GoodStart® Genetics

Patient Results Report

(855) 765-0845
www.goodstartgenetics.com

Patient Name: Donor 5118
D.O.B: 09/[redacted]1992
GSG Specimen #: PAT133681
Ordering Physician: Lorraine Bonner, MD

Clinical Indication for Testing: Gamete donor

Final Report 04/21/2015

Results Summary

No mutations detected; test values in normal range. See Results Interpretation on the following page(s) for test values and residual risk information.

Tests Ordered

	Carrier	Reduced Risk
Cystic Fibrosis (CF)	☐	☒
Spinal Muscular Atrophy (SMA)	☐	☒

APR 27 2015
29 LB 4/29/15

Patient

Name:	Donor 5118
Date of Birth:	09/[redacted]1992
Gender:	Male
Race / Ethnicity:	African American
MRN:	

Specimen

GSG Specimen #:	PAT133681
Date Collected:	04/13/2015
Date Received:	04/14/2015
Specimen Type:	Blood
Ordering Site:	
Performing Site:	GSG-01

Physician

Name:	Lorraine Bonner, MD
GSG Account #:	SBCX01CA
Address:	The Sperm Bank of California 2115 Milvia Street Berkeley CA 94704

verview AR 4/22/15

Electronically signed by:

Stephanie Hallam, Ph.D., FACMG
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Testing Performed at Good Start Genetics, Inc., 237 Putnam Avenue, Cambridge, MA 02139

CLIA #: 22D2025627

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Results Interpretation

Cystic Fibrosis (CF): No mutation detected. A negative result reduces, but does not eliminate, the chance that this individual is a carrier of cystic fibrosis. Presuming a negative family history, residual risks are as follows by ethnicity: Ashkenazi Jewish 1/699, Caucasian 1/420, Hispanic 1/427, African American 1/462, Asian 1/267.

Spinal Muscular Atrophy (SMA): Two copies of SMN1 detected.

This result reduces, but does not eliminate, the chance that this individual is a carrier of SMA. Presuming a negative family history, residual risks are as follows by ethnicity: Ashkenazi Jewish 1/611, Caucasian 1/834, Hispanic 1/579, African American 1/130, Asian 1/806.

This analysis does not differentiate carriers of SMA 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.

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; analytic sensitivity and specificity for detection of the SMN1 exon 7 deletion are >99%.

Non-NGS methodology is utilized for a subset of disorders. In some of these cases only a targeted set of mutations is genotyped. Targeted analysis focuses on those variants generally accepted to be important and prevalent, such as Ashkenazi Jewish founder mutations. Analytic sensitivity and specificity for fragile X (FMR1 gene) CGG repeat and alpha-thalassemia (HBA1/2; the common deletions and the Constant Spring mutation) analyses are >99%. 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.

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. 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 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, and the FKTN c.1167dupA mutation causing Walker-Warburg syndrome. 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. Positive results and certain negative results may be confirmed by methylation analysis at Good Start Genetics or in some instances 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.

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• 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 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. Some samples referred for beta-thalassemia / sickle cell disease evaluation may be tested at either Mayo Clinic, Department of Laboratory Medicine and Pathology, 200 First St. SW, Rochester, MN 55905; phone number (800) 533-1710 or ARUP Laboratories, 500 Chipeta Way, Salt Lake City, UT 84108-1221; phone number (800) 242-2787.

• Enzyme analysis is used to determine the percent hexosaminidase A activity in white blood cells. Testing is performed at the Mayo Clinic, Department of Laboratory Medicine and Pathology, 200 First St. SW, Rochester, MN 55905; phone number (800) 533-1710 or at Mount Sinai Genetic Testing Laboratory, 1428 Madison Avenue, Atran Laboratory Building AB 225, New York, NY 10029; phone number (212) 241-7518.

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.

• 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.

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Although this testing is highly accurate, false positive or negative diagnostic errors may occur due to one or more of the following: sample mix-up or misidentification, blood transfusion, bone marrow transplantation, technical errors, sample aging/degradation, interfering substances or 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 erthyrocytosis (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.

Number of Variants Tested, by Disease

The following table lists the number of variants being analyzed on samples received as of August 18, 2014, and is subject to change. Please contact Good Start Genetics if additional information is needed.

Disease Name	Gene Name	Number of Variants Tested
Alpha-Thalassemia	*HBA1, HBA2*	10
Beta-Thalassemia / Sickle Cell Disease	*HBB*	%A2 and Hb variants
Bloom's Syndrome	*BLM*	51*
Canavan Disease	*ASPA*	44*
Cystic Fibrosis	*CFTR*	569*
Dihydrolipoamide Dehydrogenase Deficiency	*DLD*	3*
Familial Dysautonomia	*IKBKAP*	2*
Familial Hyperinsulinism	*ABCC8*	65*
Fanconi Anemia Group C	*FANCC*	27*
Fragile X Syndrome	*FMR1*	CGG repeat size
Gaucher Disease	*GBA*	19
Glycogen Storage Disease Type 1a	*G6PC*	69*

Disease Name	Gene Name	Number of Variants Tested
Joubert Syndrome 2	*TMEM216*	1
Maple Syrup Urine Disease Type 1A	*BCKDHA*	19*
Maple Syrup Urine Disease Type 1B	*BCKDHB*	21*
Mucolipidosis Type IV	*MCOLN1*	9*
Nemaline Myopathy	*NEB*	1
Niemann-Pick Disease Type A/B	*SMPD1*	45*
Spinal Muscular Atrophy	*SMN1*	copy number
Tay Sachs Disease	*HEXA*	73*
Usher Syndrome Type 1F	*PCDH15*	16*
Usher Syndrome Type III	*CLRN1*	5*
Walker Warburg Syndrome	*FKTN*	1

* novel truncating mutations may also be detected