Research Report

Whole Exome Sequencing Diagnostic Panel

Subject ID:	[redacted]	**Date Test Started:**	**2013-01-23**
Specimen Type:	**Peripheral Blood**	**Date of Report:**	**2013-04-16**
Date Specimen Obtained:	**2012-09-12**		

TEST PERFORMED	Whole exome sequencing (WES) with Focused Diagnostic Panel(s): Neuropathy, Leukodystrophy, Myopathy See below for a complete list of genes analyzed.
RESULTS	**1 sequence variant with potential clinical relevance was identified in disease-related genes.**
	These sequence variants were submitted for confirmation in the CLIA-approved UNC Molecular Diagnostic Laboratory. See separate report for interpretation and recommendations.
INTERPRETIVE NOTES	This report details findings from WES and targeted informatics analysis performed on a research basis. ***Absence of a definitive disease-causing variant does not exclude the possibility of a genetic basis for the subject's medical condition.*** Only variants in genes associated with the medical condition, or thought to be potentially clinically relevant to the medical condition, were analyzed. The clinical implications of most genomic variations are not known at the time of this report. Specific limitations of the WES technique are as follows: • Some types of genetic abnormalities may not be detectable with the technologies used in this test. For example, this assay is not designed to detect large chromosomal aberrations, such as larger deletions and duplications (larger than ~20bp) or rearrangements. This assay also cannot detect repeat expansions. • It is possible that the disease causing mutation(s): ◦ exists in a region of the genome that was not included by the exome capture reagents, ◦ exists in a gene that was not included in the diagnostic panel analyzed due to incomplete scientific knowledge about the genes that cause human diseases, ◦ is not recognized as a type of genetic variant that causes genetic disorders due to incomplete scientific knowledge about the causation of human diseases, ◦ or exists in an exon that had low coverage or base quality in the assay performed in this subject, such that a mutation exists but was not detected. The exome data generated here will be reanalyzed on an annual basis during the course of the NCGENES research study. The data will be reassessed if newly characterized genes and/or disorders identified since the date of this report have been added to the diagnostic panel, or new algorithms are developed for more accurate base calling. This exome data will also be used for research analyses intended to examine potentially novel causes of human disease. The likelihood of success is small, but it is possible that this report would be revised if a genetic variant were found in a gene that was subsequently determined to be implicated in a disorder consistent with the subject's presenting symptoms.

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SUMMARY

The overall results of the WES analysis are as follows:

Total reads: 65084795
- mapped: 98.75 %
- paired: 97.92 %

Average coverage: 56.87

Total Number of Variants Detected: 82605

By location	By type	By effect
Coding: 18390	Substitution: 73572	Intergenic: 4396
Non-Coding: 61109	Small indel: 9033	Intronic: 50595
Transcript-dependent: 3106		Untranslated: 6024
		Synonymous: 11547
		Missense: 9348
		Non-frameshifting indel: 243
		Frameshifting indel: 226
		Splice site: 112
		Nonsense: 81
		Stoploss: 11
		Other: 22

The focused analysis examined variants in the Neuropathy, Leukodystrophy, Myopathy diagnostic list(s) consisting of 211 genes:

Total Number of Variants Analyzed: 3600
- Reported mutations: 3
- Novel/rare truncating variants: 1
- Novel/rare missense variants: 5
- Uncommon protein-coding or rare intronic variants: 328
- Common protein-coding or uncommon intronic variants: 106
- Common intronic variants: 356

METHODS

Genomic DNA was extracted from the submitted specimen and the Agilent SureSelect v4 kit was used to capture the protein-coding regions of the genome. The enriched fraction of the exome was sequenced using the Illumina HiSeq 2000 or HiSeq 2500 sequencing system with 100bp paired-end reads. Raw DNA sequence reads were mapped to the human reference genome sequence NCBI 37.1 (hg19) using bwa and aligned using the Genome Analysis Toolkit. Variants were identified using version 13 of the NCGENES variant calling pipeline.

The targeted coding exons and splice junctions of the known protein-coding RefSeq genes were assessed for the average depth of coverage and data quality threshold values. The following values represent metrics from this subject's WES analysis:

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Quality Metrics		Result
Average number of sequence reads across the entire region targeted for enrichment:		56.87
Percentage of coding nucleotides within targeted region covered with:	At least 2x coverage	99.45
	At least 8x coverage	97.99
	At least 20x coverage	92.89

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DIAGNOSTIC PANEL AND COVERAGE DETAILS

A targeted informatics analysis of the WES variant data from this subject was performed analyzed using the Neuropathy, Leukodystrophy, Myopathy diagnostic panel(s) consisting of 211 genes that are potentially implicated in this subject's clinical features.

AAAS	AARS	ABAT	ABCD1*	ABHD12*	ACADM
ACADS	ACADVL	ACOX1	ACP5	ACTA1	AFG3L2
AGL	AIFM1	AIMP1	AMPD1	ARHGEF10	ARSA
ASPA	ATL1	ATP2A1	ATP7A	ATXN1	ATXN2*
ATXN3	BAG3	BIN1	BSCL2	C10orf2	CACNA1A
CAV3	CCT5	CFL2	CNBP	CNTN1	COX14
CPT1B	CPT2	CRYAB	CTC1	CTDP1	DCTN1
DDC	DES	DHH	DMD	DMPK	DNM2
DNMT1	DST	DYNC1H1	EGR2	EIF2B1	EIF2B2
EIF2B3	EIF2B4	EIF2B5	ENO3	ETFA	ETFB
ETFDH	ETHE1	FA2H*	FAM126A	FAM134B	FASTKD2
FBLN5	FGD4	FGF14	FHL1	FIG4	FLVCR1
FOLR1	FOXRED1	GAA	GALC	GAN	GARS
GBE1	GDAP1	GFAP	GFER*	GJB1	GJC2*
GNE	GYG1	GYS1	HADHA	HADHB	HEPACAM*
HOXD10	HRAS	HSPB1	HSPB3	HSPB8	HSPD1*
HSPG2	IKBKAP	ISCU	KARS	KBTBD13*	KIF1A
KIF1B	KLHL9	LAMP2	LDHA*	LITAF	LMNA
LMNB1	LPIN1	MATR3	MCCC1	MED25	MFN2
MLC1	MPV17	MPZ	MRPS22	MSTN	MTM1
MTMR2	MYH2	MYH7	MYOT	NDRG1	NDUFA1
NDUFA11	NDUFAF1	NDUFAF2	NDUFAF3	NDUFAF4	NDUFS1
NDUFS2	NDUFS4	NDUFS6	NDUFV1	NDUFV2	NEFL
NGF	NTRK1	NUBPL	OPA1	OPA3	PABPN1*
PEX16	PEX7	PFKM	PGAM2	PGK1*	PGM1
PHKA1	PHYH	PLEKHG4	PLEKHG5	PLP1	PMP22
PNPLA2	POLG	POLG2	POLR3A	POLR3B	PPP2R2B

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PRKCG	PRNP	PRPS1	PRX	PSAP	PUS1
PYGM	RAB7A	RNASEH2A	RNASEH2B	RNASEH2C	RNF170
RRM2B	RYR1	SAMHD1	SBF2	SCN9A	SCO2
SDHA*	SEPN1*	SEPT9	SH3TC2	SLC12A6	SLC16A2
SLC22A5	SLC25A20	SLC25A3	SLC25A4	SOX10	SPTLC1
SUCLA2	SUCLG1	SUMF1	TDP1	TK1	TK2
TMEM70	TNNT1	TPM3	TREX1	TRIM32	TSFM
TTN	TTR	TUFM	TYMP*	WNK1	YARS
YARS2					

Genes labeled with an asterisk did not achieve at least 8x coverage for 90% of coding nucleotides.