Supplementary Methods

Assay design and analysis

This study was implemented as part of a research project involving genetic causes of Monogenic Diabetes.

Formerly, *HNF1A*, one of the most frequent MODY genes, was analyzed by Sanger sequencing. No pathogenic variant was identified in the coding region.

Due to negative result, next-generation sequencing (NGS) was performed. We designed a customized NGS panel including **51 nuclear genes** [**7 MODY genes** (*HNF4A*, *HNF1A, KLF11*, *CEL*, *PAX4*, *BLK*, *APPL1*), **7 genes** related to **MODY and Neonatal Diabetes** (*GCK*, *PDX1*, *HNF1B*, *NEUROD1*, *INS*, *ABCC8*, *KCNJ11*), **17 Neonatal Diabetes genes** (*PTF1A, NEUROG3, RFX6, ZFP57, GLIS3, IER3IP1, GATA6, GATA4, EIF2AK3, SLC19A2, SLC2A2, CP, PLAGL1, HYMAI, NKX2-2, MNX1, PAX6*), **3 Monogenic Autoimmune Diabetes genes** (*FOXP3, AIRE, STAT3*), **2 Wolfram Syndrome genes** (*WFS1, CISD2*), **1 gene** associated with **Insulin Resistance Syndromes** (*INSR*), **11 Lipodystrophy genes** (*AGPAT2, BSCL2, CAV1, PTRF, LMNA, PLIN1, ZMPSTE24, AKT2, CIDEC, TBC1D4, PPARG*), **3 Familial Hyperinsulinemic Hypoglycemia genes** (*HADH*, *GLUD1*, and *SLC16A1*)], in addition to **mitochondrial genome**. Target region comprised all exons, intron-exon boundaries, and promoter regions. After sequencing, bioinformatics analysis of the generated genomic data was implemented using in-house pipelines (based on BWA-MEM, FreeBayes, and ANNOVAR), comprising steps such as sequence alignment, calling variants and genomic annotation. Investigation of sequence variants included: 1- filtering called variants; 2- visualization by Integrative Genomics Viewer software (IGV); and 3- analysis of copy number variation (CNV) using Copy Number Targeted Resequencing Analysis (CONTRA). The analysis was performed considering all genes possibly associated with the patient's phenotype.