

Whole Exome Sequencing Analysis

Patient name	: Ms. XXX	PIN	: XX
Gender/ Age	: Female/ 12 Years	Sample number	: XX
Referring clinician	: XX	Sample collection date	: XX
Specimen	: Peripheral Blood	Sample receipt date	: XX
		Report date	: XX

Clinical history

Proband, XXX was born to non-consanguineous parents and presented with delayed gross motor milestones, short stature, knock knees, difficulty in walking, hypothyroidism, obesity, Acanthosis nigricans, Midface hypoplasia, Rhizomelic upper limbs. All fingers are short and middle phalanges bigger, waddling gait and increased urinary phosphorous. Spot urine study indicative of elevated levels of uric acid, calcium and phosphorous. Serum calcium, uric acid are within normal range and serum phosphorous level is elevated. Proband’s mother has short stature, hypothyroidism and cutaneous lipomas. Proband’s father and elder brother have very long fingers with marfanoid features, maternal father was diagnosed with psoriasis. Proband, XXX is suspected to be affected with Skeletal Dysplasia and has been evaluated for pathogenic variations.

Results

Likely pathogenic variant was identified in *TRPV4* gene

List of significant variant identified related to phenotype:

Gene	Region	Variant*	Allele Status	Disease	Classification*	Inheritance pattern
<i>TRPV4</i> (-)	Exon 15	c.2396C>G (p.Pro799Arg)	Heterozygous	?Avascular necrosis of femoral head, primary, 2 (OMIM#617383) Brachyolmia type 3 (OMIM#113500) Digital arthropathy-brachydactyly, familial (OMIM#606835)	Likely pathogenic	Autosomal Dominant

				Hereditary motor and sensory neuropathy, type IIc (OMIM#606071) Metatropic dysplasia (OMIM#156530) Neuronopathy, distal hereditary motor, autosomal dominant 8 (OMIM#600175) Parastremmatic dwarfism (OMIM#168400) Scapuloperoneal spinal muscular atrophy (OMIM#181405) SED, Maroteaux type (OMIM#184095) Spondylometaphyseal dysplasia, Kozlowski type (OMIM#184252)		
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*Genetic test results are based on the recommendation of American college of Medical Genetics [1].
No other variant that warrants to be reported for the given clinical indication was identified.

Interpretation

TRPV4: c.2396C>G

Variant summary: A heterozygous missense variation in exon 15 of the *TRPV4* gene (chr12:g.109784378G>C, NM_021625.5, Depth: 109x) that results in the amino acid substitution of Arginine for Proline at codon 799 (p.Pro799Arg) was detected.

Population frequency: This variant has not been reported in gnomAD database and 1000 genomes database.

ClinVar and Literature evidence: This variant has been classified as pathogenic in ClinVar database [3]. This variant has been previously reported in patient affected with spondyloepimetaphyseal dysplasia Maroteaux type in heterozygous state [4].

In-silico prediction: The *in-silico* predictions of the variant are damaging by SIFT, PolyPhen-2 (HumDiv), LRT and MutationTaster2. The reference codon is conserved across mammals in PhyloP and GERP++ tools.

OMIM phenotype: ?Avascular necrosis of femoral head, primary, 2 (OMIM#617383), Brachyolmia type 3 (OMIM#113500), Digital arthropathy-brachydactyly, familial (OMIM#606835), Hereditary motor and sensory neuropathy, type IIc (OMIM#606071), Metatropic dysplasia (OMIM#156530), Neuronopathy, distal hereditary motor, autosomal dominant 8 (OMIM#600175), Parastremmatic dwarfism (OMIM#168400), Scapuloperoneal spinal muscular atrophy (OMIM#181405), SED, Maroteaux type (OMIM#184095) and Spondylometaphyseal dysplasia, Kozlowski type (OMIM#184252) are caused by heterozygous mutation in the *TRPV4* gene (OMIM*605427). These diseases follow autosomal dominant pattern of inheritance [2].

Variant classification: Based on the evidence, this variant has been classified as likely pathogenic variant. **In this view, clinical correlation and familial segregation analysis are strongly recommended to establish the significance of the finding. If the results do not correlate, additional testing may be considered based on the phenotype observed.**

Recommendations

- Sequencing the variant(s) in the parents and the other affected and unaffected members of the family is recommended to confirm the significance.
- Alternative test is strongly recommended to rule out the deletion/duplication.
- Genetic counselling is advised.

Methodology

DNA extracted from the blood was used to perform whole exome using whole exome capture kit. The targeted libraries were sequenced to a targeted depth of 80 to 100X using GenoLab M sequencing platform. This kit has deep exonic coverage of all the coding regions including the difficult to cover regions. The sequences obtained are aligned to human reference genome (GRCh38.p13) using Sentieon aligner and analyzed using Sentieon for removing duplicates, recalibration and re-alignment of indels. Sentieon DNAScope has been used to call the variants. Detected variants were annotated and filtered using the VarSeq software with the workflow implementing the ACMG guidelines for variant classification. The variants were annotated using 1000 genomes (V2), gnomAD (v3.1.2.1.1), ClinVar, OMIM, dbSNP, NCBI RefSeq Genes. *In-silico* predictions of the variant was carried out using VS-SIFT, VS-PolyPhen2, PhyloP, GERP++, GeneSplicer, MaxEntScan, NNSplice, PWM Splice Predictor. Only non-synonymous and splice site variants found in the coding regions were used for clinical interpretation. Silent variations that do not result in any change in amino acid in the coding region are not reported.

Sequence data attributes

Total reads generated	7.94 Gb
Data ≥ Q30	96.69%

Genetic test results are reported based on the recommendations of American College of Medical Genetics [1], as described below:

Classification	Interpretation
Pathogenic	A disease-causing variation in a gene which can explain the patients’ symptoms has been detected. This usually means that a suspected disorder for which testing had been requested has been confirmed
Likely Pathogenic	A variant which is very likely to contribute to the development of disease however, the scientific evidence is currently insufficient to prove this conclusively. Additional evidence is expected to confirm this assertion of pathogenicity.
Variant of Uncertain Significance	A variant has been detected, but it is difficult to classify it as either pathogenic (disease causing) or benign (non- disease causing) based on current available scientific evidence. Further testing of the patient or family members as recommended by your clinician may be needed. It is probable that their significance can be assessed only with time, subject to availability of scientific evidence.

Disclaimer

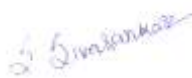
- The classification of variants of unknown significance can change over time. Anderson Diagnostics and Labs cannot be held responsible for it.
- Intronic variants, UTR, Promoter region variants and CNV are not assessed using this assay.
- Certain genes may not be covered completely, and few mutations could be missed. Variants not detected by this assay may impact the phenotype.
- The variations have not been validated by Sanger sequencing.
- The above findings and result interpretation was done based on the clinical indication provided at the time of reporting.
- It is also possible that a pathogenic variant is present in a gene that was not selected for analysis and/or interpretation in cases where insufficient phenotypic information is available.
- Genes with pseudogenes, paralog genes and genes with low complexity may have decreased sensitivity and specificity of variant detection and interpretation due to inability of the data and analysis tools to unambiguously determine the origin of the sequence data in such regions.

- Incidental or secondary findings that meet the ACMG guidelines can be given upon request [5].

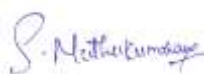
References

1. 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. Genetics in medicine: official journal of the American College of Medical Genetics. 17.5 (2015): 405-424.
2. Amberger J, Bocchini CA, Scott AF, Hamosh A. McKusick's Online Mendelian Inheritance in Man (OMIM). Nucleic Acids Res. 2009 Jan;37(Database issue):D793-6. doi: 10.1093/nar/gkn665. Epub 2008 Oct 8.
3. <https://www.ncbi.nlm.nih.gov/clinvar/variation/VCV000018432.5>
4. Cho TJ, et al. TRPV4-pathway manifesting both skeletal dysplasia and peripheral neuropathy: a report of three patients. Am J Med Genet A. 2012 Apr;158A(4):795-802. doi: 10.1002/ajmg.a.35268. Epub 2012 Mar 14. PMID: 22419508.
5. Kalia S.S. et al., Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. Genet Med., 19(2):249-255, 2017.

This report has been reviewed and approved by:



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Gene list based on phenotypes used for screening of pathogenic and likely pathogenic variants

AKT1, BMPR1A, CDKN1B, FGFR1, MEN1, PIK3CA, PTEN, ABCA4, ABCB4, ABCC8, ABHD5, ACADVL, ADCY3, ADNP, ADRB3, AFF4, AGBL5, AGRP, AHI1, AHR, AIP, AKT2, ALB, ALMS1, ANOS1, AP1S3, APOE, APPL1, ARHGEF18, ARL13B, ARL2BP, ARL3, ARL6, ARMC5, ARNT2, ARVCF, ATP10A, ATP6AP2, ATRX, BAP1, BAZ1B, BBIP1, BBS1, BBS10, BBS12, BBS2, BBS4, BBS5, BBS7, BBS9, BCL7B, BDNF, BEST1, BLK, BLM, BPTF, BRAF, BRD4, BUD23, CA4, CACNA1S, CANT1, CARTPT, CASZ1, CC2D2A, CCDC141, CCDC28B, CDH23, CDHR1, CEL, CELA2A, CEP164, CEP19, CERKL, CFAP410, CFAP418, CHD7, CHD8, CLIP2, CLRN1, CNGA1, CNGB1, CNM2, CNTNAP2, COA3, COL10A1, COMT, COPB1, CPE, CRB1, CREBBP, CRX, CTNNB1, CTSB, CUL4B, CYP19A1, CYP7A1, DCC, DDB1, DDX6, DEAF1, DHDDS, DHX38, DLK1, DMPK, DNAJC30, DNMT3A, DPYD, DUSP6, DYNC2I2, DYRK1B, EHMT1, EIF2S3, EIF4H, ELN, EMD, EP300, ERMARD, EXOC6B, EYS, FAM161A, FBXO11, FEZF1, FGF17, FGF8, FGF9, FHL1, FKBP6, FLII, FLRT3, FMR1, FOXG1, FOXP1, FSCN2, FXR1, GABRA3, GABRD, GATA4, GCK, GHR, GHRL, GJA5, GJA8, GNAI1, GNAS, GNAS-AS1, GP1BB, GTF2I, GTF2IRD1, GTF2IRD2, GUCA1B, H6PD, HACE1, HCRT, HDAC4, HDAC8, HEATR3, HECTD4, HERC2, HESX1, HGSNAT, HIRA, HLA-DQB1, HLA-DRB1, HNF1A, HNF1B, HNF4A, HS6ST1, HSD11B1, HSPG2, IDH3A, IDH3B, IER3IP1, IFT140, IFT172, IFT27, IFT74, IFT88, IGF1, IGF1R, IGFALS, IL17RD, IL36RN, IMPDH1, IMPG1, IMPG2, INPP5E, INS, IPW, IQSEC2, JMJD1C, KCNAB2, KCNJ11, KCNJ18, KDM6A, KIAA1549, KIDINS220, KIZ, KLF11, KLHL7, KMT2D, LARS2, LAS1L, LEP, LEPR, LIMK1, LIPE, LMNA, LRAT, LUZP1, LZTFL1, MAGEL2, MAK, MAN1B1, MAPK8IP3, MC4R, MCM3AP, MECP2, MEF2A, MEG3, MEGF8, MERTK, METTL27, MIA3, MKKS, MKRN3, MKS1, MLXIPL, MMP23B, MOP, MTFMT, MYT1L, NCF1, NDN, NDNF, NEK2, NEUROD1, NF2, NHLH2, NIN, NIPBL, NKAP, NPAP1, NPHP1, NROB2, NR2E3, NR3C1, NRL, NTRK2, OCA2, OFD1, OTX2, P2RY11, P4HTM, PAX4, PAX6, PCARE, PCNT, PCSK1, PDE11A, PDE4D, PDE6A, PDE6B, PDE6G, PDGFB, PDPN, PDSS1, PDX1, PGM2L1, PHF21A, PHF6, PHIP, PIGA, PIGT, PKDCC, PNKP, PNPLA2, PNPLA6, POGZ, POMC, POMGNT1, POU3F4, PPARG, PRCD, PRDM16, PRKAR1A, PRKAR1B, PRKCZ, PRMT7, PROK2, PROKR2, PROM1, PRORP, PRPF3, PRPF31, PRPF4, PRPF6, PRPF8, PRPH2, PSMD12, PWAR1, PWRN1, RAB23, RAD21, RAI1, RBMX, RBP3, RDH12, REEP6, RERE, RFC2, RGR, RHO, RLBP1, RNPC3, ROM1, RP1, RP1L1, RP2, RP9, RPE65, RPGR, RPS6KA3, RREB1, RTL1, SAG, SATB1, SCAPER, SCLT1, SDC3, SDCCAG8, SEC24C, SECISBP2, SEMA3A, SEMA4A, SETBP1, SETD1A, SETD2, SH2B1, SH3KBP1, SHANK3, SHOX, SIM1, SIN3A, SKI, SLC10A7, SLC25A4, SLC7A14, SLC7A7, SMAD4, SMARCB1, SMARCE1, SMC1A, SMC3, SMO, SNORD115-1, SNORD116-1, SNRNP200, SNRPN, SOX10, SOX2, SOX3, SPATA7, SPEN, SPG11, SPRY4, SPTBN1, SRY, STEEP1, STX16, STX1A, SUFU, SUPT16H, SYNE1, SYNE2, TACR3, TAF1, TBC1D2B, TBL2, TBX1, TBX3, TCF20, TERT, TFE3, THOC2, TMEM270, TMEM43, TMEM67, TMLHE, TNFSF4, TOGARAM1, TOPORS, TP53, TRAF3IP1, TRAF7, TRAPPC9, TRIM32, TRIP12, TTC8, TUB, TULP1, UBE3A, UBE3C, UBE4B, UCP3, UFD1, USH2A, USP48, USP8, VPS13B, VPS37D, WAC, WPCP, WDR11, WNT4, WT1, XRCC4, XYLT1, ZBTB20, ZNF365, ZNF408, ZNF513, ZNF711, ZPR1, CARMIL2, IL7R, LDHA, 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GPX4, GRHL2, GRIA3, GRIA4, GRIN1, GRIN2A, GRIN2D, GRM1, GRM7, GSC, GTF2E2, GTF2H5, GUSB, GYS2, GZF1, H19, H3-3B, H4C11, H4C5, HAAO, HADH, HBA1, HBA2, HCCS, HCFC1, HCN1, HDAC6, HELLS, HES7, HHAT, HK1, HLA-DQA1, HMGA2, HMGB3, HNRNPA1, HNRNPA2B1, HNRNPH1, HNRNPH2, HNRNPU, HOXD13, HPRT1, HRAS, HS2ST1, HSD11B2, HSD17B4, HSPA9, HUWE1, HYAL1, HYOU1, IARS2, IDH1, IDH2, IDS, IDUA, IFIH1, IFITM5, IFT122, IFT43, IFT52, IFT56, IFT57, IFT80, IGBP1, IGF2, IHH, IKBKG, IL11RA, IL6ST, INPP5K, INPPL1, INSR, INTS1, INTS8, INTU, INVS, IPO8, IQCB1, IQSEC1, IRS4, ITCH, ITGB6, JAGN1, JAK1, KANS1, KAT6A, KAT6B, KATNP1, KCNA2, KCNA4, KCNB1, KCNC3, KCNH1, KCNJ1, KCNJ10, KCNJ2, KCNJ5, KCNMA1, KCTD1, KDEL2, KDM1A, KDM3B, KDM5C, KDSR, KIAA0586, KIAA0753, KIF11, KIF14, KIF15, KIF1A, KIF1C, KIF22, KIF23, KIF7, KIFBP, KISS1R, KLF1, KLLN, KMT2A, KMT2B, KMT2C, KNL1, KRAS, KRT10, KYNU, L1CAM, LAGE3, LAMTOR2, LARGE1, LARP7, LBR, LEMD2, LEMD3, LETM1, LGI3, LHX1, LHX3, LHX4, LIFR, LIG4, LIPN, LMBR1, LMBRD1, LMNB1, LMX1B, LONP1, LRP5, LSM11, LSS, LTBP1, LTBP2, LTBP3, LYSET, LZTR1, MAB21L2, MACF1, MAD2L2, MADD, MAF, MALT1, MAP2K1, MAP2K2, MAP3K7, MAPK1, MAPKAPK5, MAPRE2, MARS2, MAST1, MATN3, MBD5, MBTPS1, MBTPS2, MCM4, MCM7, MCM9, MCPH1, MCTP2, MDM2, MED12, MED13L, MEIS2, MESD, MESP2, METTL5, MFSD2A, MGAT2, MGP, MID1, MIR140, MIR17HG, MITF, MLYCD, MMP13, MMP2, MMP9, MORC2, MPL, MPV17, MRAS, MRE11, MRPS22, MRPS25, MSH4, MSMO1, MSTO1, MTHFS, MTOR, MTX2, MVK, MYCN, MYH3, MYH7, MYH8,

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