Otogenetics® Whole Exome Sequencing

Physician Name: XXXXXXXXXX	Report Date: *mm-dd-yyyy*
Patient: T_SRVY1 Name: XXXXXX DOB: *mm-dd-yyyy* Gender: Ethnicity: XXXX	Hospital / Clinic: XXXXXXX Specimen type: Saliva/Blood Date of Sample Collection: *mm-dd-yyyy*
Prepared by:	**Date:**
(signature)	*mm-dd-yyyy*

TEST ADMINISTERED:

Otogenetics® Whole Exome Sequencing (WES). Clinical grade WES is one of the most comprehensive tools for detection of rare disease causing and associated variants in an individual's DNA. Whole Exome includes all the expressed and majority of the transcribed regions in human genome, and it is believed to cover over 85% of known and to be discovered disease causing genetic variants. Purification of genomic DNA, sample preparation, enrichment for exonic regions, Illumina HiSeq next generation sequencing, variant filtration described in Appendix A, clinical interpretation.

CLINICAL INDICATION AND NOTES

Information provided indicates that this individual might have a condition caused be germline genetic variant(s).

Patient information

	Patient Code	Sample Barcode	Gender	Date of Birth	Affe-cted	Specimen type	Date collected	Date received
Proband	XXXXX	XXXX	Female	mm-dd-yyyy	YES	Saliva/Blood	mm-dd-yyyy	mm-dd-yyyy
Father	XXXXXX	XXXXX	Male	mm-dd-yyyy	NO	Saliva/Blood	mm-dd-yyyy	mm-dd-yyyy
Mother	XXXXX	XXXXXX	Female	mm-dd-yyyy	NO	Saliva/Blood	mm-dd-yyyy	mm-dd-yyyy

Clinical indication and reported phenotypes that were considered for generating this report:

Name	Description	Suspected Mode of Inheritance
Autism	Alalia, moderate autism and intellectual disability	Unknown

TEST RESULTS SUMMARY

Primary findings of Genetic Testing

These genetic findings have been identified as the most relevant to the reported phenotype(s). These variants are deemed to be potentially pathogenic as determined by a combination of curated databases of disease association, and predicted severity of the mutations. Variants were validated using another sequencing technology (Sanger sequencing) as indicated below.

Substitutions and other small (<50 bp) genetic variants:

Variant name	Type	Zygosity	Associated phenotype/ MOI	Pop. Freq.	Sanger Validation
MT-ND4 c.259C>T p.Glu87* chrM ENST00000361381	Exonic, Stopgain	Proband:Homozygous alternate (1,706) Father_P_RUSF:Homozygous Alternate (3,2,245) Mother_P_RUSM:Homozygous Alternate (4,2,492)	[Gene]:[ORPHANET]:Maternally-inherited progressive external ophthalmoplegia; MERRF; Lethal infantile mitochondrial myopathy; Mitochondrial myopathy with reversible cytochrome C oxidase deficiency; Maternally-inherited diabetes and deafness; Myopathy and d	0.0000	YES
AL589739.1 c.13_14insCACA p.Arg5fs chr1 ENST00000600779	Exonic, Frameshift	Proband:Homozygous reference (49,0) Father_P_RUSF:Heterozygous (13,11) Mother_P_RUSM:Homozygous Reference (49,0)	[PROTEIN]:[PROTEOME]: Breast Neoplasms,Carcinoma, Hepatocellular	0.0000	
RHBDD1 c.857-6990G>A chr2 ENST00000341329	Exonic, Stopgain	Proband:Heterozygous (24,23) Father_P_RUSF:Heterozygous (11,15) Mother_P_RUSM:Homozygous Reference (35,0)	[PROTEIN]:[PROTEOME]: Macular Degeneration	0.0061	
ATP6V0A2	Exonic,	Proband:Heterozygous	[Gene]:[OMIM]:Cutis laxa,	0.0352	

c.2438C>T p.Ala813Val chr12 ENST00000330342	Nonsynonymous SNV	(20,24) Father_P_RUSF:Heterozygous (14,12) Mother_P_RUSM:Homozygous Reference (22,0)	autosomal recessive, type IIA; Wrinkly skin syndrome;[ORPHANET]:Wrinkly skin syndrome; Autosomal recessive cutis laxa type 2, classic type;[ClinVar]:Cutis laxa with osteodystrophy;[HGMD]:Cutis laxa, autosomal recessive, type 2; W		
ATP6AP2 chrX	UTR3,	Proband:Homozygous reference (43,0) Father_P_RUSF:Not covered (7,0) Mother_P_RUSM:Heterozygous (21,24)	[Regulatory][Gene]:[OMIM]: ?Mental retardation, X-linked, syndromic, Hedera type; ?Parkinsonism with spasticity, X-linked;[ORPHANET]:X-linked intellectual disability, Hedera type; X-linked parkinsonism-spasticity syndrome;[ClinVar]:Parkinsonism with spasti	0.1681	

Legend: chr – Chromosome, Zygosity: Zygosity (reference allele coverage, alternate allele coverage) MOI: Mode of Inheritance

Gross deletions and other structural (>50 bp) genetic variants:

Variant name	Type	Zygosity	Associated phenotype/ MOI	Pop. Freq.	Sanger Validation
DEL:127102869>133082104 Chr2	Gross Deletion	Proband: Homozygous Alternate Father_P_RUSF: Homozygous Reference Mother_P_RUSM: Homozygous Reference	[HGMD]:Intellectual disability	0.00000	

Legend: chr – Chromosome, MOI: Mode of Inheritance

MT-ND4 p.Glu87*

This variant is a Stopgain mutation in the MT-ND4 gene. Zygosity state is Homozygous alternate in the probnad, Homozygous Alternate in Father_P_RUSF, Homozygous Alternate in Mother_P_RUSM.
It is a variant with a 0.0000 maximal allele frequency in the population databases available for review. This variant has not been previously reported in peer reviewed clinical literature, however other variants in this gene were reported to be associated with phenotype(s) listed in the Associated phenotype field in the table above. Calculated severity score is 1.00000 on a scale of 0.0 – 1.0. According to HGMD/ClinVar, the MT-ND4 gene is associated with , Neoplasm of ovary; Familial cancer of breast; Deafness, sensorineural, with neurologic features; Mitochondrial encephalomyopathy; Multisystem disorder; Cardiomyopathy, idiopathic dilated, mitochondrial; Deafness, nonsyndromic sensorineural,

mitochondrial; Familial colorectal cancer; Myopathy; Mitochondrial myopathy; Juvenile myopathy, encephalopathy, lactic acidosis AND stroke; Myoglobinuria, recurrent; Parkinsonism/MELAS overlap syndrome; Myopathy, mitochondrial, with diabetes mellitus; Parkinson disease, mitochondrial; Mitochondrial complex I deficiency; Retinitis pigmentosa-deafness syndrome; Exercise intolerance; Myoneural gastrointestinal encephalopathy syndrome; Mitochondrial complex IV deficiency with recurrent myoglobinuria; Sideroblastic anemia, acquired idiopathic; Striatonigral degeneration, infantile, mitochondrial; Myopathy, lactic acidosis, and sideroblastic anemia 3; Infantile histiocytoid cardiomyopathy; Exercise intolerance, muscle pain, and lactic acidemia; Neuropathy ataxia retinitis pigmentosa syndrome; Striatal necrosis, bilateral, with dystonia; MERFF syndrome; Myoclonus with epilepsy with ragged red fibers; Mitochondrial myopathy, isolated; Diabetes-deafness syndrome maternally transmitted; MERRF/MELAS overlap syndrome; Mitochondrial cytochrome c oxidase deficiency; Cardiomyopathy, hypertrophic, mitochondrial; Leber's optic atrophy; Ataxia and polyneuropathy, adult-onset; Cytochrome-c oxidase deficiency; Mitochondrial myopathy, infantile, due to reversible cytochrome c oxidase deficiency; Cerebellar ataxia, cataract, and diabetes mellitus; Leigh syndrome due to mitochondrial complex I deficiency; Cytochrome c oxidase i deficiency; Primary familial hypertrophic cardiomyopathy; Pigmentary retinopathy and sensorineural deafness; Cardiomyopathy, mitochondrial; Leigh syndrome; Exercise intolerance, cardiomyopathy, and septooptic dysplasia; Cardiomyopathy and Deafness; Progressive external ophthalmoplegia with myoclonus; Leber hereditary optic neuropathy with dystonia. Additional gene description (Uniprot): Core subunit of the mitochondrial membrane respiratory chain NADH dehydrogenase (Complex I) that is believed to belong to the minimal assembly required for catalysis. Complex I functions in the transfer of electrons from NADH to the respiratory chain. The immediate electron acceptor for the enzyme is believed to be ubiquinone (By similarity). The gene has not been tested for essentiality or tolerance.
Additional comments: Primary Variant Comments 111 Primary Variant Comments 111 Primary Variant Comments 111

AL589739.1 p.Arg5fs
This variant is a Frameshift mutation in the AL589739.1 gene. Zygosity state is Homozygous reference in the probnad, Heterozygous in Father_P_RUSF, Homozygous Reference in Mother_P_RUSM.
It is a variant with a 0.0000 maximal allele frequency in the population databases available for review.
No variants in this gene have not been previously reported in peer reviewed clinical literature, however protein level alterations in the product of this gene were reported to be associated with phenotype(s) listed in the Associated phenotype field in the table above. Calculated severity score is 1.00000 on a scale of 0.0 – 1.0. The gene has not been tested for essentiality or tolerance.
Additional comments: Display comments Second Variant 222 Display comments Second Variant 222 Display comments Second Variant 222

RHBDD1
This variant is a Stopgain mutation in the RHBDD1 gene. Zygosity state is Heterozygous in the probnad, Heterozygous in Father_P_RUSF, Homozygous Reference in Mother_P_RUSM.
It is a variant with a 0.0061 maximal allele frequency in the population databases available for review.
No variants in this gene have not been previously reported in peer reviewed clinical literature, however protein level alterations in the product of this gene were reported to be associated with phenotype(s)

listed in the Associated phenotype field in the table above. Calculated severity score is 1.00000 on a scale of 0.0 – 1.0. Additional gene description (Uniprot): Intramembrane-cleaving serine protease that cleaves single transmembrane or multi-pass membrane proteins in the hydrophobic plane of the membrane, luminal loops and juxtamembrane regions. Involved in regulated intramembrane proteolysis and the subsequent release of functional polypeptides from their membrane anchors. Functional component of endoplasmic reticulum-associated degradation (ERAD) for misfolded membrane proteins. Required for the degradation process of some specific misfolded endoplasmic reticulum (ER) luminal proteins. Participates in the transfer of misfolded proteins from the ER to the cytosol, where they are destroyed by the proteasome in a ubiquitin-dependent manner. Functions in BIK, MPZ, PKD1, PTCRA, RHO, STEAP3 and TRAC processing. Involved in the regulation of exosomal secretion| inhibits the TSAP6-mediated secretion pathway. Involved in the regulation of apoptosis| modulates BIK-mediated apoptotic activity. Also plays a role in the regulation of spermatogenesis| inhibits apoptotic activity in spermatogonia.. The gene has not been tested for essentiality and its tolerance score (RVIS) is in the top 87.06%.
Additional comments: comment2

ATP6V0A2 p.Ala813Val
This variant is a Nonsynonymous SNV mutation in the ATP6V0A2 gene. Zygosity state is Heterozygous in the probnad, Heterozygous in Father_P_RUSF, Homozygous Reference in Mother_P_RUSM.
It is a variant with a 0.0352 maximal allele frequency in the population databases available for review. This variant has not been previously reported in peer reviewed clinical literature, however other variants in this gene were reported to be associated with phenotype(s) listed in the Associated phenotype field in the table above. Calculated severity score is 0.96000 on a scale of 0.0 – 1.0. According to HGMD/ClinVar, the ATP6V0A2 gene is associated with Cutis laxa, autosomal recessive, type 2; Wrinkly skin syndrome; Cutis laxa; Cutis laxa, large anterior fontanel, ASD, VSD, downslanting palpebral fissures & hernia; Cutis laxa, Dandy-Walker malformation & microcephaly; Congenital disorder of glycosylation; Cutis laxa, type 2A, Cutis laxa with osteodystrophy. Additional gene description (Uniprot): Part of the proton channel of V-ATPases. Essential component of the endosomal pH-sensing machinery. May play a role in maintaining the Golgi functions, such as glycosylation maturation, by controlling the Golgi pH.. The gene has not been tested for essentiality and its tolerance score (RVIS) is in the top 17.47%.
Additional comments: comment1

ATP6AP2
This variant is a mutation in the ATP6AP2 gene. Zygosity state is Homozygous reference in the probnad, Not covered in Father_P_RUSF, Heterozygous in Mother_P_RUSM.
It is a variant with a 0.1681 maximal allele frequency in the population databases available for review. This variant has not been previously reported in peer reviewed clinical literature, however other variants in this gene were reported to be associated with phenotype(s) listed in the Associated phenotype field in the table above. Calculated severity score is 0.00000 on a scale of 0.0 – 1.0. The variant overlaps experimentally verified miRNA (hsa-miR-152-3p) binding site and could thus affect gene regulation. Additional gene description (Uniprot): Functions as a renin and prorenin cellular receptor. May mediate renin-dependent cellular responses by activating ERK1 and ERK2. By increasing the catalytic efficiency of renin in AGT/angiotensinogen conversion to angiotensin I, it may also play a role in the renin-angiotensin system (RAS). The gene has not been tested for essentiality and its tolerance score

(RVIS) is in the top 63%.
Additional comments:

Chr2_DEL:127102869>133082104
This variant is a Gross Deletion mutation in the Chromosome 4. It includes one or more exon of the following genes: AC093875.1; RPS23P2; RP11-5K16.3; RP11-775H9.2; FTH1P24; SLC7A11-AS1; RP11-308D13.3; RP11-98O2.1; RP11-577B7.1; IL15; MGST2; RP11-173E2.2; RP11-173E2.1; RP11-793B23.1; AC060835.1; RP11-425I13.2; RP11-425I13.3; RP13-884E18.4; RP11-425I13.1; SNORD112; RP11-138I17.1; snoU13; TBC1D9; TERF1P3; ELF2; MGARP; RP11-310A13.2; RP13-884E18.2; LINC00500; INPP4B; ACA64; RP11-83A24.2; RNU6-531P; LINC00616; RNF150; CLGN; RP11-342I1.2; NDUFC1; RP11-371F15.3; TNRC18P1; RP11-102N12.2; RP11-102N12.3; Y_RNA; AC093766.1; RN7SL152P; STMN1P2; RNU6-1214P; RP11-733C7.1; RN7SL382P; ZNF330; ELMOD2; CCRN4L; LINC00498; LINC00499; RP11-362F19.3; UCP1; RP11-362F19.1; PPP1R14BP3; RP11-83A24.1; MAML3; RP11-542P2.1; RNU6-1074P; NAA15; SCOC; RN7SKP253; RP11-208N20.1; RN7SKP237; RNU6-506P; RAB33B; RN7SL311P; SLC7A11; RP11-586L23.1; RP11-392B6.1; RP11-785F11.1; RP11-362F19.2; RP11-714L20.1; PCDH18; SERF1AP1; SETD7; H3F3AP6; AC093602.1. Zygosity state is Homozygous Alternate in the probnad, Homozygous Reference in Father_P_RUSF, Homozygous Reference in Mother_P_RUSM.
It is a variant with a 0.00000 maximal allele frequency in the population databases available for review. This variant overlaps with large structural variant(s) reported in peer reviewed clinical literature to be associated with Intellectual disability. According to HGMD, the affected gene(s) associated with Diabetes, type 2, susceptibility to, association; Congenital heart disease; Increased weight gain, association with; Obesity; Psoriasis vulgaris; Waist-to-hip ratio, association with; Dyggve-Melchior-Clausen syndrome; Reduced expression; Smith-McCort dysplasia According to ClinVar, the affected gene(s) associated with Smith-McCort dysplasia 2; Long QT syndrome
Additional comments:

Secondary findings

These genetic findings have been identified as less certainly relevant to the reported phenotype(s). These variants are deemed to be potentially pathogenic as determined by a combination of curated databases of disease association, and predicted severity of the mutations. Variants were validated using another sequencing technology (Sanger sequencing) as indicated below.

Substitutions and other small (<50 bp) genetic variants:

Variant name	Type	Zygosity	Associated phenotype/ MOI	Pop. Freq.	Sanger Validation
AKR7L c.369delG p.Thr124fs chr1 ENST00000429712	Exonic, Frameshift	Proband:Heterozygous (18,20) Father_P_RUSF:Homozygous Reference (20,0) Mother_P_RUSM:Heterozygous (18,10)		0.0391	
LACTBL1 c.686C>A p.Ser229* chr1 ENST00000426928	Exonic, Stopgain	Proband:Homozygous reference (39,0) Father_P_RUSF:Heterozygous (11,10) Mother_P_RUSM:Homozygous Reference (32,0)		0.0388	
IGFBP3 c.-532C>T chr7 ENST00000381083	Exonic, Stopgain	Proband:Heterozygous (24,33) Father_P_RUSF:Heterozygous (15,9) Mother_P_RUSM:Homozygous Reference (28,0)	[Gene]:[HGMD]:Colorectal cancer, association with; Higher promoter activity, association with; Lung cancer, increased risk, association with	0.0850	
SPERT c.213C>A p.Cys71* chr13 ENST00000310521	Exonic, Stopgain	Proband:Heterozygous (21,28) Father_P_RUSF:Heterozygous (14,8) Mother_P_RUSM:Homozygous Reference (24,0)	[VARIANT]:[HGMD]:Autism spectrum disorder,	0.0749	

FANCI c.3853C>T p.Arg1285* chr15 ENST00000310775	Exonic, Stopgain	Proband:Heterozygous (33,22) Father_P_RUSF:Heterozygous (21,11) Mother_P_RUSM:Homozygous Reference (29,0)	[VARIANT]:[HGMD]:Fanconi anaemia, [ClinVar]:Fanconi anemia, complementation group I	0.0000	
PRC1 c.*510_*513delGTGT chr15 ENST00000394249	Exonic, Frameshift	Proband:Heterozygous (42,10) Father_P_RUSF:Heterozygous (14,18) Mother_P_RUSM:Homozygous Reference (24,0)	[PROTEIN]:[PROTEOME]: Breast Neoplasms,Urinary Bladder Neoplasms,Glioblastoma,Carcinoma, Non-Small-Cell Lung,Diabetes Mellitus, Type 2,Adenocarcinoma, Follicular	0.0042	YES
SORBS1 c.346-3818A>G chr10 ENST00000607232	Exonic, Nonsynonymous SNV	Proband:Homozygous reference (42,0) Father_P_RUSF:Homozygous Reference (24,0) Mother_P_RUSM:Heterozygous (14,18)	[VARIANT]:[HGMD]:Obesity and diabetes, reduced risk, association,	0.1375	YES
MBP c.609C>T p.Tyr203Tyr chr18 ENST00000355994	Exonic, Nonsynonymous SNV	Proband:Homozygous alternate (0,44) Father_P_RUSF:Homozygous Alternate (0,26) Mother_P_RUSM:Heterozygous (13,22)	[Regulatory][Gene]:[HGMD]:Schizophrenia	0.2254	YES
ZDHHC9 chrX	UTR3,	Proband:Homozygous alternate (0,43) Father_P_RUSF:Homozygous Alternate (0,11) Mother_P_RUSM:Homozygous Alternate (0,30)	[Regulatory][Gene]:[OMIM]: Mental retardation, X-linked syndromic, Raymond type;[ORPHANET]:X-linked intellectual disability with marfanoid habitus;[ClinVar]:Mental retardation, X-linked, syndromic, raymond type;[HGMD]:Mental retardation, X-linked; Intellec	0.6717	

Legend: chr – Chromosome, Zygosity: Zygosity (reference allele coverage, alternate allele coverage) MOI: Mode of Inheritance

Gross deletions and other structural (>50 bp) genetic variants:

Variant name	Type	Zygosity	Associated phenotype/ MOI	Pop. Freq.	Sanger Validation
DEL:15723626>20541422 chr17	Gross Deletion	Proband: Homozygous Alternate Father_P_RUSF: Homozygous Reference Mother_P_RUSM: Heterozygous	[HGMD]:Smith-Magenis syndrome; Birt-Hogg-Dub syndrome; Sjoegren-Larsson syndrome; Pneumothorax, primary spontaneous	0.00000	

Legend: chr – Chromosome, MOI: Mode of Inheritance

AKR7L p.Thr124fs
This variant is a Frameshift mutation in the AKR7L gene. Zygosity state is Heterozygous in the probnad, Homozygous Reference in Father_P_RUSF, Heterozygous in Mother_P_RUSM.
It is a variant with a 0.0391 maximal allele frequency in the population databases available for review.
No variants in this gene have not been previously reported in peer reviewed clinical literature. Moreover, protein level alterations in the product of this gene were not reported to be associated with any pathological phenotypes. Calculated severity score is 1.00000 on a scale of 0.0 – 1.0. Additional gene description (Uniprot): Can reduce the dialdehyde protein-binding form of aflatoxin B1 (AFB1) to the non-binding AFB1 dialcohol. May be involved in protection of liver against the toxic and carcinogenic effects of AFB1, a potent hepatocarcinogen (By similarity).. The gene has not been tested for essentiality or tolerance.
Additional comments:

LACTBL1 p.Ser229*
This variant is a Stopgain mutation in the LACTBL1 gene. Zygosity state is Homozygous reference in the probnad, Heterozygous in Father_P_RUSF, Homozygous Reference in Mother_P_RUSM.
It is a variant with a 0.0388 maximal allele frequency in the population databases available for review.
No variants in this gene have not been previously reported in peer reviewed clinical literature. Moreover, protein level alterations in the product of this gene were not reported to be associated with any pathological phenotypes. Calculated severity score is 1.00000 on a scale of 0.0 – 1.0. The gene has not been tested for essentiality or tolerance.
Additional comments:

IGFBP3
This variant is a Stopgain mutation in the IGFBP3 gene. Zygosity state is Heterozygous in the probnad, Heterozygous in Father_P_RUSF, Homozygous Reference in Mother_P_RUSM.
It is a variant with a 0.0850 maximal allele frequency in the population databases available for review.
This variant has not been previously reported in peer reviewed clinical literature, however other variants in this gene were reported to be associated with phenotype(s) listed in the Associated phenotype field in

the table above. Calculated severity score is 1.00000 on a scale of 0.0 – 1.0. According to HGMD/ClinVar, the IGFBP3 gene is associated with Colorectal cancer, association with; Higher promoter activity, association with; Lung cancer, increased risk, association with,. Additional gene description (Uniprot): IGF-binding proteins prolong the half-life of the IGFs and have been shown to either inhibit or stimulate the growth promoting effects of the IGFs on cell culture. They alter the interaction of IGFs with their cell surface receptors. Also exhibits IGF-independent antiproliferative and apoptotic effects mediated by its receptor TMEM219/IGFBP-3R.. The gene is considered non-essential and its tolerance has not been tested.
Additional comments:

SPERT p.Cys71*
This variant is a Stopgain mutation in the SPERT gene. Zygosity state is Heterozygous in the probnad, Heterozygous in Father_P_RUSF, Homozygous Reference in Mother_P_RUSM.
It is a variant with a 0.0749 maximal allele frequency in the population databases available for review. In peer reviewed clinical literature this variant has been reported to be associated with phenotype(s) listed in the Associated phenotype field in the table above. Calculated severity score is 1.00000 on a scale of 0.0 – 1.0. According to HGMD/ClinVar, the SPERT gene is associated with Autism spectrum disorder,. The gene has not been tested for essentiality and its tolerance score (RVIS) is in the top 51.92%.
Additional comments:

FANCI p.Arg1285*
This variant is a Stopgain mutation in the FANCI gene. Zygosity state is Heterozygous in the probnad, Heterozygous in Father_P_RUSF, Homozygous Reference in Mother_P_RUSM.
It is a variant with a 0.0000 maximal allele frequency in the population databases available for review. In peer reviewed clinical literature this variant has been reported to be associated with phenotype(s) listed in the Associated phenotype field in the table above. Calculated severity score is 1.00000 on a scale of 0.0 – 1.0. According to HGMD/ClinVar, the FANCI gene is associated with Progressive ataxia and palatal tremor; Progressive external ophthalmoplegia, modifer of; Distal myopathy with cachexia; Status epilepticus & epilepsy; Complex I deficiency; Developmental delay and seizures; Myocerebrohepatopathy spectrum disorders; Optic atrophy; Parkinson disease, early-onset; Oxidative phophorylation deficiency; Multiple sclerosis-like features; Sensory neuropathy; Acute liver failure; Isolated distal myopathy of upper limbs; Mitochondrial neurogastrointestinal encephalopathy-like syndrome; Progressive external ophthalmoplegia & mental retardation; Breast cancer; Fanconi anaemia with VACTERL association; Neurodegenerative disease; Fanconi anaemia; Progressive external ophthalmoplegia & ataxia; Alpers-like hepatocerebral syndrome; Mitochondrial DNA depletion syndrome, hepatocerebral; Fatigue, muscle complaints & dysarthria; Peripheral neuropathy, sensorineural hearing loss, tremor & intracranial calcifications; Depression, ataxia and cardiomyopathy; Myopathy, polyneuropathy & ataxia; Sensory-ataxic neuropathy, ophthalmoplegia, dysarthria and gastroparesis; Myoclonic epilepsy of infancy; Epilepsy and myoclonic jerks; Alpers-Huttenlocher syndrome; Ataxic neuropathy; Encephalopathy; Muscle weakness, exercise intolerance, hearing loss, arhythmia; Progressive external ophthalmoplegia and parkinsonism; Sodium valproate-induced liver toxicity, association with; Mitochondrial ataxia; SANDO with dopamine-agonist responsive parkinsonism; Progressive external ophthalmoplegia with encephalopathy; Progressive external ophthalmoplegia, parkinsonism, cognitive deficit & behavioural disturbance; Breast cancer, protection against, association with; Sensory ataxia, neuropathy,

ophthalmoparesis and stroke; Lactic acidosis; Cancer; Progressive external ophthalmoplegia; Alpers syndrome & seizures; Breast and/or ovarian cancer; Epilepsy; Ataxia, epilepsy & cataract; Mitochondrial spinocerebellar ataxia and epilepsy; Myopathy & posterior white matter mild signal alteration; POLG deficiency; SANDO; Alpers syndrome; Mitochondrial DNA depletion syndrome; Alpers-like syndrome; Mitochondrial DNA depletion syndrome, hepatic; Mitochondrial DNA depletion syndrome, myopathic; Intractable epilepsy, childhood-onset, Cerebellar ataxia infantile with progressive external ophthalmoplegia,Sensory ataxic neuropathy, dysarthria, and ophthalmoparesis; Cerebellar ataxia infantile with progressive external ophthalmoplegia; Autosomal dominant progressive external ophthalmoplegia with mitochondrial DNA deletions 1; Fanconi anemia, complementation group I; Sensory ataxic neuropathy, dysarthria, and ophthalmoparesis; Mitochondrial diseases; Camptocormism; Progressive sclerosing poliodystrophy; Myoclonic epilepsy myopathy sensory ataxia; Mitochondrial DNA depletion syndrome 4B, MNGIE type; Myoneural gastrointestinal encephalopathy syndrome; PROGRESSIVE EXTERNAL OPHTHALMOPLEGIA WITH MITOCHONDRIAL DNA DELETIONS, DIGENIC; Fanconi anemia. Additional gene description (Uniprot): Plays an essential role in the repair of DNA double-strand breaks by homologous recombination and in the repair of interstrand DNA cross-links (ICLs) by promoting FANCD2 monoubiquitination by FANCL and participating in recruitment to DNA repair sites. Required for maintenance of chromosomal stability. Specifically binds branched DNA: binds both single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA). Participates in S phase and G2 phase checkpoint activation upon DNA damage.. The gene has not been tested for essentiality and its tolerance score (RVIS) is in the top 44.54%.
Additional comments: comment sec1

PRC1
This variant is a Frameshift mutation in the PRC1 gene. Zygosity state is Heterozygous in the probnad, Heterozygous in Father_P_RUSF, Homozygous Reference in Mother_P_RUSM.
It is a variant with a 0.0042 maximal allele frequency in the population databases available for review. No variants in this gene have not been previously reported in peer reviewed clinical literature, however protein level alterations in the product of this gene were reported to be associated with phenotype(s) listed in the Associated phenotype field in the table above. Calculated severity score is 1.00000 on a scale of 0.0 – 1.0. Additional gene description (Uniprot): Key regulator of cytokinesis that cross-links antiparrallel microtubules at an average distance of 35 nM. Essential for controlling the spatiotemporal formation of the midzone and successful cytokinesis. Required for KIF14 localization to the central spindle and midbody. Required to recruit PLK1 to the spindle. Stimulates PLK1 phosphorylation of RACGAP1 to allow recruitment of ECT2 to the central spindle. Acts as an oncogene for promoting bladder cancer cells proliferation, apoptosis inhibition and carcinogenic progression (PubMed:17409436).. The gene has not been tested for essentiality and its tolerance score (RVIS) is in the top 39.11%.
Additional comments:

SORBS1
This variant is a Nonsynonymous SNV mutation in the SORBS1 gene. Zygosity state is Homozygous reference in the probnad, Homozygous Reference in Father_P_RUSF, Heterozygous in Mother_P_RUSM.
It is a variant with a 0.1375 maximal allele frequency in the population databases available for review. In peer reviewed clinical literature this variant has been reported to be associated with phenotype(s) listed in the Associated phenotype field in the table above. Calculated severity score is 0.56000 on a scale of

0.0 – 1.0. According to HGMD/ClinVar, the SORBS1 gene is associated with Obesity and diabetes, reduced risk, association,. The variant overlaps experimentally verified Enhancer site and could thus affect gene regulation. Additional gene description (Uniprot): Plays a role in tyrosine phosphorylation of CBL by linking CBL to the insulin receptor. Required for insulin-stimulated glucose transport. Involved in formation of actin stress fibers and focal adhesions (By similarity).. The gene is considered non-essential and its tolerance score (RVIS) is in the top 79.05%.
Additional comments:

MBP p.Tyr203Tyr
This variant is a Nonsynonymous SNV mutation in the MBP gene. Zygosity state is Homozygous alternate in the probnad, Homozygous Alternate in Father_P_RUSF, Heterozygous in Mother_P_RUSM.
It is a variant with a 0.2254 maximal allele frequency in the population databases available for review. This variant has not been previously reported in peer reviewed clinical literature, however other variants in this gene were reported to be associated with phenotype(s) listed in the Associated phenotype field in the table above. Calculated severity score is 0.00000 on a scale of 0.0 – 1.0. The variant overlaps experimentally verified Transcription Factor (RelA-p65-isoform1) binding site and could thus affect gene regulation. Additional gene description (Uniprot): The classic group of MBP isoforms (isoform 4-isoform 14) are with PLP the most abundant protein components of the myelin membrane in the CNS. They have a role in both its formation and stabilization. The smaller isoforms might have an important role in remyelination of denuded axons in multiple sclerosis. The non-classic group of MBP isoforms (isoform 1-isoform 3/Golli-MBPs) may preferentially have a role in the early developing brain long before myelination, maybe as components of transcriptional complexes, and may also be involved in signaling pathways in T-cells and neural cells. Differential splicing events combined with optional post-translational modifications give a wide spectrum of isomers, with each of them potentially having a specialized function. Induces T-cell proliferation.. The gene is considered non-essential and its tolerance score (RVIS) is in the top 67.19%.
Additional comments:

ZDHHC9
This variant is a mutation in the ZDHHC9 gene. Zygosity state is Homozygous alternate in the probnad, Homozygous Alternate in Father_P_RUSF, Homozygous Alternate in Mother_P_RUSM.
It is a variant with a 0.6717 maximal allele frequency in the population databases available for review. This variant has not been previously reported in peer reviewed clinical literature, however other variants in this gene were reported to be associated with phenotype(s) listed in the Associated phenotype field in the table above. Calculated severity score is 0.00000 on a scale of 0.0 – 1.0. The variant overlaps predicted miRNA (miR-299-5p) binding site and could thus affect gene regulation. Additional gene description (Uniprot): The ZDHHC9-GOLGA7 complex is a palmitoyltransferase specific for HRAS and NRAS.. The gene has not been tested for essentiality and its tolerance score (RVIS) is in the top 44.54%.
Additional comments:

chr17_DEL:15723626>20541422
This variant is a Gross Deletion mutation in the Chromosome 17. It includes one or more exon of the following genes: RNU6-767P; CENPV; TRIM16L; AC005822.1; SNORD3B-1; SNORD3B-2; AC015818.3;

MFAP4; MTND1P14; RP11-135L13.4; RP11-45M22.3; LRRC48; AL353997.11; PIGL; RP11-45M22.5; RP11-45M22.4; SPECC1; RNASEH1P2; RP11-524F11.2; RP11-524F11.1; RP11-28B23.1; UBE2SP2; ZSWIM7; MEIS3P2; RP11-92B11.4; RNU6-314P; AC122129.1; FTLP12; SLC47A1; RAI1-AS1; AC004702.2; TRPV2; ATPAF2; FLCN; PRPSAP2; Y_RNA; RN7SL775P; TNPO1P2; LGALS9B; RP1-178F10.3; AC055811.1; RP1-178F10.1; AC055811.5; SHMT1; PLD6; TBC1D27; RNU6-1057P; MIR1288; RP11-209D14.4; RP11-78O7.2; RP1-37N7.1; RP1-37N7.6; RP1-37N7.5; NOS2P3; AL353997.3; FAM106CP; MYO15A; TTC19; RN7SL627P; FBXW10; AKAP10; RASD1; ALDH3A2; COPS3; U6; AC015726.1; RP11-209D14.2; RP11-416I2.1; CCDC144CP; RP11-138I1.4; CTC-457L16.2; B9D1; AC005722.4; ADORA2B; NT5M; SRP68P1; SRP68P2; SRP68P3; FAM211A-AS1; RNF112; SLC5A10; AC087499.10; AC107983.4; RP11-258F1.1; LGALS9C; ZNF287; TBC1D28; FLII; GID4; TRNAQ41P; MIR33B; AC025627.9; UBB; AC106017.1; RP1-37N7.3; RNU6-468P; AC025627.4; GRAPL; USP32P1; USP32P2; USP32P3; RN7SL639P; RN7SL442P; RP11-1113L8.6; RP11-1113L8.7; AC008088.4; RP11-1113L8.1; RP11-1113L8.2; AC022596.2; AC022596.6; RP11-219A15.1; RP11-219A15.3; RP11-219A15.2; RP11-219A15.4; RNU6-862P; GRAP; AC004448.2; ALKBH5; TOP3A; RPL17P43; TVP23B; AC025627.7; NOS2P2; TSEN15P1; RN7SL620P; snoMBII-202; RP11-434D2.8; RP11-434D2.9; AC087499.5; AC087499.4; RP11-434D2.2; RP11-434D2.3; AC087499.9; AC104024.2; RP11-434D2.6; RP11-434D2.7; NCOR1; RP11-160E2.17; RP11-160E2.16; KRT17P2; RP11-160E2.11; AC026271.5; ZNF624; UPF3AP1; UPF3AP2; AC090286.2; AC090286.4; RP11-160E2.19; RP11-815I9.5; RP11-815I9.4; snoMe28S-Am2634; RPL13P12; MAPK7; RP11-815I9.3; RP11-434D2.10; RP11-434D2.11; RP11-434D2.12; FAM106B; SMCR2; FAM106A; CTD-2145A24.3; ALDH3A1; MED9; COTL1P1; CTD-2145A24.4; AC087163.3; AC087163.2; RPL7AP65; YWHAEP3; YWHAEP2; SLC47A2; RNU6-405P; RP11-92B11.3; AC005730.2; TNFRSF13B; RPL21P121; ZSWIM5P2; SMCR8; RP1-253P7.1; LLGL1; RP11-138I1.3; RP11-138I1.2; RPLP1P11; MTND2P12; AL353997.4; AC015922.5; SREBF1; RP11-311F12.2; AC073621.2; RP11-311F12.1; CTB-187M2.1; CTB-187M2.2; CTB-187M2.3; AC015818.6; RP11-160E2.21; FAM211A; CCDC144B; CCDC144A; EPN2-AS1; SNORA59B; KRT16P3; KRT16P2; KRT16P1; KRT16P5; KRT16P4; SNORD3D; RP11-218E15.1; AC104024.1; SMCR5; EVPLL; SNORD3A; DRG2; KRT17P1; COTL1P2; RPS2P46; AC004448.5; FAM83G; SNORD3C; MPRIP; RAI1; MPRIP-AS1; TBC1D3P4; FOXO3B; TBC1D3P3; ULK2; KCTD9P1; SNORA31; CTD-2104P17.1; snoU13; AC007952.5; AC007952.4; AC002553.1; AC007952.6; AC007952.1; AC002553.4; NEK4P2; RNU6-258P; MIR1180; KYNUP1; KYNUP3; KYNUP2; RN7SL17P; RP1-48J14.1; PEMT; EPN2; CTD-2303H24.2; CDRT15L2; AC093484.4; TOM1L2; MIEF2; RP1-77H15.1; AC107982.4; ZNF286B.
Zygosity state is Homozygous Alternate, Heterozygous in Mother_P_RUSM.
It is a variant with a 0.00000 maximal allele frequency in the population databases available for review.
This variant overlaps with large structural variant(s) reported in peer reviewed clinical literature to be associated with Smith-Magenis syndrome; Birt-Hogg-Dub syndrome; Sjoegren-Larsson syndrome; Pneumothorax, primary spontaneous. According to HGMD, the affected gene(s) associated with Birt-Hogg-Dub syndrome; Sjoegren-Larsson syndrome; Deafness, non-syndromic, autosomal recessive; Hearing loss; Smith-Magenis syndrome; Hearing loss, non-syndromic; Immunodeficiency, common variable; Hearing loss, autosomal recessive; Antibody deficiency; Reduced transport activity and 54 additional diseases with fewer reported genetic variants. According to ClinVar, the affected gene(s) associated with Multiple fibrofolliculomas; Hereditary cancer-predisposing syndrome; Deafness, autosomal recessive 3; Zunich neuroectodermal syndrome; Non-syndromic genetic deafness; Sjgren-Larsson syndrome; Common variable immunodeficiency 2; Smith-Magenis syndrome; Pneumothorax, primary spontaneous; Joubert syndrome and 20 additional diseases with fewer reported genetic variants.
Additional comments:

Follow up Recommendations

Recommendation for follow up actions based on this screening process include:

- Follow up with physicians/medical geneticists for updated genetic risk information. Future findings may provide new clinical interpretation of certain variants.

Test Statistics

Sample Identifier (Barcode)	XXXXX
Patient ID	XXXXXX
Amounts of DNA read	1,404,696,081
Targeted capture region	2,862,223,786
Total number of mutations compared to HG19	6,760,068
Bases in targeted exons with < 8X coverage	28,339,938
HGMD or ClinVar annotated locations < 8X coverage	509
Average coverage	57
Median coverage	56

Appendix A

Test Details - Methods and Limitations

General information

This genetic test report is based on analysis of raw data of Whole Exome Sequencing performed by Otogenetics. The report is intended for clinical diagnostics use. The sequencing protocol performed on the samples is of clinical grade, it is CLIA/CAP compliant, and is certified for diagnosis use. The primary purpose of this report is to communicate variants with strong evidence supporting their association to the reported phenotype(s). Incidental germline findings that do not correlate with the provided phenotype(s) are included in this report in the Incidental Findings section, if elected to be included in the report by the patient or legal guardian. Not all detected variants have been analyzed, and not all regions of the genome have been adequately sequenced. These results should be interpreted in the context of the patient's medical evaluation, family history, and genealogy. Please note that variant classification and/or interpretation may change over time as more information becomes available. Sanger validation status, when applicable, is indicated per variant in the tables above.

Sequencing and Variant Detection

Genomic DNA was extracted from clinical sample (saliva or blood), library preparation via Illumina protocols, capture based enrichment of a targeted region was performed by solution-based hybridization which enriches for coding regions of targeted genes with specific probes. Multiple quality control steps were performed for sample and derivative quality evaluation. Sequencing was performed using the Illumina HiSeq 2500, with 100-125 bp reads, sequence QC metrics were required, and a

minimum average coverage depth of 100X was required. Sequencing reads were aligned to the reference genome (UCSC hg19) by BWA-MEM and variants were called using GATK 3.6. The minimum sequence depth for all targeted exons was evaluated; further validation is recommended for exons with depth of coverage <10x. We recommend that variants of interest which do not meet the coverage minimum be confirmed clinically before treatment is undertaken.

Variant Analysis and Report Generation

Reported variants were filtered to include those present in the exonic regions and adjacent splice sites. Resulting variants were analyzed and reported using the Variantyx Genomic Intelligence platform. To maintain most up-to-date annotations, the Variantyx database is updated quarterly. As a result, variant classification and/or interpretation may change over time as more information becomes available.

The following databases and tools are included in Variantyx Genomic Intelligence platform:

1. Disease association: HGMD Professional (http://www.hgmd.cf.ac.uk/), Genome Trax (http://www.biobase-international.com/product/genome-trax), ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/), OMIM (http://www.omim.org/), Orphanet (www.orpha.net/), GeneTests (https://www.genetests.org/).
2. Population frequencies: dbSNP (http://www.ncbi.nlm.nih.gov/SNP/), ensemble (www.ensembl.org/), 1000 Genomes Project (www.1000genomes.org/), ExAC (http://exac.broadinstitute.org/), NHLBI Exome Sequencing Project (http://evs.gs.washington.edu/EVS/) and the Variantyx allele frequency database (http://variantyx.com/).
3. Severity predict ion: SIFT, MutationAssessor, Mutation Taster, GWAVA, PolyPhen2, FATHMM, Silva, LRT.
4. Conservation prediction: SiPhy, GERP++, PhyloP and PhastCons.
5. Gene Essentiality: According to published work 10.1371/journal.pgen.1003484
6. Gene tolerance: RVIS score, according to published work 10.1371/journal.pgen.1003709

Secondary/Incidental Sequence Variant(s) based on ACMG guidelines are not included in this report.

Not all mutations compared to the reference sequence have been listed on this report. Mutations were identified using the filters described below. These mutations were further reviewed by a medical geneticist, and only variations of clinical significance (primary findings) are included in this report.

Filters included:
Description of the filters used in preparation of this report can be for at the following url:
http://www.otogenetics.com/inherited-cancer-testing

Limitations

Absence of a primary diagnostic finding identified by this test does not exclude the possibility of a genetic basis for the clinical condition for this proband. Variants in the intronic, UTR and promoter regions and other copy number variants are not intended to be detected by this assay.

Specifically, detection of abnormal variants depends on the presence of these sequence variants in the targeted region that was sequenced. It is possible that the gene region where a disease causing mutation exists in the patient was not captured using the current technologies of this test and therefore was not detected.
This sequence test is designed to evaluate single nucleotide variants, 1-3 nucleotide variants and small insertions and deletions (<10 nucleotides) within the targeted region. The current technology targets the coding exonic regions of the 39 genes and not the 5' or 3' untranslated regions, promoter or splice sites of these genes. Thus, a variant in these non-coding exonic regions will not be sequenced at high depth, and may not be identified in this test.

Additionally, certain types of genetic abnormalities are difficult to identify in sequencing data and have not been validated for clinical use including but not limited to insertions, deletions, copy number alterations, long repetitive sequences, triplet repeat expansions, chromosomal rearrangements, polyploidy, repetitive regions including mono-, di- and tri-nucleotide repeats, GX rich regions, intronic variants outside the splice-site and epigenetic effects.

It is possible that a particular genetic abnormality may not be recognized as the underlying cause of the genetic disorder due to incomplete scientific knowledge about the function of all genes in the human genome and the impact of variants in those genes. Clinical correlation and periodic review of scientific and medical literature is recommended to determine whether Variants of Unknown Significance may be consistent with the patient's phenotype.

CLIA Statement

Otogenetics: This Laboratory Developed Test for Next-Generation sequencing of genomic DNA was developed and its performance characteristics established by Otogenetics Corporation, Atlanta, GA. This laboratory is regulated under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) as qualified to perform high-complexity clinical testing and has validated the test's accuracy according to CAP proficiency testing. This test has not been cleared nor approved by the U.S. Food and Drug Administration (FDA). The FDA has determined that such clearance or approval is not necessary. CLIA number – 11D2066426.

Otogenetics

4553 Winters Chapel Road, Suite 100
Atlanta, GA 30360
www.otogenetics.com
Customer support: support@otogenetics.com