

SKIV2L Mutations Cause Syndromic Diarrhea, or Trichohepatoenteric Syndrome

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Syndromic diarrhea (or trichohepatoenteric syndrome) is a rare congenital bowel disorder characterized by intractable diarrhea and woolly hair, and it has recently been associated with mutations in *TTC37*. Although databases report *TTC37* as being the human ortholog of Ski3p, one of the yeast Ski-complex cofactors, this lead was not investigated in initial studies. The Ski complex is a multiprotein complex required for exosome-mediated RNA surveillance, including the regulation of normal mRNA and the decay of nonfunctional mRNA. Considering the fact that *TTC37* is homologous to Ski3p, we explored a gene encoding another Ski-complex cofactor, *SKIV2L*, in six individuals presenting with typical syndromic diarrhea without variation in *TTC37*. We identified mutations in all six individuals. Our results show that mutations in genes encoding cofactors of the human Ski complex cause syndromic diarrhea, establishing a link between defects of the human exosome complex and a Mendelian disease.

Syndromic diarrhea (SD) is a rare and severe disease characterized by intractable diarrhea, facial dysmorphism, intra-uterine growth retardation, immunodeficiency, and hair abnormalities.¹ Trichohepatoenteric (THE [MIM 222470]) syndrome, described initially by Verloes et al. in 1997² as a different syndrome, has since been grouped with syndromic diarrhea because the main clinical features in both syndromes are identical³ (for simplicity, we now refer to both syndromes as a singular disorder, SD/THE syndrome). After a homozygosity-mapping analysis, we and others recently identified *TTC37* mutations as being responsible for this syndrome in 21 individuals.^{4,5} The precise function of *TTC37* (also called Thespin) was not elucidated even after the description of its involvement in SD/THE. This protein shares no sequence homology with other human proteins and shows no known functional domains except several tetratricopeptide-repeat (TPR) domains that are structural motifs found in over 300 human proteins.⁶ In some databases, *TTC37* is reported as being the ortholog of yeast *SKI3*, which encodes a key component of the Ski complex, a multiprotein complex required for exosome-mediated RNA surveillance.⁷ However, this lead was not explored in the initial studies. In our series, 6 out of 15 individuals did not carry a mutation in *TTC37* but presented with typical characteristics of SD/THE syndrome. Considering the fact that *TTC37* is homologous to Ski3p, we assumed that other genes encoding Ski-complex proteins might be responsible for SD/THE syndrome in these individuals; we confronted

this hypothesis with the results of the linkage analysis of one of the consanguineous families.

Out of the six individuals with typical SD/THE syndrome and no mutation in *TTC37*, only one was previously reported.⁸ All procedures followed for clinical and genetic analyses in this study were in accordance with the ethical standards of the institutional and national committees on human experimentation, and proper informed consent was obtained from the parents of the affected children. All individuals presented with severe and intractable diarrhea that occurred between 1 and 12 weeks after birth, hair abnormalities (sparse, fragile, and uncombable hair and trichorrhexis nodosa), and facial dysmorphism characterized by hypertelorism, a broad flat nasal bridge, and a prominent forehead (Figure 1). All children received parenteral nutrition, but the amount of time varied between individuals and ranged from a few weeks to several years. Immunodeficiency was mostly due to low immunoglobulin levels and to the absence of an immune response to vaccines. One individual out of the three with immunodeficiency died from a measles infection. These six individuals harbor no mutation in *TTC37*, but their clinical presentation is undistinguishable from those who have *TTC37* mutations (Table 1).

Searching for a candidate gene in this group, we investigated the possible homology (reported in the Ensembl database) between *TTC37* and yeast *SKI3*. Interspecies sequence-alignment analysis (with the bioinformatics prediction software BLAST) revealed that *TTC37* shares significant amino-acid-sequence similarity with yeast

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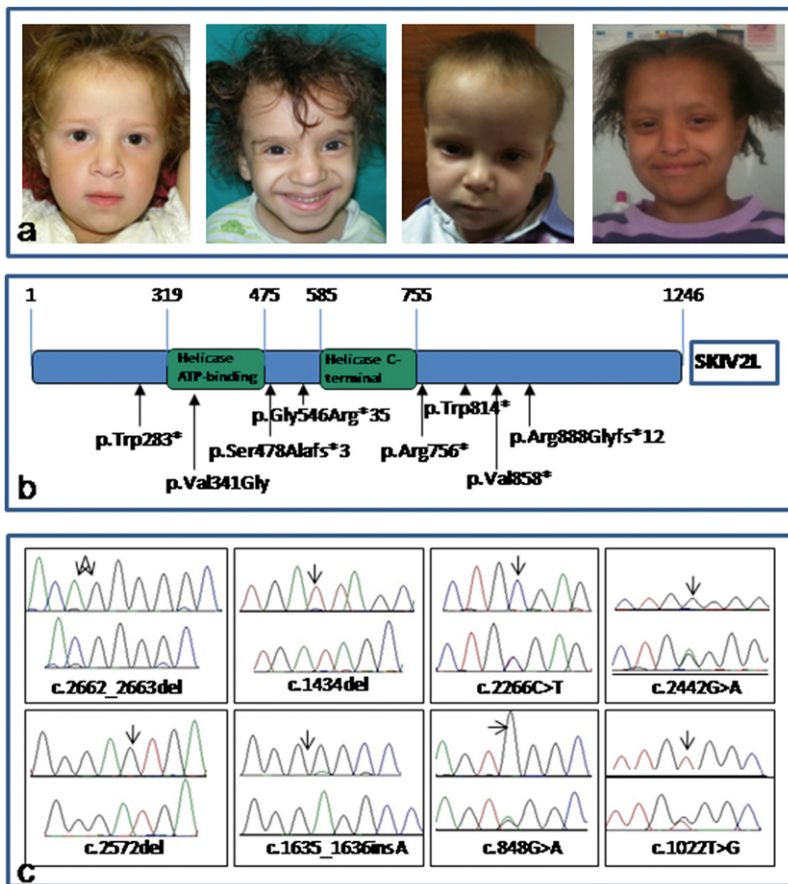


Figure 1. Clinical Presentation of Individuals with SD/THE Syndrome and *SKIV2L* Mutations

(A) Clinical presentation of four individuals with SD/THE syndrome. One individual has mutations in *TTC37* (first picture on the left), and three others have mutations in *SKIV2L* (right panel).

(B) A schematic overview of the predicted *SKIV2L* structure with a helicase ATP-binding domain and a helicase C-terminal domain (UniProt). The location of the domains is annotated by amino acid position. The eight amino acid substitutions are indicated by arrows.

(C) Nucleotide sequences are shown for the controls (upper sequence) and for each *SKIV2L* variant (lower sequence). Arrows indicate mutation positions on the reference sequences.

c.848G>A (p.Trp283*), c.1434del (p.Ser479-Alafs*3), c.1635_1636insA (p.Gly546Argfs*35), c.226C>T (p.Arg756*), c.2442G>A (p.Trp814*), c.2572del (p.Val858*), and c.2662_2663del (p.Arg888Glyfs*12). The mutation types suggest that the disease mechanism is loss of function. We identified one missense mutation, c.1022T>G (p.Val341Gly), that is located in the helicase ATP-binding domain (Figure 1) and concerns a highly conserved residue. PolyPhen predicted that this specific mutation is probably damaging (the prediction score was 2.8

[mutations predicted to be pathological have an index above 0.5]).

It is now clear that the majority of genomic information is transcribed into RNA molecules; this process generates very abundant and complex pools of RNAs that the cells have to control. While performing numerous RNA-processing reactions, the cell must, at the same time, eradicate surplus and aberrant material.¹¹ This task is, at least partially, performed by the RNA exosome multiprotein complex that contains both 3'-to-5' exonuclease and 3'-to-5' endonuclease activity and acts in both the nucleus and the cytoplasm.¹² Initially discovered in yeast but also present in higher eukaryotes, including humans, this exosome complex is involved in the decay pathways of normal mRNA but is also required to maintain the fidelity of gene expression through RNA surveillance processes, such as nonsense-mediated decay, nonstop decay, and no-go decay.¹³⁻¹⁶ Genetic screens in yeast have identified three proteins (Ski2p, Ski3p, and Ski8p) that form the Ski (Superkiller) complex and act as specific cofactors required for all the functions of the cytoplasmic exosome but not the nuclear exosome.¹⁷ The putative DEXH-box RNA helicase activity of Ski2 suggests that it is the only Ski protein with a catalytic function, whereas Ski3p and Ski8p contain repeated domains thought to be needed for protein-protein interactions.¹⁸ In humans, the cytoplasmic exosome is involved in various mRNA decay pathways and is required for normal cell growth.¹⁹

Ski3p. Between *TTC37* residues 5 and 84, *TTC37* and Ski3p were 35% identical and 58% similar, and between residues 488 and 1223, they were 20% identical and 38% similar, indicating that *TTC37* is the human ortholog of *SKI3*. Because Ski3p is part of a multiprotein complex, this finding prompted us to test whether mutations in other human orthologs of the Ski proteins could be associated with SD/THE syndrome. For one consanguineous family, we performed a linkage analysis in which the *TTC37* critical interval was excluded, and this analysis revealed that there was a region of homozygosity spanning nucleotides 9,138,488–40,453,008 and containing 856 genes in chromosomal region 6p21.2–6p24.3. When looking for functional candidate genes in this region, we noticed that *SKIV2L* (RefSeq accession number NM_006929.4), a gene described as being the human ortholog of the *SKI2*,^{9,10} was present in region 6p21.3. Thus, we performed direct sequencing of *SKIV2L* in our cohort of six individuals affected by typical SD/THE syndrome and found the presence of mutations predicted to be deleterious in all six. The identified mutations and their mode of inheritance are described in Table 2. All of the unaffected parents available for analysis were heterozygous for one of the mutations identified in their child. We identified eight different variations distributed throughout the protein (Figure 1). Seven of these variations correspond to nonsense or frameshift mutations that introduce a premature termination codon, and they are

Table 1. Clinical Data of Individuals Affected by SD/THE Syndrome

	Individuals with Mutations in <i>TTC37</i> (n = 18)	Individuals with Mutations in <i>SKIV2L</i> (n = 6)
Premature birth (<37 weeks)	9/17	2/5
Intrauterine growth restriction	14/17	4/6
Birth weight (median and mean) in kg	1.84 (0.78–3.58); 1.868	1.6 (1.01–2.00); 1.47
Intractable diarrhea	18/18	6/6
Onset of diarrhea (median and mean) in weeks	3.5 (1–32); 7.75	2.5 (1–12); 3.8
Villous atrophy	16/18	3/5
Colitis	5/6	3/3
Facial dysmorphism	18/18	6/6
Hair abnormalities	18/18	6/6
Trichorrhexis nodosa	17/18	5/5
Immune deficiency	17/18	3/6
Liver disease	9/16	3/6
Siderosis	3/14	1/3
Cirrhosis	7/15	2/3
Skin abnormalities	7/16	3/4
Platelet abnormalities	7/17	0/2
Cardiac abnormalities	4/16	2/4
Outcome (deceased/alive)	4/14	2/4

Here, we show that in six families, molecular defects in the cytoplasmic-exosome cofactor SKIV2L cause SD/THE syndrome, a severe autosomal-recessive condition mainly characterized by intractable diarrhea and woolly hair. This study establishes a formal link between a constitutional congenital disease and defects of human cytoplasmic-exosome cofactors. Although SD/THE syndrome is genetically heterogeneous and is associated with at least two different genes, it is extremely homogenous at the clinical level (Table 1), suggesting that a defect in Ski-complex function or structure is a key mechanism responsible for the main clinical features. Consequently, *WDR61*, the human ortholog of the third cofactor *SKI8*, will be a relevant candidate

gene to be tested in persons that are affected by THE syndrome and that have no mutation in *TTC37* or *SKIV2L* sequences (a situation not encountered in our series).

In human pathology, the exosome complex has previously been involved in autoimmune diseases, in which components of the nuclear or cytoplasmic exosome are the target of autoimmune response, or in cancer.²⁰ Here, we point out that exosome dysfunction must be considered a cause of Mendelian disorders. The association between mutations in exosome-cofactor-encoding genes and human diseases provides a valuable model for investigating the role of this structure in human pathology but also in normal cellular function. The mechanism by which mRNA-surveillance defects lead to various clinical symptoms, such as severe diarrhea, hair abnormalities, or immunodeficiency, will need to be investigated in further studies.

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Web Resources

The URLs for the data presented herein are as follows:

BLAST, <http://blast.ncbi.nlm.nih.gov/Blast.cgi>

Ensembl, <http://www.ensembl.org>

Homozygositymapper, <http://www.homozygositymapper.org>

Online Mendelian Inheritance in Man (OMIM), <http://www.omim.org>

Polyphen, <http://genetics.bwh.harvard.edu/pph/>

UniProt, <http://www.uniprot.org>

Table 2. Mutations in SKIV2L^a, Geographical Origins, and Consanguinity in the Families of Individuals with SD/THE Syndrome

Individual	Mutation 1	Mutation 2	Consanguinity	Geographical Origin
1	c.1635_1636insA (p.Gly546Argfs*35)	c.1635_1636insA (p.Gly546Arg*35)	yes	North Africa
2	c.2266C>T (p.Arg756*)	c.2442G>A (p.Trp814*)	no	France
3	c.848G>A (p.Trp283*)	c.1022T>G (p.Val341Gly)	no	France
4	c.2572del (p.Val858*)	c.2572del (p.Val858*)	yes	Turkey
5	c.2662_2663del (p.Arg888Glyfs*12)	c.2662_2663del (p.Arg888Glyfs*12)	yes	Turkey
6	c.1434del (p.Ser479Alafs*3)	c.1434del (p.Ser479Alafs*3)	yes	Turkey

^aRefSeq accession numbers NP_008860.4 and NM_006929.4.

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