

Patient Information

Name: 5678 Donor

Date of Birth: 05/1993

Sema4 ID: 19178651CF

Client ID: TSBCA-S4DONOR5678

Indication: Carrier Testing

Specimen Information

Specimen Type: Saliva

Date Collected: 07/17/2019

Date Received: 07/18/2019

Final Report: 07/29/2019

Referring Provider

Lorraine Bonner, M.D.

The Sperm Bank of California

2115 Milvia Street

Berkeley, CA, 94704

Fax: 510-841-0332

Custom Carrier Screen (ECS)

Number of genes tested: 2

SUMMARY OF RESULTS AND RECOMMENDATIONS

Negative

Negative for all genes tested: CFTR, and SMN1

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 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.

Anastasia Larmore, Ph.D., Assistant Laboratory Director

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

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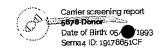
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Genes and diseases tested

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
0	Negative				
- ,;	Cystic Fibrosis	CFTR	AR	Reduced Risk (see table below)	
	Spinal Muscular Atrop	iny SMN1	AR	Reduced Risk (see table below)	SMN2 copy number: 2 SMN2 copy number: 1 c."3+80T>Q 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
Cystic Fibrosis (AR)	CFTR	African	1 in 58	91%	1 in 630	99%
•	• • • • • • • • • • • • • • • • • • • •	Ashkenazi Jewish	1 in 24	98%	1 in 1,200	
NM_000492.3		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	
		Worldwide	1 in 33	94%	1 in 500	
Exception: Exon 30						

Soinal	Muscular	Atrophy	(AR)	

NM_000344.3

Ethnicity	Carrier Frequency	Detection rate	Residual risk after negative result*	Detection rate with SMN1 c."3+80T>G	Residual risk c.*3+80T>G negative	Residual risk c. 3+80T>G positive	Residual Risk with ≥3 Copies of SMN1
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
Caucasian	1in48	95%	1in803	95%	1 in 894	1in23	1 in 4,900
Latino	1in63	91%	1in609	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	1in34	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.

SMN1

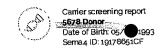
^{*} Carrier detection by HEXA enzyme analysis has a detection rate of approximately 98% (Applies to HEXA gene testing only).

⁺ Carrier frequencies include milder and reduced penetrance forms of the disease. Therefore, carrier frequencies may appear higher than reported in the literature (Applies to BTD, Fg. GJB2,

[‡] Please note that G/Bz testing includes testing for the two upstream deletions, del(GJB6-D13S1830) and del(GJB6-D13S1854) (PMID::1807148 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.

Agillent SureSelectTMQXT 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 $\Delta\Delta$ Ct formula.

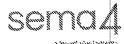
Long-Range PCR (Analytical Detection Rate >99%)

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.

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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 priorir sk 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.





Janine Mash, LCGC
Licensed, Certified Genetic Counselor
San Francisco Genetic Counseling
genetics@tsbca.org
June 26, 2019

DIS 5678 CARRIER SCREENING

Donor in screening (DIS) 5678 is of **Portuguese/Spanish & Native American descent**. Based on his ethnicity, the diseases it is recommended he be screened for: **cystic fibrosis and spinal muscular atrophy.**

PREVIOUS SCREENING COMPLETED:

DIS 5678 had a complete blood count (CBC) on 6/4/2019. From this result his mean corpuscular volume (MCV) was 86.4, and his hemoglobin electrophoresis concluded "consistent with a normal hemoglobin phenotype".

I reviewed this report on 06/26/19.

No further DNA screening is indicated.

CARRIER SCREENING CONSENT

On 06/26/19, I provided genetic counseling for carrier screening and genetic testing. Donor 5678 provided verbal consent to test for cystic fibrosis and spinal muscular atrophy.

Sincerely,

JUL 3 1 2019

Janine Mash, LCGC Certified Genetic Counselor San Francisco Genetic Counseling