GoodStart Genetics

Patient Name: Donor 5318
D.O.B.: 02/[redacted]/1995
GSG Specimen #: PAT1043906
Final Report: 11/21/2016

Results Report

Patient
MRN:
Gender: Male
Ethnicity: Hawaiian, Filipino

Specimen
Specimen Type: Blood
Date Collected: 11/10/2016
Date Received: 11/11/2016

Physician
Name: Lorraine Bonner, MD
GSG Account: SBCX01CA
Clinic Name: The Sperm Bank of California

Clinical Indication for Testing: Not Provided

RESULTS SUMMARY

OK 11/22/16

No mutations were detected and/or all values are within normal range(s) for the disorders tested and listed in the Reduced Risk Table.

TESTS ORDERED

	Carrier	Reduced Risk		Carrier	Reduced Risk
Bloom's Syndrome	☐	☑	Gaucher Disease	☐	☑
Canavan Disease	☐	☑	Mucolipidosis Type IV	☐	☑
Cystic Fibrosis (CF)	☐	☑	Niemann-Pick Disease Type A/B	☐	☑
Familial Dysautonomia	☐	☑	Spinal Muscular Atrophy (SMA)	☐	☑
Fanconi Anemia Group C	☐	☑	Tay-Sachs Disease (DNA)	☐	☑

REDUCED RISK TABLE

No mutations were detected and/or all values are within normal range(s) for the disorders tested and listed in the table. These results reduce but do not eliminate the chance that this individual is a carrier of one of the following disorders. To see specific reference ranges and testing sites, please see methods section below.

Disorder	Gene	Ethnicity	Residual Risk
Bloom's Syndrome	*BLM*	Ashkenazi Jewish	1/10457
Canavan Disease	*ASPA*	Ashkenazi Jewish	1/5489
Cystic Fibrosis	*CFTR*	African American	1/462
		Ashkenazi Jewish	1/699
		Asian	1/267
		Caucasian	1/420
		Hispanic	1/427
Familial Dysautonomia	*IKBKAP*	Ashkenazi Jewish	1/6001
Fanconi Anemia Group C	*FANCC*	Ashkenazi Jewish	1/9901
Gaucher Disease	*GBA*	Ashkenazi Jewish	1/281
		Pan-ethnic	1/735
Mucolipidosis Type IV	*MCOLN1*	Ashkenazi Jewish	1/3488
Niemann-Pick Disease Type A/B	*SMPD1*	Ashkenazi Jewish	1/4962
Spinal Muscular Atrophy Two copies of SMN1 detected. Residual risk by ethnicity: African American 1/130, Ashkenazi Jewish 1/611, Asian 1/806, Caucasian 1/834, Hispanic 1/579.	*SMN1*	Pan-ethnic	Reduced

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Disorder	Gene	Ethnicity	Residual Risk
Tay-Sachs Disease DNA analysis: No mutation detected. Residual risk by ethnicity: Ashkenazi Jewish 1/647, French Canadian 1/866, other ethnicities 1/899.	*HEXA*	Pan-ethnic	Reduced

Comments

The results and interpretation in this report are based on the information provided about the tested individual, and the currently available information regarding the disorder and, when applicable, the sequence variations of the genes tested. Individuals are presumed to be asymptomatic with a negative family history unless otherwise indicated on the test requisition form. Genetic counseling is a recommended option for all patients undergoing testing. Testing of the reproductive partner increases the accuracy of the risk assessment. For additional information about the disorders tested, please contact Good Start Genetics™, Inc. (Good Start Genetics).

Next-generation sequencing (or NGS) technology is used to evaluate genetic variants. The variants reported include all mutations (pathogenic variants) recommended for testing by ACOG/ACMG, those that have been previously determined to be pathogenic by Good Start Genetics (the GSG pathogenic mutation set), and those that are commonly assessed and meet our pathogenicity criteria (GSG class 1 variants). Novel variants, i.e., those not in the GSG pathogenic mutation set, but expected to be pathogenic based on the nature of the sequence change, are also reported. Benign variants, variants of unknown clinical significance, variants with insufficient published information to adequately assess the pathogenic nature or determine the exact genomic location, and those not reported to be associated with the appropriate phenotype are not included. For the purposes of this report, the term mutation is used interchangeably with pathogenic variant. Analytic sensitivity and specificity for the GSG pathogenic mutation set are >99%. For novel variants detected by NGS, the analytic sensitivity and specificity are >98% and >99%, respectively.

NGS is also used for copy number determination for spinal muscular atrophy (SMA); analytic sensitivity and specificity for detection of the SMN1 exon 7 deletion are >99%. SMA analysis does not differentiate carriers with two or more copies of the SMN1 gene on one chromosome and no copies on the other chromosome, from non-carriers with one copy on each chromosome. Additionally, carriers of small intragenic changes are not detected. Approximately 2% of patients with SMA have de novo mutations (new mutations in the germline that are not detected on blood analysis); this assay does not detect germline mosaicism for SMN1 mutations.

Targeted NGS or non-NGS methodology is utilized for a subset of disorders. In these cases only a targeted mutation or set of mutations is assessed with a focus on those variants generally accepted to be important and prevalent, such as founder mutations in specific ethnic groups. Fragile X (FMR1 gene) analysis consists of assessment for the number of CGG repeats present; methylation analysis may also be completed when indicated. Alpha-thalassemia (HBA1/2 gene) analysis evaluates the common deletions and the Constant Spring mutation. For HBA1/2 deletion assessment, carriers of alpha-thalassemia with three or more copies of the alpha-globin gene on one chromosome, and one or no copies on the other chromosome may not be detected. Analytic sensitivity and specificity for these assays are >99%. All DNA results for alpha-thalassemia should be interpreted in conjunction with a Complete Blood Count (CBC). Hemoglobin electrophoresis and evaluation for anemia may also be warranted.

Identified mutations are reported using HGVS approved cDNA nomenclature, whenever possible. Widely used common names are put in parentheses, if available. If subsequent testing will be undertaken at another laboratory, for the reported mutation(s), please contact Good Start Genetics to obtain the exact genomic position.

Hemoglobin electrophoresis for assessment of beta-thalassemia or sickle cell carrier status detects abnormally migrating hemoglobins and the relative amount of A2 hemoglobin (%A2). The coefficient of variation (CV) for the precision of %A2 determination is ≤2.8%. Variant hemoglobins are detected when their migration pattern is distinct from that of normal hemoglobin (Hb A; some variants overlap and cannot be readily differentiated). All hemoglobin results should be interpreted in conjunction with hematological information [in particular, patients with low mean corpuscular volume (MCV), low mean corpuscular hemoglobin (MCH) and low hemoglobin levels may warrant additional evaluation even when hemoglobin electrophoresis results are normal]. Samples suspected to be positive for Hb S will be assessed by a chemical precipitation assay or by Sanger sequencing for confirmation. Other suspected variants may be assessed using Sanger sequencing, or a second sample separation methodology. Hemoglobin variants not already characterized by Sanger sequencing should be confirmed by molecular genetic analysis of the appropriate globin genes for reproductive testing purposes (e.g. prenatal diagnosis or preimplantation genetic diagnosis).

Tay-Sachs enzyme analysis determines the level of total hexosaminidase and the percentage of hexosaminidase A (%HexA). A small percentage of carriers (<0.5%) may exhibit normal hexosaminidase A activity and will not be detected by enzyme analysis alone. In addition, patients with the AB variant will not be detected by this assay.

Tay-Sachs analysis may detect the presence of pseudodeficiency variants and B1 mutations. Pseudodeficiency variants cause a false positive result on HexA enzyme analysis and do not confer a risk for Tay-Sachs disease. B1 mutations cause a false negative result on HexA enzyme analysis and do confer a risk for Tay-Sachs disease. DNA analysis detects the two frequent pseudodeficiency

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variants (R247W and R249W) and the known B1 mutations (R178H, R178C, R178L, D258H).

Methods

Genomic DNA is isolated and quantified using standard, high purity methods and subsequently analyzed by one or more of the following processes, depending on the tests ordered.

- Next-generation sequencing (NGS): Exons, selected intronic regions, and the 2 bp conserved acceptor / donor splice sites are selectively amplified and subsequently sequenced on a next-generation DNA sequencing platform. The resulting reads are integrated to define genotypes within the amplified regions and compared to human reference genome hg18 to identify variants. Mutations that fall in low coverage or challenging regions (such as SMPD1 exon 1) and certain deletions, insertions or indels may not be assessed by NGS. In addition, certain novel mutations in CFTR exon 10 (legacy name, exon 9) may not be detected.
- Targeted NGS Analysis: A targeted NGS approach is used for detection of the TMEM216 c.218G>T mutation causing Joubert syndrome 2, the FKTN c.1167dupA mutation causing Walker-Warburg syndrome, and the NEB delExon55 mutation causing Nemaline Myopathy. Analytic sensitivity and specificity for these tests are both ≥99%.
- NGS-based copy number determination: The loci of interest as well as a set of control loci (used for normalization) are selectively amplified and sequenced on a next-generation DNA sequencing platform. The resulting normalized read-count frequencies for each of the loci of interest are subsequently utilized to infer copy number.
- Allele-specific primer extension (ASPE): GSG class 1 variants not amenable to NGS analysis are amplified by PCR using gene-specific primers. The resulting PCR fragments are either fluorescently labeled or subsequently used in one or more reactions with fluorescently labeled nucleotides. The products of the PCR, OLA or ASPE reactions are then measured by capillary electrophoresis.
- Sanger sequencing is used to assess some repetitive genomic regions (e.g., CFTR polyT) and for confirmation of some mutations found by NGS or capillary electrophoresis.
- Multiplex ligation-dependent probe amplification (MLPA): Paired oligonucleotides are hybridized to the target of interest, as well as other genomic regions for normalization of the data. After hybridization, adjacent oligonucleotides are ligated together. PCR amplification of the ligated oligonucleotides is followed by capillary electrophoresis to determine the presence or absence of each target sequence. MLPA is also used for confirmation of the spinal muscular atrophy SMN1 exon 7 deletion when detected by NGS.
- Triplet repeat detection: This technique involves PCR with fluorescently labeled primers, followed by capillary electrophoresis. Based on internal validation data, sizing accuracy is expected to be ±1 for CGG repeat alleles ≤90 and ±3 for CGG repeat alleles >90. If the two CGG repeats listed are the same, this may indicate that both alleles are the same size, or that one allele is the reported size and the other allele is too small to be detected by this analysis. Reference ranges: normal: <45 CGG repeats, intermediate: 45-54 CGG repeats, premutation: 55-200 CGG repeats, full mutation: >200 CGG repeats. When indicated, certain positive and negative results may be confirmed by methylation analysis at ARUP Laboratories, 500 Chipeta Way, Salt Lake City, UT 84108-1221; phone number (800) 242-2787.

Non-DNA based methods are performed as described below.

- Capillary electrophoresis is used to separate hemoglobin fractions directly from whole blood based on charge and mass. Potential beta-thalassemia carriers are identified based on elevation of the A2 hemoglobin fraction. The Hb S allele and certain other variant hemoglobins are detected by abnormal migration patterns. Suspected hemoglobin variant carriers identified by the capillary electrophoresis method are confirmed using either Sanger sequencing or a standard qualitative solubility assay. If the sample originated in New York state, beta-thalassemia / sickle cell disease evaluation is performed at ARUP Laboratories, 500 Chipeta Way, Salt Lake City, UT 84108-1221; phone number (800) 242-2787 (reference ranges: Hb A: 95.0-97.9%, Hb A2: 2.0-3.5%, Hb F: 0.0-2.1%). For all other samples, testing is performed at Good Start Genetics (reference ranges: Hb A: 96.5-98.4%, Hb A2: 2.0-3.2%, Hb F: 0.0-1.8%).
- Enzyme analysis is used to determine the percent hexosaminidase A activity in white blood cells. Unless otherwise noted, testing is performed at Mount Sinai Genetic Testing Laboratory, 1428 Madison Avenue, Atran Laboratory Building AB 225, New York, NY 10029; phone number (212) 241-7518 (reference ranges: normal: 55.0-72.0%, indeterminate: 50.0-54.9%, carrier: <50.0%).

References

Professional Society Guidelines

- ACOG guidelines: Update on carrier screening for cystic fibrosis. Obstetrics & Gynecology, Vol. 117, No. 4, April 2011.
- ACMG guidelines: Technical standards and guidelines for spinal muscular atrophy testing. Prior et al., Genetics in Medicine, Vol. 13, No. 7, July 2011.
- ACMG guidelines: Fragile X syndrome: diagnostic and carrier testing. Sherman et al., Genetics in Medicine, Vol. 7, No. 8, October 2005.
- ACMG practice guidelines: Carrier screening in individuals of Ashkenazi Jewish descent. Gross et al., Genetics in Medicine, Vol. 10, No. 1, January 2008.

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- ACOG committee opinion: Preconception and prenatal carrier screening for genetic diseases in individuals of Eastern European Jewish descent. Obstetrics & Gynecology, Vol. 114, No. 4, October 2009.
- ACOG committee opinion: Genetic screening for hemoglobinopathies. International Journal of Gynecology & Obstetrics, Vol. 74, No. 3, September 2001.
- Joint SOGC-CCMG clinical practice guideline: Carrier screening for thalassemia and hemoglobinopathies in Canada. Langlois et al., Journal of Obstetrics and Gynaecology Canada, Vol. 30, No. 10, Pages 950-959, October 2008.

Hemoglobinopathies

- Significant haemoglobinopathies: guidelines for screening and diagnosis. Ryan et al., British Journal of Haematology, Vol. 149, No. 1, April 2010.

Selected Ashkenazi Jewish disorders

- Experience with carrier screening and prenatal diagnosis for 16 Ashkenazi Jewish genetic diseases. Scott et al., Human Mutation, Vol. 31, No. 11, Pages 1240-1250, November 2010.
- Mutations in TMEM216 perturb ciliogenesis and cause Joubert, Meckel and related syndromes. Valente et al., Nature Genetics, Vol. 42, No. 7, Pages 619-625, July 2010.
- ABCC8 mutation allele frequency in the Ashkenazi Jewish population and risk of focal hyperinsulinemic hypoglycemia. Glaser et al., Genetics in Medicine, Vol. 13, No. 10, Pages 891-894, October 2011.
- Founder Fukutin mutation causes Walker-Warburg syndrome in four Ashkenazi Jewish families. Chang et al., Prenatal Diagnosis, Vol. 29, No. 6, Pages 560-569, June 2009.

Technical & General

- Additional references and disorder-specific information can be found on the Good Start Genetics website (www.goodstartgenetics.com).
- HGVS nomenclature reference: http://www.hgvs.org/mutnomen/ (version 1).
- A novel next-generation DNA sequencing test for detection of disease mutations in carrier and affected individuals. Porreca et al., Fertility and Sterility, Vol. 96, No. 3, Page S60, September 2011.

Disclaimer

These tests were developed and their performance characteristics determined by Good Start Genetics™, Inc. They have not been cleared or approved by the U.S. Food and Drug Administration. However, the laboratory is regulated under the Clinical Laboratory Improvement Amendments (CLIA) as qualified to perform high complexity clinical testing and the tests have been analytically validated in accordance with CLIA standards.

These tests analyze only mutations determined to be pathogenic by Good Start Genetics™, Inc., hence a negative result does not rule out the possibility that an individual carries a pathogenic mutation.

Although this testing is highly accurate, false positive or negative diagnostic errors may occur. Possible causes include but are not limited to: sample mix-up or misidentification, blood transfusion, bone marrow transplantation, technical errors, sample aging/degradation, interfering substances, conditions or genetic variants that interfere with one or more of the analyses. False negative results for beta-thalassemia may occur if Hb A2 is decreased due to iron deficiency anemia, reduced production or availability of alpha-globin, and delta-globin mutations. The chemical assay for Hb S confirmation may give false positive results in patients with erythrocytosis (elevated hematocrit), hyperglobinemia (elevated IgG or protein), extreme leukocytosis (highly elevated Leukocyte numbers), or hyperlipidemia. False positive results are also possible when extreme anemia is present or in patients with certain hemoglobin variants (Hb C-Harlem).

Residual risk values are inferred from published carrier frequencies, mutation detection rates, and mutation types per gene, in individuals of self-declared ethnicity. They are provided only as a guide for assessing approximate risk given a negative result, and values will vary based on the exact ethnic background of an individual. This report does not represent medical advice but should be interpreted by a genetic counselor, medical geneticist or physician skilled in genetic result interpretation and the relevant medical literature.

Electronically signed by:

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