Fairfax Cryobank
The Trusted Choice for Donor Sperm

Donor 4369

Genetic Testing Summary

Fairfax Cryobank recommends reviewing this genetic testing summary with your healthcare provider to determine suitability.

Last Updated: 02/15/19

Donor Reported Ancestry: Russian, German Jewish Ancestry: Yes

Genetic Test*	Result	Comments/Donor's Residual Risk**
Chromosome analysis (karyotype)	Normal male karyotype	No evidence of clinically significant chromosome abnormalities
Hemoglobin evaluation	Normal hemoglobin fractionation and MCV/MCH results	Reduced risk to be a carrier for sickle cell anemia, beta thalassemia, alpha thalassemia trait (aa/-- and a-/a-) and other hemoglobinopathies
Cystic Fibrosis (CF) carrier screening	Negative for 99 mutations in the CFTR gene	1/300
Spinal Muscular Atrophy (SMA) carrier screening	Negative for deletions of exon 7 in the SMN1 gene	1/4,700
Hb Beta Chain-Related Hemoglobinopathy (including Beta Thalassemia and Sickle Cell Disease)	Negative for 28 mutations tested by genotyping in the HBB gene	1/930
Tay Sachs Disease enzyme analysis	Non-carrier by Hexosaminidase A activity	
ABCC8-Related Hyperinsulinism	Negative for 3 mutations in the ABCC8 gene	1/670
Bloom Syndrome	Negative for 1 mutations in the BLM gene	1/11,000
Canavan Disease	Negative for 4 mutations in the ASPA gene	1/2,700
Familial Dysautonomia	Negative for 2 mutations in the IKBKAP gene	1/6,100

Fanconi Anemia Type C	Negative for 3 mutations in the FANCC gene	1/8,900
Gaucher Disease	Negative for 10 mutations in the GBA gene	1/310
Glycogen Storage Disease Type 1 A	Negative for 7 mutations in the G6PC gene	1/7,000
Hexosaminidase A Deficiency (including Tay Sachs Disease)	Negative for 9 mutations in the HEXA gene	1/370
Lipoamide Dehydrogenase Deficiency	Negative for 2 mutations in the DLD gene	1/93,000
Maple Syrup Urine Disease Type 1B	Negative for 3 mutations in the BCKDHB gene	1/9,600
Mucolipidosis IV	Negative for 2 mutations in the MCOLN1 gene	1/2,700
Niemann-Pick Disease, SMPD1-Associated	Negative for 4 mutations in the SMPD1 gene	1/3,300
Usher Syndrome Type 1F	Negative for 1 mutations in the PCDH15 gene	1/400
Usher Syndrome Type 3	Negative for 1 mutations in the CLRN1 gene	1/6,000
Special Testing		
GJB2 Nonsyndromic Hearing Loss	Negative for 29 mutation in the GJB2 gene	1/320
Retinitis Pigmentosa FAM161A Related (Type 28)	Negative for 5 mutation in the FAM161A gene	Unknown residual risk
Alpha Thalassemia	Negative by copy number and gene sequencing aa/aa	1/10,000

*No single test can screen for all genetic disorders. A negative screening result significantly reduces, but cannot eliminate, the risk for these conditions in a pregnancy.

**Donor residual risk is the chance the donor is still a carrier after testing negative.

Patient	Sample	Referring Doctor
Patient Name: Donor 4369 **Date of Birth:** [redacted] **Reference #:** FFAXCB-S44369 **Indication:** Carrier Testing **Test Type:** Alpha Thalassemia Carrier Screen	**Specimen Type:** Purified DNA **Lab #:** [redacted] **Date Collected:** 12/28/2018 **Date Received:** 12/29/2018 **Final Report:** 1/12/2019	[redacted] **Fairfax Cryobank, Inc.** [redacted] [redacted] [redacted] Fax: [redacted]

RESULTS

NEGATIVE for alpha-thalassemia
HBA1 copy number: 2
HBA2 copy number: 2
No pathogenic copy number variants detected
HBA1 and *HBA2* sequence analysis: No pathogenic or likely pathogenic variants identified
Reduced risk of being an alpha-thalassemia carrier (aa/aa)

Genes analyzed: *HBA1* (NM_000558.4) and *HBA2* (NM_000517.4)
Inheritance: Autosomal Recessive

Recommendations
Individuals of Asian, African, Hispanic and Mediterranean ancestry should also be screened for hemoglobinopathies by CBC and hemoglobin electrophoresis.

Interpretation
No pathogenic or likely pathogenic copy number variants or sequence variants were detected in this patient, suggesting that four copies of the alpha-globin gene are present (aa/aa). Typically, individuals have four functional alpha-globin genes: 2 copies of *HBA1* and 2 copies of *HBA2*, whose expression is regulated by a cis-acting regulatory element HS-40. Alpha-thalassemia carriers have three (silent carrier) or two (carrier of the alpha-thalassemia trait) functional alpha-globin genes with or without a mild phenotype. Individuals with only one functional alpha-globin gene have HbH disease with microcytic, hypochromic hemolytic anemia and hepatosplenomegaly. Loss of all four alpha-globin genes results in Hb Barts syndrome with the accumulation of Hb Barts in red blood cells and hydrops fetalis, which is fatal in utero or shortly after birth.

This individual was negative for all *HBA* deletions, duplications and variants that were tested. These negative results reduce but do not eliminate the possibility that this individual is a carrier. See *Table of Residual Risks Based on Ethnicity*. With individuals of mixed ethnicity, it is recommended to use the highest residual risk estimate.

Patient:	Donor 4369	DOB:	[redacted]	Lab #:	[redacted]

Table of Residual Risks Based on Ethnicity

Ethnicity	Carrier Frequency	Detection Rate	Residual Risk
Caucasian	1 in 500	95%	1 in 10,000
African American	1 in 30	95%	1 in 580
Asian	1 in 20	95%	1 in 380
Worldwide	1 in 25	95%	1 in 480

Test Methods and Comments

Genomic DNA isolated from this patient was analyzed by one or more of the following methodologies, as applicable:

Multiplex Ligation-Dependent Probe Amplification (MLPA) (Analytical Detection Rate >99%)
MLPA® probe sets and reagents from MRC-Holland were used for copy number analysis of specific targets versus known control samples. False positive or negative results may occur due to rare sequence variants in target regions detected by MLPA probes. Analytical sensitivity and specificity of the MLPA method are both 99%.

The copy numbers of the *HBA1* and *HBA2* genes were analyzed. Alpha-globin gene deletions, triplications, and the Constant Spring (CS) mutation are assessed. This test is expected to detect approximately 90% of all alpha-thalassemia mutations, varying by ethnicity. Carriers of alpha-thalassemia with three or more *HBA* copies on one chromosome, and one or no copies on the other chromosome, may not be detected. With the exception of triplications, other benign alpha-globin gene polymorphisms will not be reported. Analyses of *HBA1* and *HBA2* are performed in association with long-range PCR of the coding regions followed by short-read sequencing.

Long-Range PCR (Analytical Detection Rate >99%)
A long-range PCR was performed to generate a locus-specific amplicon for *HBA1* and *HBA2*. The PCR product was then prepared for short-read NGS sequencing as described below and sequenced. Sequenced reads were mapped back to the original genomic loci and converted to VCF files as described below.

Next Generation Sequencing (NGS) (Analytical Detection Rate >95%)
Samples sequenced on the Illumina HiSeq 2500 platform in the Rapid Run mode or the Illumina NovaSeq platform in the Xp workflow, using 100 bp paired-end reads. The sequencing data was analyzed using a custom bioinformatics algorithm designed and validated in house.

The coding exons and splice junctions of the tested genes were assessed for the average depth of coverage (minimum of 20X) and data quality threshold values. Exons not meeting a minimum of >20X read depth across the exon are further analyzed by Sanger sequencing.

This test will detect variants within the exons and the intron-exon boundaries of the target regions. Variants outside these regions may not be detected, including, but not limited to, UTRs, promoters, and deep intronic areas. This technology may not detect all small insertion/deletions and is not diagnostic for repeat expansions and structural genomic variation. In addition, a mutation(s) in a gene not included on the panel could be present in this patient.

Variant interpretation and classification was performed based on the American College of Medical Genetics Standards and Guidelines for the Interpretation of Sequence Variants (Richards et al, 2015). All potentially pathogenic variants may be confirmed by either a specific genotyping assay or Sanger sequencing, if indicated. Any benign variants, likely benign variants or variants of uncertain significance identified during this analysis will not be reported.

Sanger Sequencing (Confirmation method) (Accuracy >99%)
Sanger sequencing, as indicated, was performed using BigDye Terminator chemistry with the ABI 3730 DNA analyzer with target specific amplicons. It also may be used to supplement specific guaranteed target regions that fail NGS sequencing due to poor quality or low depth of coverage (<20 reads) or as a confirmatory method for NGS positive results. False negative results may occur if rare variants interfere with

amplification or annealing.

Please note these tests were developed and their performance characteristics were determined by Mount Sinai Genomics, Inc. They have not been cleared or approved by the FDA. These analyses generally provide highly accurate information regarding the patient's carrier or affected status. Despite this high level of accuracy, it should be kept in mind that there are many potential sources of diagnostic error, including misidentification of samples, polymorphisms, or other rare genetic variants that interfere with analysis. Families should understand that rare diagnostic errors may occur for these reasons.

SELECTED REFERENCES

Carrier Screening
Grody W et al. ACMG position statement on prenatal/preconception expanded carrier screening. *Genet Med.* 2013 15:482-3.
Variant Classification:
Richards S et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med.* 2015 May;17(5):405-24

Additional disease-specific references available upon request.

This case has been reviewed and electronically signed by Lisa Edelmann, Ph.D., FACMG, Laboratory Director

Laboratory Medical Consultant: George A. Diaz, M.D., Ph.D.