Whole Exome Sequencing Analysis

Patient name	:	**Miss. XXX**	**PIN**	:	**XX**
Gender/ Age	:	**Female/ 18 Years**	**Sample number**	:	**XX**
Referring clinician	:	**XX**	**Sample collection date**	:	**XX**
Specimen	:	**Peripheral Blood**	**Sample receipt date**	:	**XX**
			Report date	:	**XX**

Clinical history

Proband, Miss. XXX is presented with chief complaints of hypothyroidism, obesity, prediabetic, PCOD, severe vitamin D deficiency, fatty liver and asymmetry in right breast size with dark axillary pigmentation. Her past blood investigations indicative of hyperinsulinemia, high apolipoprotein A1, low alkaline phosphatase, low calcium, low phosphorous and urinary iodine low. She has a history of frequent leg sprains and cramps. She has a significant family history of maternal side with multiple neurofibromatosis, fibrocystic disease of breast and prostate malignancy, her paternal side with history of breast cancer, deafness and psychosomatic disorders. She is diagnosed with metabolic bone disease and suspected to be affected with fibroadenomatosis breast and MEN syndrome. Proband, Miss. XXX has been evaluated for pathogenic variations.

Results

Variants of uncertain significance was identified related to the phenotype

List of uncertain significant variants identified related to the phenotype:

Gene	Region	Variant*	Allele Status	Disease	Classification*	Inheritance pattern
SQSTM1 (+)	Exon 7	c.986A>G **(p.Asp329Gly)**	Heterozygous	Paget disease of bone 3 **(OMIM#167250)**	**Uncertain Significance**	Autosomal Dominant

MC4R (-)	Exon 1	c.899T>C **(p.Leu300Pro)**	Heterozygous**	Obesity (BMIQ20) **(OMIM#618406)**	**Uncertain Significance**	Autosomal Dominant, Autosomal Recessive

*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.

****Autosomal recessive disorder is caused by biallelic (homozygous or compound heterozygous) pathogenic/likely pathogenic variant in the *MC4R* gene. We have detected a single heterozygous variant mentioned in the results table above. However, a second significant heterozygous variant in the *MC4R* gene was not detected. The single heterozygous variant in presence of partially matching phenotype needs to be carefully correlated. The sensitivity of NGS based assays to detect large heterozygous deletions/duplications is low and an alternate method is recommended.**

Interpretation

***SQSTM1*: c.986A>G**

Variant summary: A heterozygous missense variation in exon 7 of the *SQSTM1* gene (chr5:g.179833603A>G; NM_003900.5, Depth: 141x) that results in the amino acid substitution of Glycine for Aspartic acid at codon 329 (p.Asp329Gly) was detected.

Population frequency: This variant in has minor allele frequency of 0.004% in gnomAD database and has not been reported 1000 genomes database.

Clinical evidence: This variant has been previously classified as uncertain significance in ClinVar database [3].

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

OMIM phenotype: Paget disease of bone 3 (OMIM#167250) is caused by heterozygous mutations in the *SQSTM1* gene (OMIM*601530). Paget disease is a metabolic bone disease characterized by focal abnormalities of increased bone turnover affecting one or more sites throughout the skeleton, primarily the axial skeleton. Bone lesions in this disorder show evidence of increased osteoclastic bone resorption and disorganized bone structure. This disease follows autosomal dominant pattern of inheritance [2].

Variant classification: Based on the evidence, this variant is classified as a variant of uncertain significance. **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.**

***MC4R*: c.899T>C**

Variant summary: A heterozygous missense variation in exon 1 of the *MC4R* gene (chr18:g.60371451A>G; NM_201548.5, Depth: 186x) that results in the amino acid substitution of Proline for Leucine at codon 300 (p.Leu300Pro) was detected.

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

Clinical and Literature evidence: This variant has been previously classified as uncertain significance in ClinVar database [4]. *In vitro* functional studies suggest this variant results in the decreased cell surface expression, with corresponding decrease in ligand binding [5].

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

OMIM phenotype: Obesity (BMIQ20) (OMIM#618406) is caused by heterozygous or homozygous mutations in the *MC4R* gene (OMIM*155541). Obesity due to mutation in the *MC4R* gene is the most common cause of monogenic obesity. Patients have early-onset severe obesity and hyperphagia. This disease follows autosomal dominant and autosomal recessive pattern of inheritance [2].

Variant classification: Based on the evidence, this variant is classified as a variant of uncertain significance. **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.**

****Autosomal recessive disorder is caused by biallelic (homozygous or compound heterozygous) pathogenic/likely pathogenic variant in the *MC4R* gene. We have detected a single heterozygous variant mentioned in the results table above. However, a second significant heterozygous variant in the *MC4R* gene was not detected. The single heterozygous variant in presence of partially matching phenotype needs to be carefully correlated. The sensitivity of NGS based assays to detect large heterozygous deletions/duplications is low and an alternate method is recommended.**

Recommendations

- The *SQSTM1* gene has pseudogene in the human genome. Validation of the variant(s) by Sanger sequencing is strongly recommended to rule out false positives.
- 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	13.32 Gb
Data ≥ Q30	96.71%

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 [6].

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/VCV000650222.9
4. https://www.ncbi.nlm.nih.gov/clinvar/variation/VCV002584829.2
5. Wang ZQ, Tao YX. Functional studies on twenty novel naturally occurring melanocortin-4 receptor mutations. Biochim Biophys Acta. 2011 Sep;1812(9):1190-9. doi: 10.1016/j.bbadis.2011.06.008. Epub 2011 Jun 30. PMID: 21729752; PMCID: PMC3155388.
6. 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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