be fully clarified. The increased cytoplasmic concentrations of fumarate or succinate substrates, due to low or absent FH/SDH activity, inhibit the degradation of HIF. Persistence of HIF induces activation of sustained hypoxic response signals, which are considered to be involved in hypervascularization or neoplastic growth (Pollard, 2005). However, some major questions still remain unanswered. Why, for example, do mutations in FH, SDH or VHL genes, that are components of the same pathway of hypoxia detection, cause different tumors? Moreover, as a unique mutation should lead to a unique phenotype, could the specific duplication of exon 7, which we detected in this family, be in some way related to cerebral cavernomas? To our knowledge this is the first time that cerebral angiomatous lesions are described in MCUL patients. We believe that this finding adds new insight into the genetic background of this syndrome, suggesting that a reassessment of the overall clinical spectrum of MCUL might be required.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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A Novel S115G Mutation of CGI-58 in a Turkish Patient with Dorfman–Chanarin Syndrome

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TO THE EDITOR

Dorfman–Chanarin syndrome (DCS: OMIM No. 275630), also referred to as neutral lipid storage disease, is a recessive disorder characterized by ichthyosiform erythroderma variably associated with liver steatosis, muscle weakness, neurosensory deafness, and subcapsular cataracts. DCS is associated with accumulation of lipid vacuoles in many tissues (Chanarin *et al.*, 1975) and related to a blockage in the catabolism of triacylglycerol (Igal and Coleman, 1996). We previously demonstrated that mutations in a new gene *CGI-58* underlie DCS (Lefèvre *et al.*, 2001). In this study, we report a novel mutation of *CGI-58* (S115G) in a patient with DCS of Turkish origin.

An 18-month-old Turkish girl was referred to a department of haematology for workup of splenomegaly. At birth, she presented with fine scales and erythema over her entire body, consistent with congenital non-bullous ichthyosiform erythroderma. At age 18 months, multiple laboratory abnormalities were present: elevated liver enzymes related to hepatic steatosis

Abbreviations: ATGL, adipose triglyceride lipase; DCS, Dorfman–Chanarin syndrome; OMIM, Online Mendelian Inheritence in Man; RT-PCR, reverse transcription-PCR

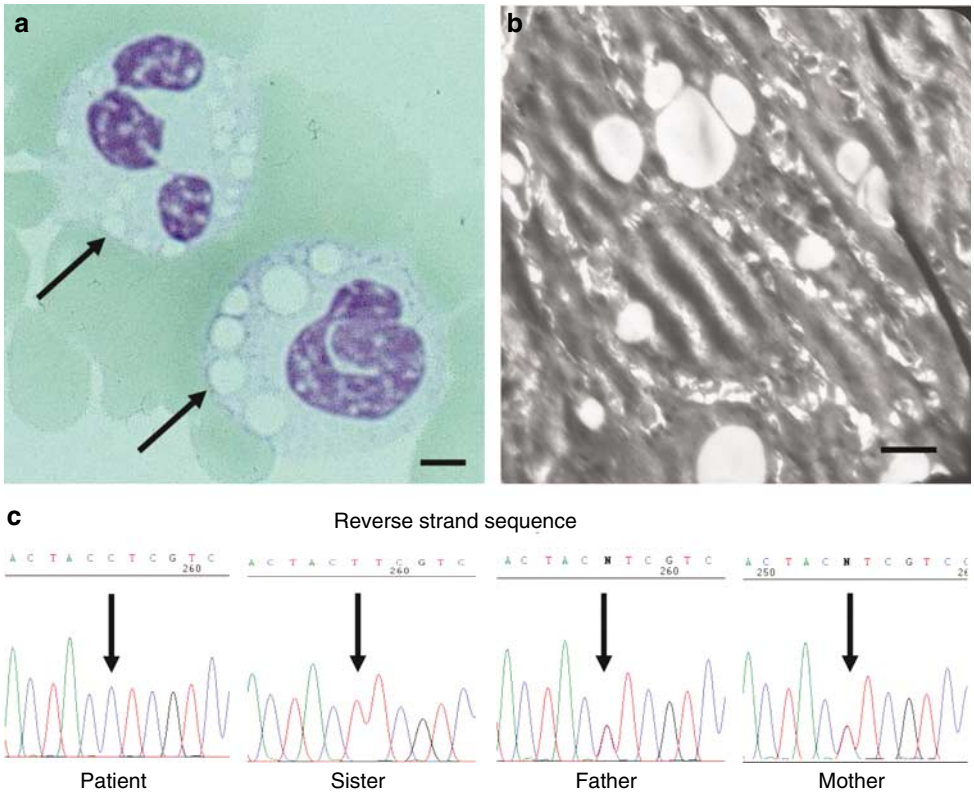

Figure 1. Lipid vacuoles in granulocytes and keratinocytes and CGI-58 mutation in the DCS patient. (**a**) Peripheral blood smear revealed cytoplasmic lipid droplets (arrow) in granulocytes (Jordans' anomaly). Bar = 2.5 μm. (**b**) Ultrastructural analysis of skin biopsy demonstrated lipid droplets devoid of membrane in keratinocytes. Bar = 2 μm. (**c**) Sequence analysis of *CGI-58* revealed a homozygous missense mutation (343A > G in exon 3 leading to S115G) in the patient, a heterozygous mutation in both parents, and a normal sequence in a sister (sequencing of the reverse strand; GenBank™ accession numbers: AF151816).

and fibrosis, raised levels of muscle enzymes, lipid vacuoles in leukocytes (Figure 1a), and in precursor myeloid cells in blood and bone marrow smears. Light microscopy of skin biopsy revealed parakeratosis, and lipid droplets were seen in keratinocytes by electron microscopy in accordance with DCS (Figure 1b). Her consanguineous parents, two sisters, and one brother were free from ichthyosis, and examination of their granulocytes revealed no vacuoles.

We searched for *CGI-58* mutation in the patient, her parents, two sisters, and one brother. These studies were conducted according to the Declaration of Helsinki Principles. The patient's parents gave their written informed consent. The medical ethical committee of the Dokuz Eylül University approved all described studies. Genomic DNA was isolated from peripheral blood and subjected to PCR amplification, followed by direct sequencing using an ABI PRISM 3100 genetic analyzer (Applied Biosystems, Courtaboeuf, France). The oligonucleotide primers and PCR conditions used have been previously described (Lefèvre *et al.*, 2001). Both DNA strands from the proband, her family members, and 49 healthy Turkish controls were entirely sequenced for the seven exons and for the intron/exon boundaries of *CGI-58*. In the patient, a homozygous A-to-G transition in exon 3 at position 343 was identified (Figure 1c). It was present at a heterozygous state in both parents (Figure 1c) and her brother. This 343A > G transition, which has not been previously reported, was neither found in both sisters nor in 49 normal Turkish subjects. To determine if this nucleotide variation altered splicing of mRNA, we performed *in silico* splice site analysis (https://splice.cmh.edu), which predicted the appearance of a cryptic splice site at nucleotide 344 (Figure 2a). Reverse transcription-PCR (RT-PCR) experiments using a forward primer from exon 3 and reverse primers from exons 3 and 6 refuted this prediction showing a normal size for both *CGI-58* transcripts (Figure 2b). This 343A > G transition substituted serine for glycine at position 115 (S115G). This novel S115G mutation was neither found in both sisters of the patient nor in 98 unrelated Turkish alleles, and was unlikely to be a polymorphism. In addition, several data suggested that the S115G mutation may have functional consequences. It changed a polar (serine) for an apolar (glycine) amino acid; this serine 115 is the first amino acid of a motif [S/T]- × -[R/K] predicting to be a site of protein kinase C phosphorylation (Woodgett *et al.*, 1986). In addition, conversion of serine 115 to glycine would be expected to affect the conformation of CGI-58 protein since *in silico* analysis (http://bioweb.pasteur.fr) predicted a structural change of leucine 110 from coil to

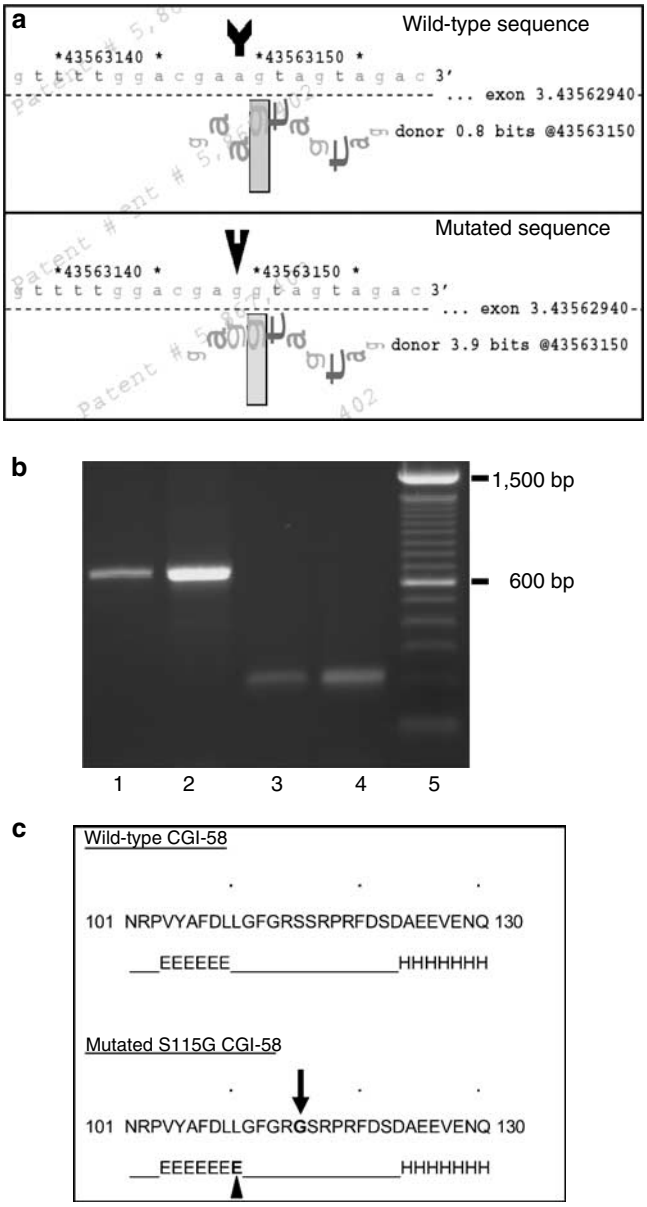

Figure 2. Consequences of the *CGI-58* mutation on splicing and secondary structure and sequence alignments around serine 115. (**a**) *In silico* analysis of the 343A>G transition showed an increase of the R_i value from 0.8 to 3.9, predicting the appearance of a cryptic splice site one nucleotide after the mutation (https://splice.cmh.edu). (**b**) RT-PCR using the forward primer from exon 3 (nucleotides 246–265) and the reverse primers from exon 3 (nucleotides 443–462) (lanes 1 and 2) and from exon 6 (nucleotides 922–941) (lanes 3 and 4) produced respectively a 217-bp and a 696-bp fragments, which were similar in the patient (lanes 2 and 4) and in a control (lanes 1 and 3). Molecular weight markers were in lane 5. (**c**) *In silico* analysis of the conversion of serine 115 to glycine (arrow) predicted a structural change (arrowhead) of leucine 110 from coil (_) to sheet (E) (http://bioweb.pasteur.fr). H designated helix structure.

sheet (Figure 2c) (Frishman and Argos, 1997). Moreover, CGI-58 amino-acid sequence alignment demonstrated that serine 115 is highly conserved among all studied species (Figure S1). Conservation of serine 115 was also observed among five of the family members of CGI-58 (ABHD 4, 8, 9, 10, and 12) (data not shown). Finally, recent functional analyses demonstrated that a close mutation (Q130P) induces an intracytoplasmic mislocalization of CGI-58, that is, abolition of recruitment of CGI-58 to lipid droplets in rat adipocytes and HeLa cells. Also, the Q130P mutated protein lost its ability to interact with perilipin, a partner located at the surface of lipid droplets (Yamaguchi *et al.*, 2004). Ultimately, CGI-58 has recently been identified as a coactivator of adipose triglyceride lipase (ATGL) and the Q130P mutation completely abrogated this function of coactivation (Lass *et al.*, 2006). All these findings strongly suggest that the S115G missense mutation alters both CGI-58 structure and function.

The *CGI-58* gene is located on 3p21, has seven exons, and its mRNA is expressed in many tissues including skin. Recently, we described eight distinct mutations of *CGI-58* in 13 patients with DCS of nine families from various countries (Lefèvre *et al.*, 2001). To date, only seven additional mutations in five patients have been reported (Akiyama *et al.*, 2003; Srinivasan *et al.*, 2004; Pujol *et al.*, 2005; Schleinitz *et al.*, 2005; Lass *et al.*, 2006). These mutations were distributed worldwide and homozygous in all cases except compound heterozygous in two patients. They included five missense mutations, five nonsense mutations, two splice site mutations, one insertion, one deletion, and one insertion/deletion. In the Turkish population, only two mutations including an E260K missense mutation and an insertion (594insC) leading to premature stop codon at position 209 were reported (Lefèvre *et al.*, 2001). We described here a third mutation, S115G, hence excluding a founder effect in Turkish patients with DCS.

CGI-58 is a 349-amino-acid protein coactivating a newly identified lipase, ATGL, which hydrolyzes triacylglycerol to yield diacylglycerol and fatty acid (Lass *et al.*, 2006). Thus, CGI-58 mutations abrogated lipolysis and induced a systemic accumulation of lipids droplets. In epidermis, lipids vacuoles were associated with a defective formation of lamellar granules (Akiyama *et al.*, 2003). Lipid microinclusions in lamellar granules formed a non-lamellar phase within the stratum corneum interstices and may explain the permeability barrier abnormality (Demerjian *et al.*, 2006).

We reported here the finding of a new pathogenic *CGI-58* mutation in a Turkish patient with DCS. This S115G mutation further illustrates the functional importance of CGI-58 in lipid metabolism and epidermal differentiation.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Figure S1. CGI-58 amino-acid sequence alignment in 13 species (ClustalW).

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Psoriasis Autoantigens in Normal Scalp Skin—Identification by Expression Cloning

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TO THE EDITOR

Psoriasis vulgaris is a common chronic inflammatory skin disease, possibly of autoimmune etiology, and characterized by erythematous, scaly lesions with a predilection for the scalp and extensor surfaces of the extremities, whereas the palms and soles are usually not primarily involved.

The inflammation is manifested by vasodilation, with infiltrates of T lymphocytes, mainly $CD4^+$ in the papillary dermis and $CD8^+$ in the epidermis. In the papillary dermis there is also an increased number of mast cells (Harvima *et al.*, 1993), natural killer (NK) cells (Cameron *et al.*, 2002) and plasmacytoid dendritic cells (Nestle *et al.*, 2005), the latter producing large amounts of IFNα, which is now considered to be of particular relevance for initiation of the inflammation. Migration of neutrophil granulocytes into the epidermis, with formation of microabscesses, is another typical feature. The possibility of dysregulation of innate immunity in psoriasis is currently under discussion (Bos *et al.*, 2005).

Although there is evidence that psoriasis is an autoimmune disease, few, if any, autoantigens have been defined and no psoriasis-specific antigen has been found (reviewed in Bos *et al.*, 2005). To reveal antigens recognized by T lymphocytes in human disease has been found to be a difficult task. There is, however, an interaction between T cells and B cells in autoimmune diseases. Antigen-specific autoreactive T lymphocytes (Th2) play a central role in autoimmunity, in which they activate B cells to produce antibodies (Nishifuji *et al.*, 2000). Hence, identification of autoantibodies against potential psoriasis-related antigens would also yield important knowledge regarding T-cell-mediated mechanisms in this disease.

We have tried to identify autoantigens in psoriasis by recombinant expression cloning, a method which is well established in our laboratory and