Patient Name	DOB	Sex	MRN	Invitae #
Ivan Opalenko	12.20.2018	Male		RQ791606

Clinical Team	Report Date	Sample Type	Sample Collection Date	Sample Accession Date
Miroshnykov Oleksandr	06.24.2019	Blood	06.10.2019	06.13.2019

Test Performed
Sequence analysis and deletion/duplication testing of the 123 genes listed in the results section below.
- Invitae Spinal Muscular Atrophy Panel
- Invitae Comprehensive Neuromuscular Disorders Panel
- Add-on Preliminary-evidence Genes for Neuromuscular Disorders
- Add-on Facioscapulohumeral Muscular Dystrophy Type 2 (FSHD2) Gene

Reason for Testing

Summary

Positive result. Homozygous Pathogenic variant identified in SMN1.
SMN2 copy number: 3
Variant of Uncertain Significance identified in CHRNE.

Clinical Summary

- A Pathogenic variant, Deletion (Entire coding sequence) (homozygous), was identified in SMN1.
    - The SMN1 gene is associated with autosomal recessive spinal muscular atrophy (SMA) (MedGen UID: 7755).
    - This result is consistent with a diagnosis of spinal muscular atrophy.
    - Spinal muscular atrophy (SMA) is a neuromuscular disorder caused by the loss of neurons within the spinal cord, which results in progressive muscle weakness and atrophy (PMID: 10679938, 18572081). Other features of SMA may include muscle fasciculations, tremor, poor weight gain, sleeping difficulties, pneumonia, scoliosis, joint contractures, and congenital heart disease (PMID: 18662980, 7658877). Four clinical SMA subtypes have been distinguished: severe infantile acute SMA type I (also referred to as Wednig-Hoffman disease), infantile chronic SMA type II, juvenile SMA type III (also referred to as Wohlfard-Kugelberg-Welander disease), and adult-onset SMA type IV (PMID: 10679938, 18572081). The age of onset, phenotype, prognosis, and life expectancy display marked inter- and intrafamilial variability, between and also within subtypes (PMID: 6855803). For SMA clinical management guidelines, refer to PMID: 17761659.
    - Biological relatives have a chance of being a carrier for autosomal recessive spinal muscular atrophy. The chance of having a child with autosomal recessive spinal muscular atrophy depends on the carrier state of this individual's partner.
- This individual has 3 copies of SMN2 exon 8, typically referred to as exon 7, which is expected to reflect SMN2 copy number.
    - While the SMN2 gene alone is not associated with disease, variation in SMN2 copy number can modify the phenotype of SMN1-related SMA (PMID: 15378550). Accumulating evidence indicates that, in individuals with homozygous Pathogenic deletions in SMN1, the presence of two or fewer copies of SMN2 may be associated with a more severe phenotype compared to the presence of three or more copies of SMN2, which may be associated with a milder phenotype (PMID: 8824882, 9199562, 9837824, 11839954). Unaffected individuals with five copies of SMN2 and a homozygous deletion in SMN1 have been reported, suggesting that five copies of SMN2 may compensate for the lack of SMN1 expression (PMID: 15378550).

Laboratory Director Tina Hambuch, Ph.D., FACMG
NY Laboratory Director Swaroop Aradhya, Ph.D., FACMG
Invitae 1400 16th Street, San Francisco, CA 94103
E: clientservices@invitae.com P: 415.374.7782 or 800.436.3037

- A Variant of Uncertain Significance, c.1262G>A (p.Arg421His), was identified in CHRNE.
    - The CHRNE gene is associated with autosomal recessive and dominant forms of congenital myasthenic syndrome (CMS) (MedGen UIDs: 373251, 344169, 833673).
    - The clinical significance of this result is uncertain. Until this uncertainty can be resolved, caution should be exercised before using this result to inform clinical management decisions.
    - Familial VUS testing is not offered. Testing family members for this variant will not contribute evidence to allow variant reclassification. Details on our VUS Resolution and Family Variant Testing Programs can be found at https://www.invitae.com/en/family/.
- These results should be interpreted within the context of additional laboratory results, family history, and clinical findings. Genetic counseling is recommended to discuss the implications of this result. For access to a network of genetic providers, please contact Invitae at clientservices@invitae.com or visit www.nsgc.org.

Complete Results

Gene	Variant	Zygosity	Variant Classification
SMN1	Deletion (Entire coding sequence)	homozygous	PATHOGENIC
CHRNE	c.1262G>A (p.Arg421His)	heterozygous	Uncertain Significance

The following genes were evaluated for sequence changes and exonic deletions/duplications:
ACTA1, AGRN, ALG14, ALG2, ANO5, ATP2A1, B3GALNT2, B4GAT1, BAG3, BIN1, CACNA1S, CAPN3, CAV3, CCDC78, CFL2, CHAT, CHKB, CHRNA1, CHRNB1, CHRND, CHRNE, CLCN1, CNTN1, COL12A1, COL6A1, COL6A2, COL6A3, COLQ, CPT2, CRYAB, DAG1, DES, DMD, DNAJB6, DNM2, DOK7, DPAGT1, DPM1, DPM2, DPM3, DYSF, EMD, FHL1, FKBP14, FKRP, FKTN, FLNC, GAA, GFPT1, GMPPB, GNE, GYS1, HNRNPA2B1, HNRNPDL, ISPD, ITGA7, KBTBD13, KCNJ2, KLHL40, KLHL41, LAMA2, LAMB2, LAMP2, LARGE1, LDB3, LIMS2, LMNA, LMOD3, LRP4, MATR3, MEGF10, MTM1, MUSK, MYF6, MYH2, MYH7, MYL2, MYOT, MYPN, NEB*, PLEC, PNPLA2, POMGNT1, POMGNT2, POMK, POMT1, POMT2, PREPL, RAPSN, RXYLT1, RYR1, SCN4A, SELENON, SGCA, SGCB, SGCD, SGCG, SLC5A7, SMCHD1, SMN1, SMN2, SNAP25, SQSTM1, STAC3, STIM1, SUN1, SUN2, SYNE1, SYNE2, TAZ, TCAP, TIA1, TMEM43, TNNT1, TNPO3, TOR1AIP1, TPM2, TPM3, TRAPPC11, TRIM32, TTN*, VCP, VMA21

Results are negative unless otherwise indicated

Benign and Likely Benign variants are not included in this report but are available upon request. An asterisk (*) indicates that this gene has a limitation. Please see the Limitations section for details.

Variant Details

SMN1, Deletion (Entire coding sequence), homozygous, PATHOGENIC

- This variant is a gross deletion of the genomic region encompassing exon 8 (conventionally referred to as exon 7) of the SMN1 gene. Due to the sequence similarity between other exons of SMN1 and SMN2, the presence of this variant is used to infer a whole-gene deletion of SMN1.
- This variant is clearly defined as a spinal muscular atrophy (SMA) causative allele (PMID: 11839954, 18572081). It has been reported in the homozygous state in approximately 96.4% of individuals affected with 5q13-linked SMA, and in the compound heterozygous state with a second loss-of-function SMN1 allele in the remaining 3.6% of affected individuals (PMID: 10679938).
- For these reasons, this variant has been classified as Pathogenic.

CHRNE, Exon 11, c.1262G>A (p.Arg421His), heterozygous, Uncertain Significance

- This sequence change replaces arginine with histidine at codon 421 of the CHRNE protein (p.Arg421His). The arginine residue is weakly conserved and there is a small physicochemical difference between arginine and histidine.
- This variant is present in population databases (rs144952259, ExAC 0.009%).
- This variant has not been reported in the literature in individuals with CHRNE-related disease.
- Algorithms developed to predict the effect of missense changes on protein structure and function do not agree on the potential impact of this missense change (SIFT: "Tolerated"; PolyPhen-2: "Probably Damaging"; Align-GVGD: "Class C0").
- In summary, the available evidence is currently insufficient to determine the role of this variant in disease. Therefore, it has been classified as a Variant of Uncertain Significance.

Methods

- Genomic DNA obtained from the submitted sample is enriched for targeted regions using a hybridization-based protocol, and sequenced using Illumina technology. Unless otherwise indicated, all targeted regions are sequenced with ≥50x depth or are supplemented with additional analysis. Reads are aligned to a reference sequence (GRCh37), and sequence changes are identified and interpreted in the context of a single clinically relevant transcript, indicated below. Enrichment and analysis focus on the coding sequence of the indicated transcripts, 10bp of flanking intronic sequence (20bp for BRCA1/2), and other specific genomic regions demonstrated to be causative of disease at the time of assay design. Promoters, untranslated regions, and other non-coding regions are not otherwise interrogated. For some genes only targeted loci are analyzed (indicated in the table above). Exonic deletions and duplications are called using an in-house algorithm that determines copy number at each target by comparing the read depth for each target in the proband sequence with both mean read-depth and read-depth distribution, obtained from a set of clinical samples. All clinically significant observations are confirmed by orthogonal technologies, except individually validated variants and variants previously confirmed in a first-degree relative. Confirmation technologies include any of the following: Sanger sequencing, Pacific Biosciences SMRT sequencing, MLPA, MLPA-seq, Array CGH. Array CGH confirmation of NGS CNV calling performed by Invitae Corporation (1400 16th Street, San Francisco, CA 94103, #05D2040778). The following analyses are performed if relevant to the requisition. For PMS2 exons 12-15, the reference genome has been modified to force all sequence reads derived from PMS2 and the PMS2CL pseudogene to align to PMS2, and variant calling algorithms are modified to support an expectation of 4 alleles. If a rare SNP or indel variant is identified by this method, both PMS2 and the PMS2CL pseudogene are amplified by long-range PCR and the location of the variant is determined by Pacific Biosciences (PacBio) SMRT sequencing of the relevant exon in both long-range amplicons. If a CNV is identified, MLPA or MLPA-seq is run to confirm the variant. If confirmed, both PMS2 and PMS2CL are amplified by long-range PCR, and the identity of the fixed differences between PMS2 and PMS2CL are sequenced by PacBio from the long-range amplicon to disambiguate the location of the CNV. Technical component of confirmatory sequencing is performed by Invitae Corporation (1400 16th Street, San Francisco, CA 94103, #05D2040778).
- The following transcripts were used in this analysis. If more than one transcript is listed for a single gene, variants were reported using the first transcript listed unless otherwise indicated in the report: ACTA1 (NM_001100.3), AGRN (NM_198576.3), ALG14 (NM_144988.3), ALG2 (NM_033087.3), ANO5 (NM_213599.2), ATP2A1 (NM_173201.3), B3GALNT2 (NM_152490.4), B4GAT1 (NM_006876.2), BAG3 (NM_004281.3), BIN1 (NM_139343.2), CACNA1S (NM_000069.2), CAPN3 (NM_000070.2), CAV3 (NM_033337.2), CCDC78 (NM_001031737.2), CFL2 (NM_021914.7), CHAT (NM_020549.4), CHKB (NM_005198.4), CHRNA1 (NM_000079.3), CHRNB1 (NM_000747.2), CHRND (NM_000751.2), CHRNE (NM_000080.3), CLCN1 (NM_000083.2), CNTN1 (NM_001843.3), COL12A1 (NM_004370.5), COL6A1 (NM_001848.2), COL6A2 (NM_001849.3), COL6A3 (NM_004369.3), COLQ (NM_005677.3), CPT2 (NM_000098.2), CRYAB (NM_001885.2), DAG1 (NM_004393.5), DES (NM_001927.3), DMD (NM_004006.2), DNAJB6 (NM_058246.3), DNM2 (NM_001005360.2), DOK7 (NM_173660.4), DPAGT1 (NM_001382.3), DPM1 (NM_003859.1), DPM2 (NM_003863.3), DPM3 (NM_153741.1), DYSF (NM_003494.3), EMD (NM_000117.2), FHL1 (NM_001449.4), FKBP14 (NM_017946.3), FKRP (NM_024301.4), FKTN (NM_001079802.1), FLNC (NM_001458.4), GAA (NM_000152.3), GFPT1 (NM_001244710.1), GMPPB (NM_021971.2), GNE (NM_001128227.2), GYS1 (NM_002103.4), HNRNPA2B1 (NM_031243.2), HNRNPDL (NM_031372.3), ISPD (NM_001101426.3), ITGA7 (NM_002206.2), KBTBD13 (NM_001101362.2), KCNJ2 (NM_000891.2), KLHL40 (NM_152393.3), KLHL41 (NM_006063.2), LAMA2 (NM_000426.3), LAMB2 (NM_002292.3), LAMP2 (NM_002294.2), LARGE1 (NM_004737.4), LDB3 (NM_001080116.1;NM_001171610.1;NM_007078.2), LIMS2 (NM_001136037.2), LMNA (NM_170707.3), LMOD3 (NM_198271.4), LRP4 (NM_002334.3), MATR3 (NM_199189.2), MEGF10 (NM_032446.2), MTM1 (NM_000252.2), MUSK (NM_005592.3), MYF6 (NM_002469.2), MYH2 (NM_017534.5), MYH7 (NM_000257.3), MYL2 (NM_000432.3), MYOT

(NM_006790.2), MYPN (NM_032578.3), NEB (NM_001271208.1), PLEC (NM_000445.4;NM_201378.3), PNPLA2 (NM_020376.3), POMGNT1 (NM_017739.3), POMGNT2 (NM_032806.5), POMK (NM_032237.4), POMT1 (NM_007171.3), POMT2 (NM_013382.5), PREPL (NM_006036.4), RAPSN (NM_005055.4), RXYLT1 (NM_014254.2), RYR1 (NM_000540.2), SCN4A (NM_000334.4), SELENON (NM_020451.2), SGCA (NM_000023.2), SGCB (NM_000232.4), SGCD (NM_000337.5), SGCG (NM_000231.2), SLC5A7 (NM_021815.2), SMCHD1 (NM_015295.2), SMN1 (NM_000344.3), SMN2 (NM_017411.3), SNAP25 (NM_130811.2), SQSTM1 (NM_003900.4), STAC3 (NM_145064.2), STIM1 (NM_003156.3), SUN1 (NM_001130965.2), SUN2 (NM_015374.2), SYNE1 (NM_033071.3), SYNE2 (NM_182914.2), TAZ (NM_000116.4), TCAP (NM_003673.3), TIA1 (NM_022173.2), TMEM43 (NM_024334.2), TNNT1 (NM_003283.5), TNPO3 (NM_012470.3), TOR1AIP1 (NM_001267578.1), TPM2 (NM_003289.3), TPM3 (NM_152263.3), TRAPPC11 (NM_021942.5), TRIM32 (NM_012210.3), TTN (NM_001267550.2), VCP (NM_007126.3), VMA21 (NM_001017980.3).

- A PMID is a unique identifier referring to a published, scientific paper. Search by PMID at http://www.ncbi.nlm.nih.gov/pubmed.
- An rsID is a unique identifier referring to a single genomic position, and is used to associate population frequency information with sequence changes at that position. Reported population frequencies are derived from a number of public sites that aggregate data from large-scale population sequencing projects, including ExAC (http://exac.broadinstitute.org), gnomAD (http://gnomad.broadinstitute.org), and dbSNP (http://ncbi.nlm.nih.gov/SNP).
- A MedGen ID is a unique identifier referring to an article in MedGen, NCBI's centralized database of information about genetic disorders and phenotypes. Search by MedGen ID at http://www.ncbi.nlm.nih.gov/medgen. An OMIM number is a unique identifier referring to a comprehensive entry in Online Mendelian Inheritance of Man (OMIM). Search by OMIM number at http://omim.org/.
- Invitae uses information from individuals undergoing testing to inform variant interpretation. If "Invitae" is cited as a reference in the variant details this may refer to the individual in this requisition and/or historical internal observations.

Limitations

- Based on validation study results, this assay achieves >99% analytical sensitivity and specificity for single nucleotide variants, insertions and deletions <15bp in length, and exon-level deletions and duplications. Invitae's methods also detect insertions and deletions larger than 15bp but smaller than a full exon but sensitivity for these may be marginally reduced. Invitae's deletion/duplication analysis determines copy number at a single exon resolution at virtually all targeted exons. However, in rare situations, single-exon copy number events may not be analyzed due to inherent sequence properties or isolated reduction in data quality. Certain types of variants, such as structural rearrangements (e.g. inversions, gene conversion events, translocations, etc.) or variants embedded in sequence with complex architecture (e.g. short tandem repeats or segmental duplications), may not be detected. Additionally, it may not be possible to fully resolve certain details about variants, such as mosaicism, phasing, or mapping ambiguity. Unless explicitly guaranteed, sequence changes in the promoter, non-coding exons, and other non-coding regions are not covered by this assay. Please consult the test definition on our website for details regarding regions or types of variants that are covered or excluded for this test. This report reflects the analysis of an extracted genomic DNA sample. In very rare cases, (circulating hematolymphoid neoplasm, bone marrow transplant, recent blood transfusion) the analyzed DNA may not represent the patient's constitutional genome.
- SMN1 or SMN2: Systematic exon numbering is used for all genes, including SMN1 and SMN2, and for this reason the exon typically referred to as exon 7 in the literature (PMID: 8838816) is referred to as exon 8 in this report. This assay unambiguously detects SMN1 and SMN2 exon 8 copy number. Copy number of SMN2 exon 8 is expected to represent copy number for the entire SMN2 gene, and is reported only in individuals with a positive result in SMN1. Due to sequence similarity between SMN1 and SMN2, variants in exons 1-7 cannot be unambiguously mapped, and are therefore reported as occurring in "SMN1 or SMN2". Variants in all exons with no evidence towards pathogenicity are not included in this report, but are available upon request. NEB: Deletion/duplication analysis is not offered for exons 82-105. NEB variants in this region with no evidence towards pathogenicity are not included in this report, but are available upon request. TTN: Exons 153-155 (NM_133378.4) are excluded from analysis. TTN variants are reported in the primary report based on functional effect and/or location. A complete list of variants of uncertain significance, likely benign and benign variants in TTN is available upon request. Variants are named relative to the NM_001267550.2 (meta) transcript, but only variants in the coding sequence and intronic boundaries of the clinically relevant NM_133378.4 (N2A) isoform are reported (PMID: 25589632).

This report has been reviewed and approved by:

Thomas L. Winder, Ph.D., FACMG
Clinical Molecular Geneticist

Disclaimer

DNA studies do not constitute a definitive test for the selected condition(s) in all individuals. It should be realized that there are possible sources of error. Errors can result from trace contamination, rare technical errors, rare genetic variants that interfere with analysis, recent scientific developments, and alternative classification systems. This test should be one of many aspects used by the healthcare provider to help with a diagnosis and treatment plan, but it is not a diagnosis itself. This test was developed and its performance characteristics determined by Invitae. It has not been cleared or approved by the FDA. The laboratory is regulated under the Clinical Laboratory Improvement Act (CLIA) as qualified to perform high-complexity clinical tests (CLIA ID: 05D2040778). This test is used for clinical purposes. It should not be regarded as investigational or for research.

Laboratory Director Tina Hambuch, Ph.D., FACMG
NY Laboratory Director Swaroop Aradhya, Ph.D., FACMG
Invitae 1400 16th Street, San Francisco, CA 94103
E: clientservices@invitae.com **P:** 415.374.7782 or 800.436.3037

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Questions? Contact the PIN coordinator:
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HEADQUARTERS & LABORATORY | 1400 16th Street, San Francisco, CA 94103 | www.invitae.com | **IN THE US** | clientservices@invitae.com | p: 800-436-3037 | f: 415-276-4164
OUTSIDE THE US | globalsupport@invitae.com or visit www.invitae.com/contact | P/N F198-2