



Donor 4598

Genetic Testing Summary

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

Last Updated: 09/16/21

Donor Reported Ancestry: Italian, Irish, Norwegian

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 for 99 variants in the CFTR gene	1/300
Spinal Muscular Atrophy (SMA) carrier screening	Negative for deletions of exon 7 in the SMN1 gene	1/610
Hb Beta Chain-Related Hemoglobinopathies (including Beta Thalassemia and Sickle Cell Disease)	Negative by genotyping for 28 variants in the HBB gene	1/290
Tay Sachs Enzyme Analysis	Non-Carrier	
Special Testing		
Pseudocholinesterase Deficiency (BCHE)	Carrier	
Gitelman Syndrome (SLC12A3)	Negative by gene sequencing	1/290
Glycine Encephalopathy (GLDC)	Negative by gene sequencing	1/760
Renal Tubular Acidosis and Deafness (ATP6V1B1)	Negative by gene sequencing	1/6600
Spinal Muscular Atrophy (SMN1)	Negative by gene sequencing and variant analysis	1/894
Glycogen Storage Disease Type II (GAA)	Negative by gene sequencing	1/520

*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.



Results Recipient



Report Date: 05/16/2013

Male

Name: DONOR 4598

DOB: [REDACTED]

Ethnicity: Northern European

Sample Type: OG-510 Saliva

Date of Collection: 05/13/2013

Date Received: 05/14/2013

Barcode: [REDACTED]

Indication: Egg or Sperm Donor

Female

Not tested

Counsyl Test Results Summary (Egg or Sperm Donor)

The Counsyl test (Fairfax Cryobank Fundamental Panel) uses copy number analysis and targeted genotyping as described in the methods section on page 2 to determine carrier status associated with 3 diseases. Please refer to page 3 for a complete list of diseases and genes included in this panel.



DONOR 4598



DONOR 4598's DNA test shows that he is not a carrier of any disease-causing mutation tested.



Partner

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

Reproductive Risk Summary

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

Clinical Notes

- If necessary, patients can discuss residual risks with their physician or a genetic counselor. To schedule a complimentary appointment to speak with a genetic counselor about these results, please visit counsyl.com/counseling/.



Male

Name: DONOR 4598

DOB: [REDACTED]

Female

Not tested

Methods and Limitations

DONOR 4598: targeted genotyping and copy number analysis.

Targeted genotyping: Targeted DNA mutation analysis is used to simultaneously determine the genotype of 127 variants associated with 2 diseases. The test is not validated for detection of homozygous mutations, and although rare, asymptomatic individuals affected by the disease may not be genotyped accurately.

Copy number analysis: Targeted copy number analysis is used to determine the copy number of exon 7 of the SMN1 gene relative to other genes. Other mutations may interfere with this analysis. Some individuals with two copies of SMN1 are carriers with two SMN1 genes on one chromosome and a SMN1 deletion on the other chromosome. In addition, a small percentage of SMA cases are caused by nondeletion mutations in the SMN1 gene. Thus, a test result of two SMN1 copies significantly reduces the risk of being a carrier; however, there is still a residual risk of being a carrier and subsequently a small risk of future affected offspring for individuals with two or more SMN1 gene copies. Some SMA cases arise as the result of de novo mutation events which will not be detected by carrier testing.

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, bone marrow transplantation, blood transfusions and technical errors. The Counsyl test does not fully address all inherited forms of intellectual disability, birth defects and genetic disease. A family history of any of these conditions may warrant additional evaluation. Furthermore, not all mutations will be identified in the genes analyzed and additional testing may be beneficial for some patients. For example, individuals of African, Southeast Asian, and Mediterranean ancestry are at increased risk for being carriers for hemoglobinopathies, which can be identified by CBC and hemoglobin electrophoresis or HPLC (*ACOG Practice Bulletin No. 78. Obstet Gynecol 2007;109:229-37*).

This test was developed and its performance characteristics determined by Counsyl, Inc. It has not been cleared or approved by the US Food and Drug Administration (FDA). The FDA does not require this test to go through premarket review. This test is used for clinical purposes. It should not be regarded as investigational or for research. This laboratory is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) as qualified to perform high-complexity clinical testing. These results are adjunctive to the ordering physician's workup. CLIA Number: #05D1102604.

Lab Director:

H. Peter Kang, MD



Male

Name: DONOR 4598

DOB [REDACTED]

Female

Not tested

ENTERED
Nov 5/28/13

Diseases Tested

✓ **Cystic Fibrosis** - Gene: CFTR. Variants (99): 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, 2143delT, 394delTT, 444delA, 1078delT, 3876delA, 3905insT, 1812-1G>A, 3272-26A>G, 2183AA>G, S549R(A>C), R117C, L206W, G330X, T338I, R352Q, S364P, G480C, C524X, S549R(T>G), Q552X, A559T, G622D, R709X, K710X, R764X, Q890X, R1066C, W1089X, Y1092X, R1158X, S1196X, W1204X(c.3611G>A), Q1238X, S1251N, S1255X, 3199delG, 574delA, 663delT, 935delA, 936delTA, 1677delTA, 1949delB4, 2043delG, 2055delG>A, 2108delA, 3171delC, 3667delA, 3791delC, 1288insTA, 2184insA, 2307insA, 2869insG, 296+12T>C, 405+1G>A, 405+3A>C, 406-1G>A, 711+5G>A, 712-1G>T, 1898+1G>T, 1898+5G>T, 3120G>A, 457TAT>G, 3849+4A>G, Q359K/T360K. Detection rate: Northern European 91%.

✓ **Hb Beta Chain-Related Hemoglobinopathy (Including Beta Thalassemia and Sickle Cell Disease)** - Gene: HBB. Variants (28): Hb S, K17X, Q39X, 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), -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, W15X, Gly16fs, Glu6fs, Hb E, Hb D-Punjab, Hb O-Arab. Detection rate: Northern European 83%.

✓ **Spinal Muscular Atrophy (copy number analysis only)** - Gene: SMN1. Variant (1): SMN1 copy number. Detection rate: Northern European 95%.



Male

Name: DONOR 4598

DOB: [REDACTED]

Female

Not tested

Risk Calculations

Below are the risk calculations for all diseases tested. Since negative results do not completely rule out the possibility of being a carrier, the **residual risk** represents the patient's post-test likelihood of being a carrier and the **reproductive risk** represents the likelihood the patient's future children could inherit each disease. These risks are inherent to all carrier screening tests, may vary by ethnicity, are predicated on a negative family history and are present even after a negative test result. Inaccurate reporting of ethnicity may cause errors in risk calculation.

Disease	DONOR 4598 Residual Risk	Reproductive Risk
Cystic Fibrosis	1 in 300	1 in 33,000
Hb Beta Chain-Related Hemoglobinopathy (Including Beta Thalassemia and Sickle Cell Disease)	1 in 290	1 in 58,000
Spinal Muscular Atrophy	SMN1: 2 copies 1 in 610	1 in 84,000

Ordering Practice:

Practice Code

Fairfax Cryobank

Report Generated: 2015-09-01

4598 4598

DOB:

Gender: Male

Ethnicity: European

Procedure ID: 28698

Kit Barcode:

Method: Genotyping

Specimen: Blood, #30116

Specimen Collection: 2015-08-24

Specimen Received: 2015-08-25

Specimen Analyzed: 2015-09-01

Partner Not Tested

SUMMARY OF RESULTS

MUTATION(S) IDENTIFIED

Disease

4598 4598

Partner Not Tested

Pseudocholinesterase
Deficiency

● Treatment Benefits

● Moderate Impact

Carrier (1 abnormal copy)

Mutation: c.A293G (p.D98G)

Gene: BCHE

Method: Genotyping



Reproductive risk detected. Consider partner testing.



All other mutations analyzed were not detected, reducing but not eliminating your chance to be a carrier for the associated genetic diseases. A list of all the diseases and mutations you were screened for is included later in this report. The test does not screen for every possible genetic disease.

For disease information, please visit www.recombine.com/diseases. To speak with a Genetic Counselor, call [855.OUR.GENES](tel:855.OUR.GENES).

♂ Male

Panel: Smith-Lemli-Opitz Syndrome, Hereditary Fructose Intolerance, Pseudocholinesterase Deficiency, Diseases

Tested: 3, Mutations Tested: 32, Genes Tested: 3, Null Calls: 0

Assay performed by

Reprogenetics

CLIA ID: 31D1054821

Lab Technician Bo Chu

Reviewed by Pere Colls, PhD, HCLD, Lab Director

ENTERED
2015-09-04

Pseudocholinesterase Deficiency

Pseudocholinesterase Deficiency occurs when the BCHE gene, which is involved in the breakdown of certain drugs in the body, is defective. The condition results in an increased sensitivity to certain muscle relaxant drugs used during general anesthesia, called choline esters. Individuals with this condition typically do not have any symptoms, but may experience temporary paralysis during administration of these drugs and may require a ventilator to assist with breathing until the drugs are cleared from the body.

Clinical Information

Physical Impairment	●
Cognitive Impairment	
Shortened Lifespan	
Effective Treatment	●

- Treatment Benefits
- Moderate Impact

Status

4598 4598:

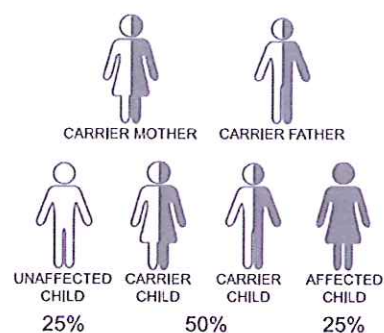
Carrier (1 abnormal copy)

Mutation: c.A293G (p.D98G)

Gene: BCHE

Method: Genotyping

Inheritance



Treatment

Treatment is only necessary during administration of choline ester drugs. Affected individuals should be placed on a ventilator during choline ester administration.

Prognosis

There are no associated symptoms with this disease other than the response to choline ester medications. Affected individuals are at risk for respiratory arrest when these medications are used, and therefore use of a ventilator during drug administration may be necessary.

To learn more visit <http://recombine.com/diseases/pseudocholinesterase-deficiency>

Carrier Risk Assessment

Ethnicity	Detection Rate	Pre-Test Risk	Post-Test Risk
General	65%	1/33	1/94
Iranian Jewish	>99%	1/9	1/900

Methods and Limitations

Genotyping: Genotyping is performed using the Illumina Infinium Custom HD Genotyping assay to identify mutations in >200 genes. The assay is not validated for homozygous mutations, and it is possible that individuals affected with disease may not be accurately genotyped.

Limitations: In some cases, genetic variations other than that which is being assayed may interfere with mutation detection, resulting in false-negative or false-positive results. Additional sources of error include, but are not limited to: sample contamination, sample mix-up, bone marrow transplantation, blood transfusions, and technical errors.

The test does not test for all forms of genetic disease, birth defects, and intellectual disability. All results should be interpreted in the context of family history; additional evaluation may be indicated based on a history of these conditions. Additional testing may be necessary to determine mutation phase in individuals identified to carry more than one mutation in the same gene. All mutations included within the genes assayed may not be detected, and additional testing may be appropriate for some individuals.

Diseases & Mutations Assayed

● High Impact
 ● Treatment Benefits
 ● X-Linked
 ● Moderate Impact

H	T	X	M	Disease	#	Mutations
<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	Hereditary Fructose Intolerance	10	♂ Genotyping c.357_360delAAAC, c.C1005G (p.N335K), c.C524A (p.A175D), c.G448C (p.A150P), c.T612G (p.Y204X), c.865_867delCTT (p.289delI), c.C720A (p.C240X), c.T442C (p.W148R), c.C178T (p.R60X), c.C10T (p.R4X)
<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	Pseudocholinesterase Deficiency	1	♂ Genotyping c.A293G (p.D98G)
<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	Smith-Lemli-Opitz Syndrome	21	♂ Genotyping c.964-1G>C, c.A356T (p.H119I), c.C1054T (p.R352W), c.C1210T (p.R404C), c.C278T (p.T93M), c.G1055A (p.R352Q), c.G1139A (p.C380Y), c.G1337A (p.R446Q), c.G452A (p.W151X), c.G453A (p.W151X), c.G744T (p.W248C), c.G976T (p.V326I), c.T326C (p.L109P), c.T470C (p.L157P), c.1342G>A (p.E448K), c.1228G>A (p.G410S), c.906C>G (p.F302I), c.725G>A (p.R242H), c.724C>T (p.R242C), c.506C>T (p.S169I), c.A1G (p.M1V)

ENTERED
609-04-15

Patient Information

Name: Donor 4598

Date of Birth: [REDACTED]

Sema4 ID: [REDACTED]

Client ID: [REDACTED]

Indication: Carrier Testing

Specimen Information

Specimen Type: Purified DNA

Date Collected: 05/08/2020

Date Received: 05/12/2020

Final Report: 05/30/2020

Referring Provider

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

Custom Carrier Screen (ECS)

Number of genes tested: 4

SUMMARY OF RESULTS AND RECOMMENDATIONS

 **Negative****Negative for all genes tested: *ATP6V1B1*, *GLDC*, *SLC12A3*, and *SMN1***

To view a full list of genes and diseases tested

please see Table 1 in this report

AR=Autosomal recessive; XL=X-linked

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 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.

**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				
Gitelman Syndrome	<i>SLC12A3</i>	AR	Reduced Risk (see table below)	
Glycine Encephalopathy (GLDC-Related)	<i>GLDC</i>	AR	Reduced Risk (see table below)	
Renal Tubular Acidosis and Deafness	<i>ATP6V1B1</i>	AR	Reduced Risk (see table below)	
Spinal Muscular Atrophy	<i>SMN1</i>	AR	Reduced Risk (see table below)	<i>SMN1</i> copy number: 2 <i>SMN2</i> copy number: 1 c.*3+80T>G: Negative

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
Gitelman Syndrome (AR) NM_000339.2	SLC12A3	African	1 in 138	78%	1 in 620	98%
		Ashkenazi Jewish	1 in 121	98%	1 in 6,000	
		East Asian	1 in 28	88%	1 in 230	
		Finnish	1 in 239	46%	1 in 450	
		European (Non-Finnish)	1 in 73	75%	1 in 290	
		Native American	1 in 131	82%	1 in 730	
		South Asian	1 in 145	68%	1 in 460	
		Worldwide	1 in 82	78%	1 in 370	
Glycine Encephalopathy (GLDC-Related) (AR) NM_000170.2	GLDC	African	1 in 515	49%	1 in 1,000	95%
		East Asian	1 in 137	42%	1 in 240	
		Finnish	1 in 112	85%	1 in 740	
		European (Non-Finnish)	1 in 255	66%	1 in 760	
		Native American	1 in 323	51%	1 in 660	
		South Asian	1 in 570	56%	1 in 1,300	
		Worldwide	1 in 246	64%	1 in 690	
Exception: Exon 1						
Renal Tubular Acidosis and Deafness (AR) NM_001692.3	ATP6V1B1	African	1 in 524	92%	1 in 6,700	99%
		East Asian	1 in 719	91%	1 in 7,800	
		European (Non-Finnish)	1 in 1092	84%	1 in 6,600	
		Native American	1 in 2097	99%	1 in 210,000	
		South Asian	1 in 1282	99%	1 in 128,000	
		Worldwide	1 in 995	85%	1 in 6,500	
		Sephardic Jewish - Syrian	1 in 140	99%	1 in 13,900	
Spinal Muscular Atrophy (AR) NM_000344.3	SMN1					

Ethnicity	Carrier Frequency	Detection rate	Residual risk after negative result*	Detection rate with <i>SMN1</i> c.*3+80T>G	Residual risk c.*3+80T>G negative	Residual risk c.*3+80T>G positive	Residual Risk with ≥ 3 Copies of <i>SMN1</i>
African American	1 in 85	71%	1 in 160	91%	1 in 455	1 in 49	1 in 4,300
Ashkenazi Jewish	1 in 76	90%	1 in 672	93%	1 in 978	1 in 10	1 in 4,800
East Asian	1 in 53	94%	1 in 864	95%	1 in 901	1 in 12	1 in 4,900
European (Non-Finnish)	1 in 48	95%	1 in 803	95%	1 in 894	1 in 23	1 in 4,900
Native American	1 in 63	91%	1 in 609	94%	1 in 930	1 in 47	1 in 4,800
South Asian	1 in 103	87%	1 in 637	87%	1 in 637	1 in 608	1 in 4,700
Sephardic Jewish	1 in 34	96%	1 in 696	97%	1 in 884	1 in 12	1 in 4,900

*Residual risk with two copies *SMN1* detected using dosage sensitive methods. The presence of three or more copies of *SMN1* reduces the risk of being an *SMN1* carrier between 5-10 fold, depending on ethnicity.

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

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

§ 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™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 $\Delta\Delta C_t$ 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.

Patient Information

Name: Donor 4598

Date of Birth: [REDACTED]

Sema4 ID: [REDACTED]

Client: [REDACTED]

Indication: Carrier Screening

Specimen Information

Specimen Type: Purified DNA

Date Collected: 05/08/2020

Date Received: 05/12/2020

Final Report: 08/01/2021

Referring Provider

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

Unmask Additional Gene(s) V1E

Number of genes tested: 1

SUMMARY OF RESULTS AND RECOMMENDATIONS

 **Negative****Negative for all genes tested: GAA**To view a full list of genes and diseases tested
please see Table 1 in this report*AR=Autosomal recessive; XL=X-linked***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				
Glycogen Storage Disease, Type II	GAA	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
Glycogen Storage Disease, Type II (AR) NM_000152.3	GAA	African	1 in 71	82%	1 in 380	99%
		Ashkenazi Jewish	1 in 76	97%	1 in 3,000	
		East Asian	1 in 63	78%	1 in 280	
		Finnish	1 in 366	59%	1 in 890	
		European (Non-Finnish)	1 in 49	91%	1 in 520	
		Native American	1 in 95	86%	1 in 690	
		South Asian	1 in 133	91%	1 in 1,500	
		Worldwide	1 in 71	87%	1 in 530	

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

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

‡ 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 (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 20 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.*380T>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.*380T>G is likely indicative of a silent (20) carrier. In individuals with two copies of *SMN1* with African American, Hispanic or Caucasian ancestry, the presence or absence of c.*380T>G significantly increases or decreases, respectively, the likelihood of being a silent 20 carrier.

Pathogenic or likely pathogenic sequence variants in exon 7 may be detected during testing for the c.*380T>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).

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 $\Delta\Delta C_t$ 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.

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Additional disease-specific references available upon request.