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Clinical research

Novel nonsense mutation in the *PTRF* gene underlies congenital generalized lipodystrophy in a consanguineous Saudi family

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ABSTRACT

Congenital generalized lipodystrophies (CGLs) are a heterogeneous group of rare, monogenic disorders characterized by loss of sub-cutaneous fat, muscular hypertrophy, acanthosis nigricans, hepatomegaly, cardiac arrhythmias, impaired metabolism and mental retardation. Four different but overlapping phenotypes (CGL1-4) have been identified, which are caused by mutations in *AGPAT2* at 9q34.3, *BSCL2* at 11q13, *CAV1* at 7q31.1, and *PTRF* at 17q21.2. In this study, we performed genome-wide homozygosity mapping of two affected and one unaffected subject in a Saudi family using a 300K HumanCytoSNPs12v12.1 array with the Illumina iScan system. A common homozygous region at chromosome 17q22.1, from 34.4 to 45.3 Mb, was identified in both the affected individuals. The region is flanked by SNPs rs139433362 and rs185263326, which encompass the *PTRF* gene. Bidirectional DNA sequencing of the *PTRF* gene covering all of the coding exons and exon–intron boundaries was performed in all family members. Sequencing analysis identified a novel homozygous nonsense mutation in the *PTRF* gene (c.550G>T; p.Glu184*), leading to a premature stop codon. To the best of our knowledge, we present a novel mutation of *PTRF* from Saudi Arabia and our findings broaden the mutation spectrum of *PTRF* in the familial CGL4 phenotype. Homozygosity mapping coupled with candidate gene sequencing is an effective tool for identifying the causative pathogenic variants in familial cases.

1. Introduction

Congenital generalized lipodystrophies (CGLs) are a heterogeneous group of rare monogenic disorders that are characterized by loss of subcutaneous, intra-abdominal regions and body fat; enlarged fatty liver; impaired triglyceride metabolism; acanthosis nigricans and early diabetes. The pathogenicity of four genes, segregating the disease allele in an autosomal recessive fashion, has been reported in four clinically overlapping CGL phenotypes.

In CGL type 1 (CGL1, MIM 608594), the patients are typically deprived of fat accumulation, specifically in adipose tissues with active metabolism, and have more prominent lytic bone lesions that are usually absent in other forms of CGL [Agarwal and Garg, 2006]. Loss-of-function mutations in the 1-acylglycerol-3-phosphate-O-Acyltransferase (*AGPAT2*, MIM 603100) gene at chromosome 9q34.3 has been reported for the CGL1 phenotype [Agarwal et al., 2002; Fu et al., 2004]. In CGL type 2 (CGL2, MIM 269700), also known as a seipin-deficient phenotype, patients have a loss of subcutaneous fat, muscular hypertrophy, cardiomyopathy, hypertriglyceridemia, acanthosis nigricans and early onset of diabetes [Magre et al., 2001; Rahman et al., 2013]. Mutations in the seipin (*BSCL2*, MIM 606158) gene at chromosome 11q13 have been reported in the CGL2 phenotype [Magre et al., 2001; Rahman et al., 2013]. In CGL type 3 (CGL3,

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MIM 612526), also known as the caveolin-1 deficient phenotype, patients uniquely have a short stature, hypocalcaemia and vitamin D resistance [Kim et al., 2008]. Mutations in the caveolin 1 (*CAV1*, MIM 601047) gene at chromosome 7q31.3 are known to be responsible for the remarkable deficiency of subcutaneous fat, liver steatosis, insulin resistance, risk of early diabetes and pulmonary artery hypertension [Kim et al., 2008; Mathew, 2014].

Type 4 is the most recently identified form of CGL (CGL4, MIM 613327). These patients are characterized by muscular dystrophy, cervical spine instability and cardiac abnormalities as well as the overlapping features of classical CGL phenotypes [Hayashi et al., 2009; Rajab et al., 2010; Shastry et al., 2010]. Loss of function mutations in RNA polymerase 1 and transcript release factor (*PTRF*, MIM 603198) gene at chromosome 17q21 have been identified for CGL4 phenotypes [Hayashi et al., 2009; Rajab et al., 2010; Shastry et al., 2010]. That *PTFR* gene products are expressed in many tissues, including adipose, smooth and skeletal muscles [Hayashi et al., 2009].

In the present study, we performed genome-wide homozygosity mapping followed by candidate gene sequencing in a familial case from Saudi Arabia that demonstrates unique features of CGL4.

2. Methods

2.1. Research volunteers and ethical approval

The present four-generation family, demonstrating an autosomal recessive CGL phenotype, lives in the South-Western region of Saudi Arabia (Fig. 1). Before commencing the study, the parents signed informed consent forms and ethical approval (refer no. 24–14), according to the Declaration of Helsinki, was obtained from the Institutional Review Board (IRB), Princess Al-Jawhara Albrahim Center of Excellence in Research of Hereditary Disorders and the Unit of Biomedical Ethics Research Committee, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia. All affected and unaffected siblings were thoroughly examined by genetic consultants at the Department of Genetic Medicine, King Abdulaziz University Hospital, Jeddah. Information regarding the disease history and consanguineous relationship of the parents was obtained by interviewing the family elders.

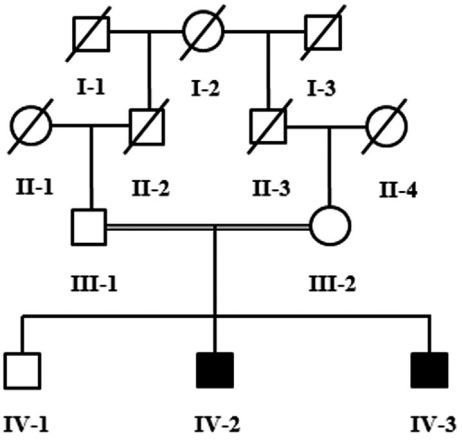

Fig. 1. Pedigree drawing of a congenital generalized lipodystrophy family. The disease phenotype is segregated in an autosomal recessive fashion. Cousin marriage is indicated by a double marriage line. Males and females are indicated by boxes and circles, respectively. Affected individuals are labelled by filled symbols.

2.2. Genomic DNA extraction and genome-wide homozygosity mapping

Peripheral blood samples from two patients (IV-2 and IV-3) and three phenotypically normal individuals (father III-1, mother III-2 and unaffected brother IV-1) in the family were collected in EDTA (BD Vacutainer® K3, USA) tubes and stored at 4 °C. Genomic DNA from whole blood was isolated using commercially available kits (QIAamp®, Qiagen, USA). The DNA was quantified by a Nanodrop-2000 spectrophotometer (Thermo Scientific, USA). A high resolution microarray (300 K HumanCytoSNP12v12.1, Illumina, USA) was performed in two affected (IV-2, IV-3) and one normal member (IV-1) of the family, according to Illumina's protocol (iScan, Illumina, USA). The entire genome data image was analysed by Illumina's GenomeStudio genotyping module 5 for detecting loss of heterozygosity mapping (LOH) regions.

2.3. Sanger sequencing

The two coding exons of the *PTRF* gene were PCR amplified and screened by DNA cycle sequencing using a Big Dye Terminator v3.1 Cycle Sequencing Kit as well as an ABI 3500 Genetic Analyzer (Life Technologies, USA) for the potential pathogenic variant. The Ensembl genome browser (asia.ensembl.org) was used to obtain the genomic sequence and coding exon information for the *PTRF* gene (ENST00000357037). Primer sequences were designed for each exon using Primer3 software [Rozen and Skaletsky, 2000] and checked for specificity using the Basic Local Alignment Search Tool (BLAST; http://www.ncbi.nlm.nih.gov/blast). Sequence variants were identified via BioEdit sequence alignment editor version 6.0.7 (www.mbio.ncsu.edu/bioedit.html).

3. Results

3.1. Clinical findings

The two affected brothers (patient IV-2 and IV-3) were referred to the Department of Genetic Medicine, King Abdulaziz University Hospital for genetic consultation in 2014. Since then, both patients have been regularly following up with a multi-disciplinary team, including clinical geneticists.

3.2. Patient 1

A 6-year-old boy (IV-2), with unremarkable antenatal history, was born at term to phenotypically unaffected, young, consanguineous parents. His birth weight was 3.2 kg (on 25th centile). In the first week of his life, he had respiratory difficulties and remained in the nursery for one week. He had feeding difficulties, with frequent regurgitation in early infancy and early childhood, which improved over time. At 3–4 months of age, he started to lose subcutaneous fat. He had stiffness in early infancy with a limited range of motions of the knee and ankle joints, with bilateral talipes equino varus, more on the right than the left side, and spastic paraplegia was suspected. At 9 months of age, he underwent a successful operation for tendon release (for talipes equino varus). He had delayed gross motor developmental milestones; he started sitting without support at 14 months, standing at 2 years and walking at 3 years of age. His formal intelligence quotient (IQ) test score was between 75 and 80. At 4 years, his abdomen sonography revealed a border line pyloric wall thickness (3 mm), but surgical intervention was not required. His thyroid stimulating hormone (TSH) reached a high level, which was diagnosed as sub-clinical hypothyroidism, and thyroxin treatment was started. His initial lipid profile showed a borderline high triglyceride level and normal