Fairfax Cryobank
The Trusted Choice for Donor Sperm

Donor 2977

Genetic Testing Summary

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

Last Updated: 03/10/20

Donor Reported Ancestry: Iranian 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 by genotyping of 97 mutations in the CFTR gene	Insufficient data to estimate residual risk
Spinal Muscular Atrophy (SMA) carrier screening	Negative for deletions of exon 7 in the SMN1 gene	1/628
Special Testing		
Phenylalanine Hydroxylase Deficiency (see attached)	Negative by gene sequencing of the PAH gene	1/279
Alpha Thalassemia (HBA1/HBA2)	Negative by gene sequencing and copy number analysis in the HBA1 and HBA2 genes	aa/aa result 1/380 residual risk
Mitochondrial Myopathy and Sideroblastic Anemia (PUS1)	Negative by gene sequencing of the PUS1 gene	1/333,000

*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.

genzyme

Cystic Fibrosis Mutation Analysis

Patient Name: Donor [redacted]
Referring Physician: [redacted]
Specimen #: [redacted] **Client #:** [redacted]
Patient ID: 6[redacted]-6 **Case #:** [redacted]

Fairfax Cryobank / Genetics and IVF Institute
Genetics and IVF Institute
3015 Williams Drive
Suite 110
Fairfax VA 22031

DOB: Not Given
Sex: M
SSN:

Date Collected: 10/05/2009
Date Received: 10/06/2009
Lab ID: 2977091005
Hospital ID:
Specimen Type: **BLDPER**

Ethnicity: Not Provided

Indication: Carrier test / Gamete donor

RESULTS: Negative for the 97 mutations analyzed

INTERPRETATION

This individual is negative for the mutations analyzed. This result reduces but does not eliminate the risk to be a CF carrier.

COMMENTS:

Mutation Detection Rates among Ethnic Groups Detection rates are based on mutation frequencies in patients affected with cystic fibrosis. Among individuals with an atypical or mild presentation (e.g. congenital absence of the vas deferens, pancreatitis) detection rates may vary from those provided here.

Ethnicity	Carrier risk reduction when no family history	Detection rate	References
African American	1/65 to 1/338	81%	Genet in Med 3:168, 2001
Ashkenazi Jewish	1/26 to 1/834	97%	Am J Hum Genet 51:951, 1994
Asian		Not Provided	Insufficient data
Caucasian	1/25 to 1/343	93%	Genet In Med 3:168, 2001; Genet In Med 4:90, 2002
Hispanic	1/46 to 1/205	78%	Genet in Med 3:168, 2001;www.dhs.ca.gov/pcfh/gdb/html/PDE/CFStudy.htm
Jewish, non-Ashkenazi		Varies by country of origin	Genet Testing 5:47, 2001, Genet Testing, 1:35, 1997
Other or Mixed Ethnicity		Not Provided	Detection rate not determined and varies with ethnicity

This interpretation is based on the clinical and family relationship information provided and the current understanding of the molecular genetics of this condition.

METHOD

DNA is isolated from the sample and tested for the 97 CF mutations listed. Regions of the *CFTR* gene are amplified enzymatically and subjected to a solution-phase multiplex allele-specific primer extension with subsequent hybridization to a bead array and fluorescent detection. The assay discriminates between ΔF508 and the following polymorphisms: F508C, I506V and I507V. In some cases, specific allele identification requires enzymatic amplification followed by hybridization to oligonucleotide probes.

Under the direction of: Ruth A Heim, PhD, FACMG

10/28/09
Date: 10/12/2009

Ruth A. Heim, Ph.D., FACMG

genzyme
GENETICS

MUTATIONS ANALYZED				
ΔF311	3120+1G>A	712-1G>T	Q359K/T360K	S549N
ΔF508	3120G>A	935delA	Q493X	S549R T>G
ΔI507	3171delC	936delTA	Q552X	T338I
1078delT	3199del6	A455E	Q890X	V520F
1288insTA	3659delC	A559T	R1066C	W1089X
1677delTA	3667del4	C524X	R1158X	W1204X
1717-1G>A	3791delC	CFTRdele2,3	R1162X	W1282X
1812-1G>A	3849+10kbC>T	D1152H	R117C	Y1092X C>A
1898+1G>A	3876delA	E60X	R117H	Y1092X C>G
1898+5G>T	3905insT	E92X	R334W	Y122X
1949del84	394delTT	G178R	R347H	
2043delG	4016insT	G330X	R347P	
2055del9>A	405+1G>A	G480C	R352Q	
2105del13ins5	405+3A>C	G542X	R553X	
2108delA	406-1G>A	G551D	R560T	
2143delT	444delA	G85E	R709X	
2183delAA>G	457TAT>G	K710X	R75X	
2184delA	574delA	L206W	R764X	
2184insA	621+1G>T	M1101K	S1196X	
2307insA	663delT	N1303K	S1251N	
2789+5G>A	711+1G>T	P574H	S1255X	
2869insG	711+5G>A	Q1238X	S364P	

False positive or negative results may occur for reasons that include genetic variants, blood transfusions, bone marrow transplantation or somatic heterogeneity of the tissue sample. This test was developed and its performance characteristics determined by Genzyme. It has not been cleared or approved by the U.S. Food and Drug Administration. The FDA has determined that such clearance or approval is not necessary. This test is used for clinical purposes. It should not be regarded as investigational or for research. The laboratory is regulated under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) as qualified to perform high complexity clinical testing.

genzyme

SMN1 Copy N nber Analysis

Patient Name: . Donor # 2977
DOB: Age:
SSN #: Gender: Male

Genzyme Specimen # [redacted]
Case #: [redacted] Patient ID #: [redacted]
Date Collected: 10/05/2009 Date Received: 10/06/2009

606452 / 310544
Fairfax Cryobank / Genetics and IVF Institute
Genetics and IVF Institute
3015 Williams Drive
Suite 110
Fairfax, VA 22031

Referring Physician: Steve Pool
Genetic Counselor:

Client Lab ID #: [redacted]
Hospital ID #:
Specimen ID #:

Specimen Type: Peripheral Blood

Specimen(s) Received: 2 - Yellow (ACD) 10 ml round bottom tube(s)

Clinical Data: Carrier Test/Gamete donor

Ethnicity: Not Provided

RESULTS: SMN1 copy number: 2 (Reduced Carrier Risk)

INTERPRETATION:

This individual has an SMN1 copy number of two. This result reduces but does not eliminate the risk to be a carrier of SMA. Ethnic specific risk reductions based on a negative family history and an SMN1 copy number of two are provided in the Comments section of this report.

COMMENT:

Spinal muscular atrophy (SMA) is an autosomal recessive disease of variable age of onset and severity caused by mutations (most often deletions or gene conversions) in the survival motor neuron (SMN1) gene. Molecular testing assesses the number of copies of the SMN1 gene. Individuals with one copy of the SMN1 gene are predicted to be carriers of SMA. Individuals with two or more copies have a reduced risk to be carriers. (Affected individuals have 0 copies of the SMN1 gene.)

This copy number analysis cannot detect individuals who are carriers of SMA as a result of either 2 (or very rarely 3) copies of the SMN1 gene on one chromosome and the absence of the SMN1 gene on the other chromosome or small intragenic mutations within the SMN1 gene. This analysis also will not detect germline mosaicism or mutations in genes other than SMN1. Additionally, de novo mutations have been reported in approximately 2% of SMA patients.

Carrier Frequency and Risk Reductions for Individuals with No Family History of SMA

Ethnicity	Detection Rate[1]	A priori Carrier Risk[1]	Reduced Carrier Risk for 2 copy result	Reduced Carrier Risk for 3 copy result
Caucasian	94.9%	1:35	1:632	1:3,500
Ashkenazi Jewish	90.2%	1:41	1:350	1:4,000
Asian	92.6%	1:53	1:628	1:5,000
Hispanic	90.6%	1:117	1:1061	1:11,000
African American	71.1%	1:66	1:121	1:3,000
Mixed Ethnicities	For counseling purposes, consider using the ethnic background with the most conservative risk estimates.			

METHOD/LIMITATIONS:
Specimen DNA is isolated and amplified by real-time polymerase chain reaction (PCR) for exon 7 of the SMN1 gene and two reference genes. A mathematical algorithm is used to calculate the number of copies of SMN1. Sequencing of the primer and probe binding sites for the SMN1 real-time PCR reaction is performed on all fetal samples, and on samples from individuals with 1 copy of SMN1 on carrier testing, to rule out the presence of sequence variants which could interfere with analysis and interpretation. False positive or negative results may occur for reasons that include genetic variants, blood transfusions, bone marrow transplantation, erroneous representation of family relationships or contamination of a fetal sample with maternal cells.

REFERENCES:
1. Carrier frequency and detection rate are calculated based on analysis of allele frequencies among > 1000 individuals from each ethnic group noted (Genzyme Genetics, data submitted for publication). 2. Online review of SMA: http://www.genereviews.org/profiles/sma

The test was developed and its performance characteristics have been determined by Genzyme. The laboratory is regulated under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) as qualified to perform high complexity clinical testing. This test must be used in conjunction with clinical assessment, when available.

10/28/09

Electronically Signed by: Hui Zhu, Ph.D. FACMG, on 10/12/2009

Reported by:

Testing performed at Genzyme Genetics 3400 Computer Drive, Westborough, MA 01581 1-800-255-7357

GENETICS & IVF
Institute

Celebrating 25 Years of Excellence

Cytogenetic Report

Client Fairfax Cryobank [redacted]

Address [redacted]

Reporting Phone # [redacted] **Fax #** [redacted] **Email** N/A

Patient name/Donor Alias Donor #2977 **Patient DOB** N/A

Donor # 2977-091015 **Specimen type** Peripheral Blood

Collection Date 10/15/2009 **Accession #** 09-061CG

Date Received 10/16/2009

RESULTS

CYTOGENETIC ANALYSIS

Cells counted	20	**Type of banding**	GTG
Cells analyzed	5	**Band resolution**	550
Cells karyotyped	2		
Modal chromosome #	46		

FISH

Probe(s) N/A

Nuclei scored N/A

KARYOTYPE 46,XY

INTERPRETATION

Normal male karyotype

No numerical or structural abnormalities were identified. This normal cytogenetic result does not exclude the possibility of the presence of subtle rearrangements beyond the technical limits of detection with this test.

Comments

Wayne S. Stanley

Wayne S. Stanley, Ph.D., FACMG
Clinical Cytogeneticist

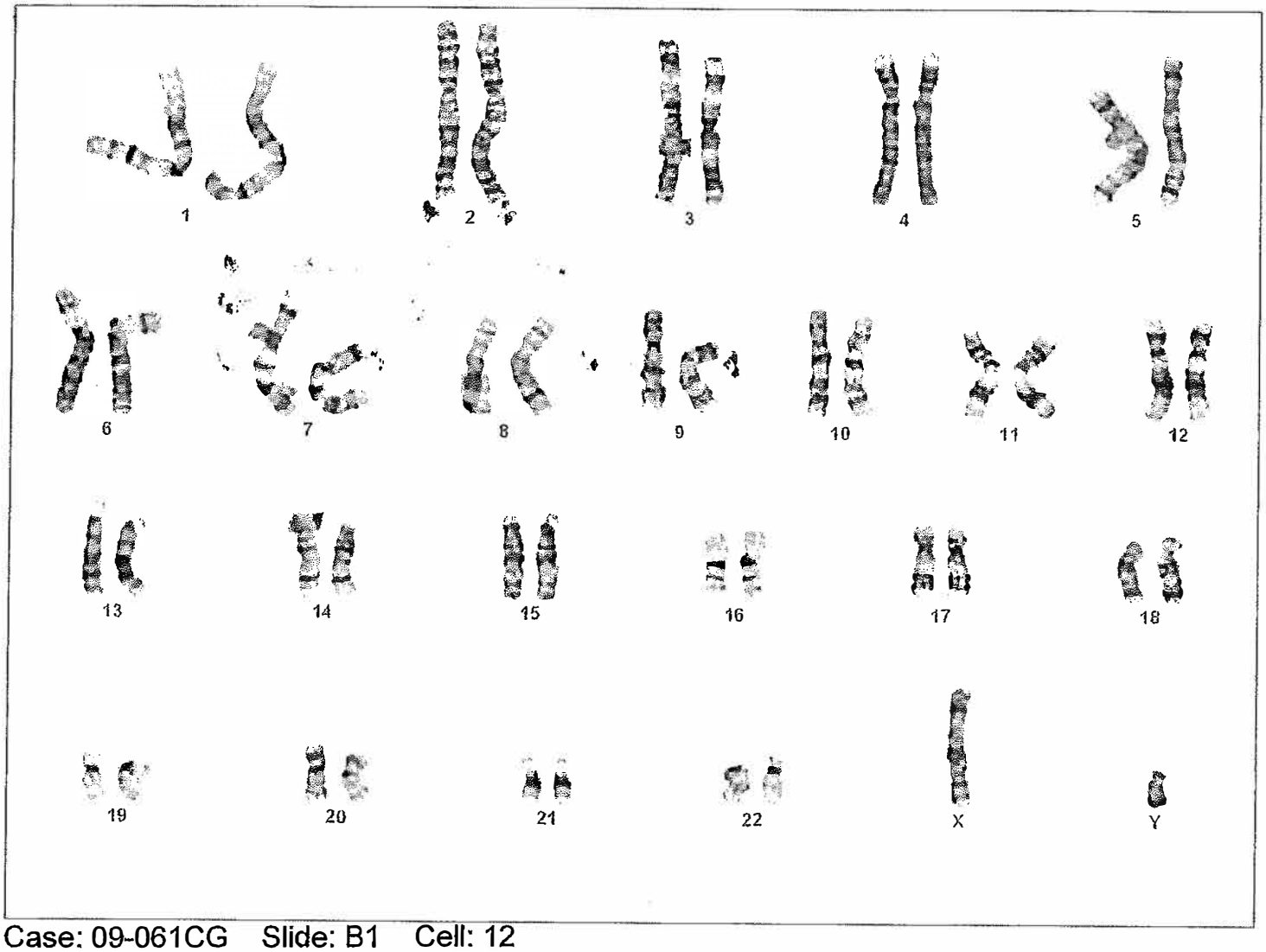
10/28/09
Date

Genetics and IVF Preimplantation Genetics Laboratory

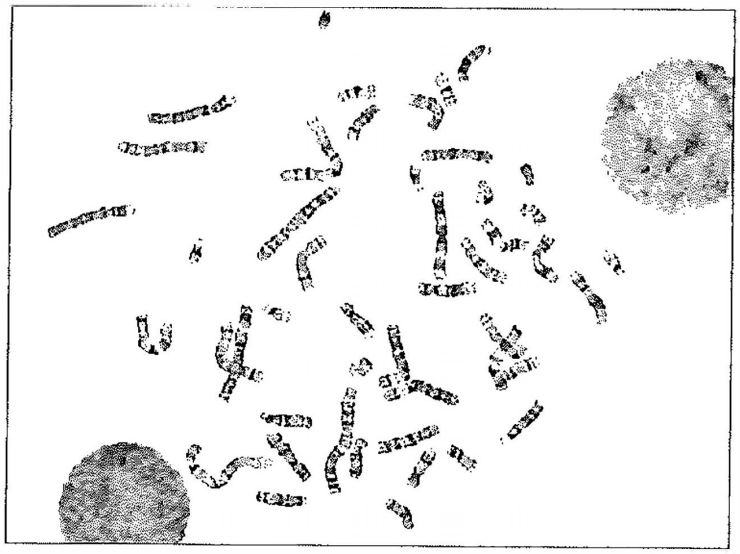
Patient name: DONOR #2977

Case name: 09-061CG

46,XY



Case: 09-061CG Slide: B1 Cell: 12



QUEST DIAGNOSTICS INCORPORATED
CLIENT SERVICE 800.758.6047

SPECIMEN INFORMATION
SPECIMEN: [redacted]
REQUISITION: [redacted]
LAB REF NO:

COLLECTED: 10/05/2009 00:00
RECEIVED: 10/06/2009 06:21
REPORTED: 10/08/2009 11:00

PATIENT INFORMATION
DONOR#2977,ONLY

DOB: Age:
GENDER: M Fasting: U

ID: 2977-091005

REPORT STATUS **Final**

ORDERING PHYSICIAN
[redacted]

CLIENT INFORMATION
41550
FAIRFAX CRYOBANK
[redacted]

Test Name	In Range	Out of Range	Reference Range	Lab
HEMOGLOBINOPATHY EVALUATION				
HEMOGLOBINOPATHY INDICES				IG
RED BLOOD CELL COUNT	5.42		4.20-5.80 Million/uL	
HEMOGLOBIN		**17.2 H**	13.2-17.1 g/dL	
HEMATOCRIT		**50.8 H**	38.5-50.0 %	
MCV	93.8		80.0-100.0 fL	
MCH	31.8		27.0-33.0 pg	
RDW	13.2		11.0-15.0 %	
HEMOGLOBINOPATHY EVALUATION				IG
HEMOGLOBIN A	97.7		>96.0 %	
HEMOGLOBIN F	<1.0		<2.0 %	
HEMOGLOBIN A2 (QUANT)	2.3		1.8-3.5 %	
INTERPRETATION	NORMAL PHENOTYPE.			
CHOLESTEROL, TOTAL	192		125-200 mg/dL	IG
AST	29		10-35 U/L	IG
ALT		**68 H**	9-60 U/L	IG
CBC (INCLUDES DIFF/PLT)				IG
WHITE BLOOD CELL COUNT	7.7		3.8-10.8 Thousand/uL	
RED BLOOD CELL COUNT	5.42		4.20-5.80 Million/uL	
HEMOGLOBIN		**17.2 H**	13.2-17.1 g/dL	
HEMATOCRIT		**50.8 H**	38.5-50.0 %	
MCV	93.8		80.0-100.0 fL	
MCH	31.8		27.0-33.0 pg	
MCHC	33.9		32.0-36.0 g/dL	
RDW	13.2		11.0-15.0 %	
PLATELET COUNT	176		140-400 Thousand/uL	
ABSOLUTE NEUTROPHILS	4135		1500-7800 cells/uL	
ABSOLUTE LYMPHOCYTES	2641		850-3900 cells/uL	
ABSOLUTE MONOCYTES	739		200-950 cells/uL	
ABSOLUTE EOSINOPHILS	154		15-500 cells/uL	
ABSOLUTE BASOPHILS	31		0-200 cells/uL	
NEUTROPHILS	53.7		%	
LYMPHOCYTES	34.3		%	
MONOCYTES	9.6		%	
EOSINOPHILS	2.0		%	
BASOPHILS	0.4		%	

10/28/09

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CARRIER SCREENING REPORT

Patient	Sample	Referring Doctor
Patient Name: Donor 2977 **Date of Birth:** [redacted] **Reference #:** [redacted] **Indication:** [redacted] **Test Type:** NGS single gene full sequencing test	**Specimen Type:** Semen **Lab #:** [redacted] **Date Collected:** 2/14/2018 **Date Received:** 2/24/2018 **Final Report:** 3/6/2018	[redacted] **Fairfax Cryobank, Inc.** [redacted] [redacted] [redacted] Fax: [redacted]

RESULT SUMMARY

NGS single gene full sequencing test

Results: No clinically significant variant(s) detected

Gene(s) analyzed: *PAH*

Interpretation: Screening for the presence of pathogenic variants in the *PAH* gene which is associated with phenylalanine hydroxylase deficiency 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. This negative result does not rule out the possibility that a pathogenic variant in the gene examined is present.

Genetic counseling is recommended.

This technology may not detect all small insertion/deletions and is not diagnostic for large duplications/deletions and structural genomic variation. The coding DNA sequence of the gene plus at least five base pairs flanking splice sites were sequenced and analyzed relative to the hg19 assembly. A mutation(s) deep in intronic sequences or in untranslated regions of the gene except a portion described above or a pathogenic variant(s) in other genes not included in this test could be present in this patient. The analytical sensitivity of this test is estimated at 99% for single base substitutions and 97% overall. All potentially pathogenic variants were subjected to Sanger sequencing or genotyping by allele specific primer extension analysis for confirmation of the result. Any benign variants identified during this analysis were not reported.

Please note that 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.

Comments: This test was developed and its performance characteristics were determined by Mount Sinai Genomics, Inc. It is considered acceptable for patient testing. It has not been cleared or approved by the FDA. The FDA has determined that such clearance or approval is not necessary.

Patient:	Donor 2977	DOB:	[redacted]	Lab #:	[redacted]

This type of mutation analysis generally provides highly accurate genotype information for point mutations and single nucleotide polymorphisms. Despite this level of accuracy, it should be kept in mind that there are many potential sources of diagnostic error, including misidentification of samples, polymorphisms, mosaicism or other rare genetic variants that interfere with analysis. In addition, families should understand the limitations of the testing and that rare diagnostic errors may occur for the reasons described.

For Disease Specific Standards and Guidelines:
https://www.acmg.net/
Additional disease-specific references available upon request.

This case has been reviewed and electronically signed by Guiqing Cai, Ph.D., DABMGG, Associate Laboratory Director

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

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
Phenylalanine Hydroxylase Deficiency (AR)	*PAH*	Caucasian	1 in 50	94%	1 in 818	>95%
NM_000277.1		African	1 in 143	87%	1 in 1093	>95%
		Asian	1 in 78	79%	1 in 368	>95%
		Ashkenazi Jewish	1 in 225	75%	1 in 897	93%
		Worldwide	1 in 65	77%	1 in 279	>95%
		Turkish	1 in 32	63%	1 in 85	>95%
		Irish	1 in 34	91%	1 in 368	92%
		Sicilian	1 in 26	48%	1 in 49	>95%
		Sephardic Jewish - Iranian, Bukharian, Kavkazi, Tunisian and Moroccan	1 in 18	88%	1 in 143	91%

AR: Autosomal Recessive

Patient Information
Name: Donor 2977
Date of Birth: [redacted]
Sema4 [redacted]
Client [redacted]
Indication: Carrier Testing

Specimen Information
Specimen Type: Purified DNA semen
Date Collected: 01/30/2020
Date Received: 02/06/2020
Final Report: 02/21/2020

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

Custom Carrier Screen (ECS)

Number of genes tested: 3

SUMMARY OF RESULTS AND RECOMMENDATIONS

Negative

Negative for all genes tested: *PUS1, and HBA1/HBA2*
To view a full list of genes and diseases tested
please see Table 1 in this report

AR=Autosomal recessive; XL=X-linked

Recommendations

- Individuals of Asian, African, Hispanic and Mediterranean ancestry should also be screened for hemoglobinopathies by CBC and hemoglobin electrophoresis.
- 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 the genes listed above using one or more of the following methodologies: target capture and short-read sequencing, long-range PCR followed by short-read sequencing, targeted genotyping, and/or 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 view the Table of Residual Risks Based on Ethnicity at the end of this report or at **go.sema4.com/residualrisk** for gene transcripts, sequencing exceptions, specific detection rates, and residual risk estimates after a negative screening result. With individuals of mixed ethnicity, it is recommended to use the highest residual risk estimate. Only known pathogenic or likely pathogenic variants are reported. This carrier screening test does not report likely benign variants and variants of uncertain significance (VUS). If reporting of likely benign variants and VUS are desired in this patient, please contact the laboratory at 800-298-6470, option 2 to request an amended report.

Rebekah Zimmerman, 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**				
Alpha-Thalassemia	*HBA1/HBA2*	AR	Reduced Risk (see table below)	*HBA1* Copy Number: 2 *HBA2* Copy Number: 2 No pathogenic copy number variants detected *HBA1/HBA2* Sequencing: Negative
Mitochondrial Myopathy and Sideroblastic Anemia 1	*PUS1*	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
Alpha-Thalassemia (AR) NM_000558.4 / NM_000517.4	*HBA1/HBA2*	European (Non-Finnish)	1 in 500	95%	1 in 10,000	99%
		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	
Mitochondrial Myopathy and Sideroblastic Anemia 1 (AR) NM_025215.5 *Exception:* chr12:132,414,446 - 132,414,532 (partial exon 2)	*PUS1*	African	1 in 2039	99%	1 in 204,000	99%
		Finnish	1 in 2001	99%	1 in 200,000	
		European (Non-Finnish)	1 in 4496	99%	1 in 449,000	
		Native American	1 in 3203	99%	1 in 320,000	
		South Asian	1 in 5130	99%	1 in 513,000	
		Worldwide	1 in 3330	99%	1 in 333,000	
		Sephardic Jewish - Iranian	N/A	99%	N/A	

* 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:

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^{TM}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.

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.