Supplementary Methods

DNA and RNA extraction. Genomic DNA purified from fresh whole blood using the Gentra Autopure method on the Qiagen Autopure LS instrument (Germantown, MD, USA) according to the manufacturer's instructions. RNA was extracted from whole blood collected in a Qiagen (Germantown, MD, USA) PAXgene collection tube and frozen using the BioRobot Universal System (Qiagen Germantown MD).

Targeted capture and exome sequencing. The Illumina Hiseq 2000 platform (Illumina, Inc. San Diego, CA) was used to perform exome-sequencing. The target regions (CCDS exonic regions and flanking intronic regions totaling ~50Mb of genomic DNA) were captured using the Agilent SureSelect Human All Exon 50Mb Kit (Agilent Technologies, Santa Clara, CA) following the vendor provided protocols. The samples were sequenced using paired 100x100 basepair reads. Each read was then aligned to the reference genome (NCBI human genome assembly build 36; Ensembl core database release 50_361 [1]) using the Burrows-Wheeler Alignment (BWA) tool [2] and single nucleotide variants (SNVs) and small insertion-deletions (indels) were identified by using SAMtools [3]. PCR duplicates were removed using the Picard software (http://picard.sourceforge.net).

Confirmation of *de novo* Variants using Sanger sequencing. The amplification was done using the ABI 9700 (Applied Biosystems, life technologies. Carlsbad, CA) and sequencing was done on the ABI 3730 (Applied Biosystems, Life technologies. Carlsbad, CA) using Life Technologies Big Dye Terminator V 1.1 cycle sequencing kit cat # 4336778. The results were analyzed using

Sequencher (Gene Codes, Ann Arbor, MI), comparing the sequence of the parents to that of the proband. Primer sequences available upon request

Real time PCR. Full-length and partial cDNA encoding the gene regions of interest was amplified from first-strand cDNA derived from the patient's mRNA with an RNeasy plus mini kit (QIAGEN, Santa Clarita, CA), High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster city, CA), PfuUltra II Fusion HS DNA Polymerase (Agilent Technologies, Palo Alto, CA). The specific primer sets are available upon request.

Plasmids. Full-length cDNAs encoding each isoform of the gene under study were subcloned into the pCR-Blunt II-TOPO vector (Invitrogen-Life Technologies, Carlsbad, CA, USA) and subjected to sequence analysis (pCR-Blunt II-WT and pCR-Blunt II-Isoform). cDNAs encoding the different isoforms were subcloned into pcDNA3.1(+) vector (Invitrogen-Life Technologies) using the Hind III and Xho I sites from pCR-Blunt II--WT or pCR-Blunt II--Isoform, respectively, and sequenced (pcDNA3.1(+)-WT or pcDNA3.1(+)-Isoform).

Western Blotting Empty pcDNA3.1 (+) vector, pcDNA3.1(+)-TCF4-WT or pcDNA3.1(+)-TCF4-Isoform were transfected into monkey kidney cell line COS-7 or human kidney cell line HEK-293 by lipofection using Lipofectamine 2000 (Invitrogen-Life Technologies). After 32h, COS-7 transfectants were incubated with 10μM of GM132 (Sigma Aldrich, St Louis, MO) or 10μM of Lactacystin (Sigma Aldrich, St Louis, MO) as the proteasome inhibitors. After 48h of transfection, the cells were lysed with RIPA buffer (Sigma Aldrich, St Louis, MO) with Protease inhibitor cocktail (Sigma Aldrich, St Louis, MO), and the lysates were subjected to SDS–PAGE gel and transferred to a polyvinylidene difluoride membrane (Millipore, Billerica, MA). The

membranes were incubated with anti-human-ITF2 antibody (N-16, Santa Cruz biotechnology, Santa Cruz, CA) or anti-GAPDH (Cell Signaling Technology, Danvers, MA). Proteins were visualized with the ECL plus western blotting detection system (GE Healthcare, Piscataway, NJ).

NGL1 Protein Expression Assay Peripheral blood mono nuclear cells (PBMCs) were derived from the whole blood of patient, both healthy parents, and three unrelated healthy controls. PBMCs were lysed by RIPA buffer with protease inhibitor cocktail, and lysates were subjected to SDS–PAGE gel and transferred to a polyvinylidene difluoride membrane. The membranes were incubated with anti-NGLY1 antibody (Sigma Aldrich) or anti-GAPDH. Proteins were visualized with the ECL plus western blotting detection system.

References

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