A patient with a novel homozygous missense mutation in *FTO* and concomitant nonsense mutation in *CETP*

Ahmet O Çağlayan1,2,6, Beyhan Tüysüz3,6, Süleyman Coşkun1, Jennifer Quon1, Akdes S Harmançılı, Jacob F Baranoski1, Burçin Baran1, E Zeynep Ersön-Omay1, Octavian Henegariu1, Shrikant M Mane4, Kaya Bilgüvar5, Katsuhito Yasuno1 and Murat Günel1

The fat mass and obesity associated (*FTO*) gene was the first gene locus reported to be associated with body weight and metabolic disorders.1-3 Since then, a cluster of common variants in the first, and largest, intron of *FTO* have been described across multiple populations of different ethnicities.6-9 Recently, Boisiel et al.10 described a consanguineous Palestinian-Arab family with a homozygous nonsynonymous *FTO* mutation that led to an inherited life-threatening disease in nine family members—this was the first report of a homozygous *FTO* mutation in the literature. The identified mutation resulted in an arginine to glutamine change at position 316 (R316Q) thereby rendering FTO catalytically inert.10 The affected family members suffered from postnatal growth retardation, head and face dysmorphisms, severe psychomotor delay, functional cognitive deficits, and, in some patients, brain malformations, cardiac defects, genital abnormalities and cleft palates. In all affected individuals, death occurred within the first 30 months of life. In this report, we describe a patient with a novel homozygous missense mutation in *FTO*. We further discuss the phenotypic expression of this mutation.

**INTRODUCTION**

The fat mass and obesity associated (*FTO*) gene was the first gene locus reported to be associated with body weight and metabolic disorders.1-3 Since then, a cluster of common variants in the first, and largest, intron of *FTO* have been described across multiple populations of different ethnicities.6-9 Recently, Boisiel et al.10 described a consanguineous Palestinian-Arab family with a homozygous nonsynonymous *FTO* mutation that led to an inherited life-threatening disease in nine family members—this was the first report of a homozygous *FTO* mutation in the literature. The identified mutation resulted in an arginine to glutamine change at position 316 (R316Q) thereby rendering FTO catalytically inert.10 The affected family members suffered from postnatal growth retardation, head and face dysmorphisms, severe psychomotor delay, functional cognitive deficits, and, in some patients, brain malformations, cardiac defects, genital abnormalities and cleft palates. In all affected individuals, death occurred within the first 30 months of life. In this report, we describe a patient with a novel homozygous missense mutation in *FTO*. We further discuss the phenotypic expression of this mutation.

**MATERIALS AND METHODS**

**Ethics statement**

The study protocol was approved by the Yale School of Medicine Human Investigation Committee (HIC) (protocol number 0908005392). Institutional review board approval for genetic and magnetic resonance imaging studies and written consent from all study subjects were obtained by the referring physicians at participating institutions.

**DNA extraction**

Blood samples were collected from patients and their parents. DNA was extracted from the blood using the commercially available Gentra Puregene Blood Kit from Qiagen (Hilden, Germany).

**Whole-genome genotyping**

Samples were analyzed using 610 K Quad Bead Chips according to the manufacturer's protocol (Illumina, San Diego, CA, USA).

**Whole-exome capture and sequencing**

Exome capture for the index case was performed using the NimbleGen 2.1 M human exome array (Roche Nimblegen, Inc., Madison, WI, USA) according to
the manufacturer’s protocol along with modifications previously described in
the literature.11,12 Exome library sequencing was performed using the
HiSeq2000 with barcoding technology, paired end analysis and six samples
per lane. Image analysis and subsequent base calling were performed using the
Illumina pipeline (version 1.8, Illumina, Inc.).

Exome data analysis
Analysis of the sequencing data was performed according to the previously
described bioinformatics pipeline devised by our research team.13

Sanger sequencing
Coding regions and exon–intron boundaries of FTO were evaluated by Sanger
sequencing using standard protocols. Amplicons were cycle sequenced on ABI
9800 Fast Thermocyclers (Applied Biosystems, Foster City, CA, USA), and post
cycle sequencing clean-up was carried out with the CleanSEQ System (Beckman
Coulter Genomics, Danvers, MA, USA). The amplicons were analyzed on
3730 × L DNA Analyzer (Applied Biosystems Inc.).

Copy number variation analysis
The depth of coverage log ratio between the patient and control samples was
calculated using the GATK-Depth of Coverage tool. Segments with copy
number variations (CNVs) were identified from the log ratio of the depth of
coverage using the ExomeCNV R package.14 False positive CNV events were
identified and corrected for by calculating minor allele frequencies (B-allele
frequency) in each CNV segment.

Co-expression analysis
Co-expression patterns were analyzed using the Database for Annotation,
Visualization and Integrated Discovery (DAVID) v6.7.13 Intergation and
procurement of results were performed using previously established
protocols.16,17

Skin biopsy and fibroblast culture
Four millimeter skin punch biopsies were obtained from the umbilical area of
the patient (NGI305-1), her parents (NGI305-2 and NGI305-3) as well as
from control individuals using a standardized procedure.18,19 Samples were
maintained in 50 ml conical tubes filled with Dulbecco’s Modified
Eagle Medium (DMEM; Gibco, Waltham, MA, USA, cat. no. 11965-084)
supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco, cat.
no. 10438-026), 1% (1 ×) l-glutamine (Gibco, cat. no. 2530-081) and 2%
(1 ×) Penicillin-Streptomycin (Gibco, cat. no. 15140-122), and subsequently
transported to the laboratory for culture. Once they arrived in the laboratory,
samples were washed at least three times in phosphate-buffered saline (PBS)
(Sigma Chemical Co., Saint Louis, MO, USA). Samples were cut into small
fragments and placed in 100 mm2 Petri dishes, which were maintained for
30 min semi-opened in laminar ow to allow specimens to adhere to the dish
surface. Afterwards, 10 ml of culture medium containing DMEM supplemented
with 10% heat-inactivated fetal bovine serum, 1% (1 ×) l-glutamine, 1% (1 ×)
Penicillin-Streptomycin at 37 °C was added to the dish. Cultures were
maintained in a humidified incubator at 37 °C, under 5% CO2 in air. The
culture medium replaced initially after 2 days, and subsequently changed three
times a week. Fibroblasts began to emerge from the biopsy fragments within in
7–9 days. Subculture (passage) of fibroblasts was performed at 70% cellular
confluence.

Once there were sufficient cells, enzymatic detachment was performed using
0.25% Trypsin-EDTA (1 × 1) (Gibco, cat. no. 25200-056) and cells were plated in
25 cm2 culture flasks for additional proliferation. Primary fibroblasts were
grown at 37 °C in 5% CO2 and medium was changed every 2 days. Cells were
passaged using trypsin at approximately 70% confluence and either harvested
for cryopreservation or reseeded for further proliferation. Early passages of
fibroblasts were used for cell-based functional assays.

Population doubling (PD) assay
Cells were trypsinized upon confluence, and 2000 cells per well were seeded
in 135 μl of medium. There were nine wells per individual

NGI305-1, NGI305-2, NGI305-3, CTRL-1, CTRL-2 and CTRL-3 included
on a 96 well-plate. A separate plate of cells was also included using serial
dilutions of cells for calibration curve. After 2nd, 5th, 7th, 9th and 11th days of
incubation, we performed a CellTiter-Glo Luminescence Cell Viability Assay
(Promega, Madison, WI, USA). Luminescence was read using the GloMax-
Multi Detection System (Promega), and data were analyzed with the Graphpad
Prism software (GraphPad Software, Inc., La Jolla, CA, USA).

Fibroblast morphology and apoptosis assay
Fibroblasts were seeded in 24-well plates, and images were obtained on the 2nd,
5th, 7th, 9th and 11th days using an inverted microscope at × 20 magnification.
Apoptosis was evaluated with an Annexin V FITC and 7-amino-actinomycin
dye kit (Ebioscience, San Diego, CA, USA) according to the manufacturer’s
protocol. Briefly, cells were harvested and washed in 1 × PBS, and then
resuspended in 1 × Binding Buffer at 1–5 × 106 cells per ml. In all, 5 μl of
fluorochrome-conjugated Annexin V was added to 100 μl of the cell suspension,
which was then incubated 10–15 min at room temperature and protected from
light. Cells were subsequently washed with 2 ml of 1 × Binding Buffer and
resuspended in 200 μl of 1 × Binding Buffer. In all, 5 μl of 7-amino-
actinomycin viability staining solution was added to the solution, which was
stored at 2–8 °C in the dark, and analyzed within 4 h using flow cytometry.

Immunohistochemistry and immunostaining
For immunohistochemistry of fibroblasts from extracted skin biopsies of index
case, her parents and control sample were fixed in 3.6% formaldehyde and
washed three times with PBS. Then, cells were exposed to 0.3% Triton X-100
(Sigma Chemical) in PBS for 1 min and then washed two times with PBS. Cells
were blocked in blocking solution containing 1 × PBS, 5% normal bovine
serum, 5% normal goat serum, 5% normal donkey serum and 0.1% Tween for
half an hour at room temperature. After blocking, specimen was extensively
washed in PBS and incubated with primary antibodies diluted in blocking
solution on a horizontal shaker overnight at 4 °C, and washed in PBS at room
temperature for three times. Cells were then incubated with secondary
antibodies in blocking solution for 1 h and washed in PBS for three times at
room temperature, then washed and imaged with the Zeiss microscopy system
(Jena, Germany). Primary antibody used for immunohistochemistry is a
custom rabbit anti-recombinant mFTO antibody (1:100 dilution), Alexa Fluor
488 Donkey Anti-Rabbit IgG (H+L) antibody (A-21206, Life Technologies,
Carlsbad, CA, USA) was used as a secondary antibody.

Western blot analysis
Protein expression was analyzed by western blot analysis as described previously.20 Proteins were extracted from fibroblasts of patient, her father
and mother and control subject, then protein blots were performed on 40 μg of
total proteins. The primary antibodies used were as follows: a custom rabbit
anti-recombinant mFTO antibody (1:100 dilution), anti-glyceroldehyde
3-phosphate dehydrogenase (1:100 dilution, Rabbit, catalog no.: sc-25778;
Santa Cruz Biotechnology, Santa Cruz, CA, USA). The horseradish peroxidase-
conjugated secondary antibody used was AffiniPure goat-anti-rabbit IgG
FC-fragment (Jackson, West Grove, PA, USA; #111-035-046).

RNA extraction, complementary DNA synthesis and quantitative PCR analysis
FTO mRNA expression in affected individual, both parents and control sample
(individual of the same ethnicity and without an FTO sequence variant is used as
a control) was assessed by real-time PCR. Total RNA was isolated using the
miRNeasy Mini Kit (Qagen; catalog no.: 24104) from fibroblasts according to
the manufacturer's instructions, and transcribed into complementary DNA as
described previously (Applied Biosystems; catalog no: 43749866). RNA quantity
was assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific
Inc, Wilmington, DE, USA) for complementary DNA synthesis. Quantitative
PCR (qPCR) analysis was performed using FastStart Universal SYBR Green
Master Mix (Roche; catalog no: 4913914001). Two different FTO and
the reference gene TBP (TATA box-binding protein) primers were used; and the
PCR efficiency of >90% (slope = −3.2 and −3.6) and R2 99% obtained.
Relative changes in gene expression were analyzed with the ΔCT method.21
Transcriptome analysis
To further characterize the effects of the FTO mutation in the family, RNA was extracted from these fibroblast cultures; and RNA expression analysis using Illumina HumanHT12.v4 chips was performed on patient, father and mother samples and analyzed using the DAVID platform. Briefly, data are normalized using normal-exponential convolution model-based background correction and quantile normalization using the limma R package. The normalized data for three samples were used to perform an unsupervised hierarchical clustering using the euclidean distance as the dissimilarity metric and the average agglomerative method for clustering.

RESULTS
Clinical report
We report the case of a 5-year-old female (NG1305-1) presented with a dysmorphic face and developmental delay. She was the first child born of a consanguineous union (parents were second cousins) (Figure 1). She was born at 40 weeks gestation with 2460 g (3rd percentile) and 50 cm long (50th percentile). Her head circumference at birth was not recorded. She was immediately admitted to the neonatal intensive care unit after birth due to respiratory distress and concern given her dysmorphic facial features. During her hospitalization, she began to have seizures that were managed with antiepileptic medications. She was subsequently discharged, but was later admitted at nine and a half months old. At this time, her weight was 7650 g (10th percentile), height was 66 cm (3rd percentile) and head circumference was 41 cm (<2nd percentile). On examination, she was hypertonic and lacked the ability to control her head movements. She was microcephalic, had sparse hair and eyebrows, a prominent metopic ridge, an asymmetric skull, and a short neck (Figure 1b). She was noted to have hepatosplenomegaly and diastasis recti. Laboratory workup revealed high aspartate aminotransferase (70 U l\(^{-1}\)) and alanine aminotransferase (57 U l\(^{-1}\)) and creatine kinase (1191 U l\(^{-1}\)) levels. Eye examination demonstrated bilateral nystagmus, iris nodules, posterior synchia and astigmatism. Assessment of auditory brainstem responses demonstrated bilateral conductive and sensorineural hearing loss. At 19 months of age, the patient’s weight was 11 kg (<3rd percentile), height was 83.5 cm (<3rd percentile) and head circumference was 45 cm (<3rd percentile). In addition to the previously noted physical exam findings, she was now also found to have difficulty swallowing. An echocardiogram was performed and demonstrated normal cardiac anatomy and function. Upper endoscopy revealed grade 1 esophageal varices. A head computed tomography demonstrated prematurely closed metopic sutures (craniosynostosis). A brain magnetic resonance imaging revealed a thin corpus callosum. Chromosomal analysis demonstrated a normal female karyotype. In addition to the physical exam abnormalities, the patient also demonstrated developmental delay with respect to reaching milestones—she did not develop head control until after 10 months of age, began sitting without support at 2 years old and walking at 4 years old. Throughout this time, her aspartate aminotransferase level was normal (29 U l\(^{-1}\)) and alanine aminotransferase (29 U l\(^{-1}\)) levels, high creatine kinase (587 U l\(^{-1}\)), normal total cholesterol (153 mg dl\(^{-1}\)), normal HDL cholesterol (127 mg dl\(^{-1}\)), and normal total cholesterol (153 mg dl\(^{-1}\)) (Figure 1). The FTO protein. This variant analysis identified only two homozygous novel mutations located within the aforementioned regions of homozygosity (Supplementary Table 4) (Figure 1). The first was a homozygous missense mutation (ENST00000471389.1:c.812A>T) in the FTO coding sequence at position 53,878,127 on chromosome 16. This mutation resulted in a histidine to proline change at position 271 (ENSP00000418823.1:p.His271Pro) in the amino-acid sequence of the FTO protein. This FTO mutation has not been previously reported in the dbSNP, NHLBI GO ESP Exome Variant Server, or 1000 Genomes
databases, nor has it been observed within the cohort of 3000 subjects with non-neurological diseases who have been whole-exome sequenced at the Yale School of Medicine. When we interrogated these databases for the mutational burden of FTO, we found no instances of homozygous deleterious mutations in FTO.

The other detected mutation (ENST00000566128.1:c.1207C>T) was in the cholesteryl ester transfer protein, plasma (CETP) gene at position 57,017,318 on chromosome 16, resulting in an early protein truncation and likely subsequent nonsense mediated decay (ENSP00000456276.1:p.Arg403X).

Figure 1 Phenotypic and molecular studies have been performed on materials obtained from index patient, parents and control samples. (a) Pedigree of the family. (b) Clinical pictures of the patient (upper right panels) when she was 9 months old (upper panels), 2 and half years old (left lower panel) and 4 and nine months old (right lower panel). (c, d) Representative sequence alignment figures cover the mutations in FTO and CETP, respectively. The top line in each panel represents the non-mutated reference sequence. The subsequent lines below the reference lines depict the results from exome sequence. Each line represents a distinct coverage read. Mean 20x coverage of all bases was above 81% for index patient. (e, f) Chromatogram illustrations of FTO and CETP obtained via Sanger sequencing analysis of the index patient analyzed via whole-exome sequencing (NG1305-1) and her parents. Note that the respective mutations identified via whole-exome sequencing were confirmed as homozygous mutations in the index patient and as heterozygous in her parents. DNA from healthy individuals was also Sanger sequenced; and these results are included as controls. (g) CNV Segment Detection of NG1305-1: The log ratio comparing NG1305-1 and control sequence depths of coverage for each exon is depicted as gray dots. The black lines demonstrate regions of segmented copy neutral events, green lines are segmented deletion events and red lines are amplification events. (h) mRNA levels of FTO in fibroblast cells were extracted from homozygous patient, heterozygous parents and control individual—same ethnicity and without a sequence variant—and without a sequence variant—were analyzed using real-time PCR (RT-PCR). (i) The figure depicts FTO staining in patient fibroblasts. Significant nuclear accumulation is noted. (j) Western blot of FTO and as an internal control GAPDH in patient, parental and control cells. No difference was detected. A full color version of this figure is available at the Journal of Human Genetics online.
Sanger sequencing of the entire coding region of FTO and CETP in the patient’s parents revealed that both were heterozygous for the identified mutations (Figure 1). The parents were then re-examined for metabolic findings previously reported in association with FTO mutations, but both were healthy and had no evidence of abnormal metabolic functioning. The patient’s exome data were analyzed for large scale CNV events, and no disease-causing large duplications or deletions within coding regions were identified (Figure 1).

Co-expression data analysis
Using the Human Brain Transcriptome database,\(^\text{16}\) we investigated the spatial and temporal changes in the FTO expression during human cortical development. FTO mRNA is expressed throughout the entire brain with marked expression in the fetal cortex and cerebellum. This expression remains robust in the adult brain (Supplementary Figure S1).\(^\text{16}\)

We next performed co-expression analysis on FTO (Supplementary Tables 5 and 6).\(^\text{16,17}\) We observed that FTO expression patterns positively correlate with the expression patterns of genes involved in the ubiquitin-mediated proteolysis pathway such as HUWE1, UBE3B and others. We also found that FTO expression patterns positively correlate with genes in which mutations cause either neurodevelopmental disorders such as MECP2 (Rett syndrome, MIM:312750) and GDI1 (Mental retardation, X-linked 41, MIM:300849), or hearing impairment such as HARS (Usher syndrome type 3B, MIM:614504).

qPCR, western blot and immunohistochemistry
We have shown that FTO is located in the nucleus of cells from the patient, her parents and non-related controls (Figure 1). Western blot analysis demonstrated no changes in patient FTO, as expected given the observed mutation. qPCR analysis revealed slightly decreased levels of FTO expression in patient cells compared with controls (Figure 1).

Transcriptome analysis
Interestingly, we found that type 2 diabetes associated genes (insulin-like growth factor binding protein 2, 36 kDa and neurocanthocytosis), retinol metabolism genes (ALDH1A1, DHR53 and RDH10), renin angiotensin system genes (AGT and MME), and genes related to metabolism of xenobiotics by cytochrome P450 (ALDH1A3, GSTT1 and MGST1) demonstrated at least a twofold increase in relative expression in the patient’s fibroblasts compared with her parents’ and controls’ fibroblasts. Conversely, cell-cycle genes (E2F2, BUB1, CDC20 and CCNB1) and obesity susceptibility genes (including ADRB2 and ENPP1) demonstrated a relatively decreased level of expression of at least twofold in the patients cells relative to her parents’ and controls’ samples.

Fibroblast morphology, PD and apoptosis assays
Fibroblast morphology was compared among the patient (NG1305-1), parent (NG1305-2 and NG1305-3) and control cultures at 11 days of incubation. No morphological differences between the patient and control fibroblasts were observed (Figure 2). The rates of apoptosis were assessed at 5 and 9 days of incubation; again, no differences were appreciated between patient and control fibroblasts (Figure 3). In addition, fibroblasts were counted on the 2nd, 5th, 7th, 9th and 11th days, and a growth curve was drawn (number of cells versus time) in order to calculate the PDs from the exponential phase of the curve.

The PD, which occurred between the 5th and 11th days, was obtained using the equation: \(\text{PD} = (\log N_1 / \log 2) - (\log N_0 / \log 2)\), where N1 was the final and N0 was the initial cell count. There were no differences in proliferation between patient and control fibroblasts (Figure 2, Supplementary Table 7).

DISCUSSION
The FTO gene encompasses a large genomic region whose nine exons span more than 400 kb on chromosome 16q12.2 and encodes 505 amino acids FTO protein composed of two domains: an N-terminal domain carrying a catalytic core and a C-terminal domain of unknown function.\(^\text{22}\) In our patient, as well as in the previously reported case, the homozygous FTO mutation affected the catalytic domain of the protein. Using both bioinformatic and in vitro biochemical data, FTO was predicted and confirmed to be an AlkB family DNA/RNA demethylase.\(^\text{23–25}\) It has been known that the FTO protein localizes to the nucleus.\(^\text{23,36,27}\) However, recently Gulati et al.\(^\text{28,29}\) demonstrated that a fraction of FTO located in the cytoplasm and N-terminus of FTO is necessary for its ability to shuttle between the nucleus and cytoplasm.\(^\text{28,29}\) Our immunohistochemistry data demonstrating that FTO was localized to the nucleus of fibroblasts are consistent with this observation.

FTO expression begins early embryogenesis when it is ubiquitously expressed throughout the body, but it most highly expressed in the brain, which is consistent with the observation that multiple organ systems are affected by FTO deficiency.\(^\text{10,23,30}\) Similar to the FTO phenotype previously reported by Boissel et al., our patient also demonstrated intrauterine growth retardation, failure to thrive, hypertonicity, seizures, severe developmental delay, postnatal micro-brachycephaly, dysmorphic craniofacial, sensory-neural hearing loss, and optic disc abnormality, a short neck, cutis marmorata, drumstick fingers and brachydactyly. Interestingly, not all of the affected family members demonstrated all of these traits and our case’s clinical findings are less aggressive than Boissel et al. cases’ especially the ones who died due to recurrent infections. Our patient additionally had abnormal behavior phenotype including repetitive hand movements, frequent laughter/smiling, apparent happy demeanor and short attention span as well as other clinical findings such as hip dislocation, strabismus, a trigonocephaly, thin upper and lower lips, a long philtrum and osteopenia.

Because of its well-known association with obesity, FTO is well studied.\(^\text{31–57}\) Previous studies have shown that even though some are predicted to have deleterious effects on FTO function, non-synonymous mutations were equally common in both the obese and lean cohorts.\(^\text{58–60}\) Other studies in lean and obese cohorts of children brought similar findings. In both African-American and Chinese Han populations, variants were identified in FTO, but the overall frequencies were similar in case and control, with none conferring risk of obesity.\(^\text{61,62}\)

Further, Boissel et al. reported none of the parents were appeared to be obese, and detailed phenotypic data on the extended family has not been reported; in our case, both of the parents of the affected children were obese, interestingly based on international diabetes foundation’s metabolic syndrome criteria, patients’ father diagnosed as a metabolic syndrome due to his increased wrist circumference and blood pressure and high triglyceride level. Since patient’s mother is also a carrier for same FTO gene variant, father’s metabolic syndrome may be caused by either environmental factors and/or sex selection for the metabolic result of FTO variant.

Variants in FTO and its association with various human phenotypes continue to emerge.\(^\text{67,9,31,63–71}\) Intriguingly, humans with loss-of-function mutations in FTO share phenotypic features of cases with partial trisomies of chromosome 16q including...
the FTO gene such as learning difficulties and behavioral problems.\textsuperscript{72,73} Boissel \textit{et al.} demonstrated changed morphology, impaired proliferation and accelerated senescence in cultured skin fibroblasts from affected subjects. Although we did not test senescence, we were not able to detect any abnormalities in either morphology or proliferation in the patient fibroblast cultures compared with either parent or control fibroblasts. Given that we did not detect any defects in proliferation, we also tested for variability in the rates of apoptosis, and again did not see any statistically significant differences between the patient and the parental.

Our patient was found to have a novel homozygous nonsense mutation in \textit{CETP}, resulting in a truncated 403 amino-acid protein (compared with the normal 476 amino-acid protein), and likely causing a complete CETP deficiency. Human CETP is a 74-kDa plasma glycoprotein that facilitates the transfer of cholesteryl esters between lipoproteins.\textsuperscript{74,75} Genetic deficiency of CETP (OMIM 607322) is a well-known cause of primary, also known as familial, hyperalphalipoproteinemia, and results in elevated plasma HDL (2–5 \times increase in the homozygous and 25–80\% greater in the heterozygous genotype) and decreased LDL;\textsuperscript{76–78} and it is mostly detected in the Japanese population than in Caucasians.\textsuperscript{78–80} In Caucasians, only a few cases of hyperalphalipoproteinemia due to \textit{CETP} mutations have been reported.\textsuperscript{81–85} Despite the high HDL-C levels in patients with CETP deficiency, an increased risk of atherosclerotic coronary artery disease has been reported.\textsuperscript{86} Our patient’s mutation in \textit{CETP} was novel and laboratory findings fit with the genetic diagnosis.

Boissel \textit{et al.} were the first to report its homozygous loss-of-function mutation as an example of a human disorder related to a defect in an AlkB-related protein. We describe a novel homozygous missense mutation in \textit{FTO} to be reported in the literature, in addition to a nonsense mutation in \textit{CETP}. We further discuss some of the phenotypic consequences of such an \textit{FTO} mutation. However, based on studies conducted in human and other organisms, fully functional FTO is critical for normal physiology.

\textbf{Figure 2} Phenotypic and proliferation comparisons of fibroblasts extracted from index case, parents and control subjects. \textit{Upper panel shows} comparison of fibroblast morphology of the patient, her parents and control fibroblasts. Images were taken after 2nd, 5th, 7th, 9th and 11th days of incubation under \times 20 objectives. \textit{Lower panel} compares number of cells versus time in each study subject, and there is no statistically difference between the patient and control samples. A full color version of this figure is available at the \textit{Journal of Human Genetics} journal online.
and there is still much to be learned about the molecular mechanisms by which a mutation in FTO leads to such a severe phenotype, as observed in our and previously reported patients.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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Figure 3 The flow-cytometric analysis of apoptosis in fibroblast cells after 3 days of incubation using FITC-annexin V and 7AAD double staining. Quadrant analysis of the gated cells in FL-1 versus FL-3 channels was from 1000 events. Annexin V+/7AAD− (lower left quadrant) areas stand for early apoptotic cells, and Annexin V+/7AAD+ (upper right quadrant) areas stand for late apoptotic or necrotic cells. A full color version of this figure is available at the Journal of Human Genetics journal online.
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