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Carrier screening report
5769 Donor
Date of Birth: 06/[redacted]/1989
Sema4 ID: 20132933CS

Patient Information
Name: 5769 Donor
Date of Birth: 06/[redacted]/1989
Sema4 ID: 20132933CS
Client ID: TSBCA-S45769
Indication: Carrier Testing

Specimen Information
Specimen Type: Saliva
Date Collected: 08/03/2020
Date Received: 08/05/2020
Final Report: 08/19/2020

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

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.

Anastasia Larmore, Ph.D., Assistant Laboratory Director
Laboratory Medical Consultant: George A. Diaz, M.D., Ph.D.

Reviewed by genetic counselor. 8-20-20 TS.

AUG 26 2020

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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
Negative				
Cystic Fibrosis	*CFTR*	AR	Reduced Risk (see table below)	
Spinal Muscular Atrophy	*SMN1*	AR	Reduced Risk (see table below)	*SMN1* copy number: 2 *SMN2* copy number: 2 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
Cystic Fibrosis (AR) NM_000492.3	*CFTR*	African	1 in 58	91%	1 in 630	99%
		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	
		European (Non-Finnish)	1 in 23	95%	1 in 440	
		Native American	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	

Spinal Muscular Atrophy (AR)
NM_000344.3 *SMN1*

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
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 *BTD, 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 followingmethodologies, 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 likelypathogenic variants.

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Agilent SureSelect™QXT technology was used with a custom capture library to target theexonic regions and intron/exon splice junctions of the relevant genes, as well as a numberof UTR, intronic or promoter regions that contain previously reported mutations. Sampleswere pooled and sequenced on the Illumina HiSeq 2500 platform in the Rapid Run mode or theIllumina NovaSeq platform in the Xp workflow, using 100 bp paired-end reads. Thesequencing data was analyzed using a custom bioinformatics algorithm designed andvalidated in house.

The coding exons and splice junctions of the known protein-coding RefSeq genes wereassessed for the average depth of coverage (minimum of 20X) and data quality thresholdvalues. Most exons not meeting a minimum of >20X read depth across the exon are furtheranalyzed by Sanger sequencing. Please note that several genomic regions presentdifficulties in mapping or obtaining read depth >20X. The exons contained within theseregions are noted within Table 1 (as 'Exceptions') and will not be reflexed to Sangersequencing if the mapping quality or coverage is poor. Any variants identified duringtesting in these regions are confirmed by a second method and reported if determined to bepathogenic or likely pathogenic. However, as there is a possibility of false negativeresults within these regions, detection rates and residual risks for these genes have beencalculated with the presumption that variants in these exons will not be detected, unlessincluded in the MassARRAY® genotyping platform.

This test will detect variants within the exons and the intron-exon boundaries of thetarget regions. Variants outside these regions may not be detected, including, but notlimited to, UTRs, promoters, and deep intronic areas, or regions that fall into theExceptions mentioned above. This technology may not detect all small insertion/deletionsand 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 ofMedical Genetics Standards and Guidelines for the Interpretation of Sequence Variants(Richards et al, 2015). All potentially pathogenic variants may be confirmed by either aspecific genotyping assay or Sanger sequencing, if indicated. Any benign variants, likelybenign variants or variants of uncertain significance identified during this analysis willnot be reported.

Copy Number Variant Analysis (Analytical Detection Rate >95%)

Large duplications and deletions were called from the relative read depths on anexon-by-exon basis using a custom exome hidden Markov model (XHMM) algorithm. Deletions orduplications determined to be pathogenic or likely pathogenic were confirmed by either acustom arrayCGH platform, quantitative PCR, or MLPA (depending on CNV size and gene content). While this algorithm is designed to pick updeletions and duplications of 2 or more exons in length, potentially pathogenicsingle-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-targetedexon-focused array capable of detecting medically relevant microdeletions andmicroduplications at a much higher resolution than traditional aCGH methods. Each arraymatrix has approximately 180,000 60-mer oligonucleotide probes that cover the entiregenome. This platform is designed based on human genome NCBI Build 37 (hg19) and the CGHprobes 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 testfor genomic imbalances, both sample DNA and reference DNA is amplified with primer/probesets that specific to the target region and a control region with known genomic copynumber. 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 thebioinformatics pipeline. If indicated, copy number from MLPA was correlated with thesequencing 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 locatedon the same chromosome in tandem, only the second copy will be amplified and assessed forpotentially 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 thetandem allele and this patient is therefore less likely to be a carrier. When anindividual carries both a duplication allele and a pathogenic variant, or multiplepathogenic variants, the current analysis may not be able to determine the phase(cisrans configuration) of the *CYP21A2*alleles identified. Family studies may be required in certain scenarios where phasing isrequired to determine the carrier status.

Residual Risk Calculations

Carrier frequencies and detection rates for each ethnicity were calculated through thecombination of internal curations of >28,000 variants and genomic frequency data from>138,000 individuals across seven ethnic groups in the gnomAD database. Additionalvariants in HGMD and novel deleterious variants were also incorporated into thecalculation. 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

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assessing approximate risk given a negative result,and values will vary based on the exact ethnic background of an individual. This reportdoes not represent medical advice but should be interpreted by a genetic counselor,medical geneticist or physician skilled in genetic result interpretation and the relevantmedical literature.

Sanger Sequencing (Confirmation method) (Accuracy >99%)

Sanger sequencing, as indicated, was performed using BigDye Terminator chemistry with theABI 3730 DNA analyzer with target specific amplicons. It also may be used to supplementspecific guaranteed target regions that fail NGS sequencing due to poor quality or lowdepth of coverage (<20 reads) or as a confirmatory method for NGS positive results. Falsenegative 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 carrierscreening. *Genet Med*.2013 15:482-3.

Variant Classification:

Richards S et al. Standards and guidelines for the interpretation of sequence variants: ajoint consensus recommendation of the American College of Medical Genetics and Genomicsand the Association for Molecular Pathology. *Genet Med*.2015 May;17(5):405-24

Additional disease-specific references available upon request.