Fairfax Cryobank
The Trusted Choice for Donor Sperm

Donor 4405

Genetic Testing Summary

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

Last Updated: 09/06/23

Donor Reported Ancestry: English, German, Portuguese Jewish Ancestry: No

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 108 mutations in the CFTR gene	1/270
Spinal Muscular Atrophy (SMA) carrier screening	Negative for deletions of exon 7 in the SMN1 gene	<1/500
Sickle Cell Disease Beta-thalassemia	Negative for 37 mutations in the HBB gene	<1/500 <1/500
Special testing		
Congenital Adrenal Hyperplasia due to 21-hydroxylase deficiency (CYP21A2)	Carrier: Classic variant in the CYP21A2 gene	Partner testing is indicated before using this donor
Cystic Fibrosis (CFTR)	Negative by gene sequencing in the CFTR gene	1/440
Retinitis Pigmentosa 28 (FAM161A)	Negative by gene sequencing in the FAM161A gene	1/34,200
Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay (SACS)	Negative by gene sequencing in the SACS gene	1/2100
Congenital Disorder of Glycosylation Type 1 A (PMM2)	Negative by gene sequencing in the PMM2 gene	1/540
Familial Dysautonomia (IKBKAP)	Negative by gene sequencing in the IKBKAP gene	1/50,500
Cockayne Syndrome (ERCC8)	Negative by gene sequencing in the ERCC8 gene	Not provided
Genes: ACSF3, GAA, SLC22A5	Negative by gene sequencing	

*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.

Counsyl

Results Recipient	Ordering Healthcare Professional	Male Details	Female Details
Fairfax Cryobank - [redacted] Attn: Dr. Harvey Stern [redacted] Phone: [redacted] Report Date: 03/27/2011	Fairfax Cryobank - [redacted] Attn: Dr. Harvey Stern [redacted] Phone: [redacted] NPI: 1417048786	Name: Donor 4405 DOB: [redacted] Ethnicity: Northern European Sample Type: Saliva (OG-300) Date of Collection: 03/10/2011 Barcode: [redacted] Indication: Egg or Sperm Donor	Not tested

Universal Genetic Test (Egg or Sperm Donor)

The Universal Genetic Test uses targeted DNA mutation analysis to simultaneously determine the carrier status of an individual for a number of Mendelian diseases. This report indicates which mutations, if any, were detected for each mutation panel. Because only select mutations are tested, the percentage of carriers detected varies by ethnicity. A negative test result does not eliminate the possibility that the individual is a carrier. Interpretation is given as an estimate of the risk of conceiving a child affected with a disease, which is based on reported ethnicity, the test results, and an assumption of no family history.*

Donor 4405

Donor 4405's DNA test shows that he is not a carrier of any disease-causing mutation tested.

Partner

The child risk presented is based on a hypothetical pairing with a partner of the same ethnic group.

Child Risk Summary

No increased child risks to highlight. Please refer to the following pages for detailed information about the results.

Note on hemoglobinopathies:
Individuals of African, Southeast Asian, and Mediterranean ancestry are at increased risk for being carriers for hemoglobinopathies and should also be offered carrier testing by CBC and hemoglobin electrophoresis.

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* **Limitations:** In an unknown number of cases, nearby genetic variants may interfere with mutation detection. Other possible sources of diagnostic error include sample mix-up, trace contamination, and technical errors. The child risk summary is provided as an aid to genetic counseling. Inaccurate reporting of ethnicity may cause errors in risk calculation.

This test was developed and its performance characteristics determined by Counsyl, Inc. The laboratory is regulated under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) as qualified to perform high-complexity clinical testing. This test is used for clinical purposes. It should not be regarded as investigational or for research. These results are adjunctive to the ordering physician's workup.

Laboratory Director: Jessica Jacobson, MD
CLIA Number: 05D1102604

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Male

Name: Donor 4405
DOB: [redacted]

Female

Not tested

Full Results

Below are the full test results for all diseases on the panel. Noted are the specific genetic mutations for which the patient tested positive or negative. If there was insufficient data to determine the genotype for any variant, this will be noted as "no call." Also listed in this section is the patient's post-test risk of being a carrier of each disease as well as the odds that his future children could inherit each disease.

Beta Thalassemia

Your child's risk: Less than 1 in 1,000,000

Risk before testing: 1 in 250,000

Reduced risk

Donor 4405: No mutations detected. This does not rule out the possibility of being a carrier of untested mutations. The post-test risk of being a carrier, assuming a negative family history, is < 1 in 500. 80% detection rate.

Gene: HBB. **Variants (35):** K17X, Q39X, 619 bp deletion, Phe41fs, Ser9fs, IVS-II-654, IVS-II-745, IVS-II-850, IVS-I-6, IVS-I-110, IVS-I-5, IVS-I-1(G>A), IVS-I-1(G>T), -88C>T, -28A>G, -29A>G, Lys8fs, Phe71fs, IVS-II-849(A>C), IVS-II-849(A>G), Gly24 T>A, -87C>G, Hb C, Poly A: AATAAA->AATGAA, Poly A: AATAAA->AATAAG, W15X, Pro5fs, Gly16fs, Glu6fs, IVS-II-705, IVS-II-844, -30T>A, CAP+1 A>C, Hb E, Hb O-Arab.

Cystic Fibrosis

Your child's risk: 1 in 30,000

Risk before testing: 1 in 3,000

Reduced risk

Donor 4405: No mutations detected. No call for 3199del6. This does not rule out the possibility of being a carrier of untested mutations. The post-test risk of being a carrier, assuming a negative family history, is 1 in 270. 90% detection rate.

Gene: CFTR. **Variants (108):** G85E, R117H, R334W, R347P, A455E, G542X, G551D, R553X, R560T, R1162X, W1282X, N1303K, F508del, I507del, 2184delA, 3659delC, 621+1G>T, 711+1G>T, 1717-1G>A, 1898+1G>A, 2789+5G>A, 3120+1G>A, 3849+10kbC>T, E60X, R75X, E92X, Y122X, G178R, R347H, Q493X, V520F, S549N, P574H, M1101K, D1152H, S1235R, 394delTT, 1078delT, 3876delA, 3905insT, 1812-1G>A, 3272-26A>G, 2183AA>G, S549R(A>C), G91R, R117C, I148T, L206W, G330X, T338I, R352Q, S364P, G480C, I506V, F508C, C524X, S549I, S549R(T>G), Q552X, A559T, G622D, R709X, K710X, Q890X, R1066C, R1070Q, W1089X, Y1092X, R1158X, S1196X, W1204X(c.3611G>A), Q1238X, S1251N, S1255X, R1283M, dele2-3 21kb, 3199del6, F311del, 574delA, 663delT, 935delA, 936delTA, 1161delC, 1609delCA, 1677delTA, 1949del84, 2043delG, 2055del9>A, 2105-2117del13insAGAAA, 3171delC, 3667del4, 3821delT, 1288insTA, 2184insA, 2307insA, 2869insG, 296+12T>C, 405+1G>A, 405+3A>C, 406-1G>A, 711+5G>A, 712-1G>T, 1811+1.6kbA>G, 1898+1G>T, 1898+5G>T, 3120G>A, 457TAT>G, W1204X(c.3612G>A).

Sickle Cell Disease

Your child's risk: Less than 1 in 1,000,000

Risk before testing: less than 1 in 1,000,000

Reduced risk

Donor 4405: No mutations detected. This does not rule out the possibility of being a carrier of untested mutations. The post-test risk of being a carrier, assuming a negative family history, is < 1 in 500. >99% detection rate.

Gene: HBB. **Variants (37):** Hb S, K17X, Q39X, 619 bp deletion, Phe41fs, Ser9fs, IVS-II-654, IVS-II-745, IVS-II-850, IVS-I-6, IVS-I-110, IVS-I-5, IVS-I-1(G>A), IVS-I-1(G>T), -88C>T, -28A>G, -29A>G, Lys8fs, Phe71fs, IVS-II-849(A>C), IVS-II-849(A>G), Gly24 T>A, -87C>G, Hb C, Poly A: AATAAA->AATGAA, Poly A: AATAAA->AATAAG, W15X, Pro5fs, Gly16fs, Glu6fs, IVS-II-705, IVS-II-844, -30T>A, CAP+1 A>C, Hb E, Hb D-Punjab, Hb O-Arab.

Spinal Muscular Atrophy

Your child's risk: 1 in 97,000

Risk before testing: 1 in 4,800

Reduced risk

Donor 4405: No mutations detected. This does not rule out the possibility of being a carrier of untested mutations. The post-test risk of being a carrier, assuming a negative family history, is < 1 in 500. 95% detection rate.

Gene: SMN1. **Variants (1):** Exon 7 deletion.

Laboratory Director: Jessica Jacobson, MD
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CARRIER SCREENING REPORT

Patient	Sample	Referring Doctor
Patient Name: Donor 4405 **Date of Birth:** [redacted] **Reference #:** FFAXCB-S44405 **Indication:** Carrier Testing **Test Type:** Custom Carrier Screen (ECS)	**Specimen Type:** Purified DNA(Semen) **Lab #:** [redacted] **Date Collected:** 5/7/2019 **Date Received:** 5/10/2019 **Final Report:** 5/23/2019	[redacted] **Fairfax Cryobank, Inc.** [redacted]

RESULTS

POSITIVE for congenital adrenal hyperplasia (due to 21-hydroxylase deficiency)
A heterozygous (one copy) pathogenic variant, c.1066C>T, p.R356W, was detected in the *CYP21A2* gene

NEGATIVE for the remaining diseases tested

Congenital Adrenal Hyperplasia (21-Hydroxylase Deficiency)

POSITIVE for congenital adrenal hyperplasia (due to 21-hydroxylase deficiency)
CYP21A2 copy number: 2
No pathogenic copy number variants detected
Sequence analysis: A heterozygous (one copy) pathogenic variant, c.1066C>T, p.R356W, was detected in the *CYP21A2* gene

Genes analyzed: *CYP21A2* (NM_000500.6)
Inheritance: Autosomal Recessive

Recommendations
Testing of the patient's partner and genetic counseling are recommended.

Interpretation for congenital adrenal hyperplasia (due to 21-hydroxylase deficiency)

A heterozygous (one copy) pathogenic missense variant, c.1066C>T, p.R356W, was detected in the *CYP21A2* gene (NM_000500.6). Please note that this variant is reported to be causative for the classic salt-wasting/severe virilizing form of congenital adrenal hyperplasia (PMID: 29450859). Variants associated with the classic form usually cause classic congenital adrenal hyperplasia when found in trans with a second classic allele, or non-classic congenital adrenal hyperplasia when found in trans with a non-classic allele (PMID: 29450859). Therefore, this individual is expected to be at least a carrier for congenital adrenal hyperplasia. Heterozygous carriers are not expected to exhibit symptoms of this disease.

Patient:	Donor 4405	DOB:	[redacted]	Lab #:	[redacted]

What is congenital adrenal hyperplasia (due to 21-hydroxylase deficiency)?

Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders resulting from deficiency in the enzymes involved in cortisol biosynthesis. The majority (95%) of CAH cases are due to 21-hydroxylase deficiency (21-OHD CAH), which is caused by homozygous or compound heterozygous pathogenic variants in the gene *CYP21A2*. Approximately 20% of mutant alleles have deletions of 30 kb that have been generated by unequal meiotic crossing-over between the two genes. Another 75% of mutant alleles are due to gene conversion events, where an inactivating mutation from the *CYP21A1P* pseudogene is introduced into one copy of the *CYP21A2* gene, thus making the gene non-functional. Three different forms of 21-OHD CAH have been reported: a classic salt wasting form, a classic simple virilizing form, and a non-classic form.

- The classic salt wasting form results from a nonfunctional enzyme and is the most severe. The phenotype includes prenatal onset of virilization and inadequate adrenal aldosterone secretion that can result in fatal salt-wasting crises.
- The classic simple virilizing form results from low levels of functional enzyme and involves prenatal virilization but no salt-wasting.
- The non-classic form, which results from a mild enzyme deficiency, occurs postnatally and involves phenotypes associated with hyperandrogenism, such as hirsutism, delayed menarche, and infertility.

Treatment for the classic forms of the disorder include glucocorticoid and mineralocorticoid replacement therapy, as well as the possibility of feminizing genitoplasty, while patients with the non-classic form usually do not require treatment. The life expectancy for this disorder can be normal with treatment, however the occurrence of salt-wasting crises can be fatal.

Custom Carrier Screen (ECS)

Negative: No clinically significant variant(s) detected

Gene(s) analyzed: *CFTR, SACS,* and *FAM161A*

Recommendations:
Consideration of residual risk by ethnicity after a negative carrier screen is recommended, especially in the case of a positive family history for a specific disorder.

Interpretation:
Screening for the presence of pathogenic variants in the *CFTR, SACS,* and *FAM161A* genes which are associated with cystic fibrosis, autosomal recessive spastic ataxia of Charlevoix-Saguenay, and retinitis pigmentosa 28, respectively, was performed by next generation sequencing and possibly genotyping for select variants on DNA extracted from this patient's sample. No clinically significant variants were detected during this analysis.

Patient:	Donor 4405	DOB:	[redacted]	Lab #:	[redacted]

Please note that negative results reduce but do not eliminate the possibility that this individual is a carrier for the disorder(s) tested. Please see table of residual risks for specific detection rates and residual risk by ethnicity. With individuals of mixed ethnicity, it is recommended to use the highest residual risk estimate. Only variants determined to be pathogenic or likely pathogenic are reported in this carrier screening test.

Comments:
This carrier screening test masks likely benign variants and variants of uncertain significance (VUS) if there are any. Only known pathogenic variants or likely pathogenic variants which are expected to result in deleterious effects on protein function are reported. If reporting of likely benign variants and VUS is desired in this patient, please contact the laboratory (tel. 212-241-2537) to request an amended report.

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.

Table of Residual Risks by Ethnicity

Please note: This table displays residual risks after a negative result for each of the genes and corresponding disorders. **If a patient is reported to be a carrier of a disease, their residual risk is 1 and this table does not apply for that disease.**

Disease (Inheritance)	Gene	Ethnicity	Carrier Frequency	Detection Rate	Residual Risk	Analytical Detection Rate
Autosomal Recessive Spastic Ataxia	*SACS*	African	1 in 201	99%	1 in 20,000	99%
Of Charlevoix-Saguenay (AR)		Ashkenazi Jewish	1 in 483	80%	1 in 2,400	
NM_014363.5		East Asian	1 in 338	84%	1 in 2,100	
		Finnish	1 in 341	99%	1 in 34,000	
		Caucasian	1 in 100	95%	1 in 2,100	
		Latino	1 in 309	88%	1 in 2,600	
		South Asian	1 in 383	97%	1 in 11,000	
		Worldwide	1 in 148	95%	1 in 2,900	
		French Canadian - Charlevoix-Saguenay	1 in 21	99%	1 in 2,000	
Classic Congenital Adrenal Hyperplasia	*CYP21A2*	Ashkenazi Jewish	1 in 40	95%	1 in 780	95%
Due to 21-Hydroxylase Deficiency (AR)		Caucasian	1 in 67	95%	1 in 1,300	
NM_000500.7		Worldwide	1 in 60	95%	1 in 1,200	
Non-Classic Congenital Adrenal Hyperplasia	*CYP21A2*	Ashkenazi Jewish	1 in 7	95%	1 in 120	95%
Due to 21-Hydroxylase Deficiency (AR)		Caucasian	1 in 11	95%	1 in 200	
NM_000500.7		Worldwide	1 in 16	95%	1 in 300	

Patient:	Donor 4405	DOB:	[redacted]	Lab #:	[redacted]

Cystic Fibrosis (AR)	*CFTR*	African	1 in 58	91%	1 in 630	99%
NM_000492.3		Ashkenazi Jewish	1 in 24	98%	1 in 1,200	
		East Asian	1 in 277	80%	1 in 1,400	
		Finnish	1 in 75	93%	1 in 1,100	
		Caucasian	1 in 23	95%	1 in 440	
		Latino	1 in 40	96%	1 in 1,000	
		South Asian	1 in 73	91%	1 in 800	
Exception: Exon 10		Worldwide	1 in 33	94%	1 in 500	
Retinitis Pigmentosa 28 (AR)	*FAM161A*	African	1 in 894	99%	1 in 89,300	99%
NM_032180.2		Ashkenazi Jewish	1 in 242	99%	1 in 24,100	
		East Asian	1 in 1450	99%	1 in 145,000	
		Finnish	1 in 656	99%	1 in 65,500	
		Caucasian	1 in 343	99%	1 in 34,200	
		Latino	1 in 442	99%	1 in 44,100	
		South Asian	1 in 795	99%	1 in 79,400	
		Worldwide	1 in 423	99%	1 in 42,200	
		Sephardic Jewish - Libyan, Moroccan, Tunisian and Bulgarian	1 in 41	99%	1 in 4,000	

AR: Autosomal Recessive

This case has been reviewed and electronically signed by Anastasia Larmore, PhD, Assistant Director

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

Patient:	Donor 4405	DOB:	[redacted]	Lab #:	[redacted]

Test Methods and Comments

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

Fragile X CGG Repeat Analysis (Analytical Detection Rate >99%)
PCR amplification using Asuragen, Inc. AmplideX® *FMR1* PCR reagents followed by capillary electrophoresis for allele sizing was performed. Samples positive for *FMR1* CGG repeats in the premutation and full mutation size range were further analyzed by Southern blot analysis to assess the size and methylation status of the *FMR1* CGG repeat.

Genotyping (Analytical Detection Rate >99%)
Multiplex PCR amplification and allele specific primer extension analyses using the MassARRAY® System were used to identify variants that are complex in nature or are present in low copy repeats. Rare sequence variants may interfere with assay performance.

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%.

For alpha thalassemia, 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.

For Duchenne muscular dystrophy, the copy numbers of all *DMD* exons were analyzed. Potentially pathogenic single exon deletions and duplications are confirmed by a second method. Analysis of *DMD* is performed in association with sequencing of the coding regions.

For congenital adrenal hyperplasia, the copy number of the *CYP21A2* gene was analyzed. This analysis can detect large deletions due to unequal meiotic crossing-over between *CYP21A2* and the pseudogene *CYP21A1P*. These 30-kb deletions make up approximately 20% of *CYP21A2* pathogenic alleles. This test may also identify certain point mutations in *CYP21A2* caused by gene conversion events between *CYP21A2* and *CYP21A1P*. Some carriers may not be identified by dosage sensitive methods as this testing cannot detect individuals with two copies (duplication) of the *CYP21A2* gene on one chromosome and loss of *CYP21A2* (deletion) on the other chromosome. Analysis of *CYP21A2* is performed in association with long-range PCR of the coding regions followed by short-read sequencing.

For spinal muscular atrophy (SMA), the copy numbers of the *SMN1* and *SMN2* genes were analyzed. The individual dosage of exons 7 and 8 as well as the combined dosage of exons 1, 4, 6 and 8 of *SMN1* and *SMN2* were assessed. Copy number gains and losses can be detected with this assay. Depending on ethnicity, 6 - 29 % of carriers will not be identified by dosage sensitive methods as this testing cannot detect individuals with two copies (duplication) of the *SMN1* gene on one chromosome and loss of *SMN1* (deletion) on the other chromosome (silent 2+0 carrier) or individuals that carry an intragenic mutation in *SMN1*. Please also note that 2% of individuals with SMA have an *SMN1* mutation that occurred *de novo*. Typically in these cases, only one parent is an SMA carrier.

The presence of the c.*3+80T>G (chr5:70,247,901T>G) variant allele in an individual with Ashkenazi Jewish or Asian ancestry is typically indicative of a duplication of *SMN1*. When present in an Ashkenazi Jewish or Asian individual with two copies of *SMN1*, c.*3+80T>G is likely indicative of a silent (2+0) carrier. In individuals with two copies of *SMN1* with African American, Hispanic or Caucasian ancestry, the presence or absence of c.*3+80T>G significantly increases or decreases, respectively, the likelihood of being a silent 2+0 silent carrier.

Pathogenic or likely pathogenic sequence variants in exon 7 may be detected during testing for the c.*3+80T>G variant allele; these will be reported if confirmed to be located in SMN1 using locus-specific Sanger primers

MLPA for Gaucher disease (*GBA*), cystic fibrosis (*CFTR*), and non-syndromic hearing loss (*GJB2/GJB6*) will only be performed if indicated for confirmation of detected CNVs. If *GBA* analysis was performed, the copy numbers of exons 1, 3, 4, and 6 - 10 of the *GBA* gene (of 11 exons total) were analyzed. If *CFTR* analysis was performed, the copy numbers of all 27 *CFTR* exons were analyzed. If *GJB2/GJB6* analysis was performed, the copy number of the two *GJB2* exons were analyzed, as well as the presence or absence of the two upstream deletions of the *GJB2* regulatory region, del(*GJB6*-D13S1830) and del(*GJB6*-D13S1854).

Patient:	Donor 4405	DOB:	[redacted]	Lab #:	[redacted]

Next Generation Sequencing (NGS) (Analytical Detection Rate >95%)
NGS was performed on a panel of genes for the purpose of identifying pathogenic or likely pathogenic variants.

Agilent SureSelect™QXT technology was used with a custom capture library to target the exonic regions and intron/exon splice junctions of the relevant genes, as well as a number of UTR, intronic or promoter regions that contain previously reported mutations. Samples were pooled and 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 known protein-coding RefSeq genes were assessed for the average depth of coverage (minimum of 20X) and data quality threshold values. Most exons not meeting a minimum of >20X read depth across the exon are further analyzed by Sanger sequencing. Please note that several genomic regions present difficulties in mapping or obtaining read depth >20X. The exons contained within these regions are noted within Table 1 (as "Exceptions") and will not be reflexed to Sanger sequencing if the mapping quality or coverage is poor. Any variants identified during testing in these regions are confirmed by a second method and reported if determined to be pathogenic or likely pathogenic. However, as there is a possibility of false negative results within these regions, detection rates and residual risks for these genes have been calculated with the presumption that variants in these exons will not be detected, unless included in the MassARRAY® genotyping platform.

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, or regions that fall into the Exceptions mentioned above. 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.

Copy Number Variant Analysis (Analytical Detection Rate >95%)
Large duplications and deletions were called from the relative read depths on an exon-by-exon basis using a custom exome hidden Markov model (XHMM) algorithm. Deletions or duplications determined to be pathogenic or likely pathogenic were confirmed by either a custom arrayCGH platform, quantitative PCR, or MLPA (depending on CNV size and gene content). While this algorithm is designed to pick up deletions and duplications of 2 or more exons in length, potentially pathogenic single-exon CNVs will be confirmed and reported, if detected.

Exon Array (Confirmation method) (Accuracy >99%)
The customized oligonucleotide microarray (Oxford Gene Technology) is a highly-targeted exon-focused array capable of detecting medically relevant microdeletions and microduplications at a much higher resolution than traditional aCGH methods. Each array matrix has approximately 180,000 60-mer oligonucleotide probes that cover the entire genome. This platform is designed based on human genome NCBI Build 37 (hg19) and the CGH probes are enriched to target the exonic regions of the genes in this panel.

Quantitative PCR (Confirmation method) (Accuracy >99%)
The relative quantification PCR is utilized on a Roche Universal Library Probe (UPL) system, which relates the PCR signal of the target region in one group to another. To test for genomic imbalances, both sample DNA and reference DNA is amplified with primer/probe sets that specific to the target region and a control region with known genomic copy number. Relative genomic copy numbers are calculated based on the standard ΔΔCt formula.

Long-Range PCR (Analytical Detection Rate >99%)
Long-range PCR was performed to generate locus-specific amplicons for *CYP21A2*, *HBA1* and *HBA2* and *GBA*. The PCR products were then prepared for short-read NGS sequencing and sequenced. Sequenced reads were mapped back to the original genomic locus and run through the bioinformatics pipeline. If indicated, copy number from MLPA was correlated with the sequencing output to analyze the results. For *CYP21A2*, a certain percentage of healthy individuals carry a duplication of the *CYP21A2* gene, which has no clinical consequences. In cases where two copies of a gene are located on the same chromosome in tandem, only the second copy will be amplified and assessed for potentially pathogenic variants, due to size limitations of the PCR reaction. However, because these alleles contain at least two copies of the *CYP21A2* gene in tandem, it is expected that this patient has at least one functional gene in the tandem allele and this patient is therefore less likely to be a carrier. When an individual carries both a duplication allele and a pathogenic variant, or multiple pathogenic variants, the current analysis may not be able to

determine the phase (cis/trans configuration) of the *CYP21A2* alleles identified. Family studies may be required in certain scenarios where phasing is required to determine the carrier status.

Residual Risk Calculations
Carrier frequencies and detection rates for each ethnicity were calculated through the combination of internal curations of >28,000 variants and genomic frequency data from >138,000 individuals across seven ethnic groups in the gnomAD database. Additional variants in HGMD and novel deleterious variants were also incorporated into the calculation. Residual risk values are calculated using a Bayesian analysis combining the *a priori* risk of being a pathogenic mutation carrier (carrier frequency) and the detection rate. 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.

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.

Tay-Sachs Disease (TSD) Enzyme Analysis (Analytical Detection Rate ≥98%)
Hexosaminidase activity and Hex A% activity were measured by a standard heat-inactivation, fluorometric method using artificial 4-MU-β-N-acetyl glucosaminide (4-MUG) substrate. This assay is highly sensitive and accurate in detecting Tay-Sachs carriers and individuals affected with TSD. Normal ranges of Hex A% activity are 55.0-72.0 for white blood cells and 58.0-72.0 for plasma. It is estimated that less than 0.5% of Tay-Sachs carriers have non-carrier levels of percent Hex A activity, and therefore may not be identified by this assay. In addition, this assay may detect individuals that are carriers of or are affected with Sandhoff disease. False positive results may occur if benign variants, such as pseudodeficiency alleles, interfere with the enzymatic assay. False negative results may occur if both *HEXA* and *HEXB* pathogenic or pseudodeficiency variants are present in the same individual.

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.
Fragile X syndrome:
Chen L et al. An information-rich CGG repeat primed PCR that detects the full range of Fragile X expanded alleles and minimizes the need for Southern blot analysis. *J Mol Diag* 2010 12:589-600.
Spinal Muscular Atrophy:
Luo M et al. An Ashkenazi Jewish SMN1 haplotype specific to duplication alleles improves pan-ethnic carrier screening for spinal muscular atrophy. *Genet Med*. 2014 16:149-56.
Ashkenazi Jewish Disorders:
Scott SA et al. Experience with carrier screening and prenatal diagnosis for sixteen Ashkenazi Jewish Genetic Diseases. *Hum. Mutat.* 2010 31:1-11.
Duchenne Muscular Dystrophy:
Flanigan KM et al. Mutational spectrum of DMD mutations in dystrophinopathy patients: application of modern diagnostic techniques to a large cohort. *Hum Mutat*. 2009 30:1657-66.
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.

Patient:	Donor 4405	DOB:	[redacted]	Lab #:	[redacted]

Patient Information
Name: Donor 4405
Date of Birth: [redacted]
Sema4 ID: [redacted]
Client ID: [redacted]
Indication: Carrier Testing

Specimen Information
Specimen Type: Purified DNA(Semen)
Date Collected: 05/07/2019
Date Received: 08/19/2019
Final Report: 09/01/2019

Referring Provider
[redacted]
Fairfax Cryobank, Inc.
[redacted]

Unmask Additional Gene(s)

Number of genes tested: 2

SUMMARY OF RESULTS AND RECOMMENDATIONS

Negative

Negative for all genes tested: *PMM2, and IKBKAP*
To view a full list of genes and diseases tested
please see Table 1 in this report

Recommendations

- Consideration of residual risk by ethnicity after a negative carrier screen is recommended for the other diseases on the panel, especially in the case of a positive family history for a specific disorder.

Test description

This patient was tested for a panel of diseases using a combination of sequencing, targeted genotyping and copy number analysis. Please note that negative results reduce but do not eliminate the possibility that this individual is a carrier for one or more of the disorders tested. Please see Table 1 for a list of genes and diseases tested, and **go.sema4.com/residualrisk** for specific detection rates and residual risk by ethnicity. With individuals of mixed ethnicity, it is recommended to use the highest residual risk estimate. Only variants determined to be pathogenic or likely pathogenic are reported in this carrier screening test.

Ruth Kornreich, Ph.D., FACMG, Laboratory Director
Laboratory Medical Consultant: George A. Diaz, M.D., Ph.D.

Genes and diseases tested

For specific detection rates and residual risk by ethnicity, please visit **go.sema4.com/residualrisk**

Table 1: List of genes and diseases tested with detailed results

Disease	Gene	Inheritance Pattern	Status	Detailed Summary
⊖ **Negative**				
Congenital Disorder of Glycosylation, Type Ia	*PMM2*	AR	Reduced Risk (see table below)	
Familial Dysautonomia	*IKBKAP*	AR	Reduced Risk (see table below)	

AR=Autosomal recessive; XL=X-linked

Table 2: Residual Risk by ethnicity for negative results

Disease (Inheritance)	Gene	Ethnicity	Carrier Frequency	Detection Rate	Residual Risk	Analytical Detection Rate
Congenital Disorder of Glycosylation, Type Ia (AR) NM_000303.2	*PMM2*	African	1 in 245	99%	1 in 24,400	99%
		Ashkenazi Jewish	1 in 66	99%	1 in 6,500	
		East Asian	1 in 133	76%	1 in 550	
		Finnish	1 in 58	99%	1 in 5,700	
		Caucasian	1 in 58	89%	1 in 540	
		Latino	1 in 114	91%	1 in 1,200	
		South Asian	1 in 278	86%	1 in 2,000	
		Worldwide	1 in 80	91%	1 in 840	
Familial Dysautonomia (AR) NM_003640.3	*IKBKAP*	African	1 in 409	99%	1 in 40,800	99%
		Ashkenazi Jewish	1 in 35	99%	1 in 3,400	
		East Asian	1 in 784	99%	1 in 78,300	
		Finnish	1 in 707	99%	1 in 70,600	
		Caucasian	1 in 506	99%	1 in 50,500	
		Latino	1 in 801	99%	1 in 80,000	
		South Asian	1 in 855	99%	1 in 85,400	
		Worldwide	1 in 345	99%	1 in 34,400	

* Carrier detection by HEXA enzyme analysis has a detection rate of approximately 98% (Applies to *HEXA* gene testing only).

ǂ Carrier frequencies include milder and reduced penetrance forms of the disease. Therefore, carrier frequencies may appear higher than reported in the literature (Applies to *BTD*, *F9*, *GJB2*, *GJB1*, *GLA*, and *MEFV* gene testing only).

ǂ Please note that *GJB2* testing includes testing for the two upstream deletions, del(GJB6-D13S1830) and del(GJB6-D13S1854) (PMID:11807148 and 15994881) (Applies to *GJB2* gene testing only).

AR: Autosomal recessive; N/A: Not available; XL: X-linked

Test methods and comments

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

Fragile X CGG

Repeat Analysis

PCR amplification using Asuragen, Inc. AmplideX® *FMR1* PCR reagents followed by capillary electrophoresis for allele sizing was performed. Samples positive for *FMR1* CGG repeats in the premutation and full mutation size range are further analyzed by Southern blot analysis to assess the size and methylation status of the *FMR1* CGG repeat.

Genotyping

Multiplex PCR amplification and allele specific primer extension analyses using the MassARRAY® System or Luminex® xMAP® technology were used to identify variants that are complex in nature or are present in low copy repeat regions and are, therefore, not amenable to Next Generation Sequencing technologies. Rare sequence variants may interfere with assay performance.

Multiplex Ligation-Dependent Probe Amplification (MLPA)

MLPA® probe sets and reagents, MRC-Holland, were used for the analysis of copy number of specific targets versus known control samples. Each target region was assayed with two adjacent oligonucleotide probes which following hybridization were ligated and used as template for subsequent rounds of amplification. Each complete probe within the assay has a unique length and amplicons are separated and identified by capillary electrophoresis. False positive or negative results may also occur due to rare sequence variants in target regions detected by MLPA probes. Analytical sensitivity and specificity of the MLPA method are both 99%.

For Alpha Thalassemia, the copy numbers of the *HBA1* and *HBA2* genes were analyzed. Alpha-globin gene deletions, triplications, and the Constant Spring (CS) mutation are assessed. However, it does not detect all known alpha-thalassemia mutations such as point mutations. In addition, carriers of alpha-thalassemia with three or more *HBA* copies on one chromosome, and one or no copies on the other chromosome, will not be detected. This test detects most alpha-globin gene deletions, triplications, and the Constant Spring (CS) mutation using Multiplex Ligation-Dependent Probe Amplification (MLPA). It is expected to detect approximately 90% of all alpha-thalassemia mutations, varying by ethnicity. Therefore, this result reduces, but does not eliminate, the chance that this patient is a carrier of alpha-thalassemia. With the exception of triplications, other benign alpha-globin gene polymorphisms will not be reported.

For Duchenne Muscular Dystrophy, the copy numbers of all *DMD* exons were analyzed. Please note that single-exon deletions and duplications will not be reported unless they are confirmed by NGS data (for example, if breakpoints occurring in an exon can be visualized).

For Spinal Muscular Atrophy (SMA), the copy numbers of the *SMN1* and *SMN2* genes were analyzed. The individual dosage of exons 7 and 8 as well as the combined dosage of exons 1, 4, 6 and 8 of *SMN1* and *SMN2* were assessed. Copy number gains and losses can be detected with this assay.

Depending on ethnicity 6 - 29 % of carriers will not be identified by dosage sensitive methods as this testing cannot detect individuals with two copies (duplication) of the *SMN1* gene on one chromosome and loss of *SMN1* (deletion) on the other chromosome (silent 2+0 carrier) or individuals that carry an intragenic mutation in *SMN1* . Please also note that 2% of individuals with SMA have an *SMN1* mutation that occurred *de novo* . Typically in these cases, only one parent is an SMA carrier.

The presence of the g.27134T>G variant allele in an individual with Ashkenazi Jewish or Asian ancestry is indicative of a duplication of *SMN1* . When present in an Ashkenazi Jewish or Asian individual with two copies of *SMN1* , g.27134T>G is likely indicative of a silent (2+0) carrier. In individuals with two copies of *SMN1* with African American, Hispanic or Caucasian ancestry, the presence or absence of g.27134T>G significantly increases or decreases, respectively, the likelihood of being a silent 2+0 silent carrier.

Next Generation Sequencing (NGS)

NGS was performed on a panel of genes for the purpose of identifying pathogenic or likely pathogenic variants.

Agilent SureSelect^{TM}QXT technology was used with custom capture library to target the guaranteed list of mutations and exonic regions of the relevant genes. These targeted regions were sequenced using the Illumina HiSeq2500 system with 100 bp paired-end reads. The DNA sequences were mapped to and analyzed in comparison with the published human genome build UCSC hg19 reference sequence. The targeted coding exons and splice junctions of the known protein-coding RefSeq genes were assessed for the average depth of coverage and data quality threshold values. This technology may not detect all small insertion/deletions and is not diagnostic for large duplications/deletions, repeat expansions, and structural genomic variation. This test will detect variants within the exons and the intron-exon boundaries of the target regions. Variants outside these regions will either not be detected or are not guaranteed to be detected. These regions include, but are not limited to, UTRs, promoters, and deep intronic areas or regions that fall within low copy repeat segments. In addition, a mutation(s) in a gene not included on the panel could be present in this patient. 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 were not reported.

Sanger Sequencing

Sanger sequencing, as indicated, was performed in both directions 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.

Tay-Sachs Disease (TSD) Enzyme Analysis

Hexosaminidase activity and Hex A % activity were measured by a standard heat-inactivation, fluorometric method using artificial 4-MU-â-N-acetyl glucosaminide (4-MUG) substrate. This assay is highly sensitive and accurate in detecting Tay-Sachs carriers and individuals affected

with TSD. I t is estimated that less than 0.5% of Tay-Sachs carriers have non-carrier levels of percent Hex A activity, and therefore may not be identified by this assay. In addition, this assay may detect individuals that are carriers of or are affected with Sandhoff Disease. False positive results, such as pseudodeficiency alleles, may occur if benign variants interfere with the enzymatic assay. False negative results may occur if both *HEXA* and *HEXB* pathogenic variants are present in the same individual.

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.

Umbarger MA. Next-generation carrier screening. *Genet Med* . 2014 16:132-40.

Alpha-thalassemia:

Galanello R et al. Gene test review: Alpha-thalassemia. *Genet Med* . 2011 13:83-8.

Waye JS et al. Diagnostic testing for á-globin gene disorders in a heterogeneous N orth American population. *Int J Lab Hematol* . 2013 35:306-13.

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ACOG Committee Opinion. Number 325, Update on carrier screening for cystic fibrosis. 2005.

Fragile X syndrome:

Chen L et al. An information-rich CGG repeat primed PCR that detects the full range of Fragile X expanded alleles and minimizes the need for Southern blot analysis. *J Mol Diag* 2010 12:589-600.

Spinal Muscular Atrophy:

Hendrickson BC et al. Differences in SMN1 allele frequencies among ethnic groups within North America. *J Med Genet* . 2009 46:641-4.

Ogino S et al. Genetic risk assessment in carrier testing for spinal muscular atrophy. *Am J Med Genet* . 2002 110:301-7.

Luo M et al. An Ashkenazi Jewish SMN1 haplotype specific to duplication alleles improves pan-ethnic carrier screening for spinal muscular atrophy. *Genet Med* . 2014 16:149-56.

Ashkenazi Jewish Disorders:

Scott SA et al. Experience with carrier screening and prenatal diagnosis for sixteen Ashkenazi Jewish Genetic Diseases. *Hum. Mutat.* 2010 31:1-11.

Duchenne Muscular Dystrophy:

Aartsma-Rus A et al. Entries in the Leiden Duchenne muscular dystrophy mutation database: an overview of mutation types and paradoxical cases that confirm the reading-frame rule. *Muscle Nerve* . 2006b 34:135-44.

Flanigan KM et al. Mutational spectrum of DMD mutations in dystrophinopathy patients: application of modern diagnostic techniques to a large cohort. *Hum Mutat* . 2009 30:1657-66.

Beta Globin-related Disorders:

Cao A et al. Beta-Thalassemia. GeneReviews (http://www.ncbi.nlm.nih.gov/books/NBK1426/)

Modell B et al. Epidemiology of haemoglobin disorders in Europe: an overview. Scand J Clin Lab Invest. 2007 67:39-69.

For further reading:

Orphanet: http://www.orpha.net/consor/cgi-bin/index.php

GeneReviews:

For Disease Specific Standards and Guidelines:

https://www.acmg.net/

Additional disease-specific references available upon request.

sema4
a Mount Sinai venture

Patient	Sample	Referring Doctor
Patient Name: Donor 4405 **Date of Birth:** [redacted] **Reference #:** [redacted] **Indication:** Carrier Testing **Test Type:** ***ERCC8*** **NGS**	**Specimen Type:** DNA from Semen **Lab #:** [redacted] **Date Collected:** 10/3/2019 **Date Received:** 10/9/2019 **Final Report:** 10/22/2019	[redacted] **Fairfax Cryobank, Inc.** [redacted]

RESULTS SUMMARY

No clinically significant variant(s) detected.

Gene(s) Analyzed:

Gene	Marker	Band/RefSeq
ERCC8	Cockayne syndrome (ERCC8-related)	NM_001007233.2

1. All coding DNA sequence of the genes corresponding to the transcripts listed plus the flanking 5 base pair splice sites are sequenced relative to the hg19 assembly.
2. Alternate transcripts may also be tested.

Recommendations

- Consideration of residual risk by ethnicity after a negative carrier screen is recommended, especially in the case of a positive family history for a specific disorder.

Interpretation

Next generation sequencing of the *ERCC8* gene was performed on DNA extracted from semen from this patient.

No clinically significant variant/(s) detected during this analysis. This negative result does not rule out the possibility that a mutation not detectable by this test may be present in this individual. **Only known pathogenic variants or likely pathogenic variants are reported in this carrier screening test.** If reporting of variant of uncertain clinical significance is desired in this patient, please contact the laboratory (tel. 212-241-2537) to request an amended report.

This case has been reviewed and electronically signed by Funda Suer, Ph.D., FACMG, Laboratory Director
Laboratory Medical Consultant: George A. Diaz, M.D., Ph.D.

METHODS

Next Generation Sequencing (NGS) (Analytical Detection Rate >95%)
Agilent SureSelect™ QXT technology is used with a custom capture library to target the exonic regions and intron/exon splice junctions of the relevant genes, as well as a number of UTR, intronic or promoter regions that contain previously reported mutations. Samples are pooled and sequenced on the Illumina NovaSeq platform in the Xp workflow, using 100 bp paired-end reads. The sequencing data are analyzed using a custom bioinformatics algorithm designed and validated in-house. In our validation, average coverage was greater than 200X per sample with >99.9% of regions covered at greater than 20X.

The coding exons and splice junctions of the known protein-coding RefSeq genes are assessed for the average depth of coverage (minimum of 20X) and data quality threshold values. Most exons not meeting a minimum of >20X read depth across the exon are further analyzed by Sanger sequencing. Please note that several genomic regions present difficulties in mapping or obtaining read depth >20X. These regions include, but are not limited to, UTRs, promoters, and deep intronic areas. Regions (hg19 coordinates) that have been excluded due to lack of amenability to NGS or Sanger sequencing, high GC content, high homology, lack of known clinically significant variants, or overlap with repetitive regions are described above. Exons contained within these regions will not be reflexed to Sanger sequencing if the mapping quality or coverage is poor due to high sequence homology. Any clinically significant variants identified during testing in these regions are confirmed by a second method and reported.

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, or regions that fall into the exceptions mentioned above. 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.

Copy Number Variant Analysis (Analytical Detection Rate >90%)
Large duplications and deletions were called from the relative read depths on an exon-by-exon basis using a custom Exome Hidden Markov Model (XHMM) algorithm. This algorithm is designed to pick up deletions and duplications of two or more exons/probed regions in length. For deletions (≥2 exons/probed regions), the analytical sensitivity and analytical specificity are >99%. For duplications (≥2 exons/probed regions), the analytical sensitivity is >80% and analytical specificity is >99%. All reported pathogenic or likely pathogenic deletions and/or duplications were confirmed by a custom aCGH platform, quantitative PCR, and/or MLPA, depending on CNV size and gene content.

Multiplex Ligation-Dependent Probe Amplification (MLPA) (Confirmation Method) (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%. MLPA for Duchenne muscular dystrophy disease (*DMD*) will only be performed if indicated. For Duchenne muscular dystrophy, the copy numbers of all *DMD* exons were analyzed. Potentially pathogenic single exon deletions and duplications are confirmed by a second method. Analysis of *DMD* is performed in association with sequencing of the coding regions.

Exon Array (Confirmation method) (Accuracy >99%)
The customized oligonucleotide microarray (Oxford Gene Technology) is a highly-targeted exon-focused array capable of detecting medically relevant microdeletions and microduplications at a much higher resolution than traditional aCGH methods. Each array matrix has approximately one hundred and eighty thousand 60-mer oligonucleotide probes that cover the entire gene panel. This platform is designed based on human genome NCBI Build 37 (hg19) and the CGH probes are selected to target the exonic regions of the genes in this panel. This test does not include analysis of *ADNP, ATRIP, COMT, CTCF, CYP2B6, CYP2D6, DEPDC5, EEF1A2, GABRB2, GATAD2B, GNAO1, GNB1, GRIK4, HTR2A, KCNA2, KCNB1, KIF2A, MTOR, NECAP1, NPRL2, NPRL3, OPRM1, PURA, SETD5, SIK1, SLC12A5, SLC13A5, SLC1A2, SLC25A1, SLC35A2, SOX11, STX1B, SZT2, TBR1, TCF12, TCF20, TUBB, TUBB2A, TUBG1, UGT2B15, WAC, ZMYND11,* and *ZNF407.*

Quantitative PCR (Confirmation method) (Analytical detection rate >99%)
The relative quantification PCR is utilized on a Roche Universal Library Probe (UPL) system, which relates the PCR signal of the target region in one group to another. To test for genomic imbalances, both sample DNA and reference DNA is amplified with primer/probe sets that specific to the target region and a control region with known genomic copy number. Relative genomic copy numbers are calculated based on the standard ΔΔCt formula.

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.

Variant Interpretation and Reporting
Variant interpretation and classification was performed based on the American College of Medical Genetics Standards and guidelines for the interpretation of sequence variants (PMID:25741868). Frequency in control populations were evaluated based on the Exome Aggregation Consortium (ExAC, http://exac.broadinstitute.org/) , and Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org/). Variants that are related to the patient's phenotype and relevant to indications were investigated. Potentially pathogenic variants may be confirmed by Sanger sequencing if indicated. Familial samples are only tested for certain variants by Sanger sequencing if indicated and tested solely for the presence or absence of the variants. The non-paternity and germline mosaicism were not ruled out. Any benign polymorphisms and likely benign variants identified during this analysis were not reported but are available upon request. Only variants determined to be pathogenic or likely pathogenic are reported in this carrier screening test. We

cannot rule out the possibility that variants classified as uncertain clinical significance may contribute to disease. Variant interpretations, based on current knowledge, may change over time as more information arises.

Technical limitations
This NGS technology may not detect all small insertions/deletions and is not diagnostic for large duplications/deletions, repeat expansions, and structural genomic variation. This test will only detect variants within the exons and the intron-exon boundaries of the target genes as listed in the report table. Variants outside these regions will not be detected. These regions include, but are not limited to, UTRs, promoters, and deep intronic, low coverage areas. In addition, a mutation(s) in a gene not included on the panel could be present in this patient.

Comments
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. 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

For Disease Specific Standards and Guidelines
https://www.acmg.net/
https://www.orpha.net/

Additional Resources: GenomeConnect is an NIH initiative created to enable individuals and families with the same genetic variant or medical history to connect and share de-identified information. If you are interested in participating, please visit www.genomeconnect.org.

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[redacted]

Patient Information:
4405, Donor
DOB: [redacted]
Sex: M
MR#: 4405
Patient#: [redacted]

Partner Information:
Not Tested

Physician:
Seitz, Suzanne
ATTN: Seitz, Suzanne
Fairfax Cryobank
3015 Williams Drive
Fairfax, VA 22031

Laboratory:
Fulgent Genetics
CAP#: 8042697
CLIA#: 05D2043189
Laboratory Director:
Dr. Hanlin (Harry) Gao
Report Date: **Aug 30,2023**

Accession:
[redacted]
Test#: [redacted]
Specimen Type: DNA
Collected: Jul 31,2023

Accession:
N/A

FINAL RESULTS

No carrier mutations identified

TEST PERFORMED

Custom Beacon Carrier Screening Panel

(3 Gene Panel: *ACSF3, GAA, and SLC22A5*; gene sequencing with deletion and duplication analysis)

INTERPRETATION:

Notes and Recommendations:

- No carrier mutations were identified in the submitted specimen. A negative result does not rule out the possibility of a genetic predisposition nor does it rule out any pathogenic mutations in areas not assessed by this test or in regions that were covered at a level too low to reliably assess. Also, it does not rule out mutations that are of the sort not queried by this test; see Methods and Limitations for more information.
- This carrier screening test does not screen for all possible genetic conditions, nor for all possible mutations in every gene tested. Individuals with negative test results may still have up to a 3-4% risk to have a child with a birth defect due to genetic and/or environmental factors.
- Patients may wish to discuss any carrier results with blood relatives, as there is an increased chance that they are also carriers. These results should be interpreted in the context of this individual's clinical findings, biochemical profile, and family history.
- X-linked genes are not routinely analyzed for male carrier screening tests. Gene specific notes and limitations may be present. See below.
- This report does not include variants of uncertain significance.
- Genetic counseling is recommended. Available genetic counselors and additional resources can be found at the National Society of Genetic Counselors (NSGC; https://www.nsgc.org)

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GENES TESTED:

Custom Beacon Carrier Screening Panel - 3 Genes

This analysis was run using the Custom Beacon Carrier Screening Panel gene list. 3 genes were tested with 100.0% of targets sequenced at >20x coverage. For more gene specific information and assistance with residual risk calculation, see the SUPPLEMENTAL TABLE.

ACSF3, GAA, SLC22A5

METHODS:

Genomic DNA was isolated from the submitted specimen indicated above (if cellular material was submitted). DNA was barcoded, and enriched for the coding exons of targeted genes using hybrid capture technology. Prepared DNA libraries were then sequenced using a Next Generation Sequencing technology. Following alignment to the human genome reference sequence (assembly GRCh37), variants were detected in regions of at least 10x coverage. For this specimen, 100.00% and 100.00% of coding regions and splicing junctions of genes listed had been sequenced with coverage of at least 10x and 20x, respectively, by NGS or by Sanger sequencing. The remaining regions did not have 10x coverage, and were not evaluated. Variants were interpreted manually using locus specific databases, literature searches, and other molecular biological principles. To minimize false positive results, any variants that do not meet internal quality standards are confirmed by Sanger sequencing. Variants classified as pathogenic, likely pathogenic, or risk allele which are located in the coding regions and nearby intronic regions (+/- 20bp) of the genes listed above are reported. Variants outside these intervals may be reported but are typically not guaranteed. When a single pathogenic or likely pathogenic variant is identified in a clinically relevant gene with autosomal recessive inheritance, the laboratory will attempt to ensure 100% coverage of coding sequences either through NGS or Sanger sequencing technologies ("fill-in"). All genes listed were evaluated for large deletions and/or duplications. However, single exon deletions or duplications will not be detected in this assay, nor will copy number alterations in regions of genes with significant pseudogenes. Putative deletions or duplications are analyzed using Fulgent Germline proprietary pipeline for this specimen. Bioinformatics: The Fulgent Germline v2019.2 pipeline was used to analyze this specimen.

LIMITATIONS:

General Limitations

These test results and variant interpretation are based on the proper identification of the submitted specimen, accuracy of any stated familial relationships, and use of the correct human reference sequences at the queried loci. In very rare instances, errors may result due to mix-up or co-mingling of specimens. Positive results do not imply that there are no other contributors, genetic or otherwise, to future pregnancies, and negative results do not rule out the genetic risk to a pregnancy. Official gene names change over time. Fulgent uses the most up to date gene names based on HUGO Gene Nomenclature Committee (https://www.genenames.org) recommendations. If the gene name on report does not match that of ordered gene, please contact the laboratory and details can be provided. Result interpretation is based on the available clinical and family history information for this individual, collected published information, and Alamut annotation available at the time of reporting. This assay is not designed or validated for the detection of low-level mosaicism or somatic mutations. This assay will not detect certain types of genomic aberrations such as translocations, inversions, or repeat expansions other than specified genes. DNA alterations in regulatory regions or deep intronic regions (greater than 20bp from an exon) may not be detected by this test. Unless otherwise indicated, no additional assays have been performed to evaluate genetic changes in this specimen. There are technical limitations on the ability of DNA sequencing to detect small insertions and deletions. Our laboratory uses a sensitive detection algorithm, however these types of alterations are not detected as reliably as single nucleotide variants. Rarely, due to systematic chemical, computational, or human error, DNA variants may be missed. Although next generation sequencing technologies and our bioinformatics analysis significantly reduce the confounding contribution

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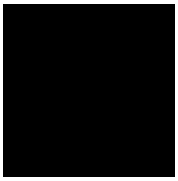

of pseudogene sequences or other highly-homologous sequences, sometimes these may still interfere with the technical ability of the assay to identify pathogenic alterations in both sequencing and deletion/duplication analyses. Deletion/duplication analysis can identify alterations of genomic regions which include one whole gene (buccal swab specimens and whole blood specimens) and are two or more contiguous exons in size (whole blood specimens only); single exon deletions or duplications may occasionally be identified, but are not routinely detected by this test. When novel DNA duplications are identified, it is not possible to discern the genomic location or orientation of the duplicated segment, hence the effect of the duplication cannot be predicted. Where deletions are detected, it is not always possible to determine whether the predicted product will remain in-frame or not. Unless otherwise indicated, deletion/duplication analysis has not been performed in regions that have been sequenced by Sanger.

Gene Specific Notes and Limitations

No gene specific limitations apply to the genes on the tested panel.

SIGNATURE:

Yan Meng, Ph.D., CGMB, FACMG on **8/30/2023 4:15 PM PDT**
Electronically signed

DISCLAIMER:

This test was developed and its performance characteristics determined by **Fulgent Genetics**. It has not been cleared or approved by the FDA. The laboratory is regulated under CLIA as qualified to perform high-complexity testing. This test is used for clinical purposes. It should not be regarded as investigational or for research. Since genetic variation, as well as systematic and technical factors, can affect the accuracy of testing, the results of testing should always be interpreted in the context of clinical and familial data. For assistance with interpretation of these results, healthcare professionals may contact us directly at **(626) 350-0537** or **info@fulgentgenetics.com**. It is recommended that patients receive appropriate genetic counseling to explain the implications of the test result, including its residual risks, uncertainties and reproductive or medical options.

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Supplemental Table

Gene	Condition	Inheritance	Ethnicity	Carrier Rate	Detection Rate	Post-test Carrier Probability*	Residual Risk*
ACSF3	Combined malonic and methylmalonic aciduria	AR	General Population	<1 in 500	98%	1 in 24,951	<1 in 10 million
GAA	Pompe disease	AR	General Population	1 in 100	98%	1 in 4,951	1 in 1,980,400
			African/African American Population	1 in 60	98%	1 in 2,951	1 in 708,240
			East Asian Population	1 in 112	98%	1 in 5,551	1 in 2,486,848
			Ashkenazi Jewish Population	1 in 76	99%	1 in 7,501	1 in 2,280,304
SLC22A5	Systemic primary carnitine deficiency	AR	General Population	1 in 129	99%	1 in 12,801	1 in 6,605,316
			African/African American Population	1 in 86	99%	1 in 8,501	1 in 2,924,344
			East Asian Population	1 in 77	99%	1 in 7,601	1 in 2,341,108
			Faroese Population	1 in 9	99%	1 in 801	1 in 28,836
			Pacific Islander Population	1 in 37	99%	1 in 3,601	1 in 532,948
			South Asian/Indian Population	1 in 51	99%	1 in 5,001	1 in 1,020,204

* For genes that have tested negative

Abbreviations: AR, autosomal recessive; XL, X-linked