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SAMPLE REPORT

FINAL REPORT - 08/25/2020

Ordered By
Medical Professional: Sample Physician, MD
Client: Sample Facility

Additional Authorized Recipient:
Sample GC, CGC

Patient Name: **Patient, Sample**
Accession #: **00-109625**
AP2 Order #: 835495

Birthdate: 01/01/1976
MRN #: N/A
Indication: Diagnostic

Specimen #: N/A
Specimen: Blood EDTA
Gender: M
Collected: 07/29/2020
Received: 07/30/2020

AutismNext®: Analyses of 72 Genes Associated with Autism Spectrum Disorders and/or Intellectual Disability

RESULTS

Gene	Inheritance	Alteration	Proband
***POGZ* (NM_015100)**	**Autosomal dominant**	**Pathogenic Mutation: c.3528C>A (p.Y1176*)**	**Heterozygous**
ANKRD11 (NM_013275)	Autosomal dominant	Variant of Uncertain Significance: c.5351C>T (p.S1784F)	Heterozygous

SUMMARY

POSITIVE: Pathogenic Mutation Detected

INTERPRETATION

- This individual is is heterozygous for the **c.3528C>A (p.Y1176*)** pathogenic mutation in the *POGZ* gene.
- This result is consistent with a diagnosis of *POGZ*-related neurodevelopmental disorder.
- The expression and severity of disease for this individual cannot be predicted.
- Genetic testing for pathogenic mutations in family members can be helpful in identifying at-risk individuals.
- Genetic counseling is a recommended option for all individuals undergoing genetic testing.

This individual is also heterozygous for the c.5351C>T (p.S1784F) variant of uncertain significance in the *ANKRD11* gene, which may or may not contribute to this individual's clinical history. Refer to the supplementary pages for additional information on these variants. No additional pathogenic mutations, variants of uncertain significance, or gross deletions or duplications were detected.

***POGZ* Additional Information**

Gene Symbol	RefSeq ID	Genomic Coordinates (GRCh37)	Genomic Size (bp)	Total Exons	Coding Exons	Number of Amino Acids
POGZ	NM_015100	chr1:151375200-151431941	56742	19	18	1410 aa

VARIANT DETAILS:

- The c.3528C>A (p.Y1176*) alteration, located in exon 19 (coding exon 18) of the *POGZ* gene, consists of a C to A substitution at nucleotide position 3528. This changes the amino acid from a tyrosine (Y) to a stop codon at amino acid position 1176.
- Based on data from the Genome Aggregation Database (gnomAD), the *POGZ* c.3528C>A alteration was not observed, with coverage at this position.
- Based on the available evidence, the *POGZ* c.3528C>A (p.Y1176*) alteration is classified as pathogenic.

GENE INFORMATION:

The *POGZ* gene is located on chromosome 1q21.3 and encodes the pogo transposable element with ZNF domain protein. Pathogenic alterations in this gene have been associated with *POGZ*-related intellectual disability, also known as White-Sutton syndrome, which is an autosomal dominant condition that generally occurs *de novo*. *POGZ*-related intellectual disability is characterized by intellectual disability, motor and speech delay, and learning difficulties. Additional features include feeding problems, vision problems, microcephaly, autism spectrum disorder/autistic features, abnormal brain imaging, and variable facial dysmorphisms including midface hypoplasia, low-set ears, and high, broad forehead or frontal bossing (Assia Batzir, 2019; Stessman, 2016). Loss of function alterations have been reported as the mechanism of disease for *POGZ*-related intellectual disability.

Order Summary: The following products were included in the test order for this individual. Please Note: tests on hold and those that have been cancelled (including reflex testing steps cancelled due to a positive result in a preceding test) are excluded. For additional information, please contact Ambry Genetics.

- AutismNext® (Product Code 6863)

Electronically Signed By Sample Director, on 8/25/2020 at 0:00:00 PM

All content hereafter is supplemental information to the preceding report.

ANKRD11 NM_013275 c.5351C>T p.S1784F

Gene Symbol	RefSeq ID	Genomic Coordinates (GRCh37)	Genomic Size (bp)	Total Exons	Coding Exons	Number of Amino Acids
ANKRD11	NM_013275	chr16:89334035-89556969	222935	13	11	2663 aa

VARIANT DETAILS:

- The c.5351C>T (p.S1784F) alteration is located in exon 9 (coding exon 7) of the *ANKRD11* gene. This alteration results from a C to T substitution at nucleotide position 5351, causing the serine (S) at amino acid position 1784 to be replaced by a phenylalanine (F).
- This amino acid position is not well conserved in available vertebrate species.
- The p.S1784F alteration is predicted to be tolerated by *in silico* analysis.
- Based on the available evidence, the clinical significance of the *ANKRD11* c.5351C>T (p.S1784F) alteration is uncertain.

FAMILY STUDIES PROGRAM:

Ambry Genetics offers complimentary genetic studies for variants of unknown significance (VUSs) meeting specific criteria in appropriate family members. Review of clinical information is required. Additional information, application instructions and required forms, and patient education materials are available at http://ambrygen.com/family-studies-program. For additional information, please email us at GeneticCounselor@ambrygen.com or call 949-900-5500 and ask to speak with a genetic counselor.

Please note that the classification of variants may change over time as additional information becomes available. Alerts are disseminated via fax and/or AmbryPort email to clinicians upon clinically relevant variant reclassifications. If no updates are received, clinicians are encouraged to contact the laboratory at 949-900-5500 once a year to review the status of previously reported variants.

GENE INFORMATION:

The *ANKRD11* gene is located on chromosome 16q24.3 encodes the ankyrin repeat domain-containing protein 11. Pathogenic alterations in this gene have been associated with KBG syndrome which is inherited in an autosomal dominant fashion and typically occurs *de novo*. KBG syndrome is characterized by intellectual disability, brachydactyly and/or clinodactyly, macrodontia, typical facial gestalt involving bracycephaly, triangular face, prominent cheekbones, synophrys, thin upper lip, and an abnormal nose that is usually prominent with a high nasal bridge, wide base, bulbous tip, thick alae nasi, and anteverted nares. Additional features seen in the majority of patients include developmental delays, short stature, behavior problems, abnormal electroencephalogram (EEG) recording, delayed bone age, and other facial features such as a long protuberant philtrum, large prominent ears, hypertelorism, and strabismus. Features seen in a substantial minority of patients include epilepsy, variable brain MRI abnormalities, mixed hearing loss, feeding difficulties, cryptorchidism, and skeletal problems such as joint stiffness as well as spinal and costovertebral abnormalities (Gnazo, 2020; Scarano, 2019; Morel Swols and Tekin, 2018; Goldenberg, 2016; Low, 2016). Loss of function has been reported as the mechanism of disease for KBG syndrome.

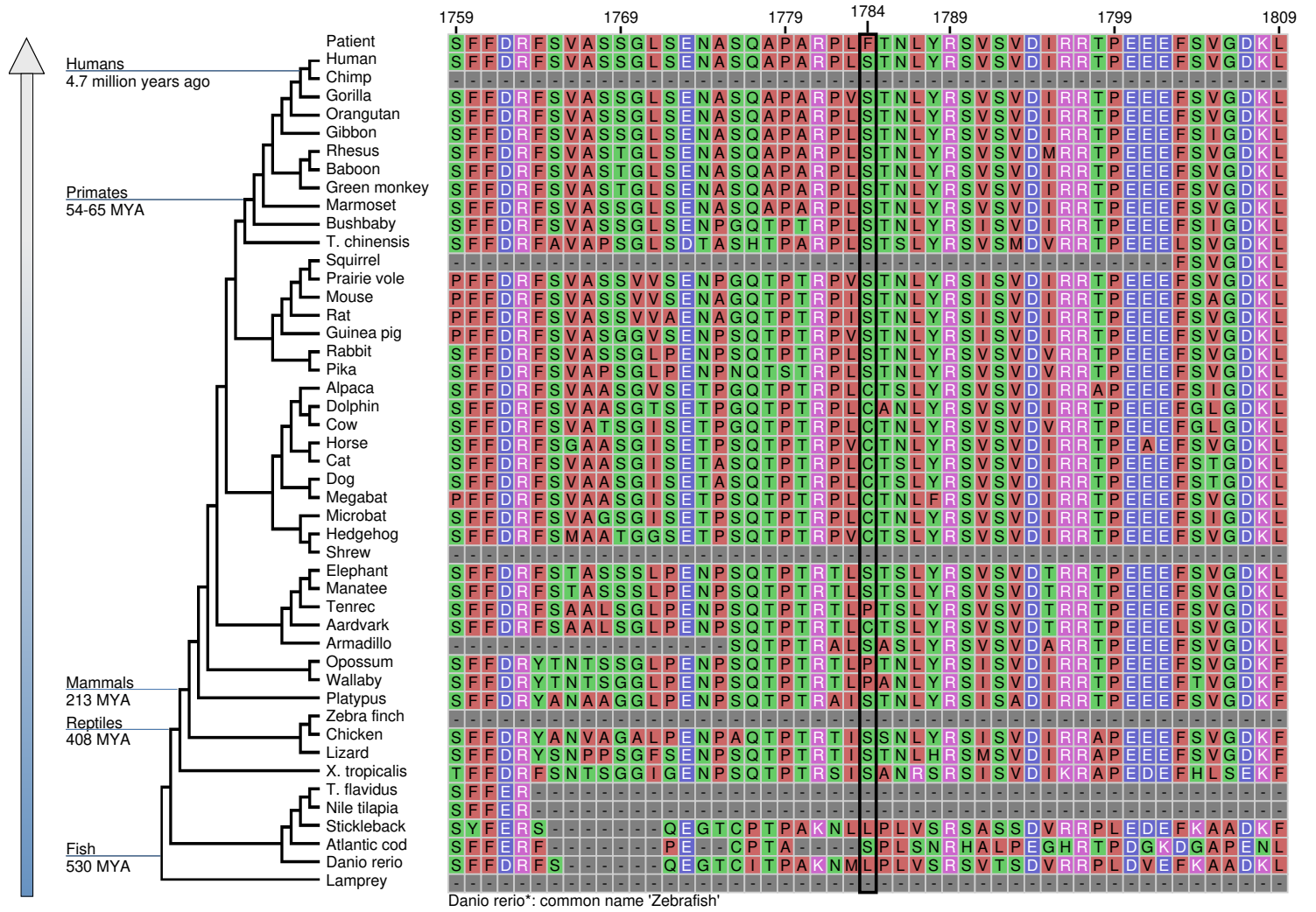
ADDITIONAL SUPPORTING INFORMATION:

Co-Segregation	Co-segregation data for this variant is currently unavailable.
Co-Occurrence	No significant co-occurrence data is currently available at our laboratory.
Frequency	Based on data from the Genome Aggregation Database (gnomAD), the *ANKRD11* c.5351C>T alteration was not observed, with coverage at this position.
in silico	The p.S1784F alteration is predicted to be tolerated by *in silico* analysis.

ANKRD11 NM_013275 c.5351C>T p.S1784F

Evolutionary conservation diagram: Amino Acid Alignment

This amino acid position is not well conserved in available vertebrate species.



Danio rerio*: common name 'Zebrafish'

Amino Acid Change:

Trait	Ser (S)	Phe (F)
Amino Acid Name	Serine	Phenylalanine
Polarity/Charge	polar	non-polar
pH	neutral	neutral
Residue Weight	87	147
Hydrophobicity Score	-0.8	2.8
Hydrophilicity Score	0.3	-2.5
Secondary Structure Propensity	α indifferent / β breaker	α former / β former

Report References

- Assia Batzir N, *et al.* (2020) *Am. J. Med. Genet. A* **182**(1):38-52. **PMID:**31782611
- Gnazzo M, *et al.* (2020) *Am. J. Med. Genet. A* **182**(5):1073-1083. **PMID:**32124548
- Goldenberg A, *et al.* (2016) *Am. J. Med. Genet. A* **170**(11):2847-2859. **PMID:**27605097
- Low K, *et al.* (2016) *Am. J. Med. Genet. A* **170**(11):2835-2846. **PMID:**27667800
- Morel Swols D and Tekin M. *GeneReviews*. 2018 Mar 22. **PMID:**29565525
- Scarano E, *et al.* (2019) *Mol Syndromol* **10**(3):130-138. **PMID:**31191201
- Stessman HA, *et al.* (2016) *Am. J. Hum. Genet.* **98**(3):541-52. **PMID:**26942287

Genes Analyzed

ACSL4, ADNP, AFF2, ANK2, ASH1L, BRWD3, CAMK2A, CAMK2B, CC2D1A, CELF4, CHAMP1, CHD2, CHD3, CHD8, CIC, CREBBP, CTNNB1, CTNND2, DLL1, DYRK1A, EHMT1, EIF3F, ELP2, FMR1, FOXP1, FOXP2, FRMPD4, GABRB3, GRIA2, GRIA3, GRIN2B, HECW2, KDM5C, KMT2C, KMT5B, MAGEL2, MAOA, MECP2, MED12, MED13, MEF2C, NLGN3, NLGN4X, OPHN1, PAK3, PHIP, POGZ, PTCHD1, PTEN, RAB39B, RAI1, RORB, SETBP1, SETD2, SETD5, SHANK2, SHANK3, SYN1, TANC2, TBR1, TCF20, TCF7L2, TRIP12, TSC1, TSC2, UBE2A, UPF3B, WDFY3, YY1, ZDHHC9, ZMIZ1 and ZNF292.

Metrics and Coverage

Complete coverage data for this proband is available for download through AmbryPort or can be e-mailed by request.

The following genes (coverage)* did not achieve 100% coverage at 10X for all nucleotides in the coding regions:

CHD3 (98.95%), ***HECW2*** (98.69%), ***PHIP*** (99.65%), ***SHANK2*** (85.29%), ***SHANK3*** (98.17%)

*percentage of the coding region covered at ≥10X

Assay Information

General Information: Autism spectrum disorder (ASD), which affects 1-2% of children in the United States, is a neurodevelopmental disability that can cause behavioral, social and communication difficulties that begin in early childhood. Genetic testing is recommended for all children with ASD and can be a critical step in providing accurate diagnosis, treatment, prognosis, and genetic counseling. **AutismNext®** is a focused panel analyzing genes primarily associated with non-syndromic presentations of ASD.

Methodology: Genomic deoxyribonucleic acid (gDNA) is isolated from the patient's whole blood. Samples are prepared using the IDT xGen Exome Research Panel v1.0 (Integrated DNA Technologies). Each DNA sample is sheared, adaptor ligated, PCR-amplified and incubated with the exome baits. Captured DNA is eluted, and PCR amplified. Final quantified libraries are seeded onto an Illumina flow cell and sequenced using paired-end, 150 cycle chemistry on the Illumina HiSeq or NextSeq. Initial data processing, base calling, alignments and variant calls are generated by various bioinformatics tools. Data is annotated with the Ambry Variant Analyzer tool (AVA), including, but not limited to, the following information: nucleotide and amino acid conservation, biochemical nature of amino acid substitutions, population frequency, and predicted functional impact. The following sites are used to search for previously described gene mutations and polymorphisms: The Human Gene Mutation Database (HGMD), the Online Mendelian Inheritance in Man (OMIM), the genome aggregation database (gnomAD), HapMap data, and online search engines (e.g., PubMed). Variants are filtered further based on likelihood of pathogenicity (Farwell K, et al., Genet Med., 2014), as well as by manual preliminary screening performed by licensed genetic counselors using criteria obtained from Ambry's General Variant Classification Scheme (https://www.ambrygen.com/science/variant-classification) to further filter alterations that are unlikely to be classified as disease-causing. The exome is targeted and sequenced, but analysis is limited to only the set of genes listed in this report. All reportable findings undergo manual review by molecular geneticists using integrated genomics software (IGV) and undergo confirmation either by automated fluorescence dideoxy (aka "Sanger") sequencing or via coverage and alternate read ratios above established confidence thresholds (heterozygous calls with 40-65% variant allele frequency and >40x coverage, hemizygous and homozygous calls with 100% variant allele frequency and >40x coverage). Co-segregation studies are performed if family members are available. Co-segregation results may be confounded by many factors which cannot be completely ruled out including reduced penetrance, age-of-onset, and/or variable expressivity. In most cases, phase cannot be determined.
New gene lists are regularly added due to proactive review of current literature using Ambry's peer-reviewed clinical validity scheme to ensure inclusion of the most up-to-date disease-associated genes (Smith ED, Radtke K, Rossi M, et al. 2017 Human mutation 38(5):600-608). As part of the Patient for Life program, Ambry will continually review past patient's data for potential mutations in newly added genes and proactively issue reclassification reports, as applicable.

Analytical range: Approximately 75% of the bases are expected to have quality scores of Q30 or higher, which translates to an expected base-calling error rate of 1:1000, or an expected base-calling accuracy of 99.9%. Additionally, 90% and 95% of the exome will be covered at ≥20x and ≥10x, respectively. Coverage is sufficient to detect >98% and up to 99.7% of disease-causing mutations (LaDuca H, Farwell KD, Vuong H, et al. 2017. PLoS ONE 12(2):e0170843). Coding exons plus at least 6 bases into the 5' and 3' ends of all the introns are analyzed and reported. Gross deletion/duplication analysis is assessed for all genes within the targeted exome using a custom pipeline based on coverage (>4 exons in size) and/or breakpoint analysis from NGS data and confirmed by targeted chromosomal microarray, SNP array or MLPA when applicable. CNVs detected by NGS pipeline for which no orthogonal method of confirmation is available will not be included.

Result Reports: In result reports, alterations in the following classifications are always reported, and are based on the following definitions and clinical recommendations:

- **Pathogenic Mutation:** Alterations with sufficient evidence to classify as pathogenic (capable of causing disease). Targeted testing for at-risk, affected, or possible carrier relatives may be warranted.
- **Variant, Likely Pathogenic (VLP):** alterations with strong evidence in favor of pathogenicity. Targeted testing for at-risk, affected, or possible carrier relatives may be warranted.
- **Variant, Unknown Significance (VUS): only if requested**, alterations with limited and/or conflicting evidence regarding pathogenicity will be reported.

Alterations of unlikely clinical significance (those with strong/very strong evidence to argue against pathogenicity) are not included on results reports. These include findings classified as "likely benign" and "benign" alterations.
A clinical report will only be generated for the proband, even when familial samples are received. Default reporting does not include variants of unknown significance (VUS), however clinicians may opt-in. If clinicians do not opt-in to receiving VUSs, family member samples will not be used for co-segregation analysis for alterations without the potential to be upgraded to VLP/Pathogenic.

Test Limitations and Disclaimer: This test was developed, and its performance characteristics were determined, by Ambry Genetics. It has not been cleared or approved by the US Food and Drug Administration. The FDA does not require this test to go through premarket FDA review. It should not be regarded as investigational or for research. This test should be interpreted in context with other clinical findings. This report does not represent medical advice. Any questions, suggestions, or concerns regarding interpretation of results should be referred to a genetic counselor, medical geneticist, or physician skilled in evaluating the relevant medical literature. This laboratory is certified under the Clinical Laboratory Improvement Amendments (CLIA) as qualified to perform high complexity clinical laboratory testing. The following types of mutations are detectable: nucleotide substitutions, small deletions, small insertions, small indels, and copy number variants (CNV). The overall coverage of each genes varies and each individual may have slightly different coverage yield. Accurate exon-level CNV detection by exome sequencing is dependent on several factors such as inherent sequence properties of the targeted regions, including shared homology and exon size, depth-of-coverage, efficiency of capture, and degree of read depth variation in the selected background samples. Therefore, the specificity and sensitivity of CNV detection by exome sequencing maybe reduced. This assay is not is not intended to systematically detect and analyze, gross rearrangements, deep intronic variations, long repeat sequences, portions of genes with highly homologous pseudogenes, repeat expansions, mutations involved in tri-allelic inheritance, mitochondrial genome mutations, epigenetic effects, oligogenic inheritance, or X-linked recessive mutations in females who manifest disease due to skewed X-inactivation and other unknown abnormalities. A negative result from the analysis cannot rule out the possibility that the tested individual carries a rare unexamined mutation or mutation in the undetectable region. Although molecular tests are highly accurate, rare diagnostic errors may occur. Possible diagnostic errors include sample mix-up, erroneous paternity identification, technical errors, clerical errors, and genotyping errors. Genotyping errors can result from trace contamination of PCR reactions, from rare genetic variants that may interfere with analysis, or from other sources. The clinical sensitivity of the test may vary widely according to the specific clinical and family history. Disorders of neurodevelopment are a complex spectrum of clinical disorders. Mutations in other genes or the regions not analyzed by this panel can also give rise to similar clinical conditions.

Resources: The following references are used in variant analysis and classification when applicable for observed genetic alterations.

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20. Grantham prediction: Grantham R. (1974) *Science* **185**(4151):862-864.
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23. HGMD® [Internet]: Stenson PD, *et al*. (2014) *Hum Genet*. **133**(1):1-9. Available from: http://www.hgmd.cf.ac.uk.
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Dear Client,

Variant(s) of unknown significance were detected in your patient. Family study of the patient's parents may be helpful for variant interpretation.

- If you wish to move forward with parental testing, please follow the instructions below.
- If you wish to discuss your case before proceeding with parental testing please call 949-900-5500 and ask to speak with a genetic counselor in the Neurology department.
- Clinically actionable alterations, such as mutations and VLPs, are not eligible for family study and may be tested through clinical diagnostic/carrier testing.
- Gross deletions and duplications are not eligible for family study.
- Please note that single VUS detected in autosomal recessive genes are not applicable for family study (unless co-occurring with a mutation or VLP in the same gene)

To move forward with Family Studies, the following items (located at https://www.ambrygen.com/science/family-studies) are needed:

1. Family Studies Requisition Form – completed and signed by the ordering provider
2. Family Studies Consent Form – signed by the parent(s)
3. Parental specimens – this can be either blood or saliva. If needed, saliva kits can be ordered through our website (instructions attached).
4. Clinical records on the patient, if not previously submitted, (and parents, if applicable) to ensure optimal variant interpretation

It is extremely important that both completed forms and the specimens are sent in together. If not, this can delay the testing and reporting process.

Once the specimen arrives you will be made aware if any missing or additional documentation is needed before Family Study testing is initiated. Please be aware that our Family Studies Program is a research based program and can take 2-3 months to report out the results, which will be issued under the patient's record.

All follow up correspondence containing patient identifiers and/or sensitive information should be sent via a secure method (fax, secure email or AP2).

If you have any questions please contact Ambry Genetics and ask to speak with one of our Family Studies team members at 949-900-5500 or email familystudies@ambrygen.com. Thank you!

Sincerely,
Ambry Family Studies Department