Figure S1

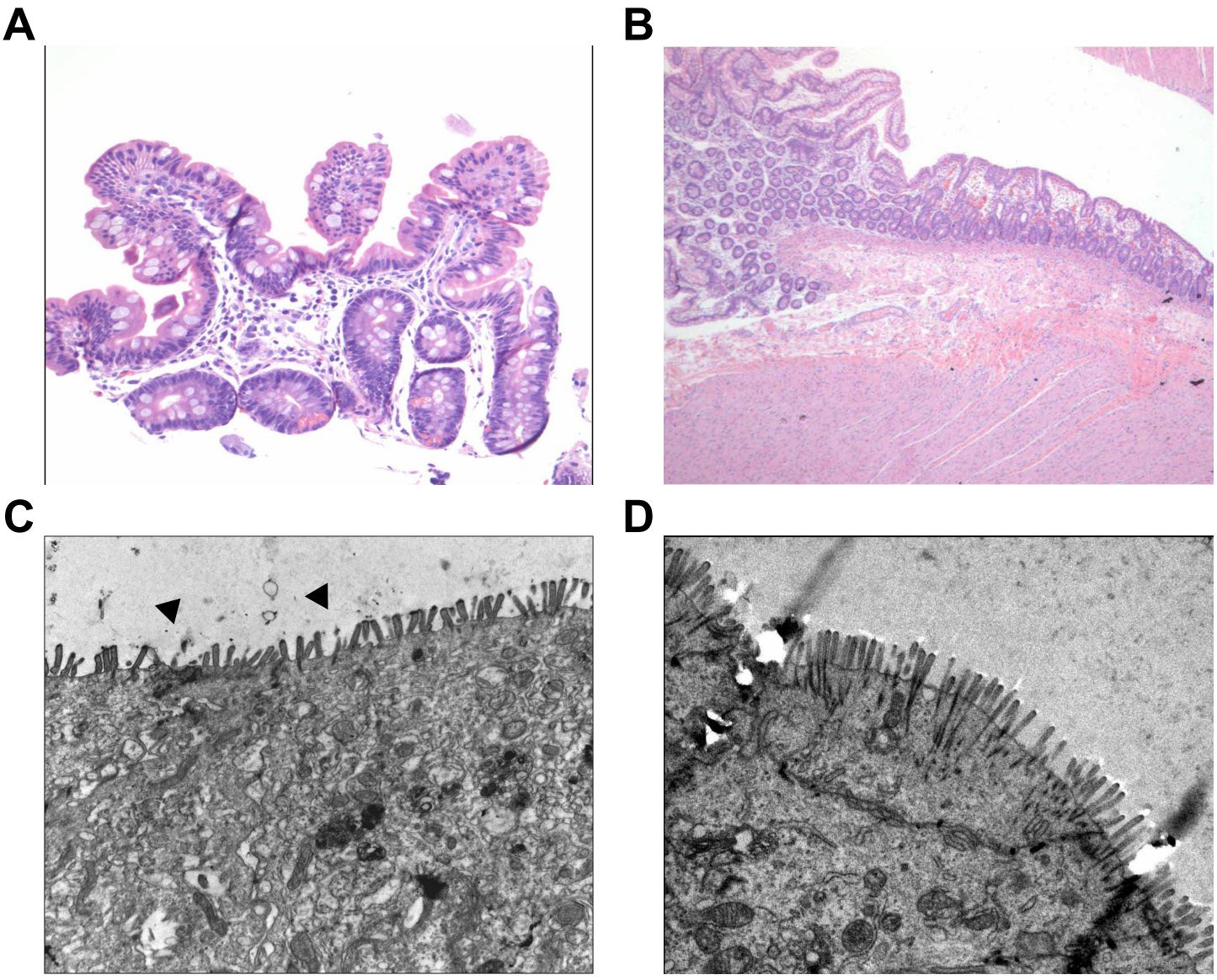

Figure S1. Histopathology of Case 1 show areas of villous atrophy and microvillous dystrophy in proximity to areas of normal morphology. A. Jejunal biopsy at 1 month of age shows preserved villi and normal crypts with Paneth cells. There was no evidence for increased intraepithelial lymphocytes. **B.** A duodenal biopsy shows an area of severe enteritis adjacent to area with intact villi. There does not appear to be increased inflammation in the lamina propria.**C.** Distorted and separated microvilli are present in some areas of the biopsy. Enterocytes show well preserved cytoplasmic organelles, including mitochondria, endoplasmic reticulum and lysosomes. There is slight dilation of the ER and an increase in number of lysosomes, but intracellular junctions and basement membranes appear intact. **D.** A second biopsy taken 11 months later still shows some areas of poorly formed microvilli, and overall cellular morphology appears unchanged from first biopsy.

Figure S2

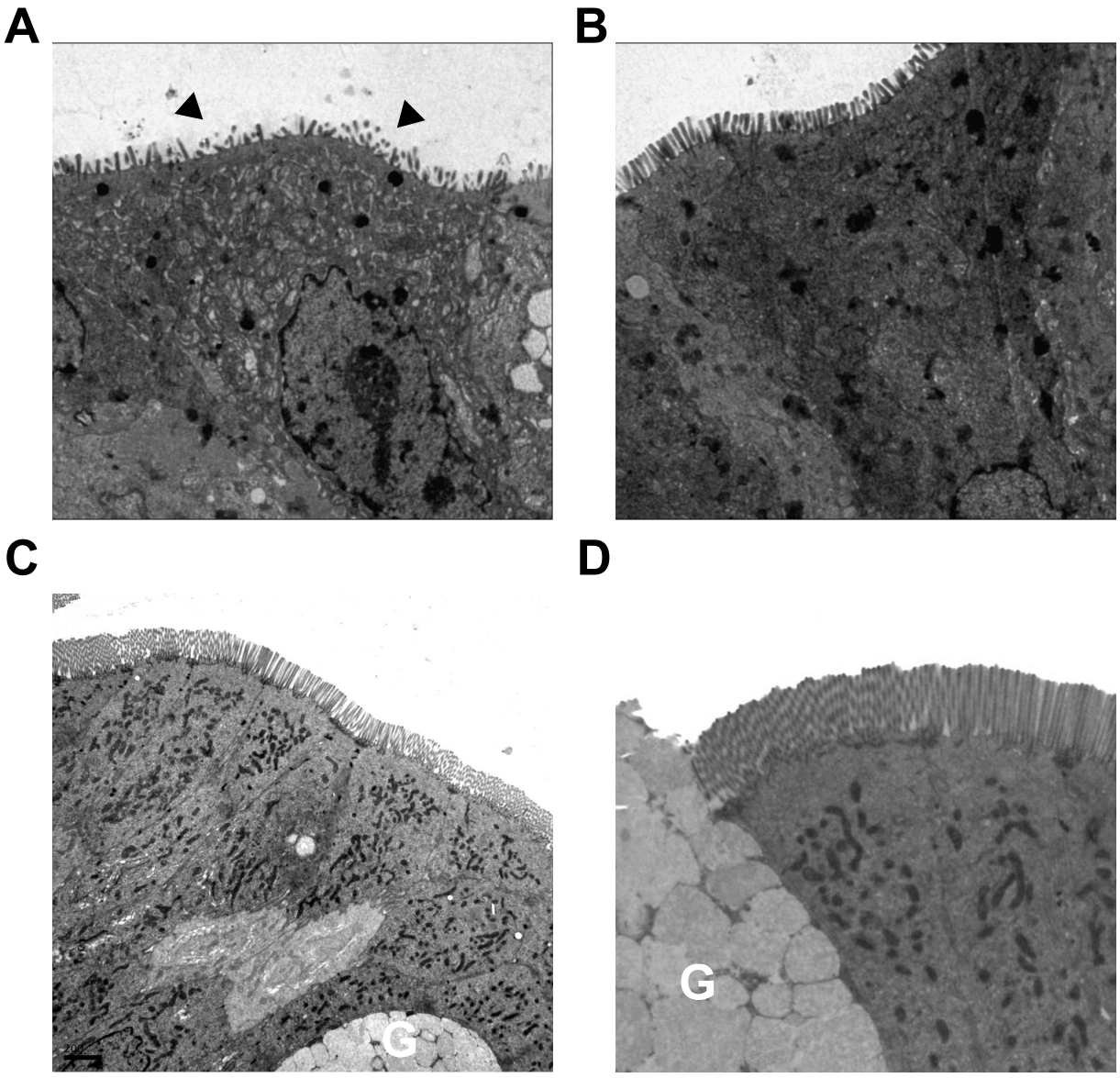

Figure S2. Electron micrographs of duodenal biopsies from Case 2 show areas of microvillous dystrophy with moderate improvement over time. A. Microvillous malformations (arrows) present in duodenal biopsy taken from Case 2 at age two months. Similar characteristics as Case 1, including slight dilation of the ER and increased lysosome numbers, but otherwise good preservation of cytoplasmic organelles, intracellular junctions and basement membranes. **B.** Another field taken at age 2 months shows better preservation of microvilli. **C.** and **D.** A second biopsy taken after improvement of symptoms (age 13 months) shows improved morphology of microvilli. Goblet cells (G) can be seen, some with depleted mucin vacuoles.

Exome Capture and Sequencing

Exome capture was performed on genomic DNA with Agilent Whole Exome SureSelect v2 kit according to manufacturer's instructions. Captured exome DNA was then subjected to Illumina sequencing. Reads were processed by Picard and aligned to the human reference genome hg19 (1) with Burrows-Wheeler Aligner (2), and then single nucleotide variant (SNV) calling on the exomes was performed by using the GATK toolkit (3). Search parameters were set for identification of rare (frequency < 1% in available exome pool) alleles with predicted nonsense, splice or frameshift, or non-synonymous and predicted deleterious (4). Criteria were further constrained by searching for mutations with expected Mendelian ratio in parents and affected child.

Restriction Fragment Length Polymorphism Assay

A restriction fragment length polymorphism assay was designed with the following primers: F-*TGAGCTCGTAGCACAAGGTG,* R-*TAGCCTCTGCAGGGAAGAAG*. PCR on genomic DNA was performed with subsequent digestion by the restriction enzyme *Fnu*4-HI. The digested products were separated by agarose gel electrophoresis. The wildtype allele yields bands sized 165, 32 and 3 bp (latter not resolved); the mutant allele yields bands sized 122, 43, 32 and 3 bp.

cDNAs and Plasmids

Wildtype *DGAT1* cDNA was isolated from HeLa cDNA by PCR and subcloned, with or without FLAG-tag sequences, into a modified pMSCV-puro vector containing a CMV promoter (Clontech). The PGK:puromycin cassette was replaced with a PGK:mRuby-T2A-Zeocin cassette encoding the red fluorescent protein mRuby (5), the T2A self cleaving peptide, and a Zeocin resistance gene. Δ8 *DGAT1* cDNA was generated as described (6). FLAG-H415A *DGAT1* was generated with Site-Directed Mutagenesis kit (Stratagene).

Cell Lines

Mouse embryonic fibroblasts (MEFs) were isolated from E14.5 embryos and immortalized by serial passaging (7). Immortalized cells were transduced with retrovirus encoding *DGAT1* cDNAs. Cells were cultured in DMEM-High Glucose medium (Invitrogen) with 10% FBS (Thermo Fisher). Stable cell lines were selected in 300 μg/mL Zeocin (Invitrogen).

DGAT1 Activity

DGAT activity was measured as described (8) with conditions specific for DGAT1. Briefly, 7.5 μg of microsomal proteins were added to a reaction mix of 100 mM Tris-HCl pH 7.5, 50 mM $MgCl_2$, 0.625 mg/mL BSA, 25 μM [^{14}C]-oleoyl-CoA, and 200 μM 1,2-dioleoyl-glycerol (DAG) in a final volume of 200 μL. The assay was carried out at 37 °C for 5 min and quenched with 2:1 chloroform:methanol. Lipids were separated by TLC and visualized and quantified with a phosphorimage screen.

RT-PCR

For cells, total RNA was isolated with Trizol (Invitrogen). For patient samples, total RNA was

isolated from whole blood collected in Paxgene (Qiagen) tubes. White cells were isolated by centrifugation and washed twice with PBS. RNA was isolated from white cells using RNEasy spin columns (Qiagen) according to manufacturer's instructions. RNA samples were reverse-transcribed (RT) using the iScript cDNA Synthesis kit (BioRad). RT reaction products were diluted 10-fold for PCR reactions.

Immunoblotting

Anti-FLAG monoclonal antibody M2 (Sigma Aldrich) was used to detect the FLAG epitope (1:2000). Other antibodies were anti-DGAT1 polyclonal antibody NB110-41487 (1:1000, Novus Biologicals) and anti-HSP90 (1:2000, BD Biosciences). Secondary antibodies conjugated to HRP (1:5000, Amersham) were used for detection with ECL reagents (Pierce).

Statistics

The probability of the Δ8 mutation causing the CDD phenotype was calculated empirically from the frequency of any homozygous or compound heterozygous and deleterious mutations (9).

Study Approval

Eight members of one family with two affected individuals were studied. Written informed consents for all adult participants and the parents of the children were obtained to permit genetic studies of their samples which are included in a biorepsitory (HSW) that was approved by the Partners Institutional Review Board.

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