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# Mutations in *TTC37* Cause Trichohepatoenteric Syndrome (Phenotypic Diarrhea of Infancy)

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**BACKGROUND & AIMS:** Trichohepatoenteric syndrome (THES) is an autosomal-recessive disorder characterized by life-threatening diarrhea in infancy, immunodeficiency, liver disease, trichorrhexis nodosa, facial dysmorphism, hypopigmentation, and cardiac defects. We attempted to characterize the phenotype and elucidate the molecular basis of THES. **METHODS:** Twelve patients with classic THES from 11 families had detailed phenotyping. Autozygosity mapping was undertaken in 8 patients from consanguineous families using 250,000 single nucleotide polymorphism arrays and linked regions evaluated using microsatellite markers. Linkage was confirmed to one region from which candidate genes were analyzed. The effect of mutations on protein production and/or localization in hepatocytes and intestinal epithelial cells from affected patients was characterized by immunohistochemistry. **RESULTS:** Previously unrecognized platelet abnormalities (reduced platelet α-granules, unusual stimulated alpha granule content release, abnormal lipid inclusions, abnormal platelet canalicular system, and reduced number of microtubules) were identified. The THES locus was mapped to 5q14.3–5q21.2. Sequencing of candidate genes showed mutations in *TTC37*, which encodes the uncharacterized tetratricopeptide repeat protein, thespin. Bioinformatic analysis suggested thespin to be involved in protein–protein interactions or chaperone. Preliminary studies of enterocyte brush-border ion transporter proteins (sodium hydrogen exchanger 2, sodium hydrogen exchanger 3, aquaporin 7, sodium iodide symporter, and hydrogen potassium adenosine triphosphatase [ATPase]) showed reduced expression or mislocalization in all THES patients with different profiles for each. In contrast the basolateral localization of Na/K ATPase was not altered. **CONCLUSIONS: THES is caused by mutations in *TTC37*. *TTC37* mutations have a multisystem effect, which may be owing to abnormal stability and/or intracellular localization of *TTC37* target proteins.**

*Keywords:* Phenotypic Diarrhea of Infancy; Platelet Alpha Granules; Ion Transporter Proteins; Thespin.

BASIC–ALIMENTARY TRACT

Diarrhea is a major cause of morbidity and mortality throughout the world: an estimated 1.87 million young children die of it each year.[1] Although infection is the most common cause of diarrhea, investigation of rarer causes of diarrhea can increase understanding of the normal gastrointestinal function and the molecular pathogenesis of diarrhea. Inherited diarrhea syndromes may be associated with phenotypic abnormalities of the enterocyte (eg, microvillous inclusion disease[2] and tufting enteropathy[3]) or with a normal cellular phenotype but abnormal solute transporter function (eg, congenital chloridorrhea[4] and congenital sodium diarrhea[5]).

Trichohepatoenteric syndrome (THES, also known as *phenotypic diarrhea of infancy*; MIM 222470) is an autosomal-recessive inherited disorder with an estimated incidence of 1 in 400,000–500,000 live births. Stankler et al[6] first described THES in 2 siblings with low birth weight, dysmorphic features, and abnormal "woolly" hair with abnormalities on microscopy indicating weakness of the hair shaft. Diarrhea began in the third postnatal week and resulted in death. The livers were fibrotic with marked hemosiderosis.[6] A description of 8 similar chil-

*Abbreviations used in this paper:* Apq7, aquaporin 7; ARC, arthrogryposis-renal dysfunction-cholestasis syndrome; H/K ATPase, hydrogen potassium adenosine triphosphatase; NHE, sodium hydrogen exchanger; NIS, sodium iodide symporter; THES, tricohepatoenteric syndrome; TPR, tetratricopeptide repeat domains.


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dren[7] and subsequent case reports have extended the clinical spectrum,[8–13] which comprises distinctive facial features (hypertelorism, broad flat nasal bridge, prominent forehead), abnormal hair (coarse, sparse, and fragile with trichorrhexis nodosa), and diarrhea that manifests not at birth but weeks to months thereafter. On microscopy, intestinal changes are nonspecific; initial subtotal villous atrophy improves with time (although severity of diarrhea does not correlate with the histologic change) and a mixed inflammatory infiltrate varies. The enterocyte brush border is normal ultrastructurally. All affected children require parenteral nutrition to maintain life and growth. In some, parenteral nutrition is a life-long requirement; in others it can be weaned to full enteral feeding.

Immunodeficiency is a consistent feature with low serum concentrations of immunoglobulins (which often, but not always, improves with age) and a poor immunologic response to childhood vaccination. Hepatic involvement is inconsistent, even among siblings, and irrespective of parenteral nutrition can include hepatomegaly, fibrosis, siderosis, and cirrhosis (even before the onset of diarrhea). Prenatal manifestations include polyhydramnios and placentomegaly, and other clinical findings include cardiac lesions (tetralogy of Fallot and ventricular and atrial septal defects), hypopigmentation of the skin, inguinal and umbilical hernia, mental retardation, and delayed puberty.

THES is life-limiting. Death results from hepatic failure or secondary to the complications associated with the need for long-term parenteral nutrition.[6–13]

Defining the molecular genetic basis of THES will facilitate diagnosis and management. It will help in counseling regarding prognosis and will enable prenatal and preimplantation diagnosis in families at risk. It also might provide critical insight into the mechanisms of diarrhea and thus treatment.

## Methods

### *Patients*

DNA was extracted from whole blood (Gentra Puregene DNA purification system; Qiagen, Crawley, West Sussex, UK) in 12 children with THES and their parents. In unaffected siblings DNA was extracted from either a blood sample or mouth swab. Lymphocyte RNA (PAXgene Blood RNA Kit; Qiagen) was available from 4 affected children. Jejunal specimens were taken at the time of initial diagnostic investigation for intractable diarrhea and liver specimens in the course of evaluation of clinical liver disease. This study was conducted according to the principles expressed in the Declaration of Helsinki and was approved by the South Birmingham Research Ethics Committee. In all cases written consent was obtained for sample collection and subsequent analysis.

### *Analytic Methods*

**Molecular genetic studies.** Genomic DNA was extracted by standard techniques. A genome-wide linkage scan was undertaken using the Affymetrix (Santa Clara, CA) 250 000 single nucleotide polymorphism microarray with DNA from 8 affected individuals in 7 consanguineous families. Regions of homozygosity greater than 3 centimorgan that were shared by all the affected children were investigated further (n = 3). Microsatellite markers were used to confirm or refute linkage within individual families and to fine-map the region of interest at 5q14.3–5q21.2. Direct sequencing of the 45 genes within the identified region was prioritized according to putative function (predicted ion transport, DNA repair, and chaperone functions), site of expression, and position. The genomic DNA sequence of candidate genes was taken from Ensembl (http://www.ensembl.org/index.html) and primer pairs for the translated exons were designed using ExonPrimer software (http://ihg2.helmholtz-muenchen.de/ihg/ExonPrimer.html). Individual exons and flanking sequences (primer details are shown in Supplementary Table 1) were amplified using standard polymerase chain reaction. Polymerase chain reaction products were sequenced directly by the Big Dye Terminator Cycle Sequencing System (Applied Biosystems, Foster City, CA) with the use of an ABI PRISM 3730 DNA Analyzer (Applied Biosystems). DNA sequences were analyzed using Chromas software (www.technelysium.com.au/chromas.html). Reverse-transcriptase polymerase chain reaction was used to identify the effect of splice site mutations on gene expression.

**Immunohistochemistry of liver specimens.** Samples of formalin-fixed, paraffin-embedded liver, sectioned at 4–5 $\mu$m and picked up on glass slides, were immunostained and counterstained as described previously[14] for the canalicular transport proteins bile salt export pump and multidrug resistance-associated protein 2.

**Immunohistochemistry of jejunal specimens.** Sections (4 $\mu$m) of archival material originally obtained for clinical diagnosis were deparaffinized and heat fixed. Slides were microwaved for antigen recovery in 10 mmol/L sodium citrate buffer, pH 6 (Sigma Chemical Company, St Louis, MO) at a power level setting of 9 (Panasonic Model NN-C980B Conventional Microwave Oven; Secaucus, NJ) for 2–5 minutes. After cooling for 30 minutes, sections were washed in phosphate-buffered saline (PBS) and blocked with 5% normal goat serum in PBS. Sections were incubated with rabbit polyclonal antibodies against the sodium hydrogen exchanger 2 (NHE2) (Ab597), NHE3 (Ab1381), sodium iodide symporter (NIS), and hydrogen potassium adenosine triphosphatase (H/K ATPase), and chicken polyclonal antibody against aquaporin 7 (Aqp7), as well as with monoclonal antibodies against villin and the Na/K ATPase (the Na/K ATPase developed by Douglas M Fam-