and *WDPCP*. These genes are associated with 28 phenotypes such as Alström syndrome; Bardet-Biedl syndrome; Leptin deficiency; Leptin receptor deficiency; Obesity due to MC3R deficiency; Insulin resistance, Lipodystrophy etc. The sequence analysis included all protein coding exons, exon-intron boundaries of each gene therein, and some mutations outside the coding region. It was used to detect mutations such as single nucleotide substitutions [SNVs], and small insertions and deletions (INDELs). The test showed an analytical sensitivity of 99.3% and 99.9% for detecting SNPs. Its sensitivity for detecting INDELS depended on the size of the alteration: 1-10bps (96.0%), 11-20 bps (88.4%) and 21-30 bps (66.7%). The test should not be used for detection of balanced translocations, complex inversions, and low-level mosaicism. Adjunct Del/Dup (CNV) analysis was available.

Total genomic DNA was extracted from saliva, and quantitated by fluorometric analysis. A ligation-based sequencing library was prepared, and amplified using PCR. Its quality and quantity were assessed using electrophoresis, and fluorometric analyses, respectively. Next, targeted sequencing was carried out. Raw sequence reads were filtered, and high-quality reads of sufficient length were compared to the human genome reference sequence [Hg19]. Single nucleotide polymorphisms [SNPs], and INDELS were identified using a sequencing data analysis pipeline. Each Pathogenic/Likely Pathogenic variant was confirmed using bidirectional Sanger sequencing.

Pathogenic potential of variants were evaluated by comparing biochemical properties of codon change, evolutionary conservation, and allelic frequencies to datasets such as the 1000 Genomes project, the ExAC consortium and ClinVar. Reporting was carried out per the Human Genome Variation Society (HGVS) and American College of Medical Genetics and Genomics (ACMG) guidelines.

FINAL DIAGNOSIS

The patient tested positive for a homozygous missense variant c.1055G>A, p.(Cys352Tyr) in the *LEPR* gene. This variant was classified as 'likely pathogenic' and the probable cause for patient's disease, considering the current evidence for the variant. Obesity due to *LEPR* mutations is inherited in an autosomal recessive [AR] manner. Additionally, we identified another variant *SDCCAG8* c.1513C>G, p.(Gln505Glu) in the patient. This was classified as a variant of uncertain significance [VUS]. Test results were interpreted in the context of clinical findings, and family history.