al. 2015). ASD concordance is as high as 70% in monozygotic twins. Familial recurrence rates are 7% if the first affected child is female and 4% if the first affected child is male (Schaefer and Mendelsohn 2008). The incidence of ASD is approximately 1 in 68 individuals with a male-to-female ratio of 4:1 (CDC 2014). Interestingly, although ~30% more males are diagnosed with ID than females, the male-to-female ratio decreases as IQ decreases (American Psychiatric Association 2000). However, co-occurring ASD and ID has a similar male-to-female prevalence ratio of ~4:1 (Christensen et al. 2016).

According to a large number of reports, chromosomal abnormalities (Fragile X syndrome, translocations, recurrent autosomal microdeletions/duplications) and pathogenic copy number variants (CNVs), both familial and de novo, can explain ~10%-15% of ASD-ID cases. The fragile X mental retardation 1 (FMR1) gene remains the most frequent candidate, explaining ~0.5%-5% of cases (Ropers and Hamel 2005; Rauch et al. 2006; Zahir and Friedman 2007; Schaefer and Mendelsohn 2013; Vissers et al. 2016). Consequently, chromosomal microarray (CMA) is recommended as the first-tier diagnostic test in these disorders, followed by screening for Fragile X syndrome (Mefford et al. 2008; Weiss et al. 2008; Miller et al. 2010; Schaefer and Mendelsohn 2013; Vissers et al. 2016). For ASD, de novo missense and likely gene disrupting variants are 15% and 75% more frequent in patients than unaffected controls, respectively (Iossifov et al. 2014). Hence, trio testing (whenever possible) is considered the most powerful approach for genetic diagnosis of ASD-ID (Lee et al. 2014; Wright et al. 2015).

This test includes 1,907 genes that through literature, OMIM, and HGMD searches have at least a potential association with ASD-ID phenotypes. Please see the test description on our website (www.preventiongenetics.com) for a full list of genes and transcripts analyzed.

Testing Strategy: For the ASD-ID comprehensive panel, Next Generation Sequencing (NGS) technologies are used to cover the coding regions of targeted genes plus ~10 bases of non-coding DNA flanking each exon. Genomic DNA is extracted from patient specimens, as required. Patient DNA corresponding to the targeted genes is captured using Agilent Clinical Research Exome hybridization probes. Captured DNA is then sequenced using Illumina’s Reversible Dye Terminator (RDT) platform NextSeq 500 using 150 by 100 bp paired-end reads (Illumina, San Diego, CA, USA). The following quality control metrics are generally achieved: >97% of target bases are covered at >20x, and mean coverage of target bases >120x. Data analysis and interpretation is performed by the internally developed software Titanium-Exome. In brief, the output data from the NextSeq 500 is converted to fastqs by Illumina Bcl2Fastq 1.8.4, and mapped by BWA. Variant calls are made by the GATK Haplotype caller and annotated using in house software and SnpEff. Variants are filtered and annotated using...
VarSeq (www.goldenhelix.com). Since de novo variants are recorded at elevated frequencies in individuals with ASDs and ID, de novo variants in all clinically-relevant genes included in our PGxome test (Test #5000) are filtered, annotated, and interpreted. All reported pathogenic, likely pathogenic, and variants of uncertain significance are confirmed by Sanger sequencing.

For Sanger sequencing, polymerase chain reaction (PCR) is used to amplify targeted regions. After purification of the PCR products, cycle sequencing is carried out using the ABI Big Dye Terminator v.3.0 kit. PCR products are resolved by electrophoresis on an ABI 3730xl capillary sequencer. In nearly all cases, cycle sequencing is performed separately in both the forward and reverse directions.

The report will not include all the observed variants in the ASD-ID comprehensive panel due to the large number of genes included. However, the list of variants is available along with our interpretations, upon request. Since this test is performed using exome capture probes, a reflex to exome sequencing may be ordered. Please see the PGxome page (Test #5000) for limitations and reporting criteria for this test.

**Indications for Test:** This test is primarily indicated for patients with ASDs and/or ID, who are negative for any kind of cytogenetic abnormalities and Fragile-X syndrome (particularly males). Whole genome chromosomal microarray (CMA) via aCGH and SNP (Test #2000) and the FMR1 CGG-repeat expansion (Test #558) tests are available for individuals who have not been previously tested.

**Sensitivity of Test:** CMA and FMR1 CGG-repeat expansion testing have a combined diagnostic yield of 20%-25% and should always be carried out as primary testing (Schaefer and Mendelsohn 2013). CMA analysis is limited in its ability to identify low-level mosaicism and balanced translocations, however, these variants are infrequently (<1%) the cause of phenotypes in ASD/ID patients (Miller et al. 2010). Genetic variants have been found responsible in 25%-50% of ID cases and this percentage increases proportionally with the severity of the phenotype (McLaren and Bryson 1987). For ASD, while heritability estimates have been reported as high as 90% (Bailey et al. 1995; Lichtenstein et al. 2010), only 20% of ASD cases can be explained to date using combined genetic approaches (Devlin et al. 2012).

Trio-based studies have reported molecular diagnostic rates as high as 30%-40% for developmental phenotypes (Lee et al. 2014; Fitzgerald et al. 2015; Wright et al. 2015). Therefore, all attempts should be made to utilize trio-based testing in order to maximize the clinical sensitivity of this test through clear identification of compound heterozygous and de novo variants in the probands (singleton studies cannot resolve either of these situations) (Lee et al. 2014; Wright et al. 2015; Retterer et al. 2016).

**Reporting:** Reports will consist of two different sections:
- Variants in genes known to be associated with phenotype
- Variants in genes possibly associated with phenotype

All differences from the reference sequences (sequence variants) are assigned to one of five interpretation categories (Pathogenic, Likely Pathogenic, Variant of Uncertain Significance, Likely Benign and Benign) per ACMG Guidelines (Richards et al. 2015). Pathogenic, Likely Pathogenic and Variants of Uncertain Significance considered to contribute to the proband’s phenotype will be reported in the first and second sections (1 & 2).

Human Genome Variation Society (HGVS) recommendations are used to describe sequence variants (http://www.hgvs.org).

**Limitations and Other Test Notes:** Interpretation of the test results is limited by the information that is currently available. Enhanced interpretation should be possible in the future as more data and knowledge about human genetics and this specific disorder accumulate.
When sequencing does not reveal any heterozygous differences from the reference sequence, we cannot be certain that we were able to detect both patient alleles. Occasionally, a patient may carry an allele which does not capture or amplify, due to a large deletion or insertion. In these cases, the report will contain no information about the second allele. Our Sanger and NGS tests (including PGxome) are generally not capable of detecting Copy Number Variants (CNVs).

We sequence coding exons for most given transcripts, plus ~10 bp of flanking non-coding DNA for each exon. Unless specifically indicated, test reports contain no information about other portions of the gene, such as regulatory domains, deep intronic regions, uncharacterized alternative exons, chromosomal rearrangements, repeat expansions, epigenetic effects, and mitochondrial genome variants.

In most cases, we are unable to determine the phase of sequence variants. In particular, when we find two likely causative variants for recessive disorders, we cannot be certain that the variants are on different alleles, unless parental specimens are also tested.

Our ability to detect minor sequence variants due to somatic mosaicism is limited. Sequence variants that are present in less than 50% of the patient's nucleated cells may not be detected.

This test targets most, but not all, of the coding parts of genes within the panel (called exons).

This test will not provide detection of specific exons of genes due to complicated technicalities (such as sequence characteristics or interfering pseudogenes). Because of these technicalities, this test is not 100% sensitive and will not identify all disease-causing variants.

Runs of mononucleotide repeats (e.g., (A)n or (T)n) with n >8 in the reference sequence are generally not analyzed because of strand slippage during amplification.

Unless otherwise indicated, DNA sequence data is obtained from a specific cell-type (usually leukocytes if taken from whole blood). Test reports contain no information about the DNA sequence in other cell-types.

We cannot be certain that the reference sequences are correct.

We have confidence in our ability to track a specimen once it has been received by PreventionGenetics. However, we take no responsibility for any specimen labeling errors that occur before the sample arrives at PreventionGenetics.

A negative finding does not rule out a genetic diagnosis.

Genetic counseling to help to explain test results to the patients and to discuss reproductive options is recommended.

References: Please see the test description for this test, as well as the test description for PGxome, on our website (www.preventiongenetics.com) for a full list of complete citations.

FDA Notes
These results should be used in the context of available clinical findings, and should not be used as the sole basis for treatment. This test was developed and its performance characteristics determined by PreventionGenetics. US Food and Drug Administration (FDA) does not require this test to go through premarket FDA review. This test is used for clinical purposes. It should not be regarded as investigational or for research. This laboratory is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) as qualified to perform high complexity clinical laboratory testing.