A novel nonsense mutation in the *ABC1* gene causes a severe syringomyelia-like phenotype of Tangier disease

Stephan Züchner, Anne D. Sperfeld, Jan Senderek, Bernd Sellhaus, Clemens Oliver Hanemann and J. Michael Schröder

Departments of 1Neuropathology and 2Human Genetics, University Hospital, Technical University of Aachen, Aachen and 3Department of Neurology, University of Ulm, Ulm, Germany

Correspondence to: Professor Dr J. Michael Schröder, Institut für Neuropathologie, Universitätsklinikum der RWTH Aachen, Pauwelsstrasse 32, 52074 Aachen, Germany

E-mail: jmschroder@ukaachen.de

Summary

Tangier disease is a rare autosomal recessive disorder caused by mutations in the recently identified ATP-binding cassette transporter 1 gene (*ABC1*). A typical clinical manifestation of Tangier disease is peripheral neuropathy. Former studies differentiated between two manifestations: the more frequent mono- or polyneuropathic form and a syringomyelia-like type. It is unknown whether specific mutations in the *ABC1* gene or a particular genetic background are responsible for either of these forms. A family is presented comprising a case with a severe syringomyelia-like phenotype of Tangier disease and absence of cardiovascular disease. Sequencing analysis of the *ABC1* gene was performed. A new homozygous C→T transition in exon 18 was found in the index patient. This mutation results in a stop codon at position 909 (R909X) leading to premature termination of translation. Her clinically asymptomatic daughters, her sister and one of her nieces were heterozygous. Sural nerve biopsies were studied in the index patient at the age of 45 and 54 years; both revealed a severe neuropathy, characterized by a subtotal and finally complete loss of nerve fibres. The entire loss of Schwann cells resulted in an extraordinary form of endoneurial sclerosis. Only rare capillaries, lipid-laden macrophages and fibroblasts had survived in the endoneurium. This case appears to be unique in respect to the underlying novel mutation in the *ABC1* gene and its association with complete endoneurial sclerosis of all fascicles in the sural nerve and absence of cardiovascular disease.

Keywords: Tangier disease; *ABC1* gene; peripheral neuropathy; syringomyelia; high-density lipoprotein

Abbreviations: ABC1 = ATP-binding cassette transporter 1; apo A-I = apolipoprotein A-I; apo B = apolipoprotein B; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; VLDL-C = very low density lipoprotein cholesterol

Introduction

Tangier disease is a rare autosomal recessive disorder caused by mutations in the ATP-binding cassette transporter 1 gene (*ABC1*) (Bodzioch et al., 1999; Brooks-Wilson et al., 1999; Rust et al., 1999). The disorder is characterized by nearly absent plasma levels of high-density lipoprotein cholesterol (HDL-C), reduced plasma levels of low-density lipoprotein cholesterol (LDL-C), low or absent plasma levels of apolipoprotein A-I (apo A-I) and, occasionally, decreased total plasma cholesterol. The identification of the responsible gene promised key insights into the pathogenesis of the cholesterol metabolism in different tissues.

Cell membranes contain large amounts of cholesterol. It has been shown that macrophages and other cells of the reticulo-endothelial system, which routinely ingest cellular membrane debris, are heavily dependent on cholesterol efflux (Lawn et al., 1999). Blocking the expression or activity of *ABC1* reduced the capacity of macrophages and fibroblasts to transfer cellular cholesterol to apo A-I, the major apolipoprotein of HDL (Francis et al., 1995; Lawn et al., 1999; Orso et al., 2000; Neufeld et al., 2001). The reduced HDL-mediated return of cholesterol from the periphery to the liver accounts for cholesterol ester accumulation in many tissues.
Ultrastructural studies revealed deposition of cholesteryl esters in the reticulo-endothelial system of different organs (Ferrans and Fredrickson, 1975). The typical clinical manifestations of Tangier disease are peripheral neuropathy, premature coronary artery disease, hepatosplenomegaly, lymphadenopathy and enlarged yellow tonsils. In peripheral nerves associated with Tangier disease, lipid-laden vacuoles are apparent in macrophages and Schwann cells, which normally synthesize the myelin sheaths as a large cellular membrane (Ferrans and Fredrickson, 1975; Marbini et al., 1985). Although peripheral neuropathy is often the presenting symptom of the disorder, much more attention has been paid to the clinical symptoms of cardiovascular disease since the identification of the responsible gene (Remaley et al., 1999; Bertolini et al., 2001; Huang et al., 2001). In reviewing 33 patients with Tangier disease, peripheral neuropathy was evident in 58%, and only 44% of the patients over 35 years of age were reported to present clinical symptoms of cardiovascular disease (Serfaty-Lacroixniere et al., 1994). Although peripheral neuropathy is often the presenting symptom of the disorder, much more attention has been paid to the clinical symptoms of cardiovascular disease since the identification of the responsible gene (Remaley et al., 1999; Bertolini et al., 2001; Huang et al., 2001). In reviewing 33 patients with Tangier disease, peripheral neuropathy was evident in 58%, and only 44% of the patients over 35 years of age were reported to present clinical symptoms of cardiovascular disease (Serfaty-Lacroixniere et al., 1994). Clinically two subtypes of neuropathy in Tangier disease were described: (i) a frequent mono/polyneuropathy with remitting loss of peripheral sensory and/or motor function manifesting during the first two decades of life; and (ii) a rare syringomyelia-like syndrome with slowly progressive dissociated sensory loss and facio-brachial muscle wasting which affects adults only (Kocen et al., 1973; Dyck et al., 1978; Pollock et al., 1983; Pietrini et al., 1985). It is not known whether different molecular defects in ABC1 are associated with either of these forms. We present a novel homozygous point mutation in ABC1 causing a severe syringomyelia-like phenotype of Tangier disease and nearly complete absence of cardiovascular disease. This is the first reported mutation in a case with this rare phenotype and the first with additionally available sural nerve biopsies.

Material and methods

Index patient and family members

The index patient was a 54-year-old female from northern Germany. Since the age of 26, she suffered from a progressive syringomyelia-like syndrome with dissociated sensory disturbances. The disease resulted in multiple mutilations, especially loss of terminal phalanges of the upper extremities. Her consanguineous parents were second-degree relatives. The index patient, her two daughters from different marriages (VI:1, VI:2), a sister (V:6) and two nieces (VI:3, VI:4) were examined in this study. Informed consent was obtained from all subjects investigated.

Apparative diagnostics and laboratory analysis

Electrophysiological examination, autonomic and cardiovascular function testing, ECG, MRI of the brain and cervical part of the spinal cord, transcranial Doppler sonography, duplex and Doppler sonography of the carotid and vertebral arteries, and abdominal sonography were performed in the index patient. Levels of serum cholesterol, HDL-C, LDL-C, very low-density lipoprotein (VLDL-C), apo A-I, apolipoprotein B (apo B), liver parameters, creatine kinase and lipid electrophoretic were estimated in the index patient and partially in her daughters (VI:1, VI:2).

Mutation analysis

Primers for the polymerase chain reaction (PCR) for all coding exons of the ABC1 gene were selected using the Primer3 algorithm (http://www.genome.wi.mit.edu/cgi-bin/ primer/primer3_-www.cgi). The primers for the mutation containing exon 18 were ABC18-F: 5'-CAGT-GCTTTCTGGGTTCA-3' and ABC18-R: 5'-ACCTCCTGTGGCTGATTCTTCT-3'.

The applied PCR reaction conditions in a GeneAmp PCR System 9700 (Perkin Elmer-Applied Biosystems, PE-ABI, Foster City, CA, USA) were 5 min at 95°C, 35 cycles of 30 s at 95°C, 30 s at 60°C, 30 s at 72°C, and a final elongation at 72°C for 7 min. PCR products were separated on 1.5% agarose gels in 1 x TBE (Tris-borate–EDTA) buffer. The individual bands were excised and eluted using a QIAquick PCR Purification Kit (Qiagen, Hilden, Germany). PCR fragments were sequenced directly using a Bigdye...
Terminator Reaction Kit (PE-ABI). Samples were run and analysed on a ABI PRISM 310 fluorescent DNA sequencer (PE-ABI). Restriction enzyme analysis was performed as recommended by the manufacturer (New England Biolabs, Beverly, MA, USA). The resulting restriction fragments were fractionated on 12% polyacrylamide gels and stained with ethidium bromide.

**Nerve biopsies**

Sural nerve biopsies were studied in the index case. These were taken at two time points, i.e. at the age of 45 and 54 years. The nerves were fixed in 3.9% phosphate-buffered glutaraldehyde, post-fixed in a 2% phosphate-buffered solution of osmium tetroxide, and embedded in epoxy resin. For light microscopy, semi-thin sections were stained with toluidine blue and paraphenylene diamine. Parafin sections were stained with haematoxylin–eosin (H & E) and antibodies against epithelial membrane antigen. Ultra-thin sections were contrast-enhanced with lead citrate and uranyl acetate, and examined with a Philips 400T electron microscope.

**Results**

**Clinical characterization of subjects**

The pedigree of this large Tangier disease family is shown in Fig. 1. The index patient, a 54-year-old female, had suffered from hepatitis B infection in infancy. Her tonsils were excised at the age of 2 years. At the age of 26 years, painless scalds with poor spontaneous recovery on forearms became evident for the first time. The patient observed mild distal weakness of the upper extremities and recurrent numbness of her fingers. Neurological investigation at the age of 30 years revealed a syringomyelia-like syndrome with dissociated sensory disturbances, weakness, and slight asymmetric atrophy of the interosseus muscles of the upper extremities. General medical examination revealed no abnormalities. At the age of 33 years, sensory disturbances, mutilation of toes, sharp paroxysmal pains in distal lower extremities and bilateral weakness of the facial muscles with incomplete closure of the eyelids became apparent. Actual clinical and neurological examination revealed facial diplegia, weakness of all extremities including steppe gait with severe muscular atrophy of the hands and forearms, and moderate muscular atrophy of distal lower extremities. Except for diminished biceps, triceps, patellar and adductor reflexes, deep tendon reflexes were absent. No pyramidal signs were detected. Sensory examination revealed complete anhidrosis, loss of pain and temperature sensation on the entire body except for the face, and severe distal symmetric hypeaesthesia up to the knees and wrists. Multiple mutilations on the tips of her fingers and loss of several endphalanges were apparent (Fig. 2). Testing of coordinative functions revealed slight sensory ataxia.

Her father had suffered from ‘muscle atrophy of the hands’ and died at the age of 32 years during the war. The two daughters from different marriages were healthy at the age of 15 and 27 years, and showed no clinical evidence of neuropathy. A 52-year-old sister of the index patient (V:6) and her two daughters (22 years, VI:3; and 19 years, VI:4) were clinically unaffected. Otherwise, family history was negative (including cardiac diseases).

**Apparative diagnostics and laboratory analysis**

Panmyelography at the age of 33 years and a recent MRI study of the cervical spinal cord showed no evidence of syringomyelia or cervical myelopathy. Electrophysiological examination revealed a sensorimotor type of polyneuropathy with predominance in the upper limbs. Compound motor action potentials of axillar and femoral nerves were slightly reduced and motor latencies were in the upper range. In the median, peroneal, sural and tibial nerves, no compound motor action or sensory action potentials were detectable. EMG showed signs of chronic and acute denervation in all examined muscles. Blood pressure response to standing and vertical tilt, respiratory heart rate variation and cardiac axon reflexes were normal. Abdominal ultrasound examination showed no evidence of hepatosplenomegaly or lymphadenopathy. Echocardiography was normal except for distinct sclerosis of the aorta and the aortic valve. MRI of the brain, duplex and Doppler sonography of the intracranial, carotid, and vertebral arteries showed no evidence of arteriosclerosis or its sequels.

The values for total plasma cholesterol, HDL-C, LDL-C, apo A-I, apo B, and VLDL-C are indicated in Table 1. In the index patient, HDL-C, LDL-C and apo A-I were not measurable and VLDL-C was markedly increased. In her younger daughter (VI:2), reduced levels of HDL-C, apo A-I and VLDL-C were apparent. The other daughter (VI:1) showed only a reduced level of VLDL-C. No laboratory results were available for the sister (V:6) and her daughters (VI:3, VI:4). In the index patient, creatine kinase level was
elevated (151 U/l, normally <80 U/l). The other parameters listed above were in the normal range.

**Mutation analysis of the ABC1 gene**

Automated fluorescent sequencing of the ABC1 gene revealed a homozygous C→T transition at nucleotide position 2665 in exon 18 of the index patient (Fig. 3). This base substitution predicts the replacement of arginine with a stop codon at position 909 (R909X), resulting in premature termination of the mRNA translation. This sequence change introduced an additional DdeI restriction site in the PCR fragment. In healthy controls, an undigested PCR product of 260 bp was observed. As expected, the daughters were heterozygous for the mutation. The sister (V:6) and one of her daughters (VI:4) were also found to be heterozygous.

**Table 1 Serum lipid findings in the Tangier disease family**

<table>
<thead>
<tr>
<th>Patients</th>
<th>Normal values</th>
</tr>
</thead>
<tbody>
<tr>
<td>V:2</td>
<td>VI:1</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>2.8</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1.6</td>
</tr>
<tr>
<td>HDL-C</td>
<td>Absent</td>
</tr>
<tr>
<td>LDL-C</td>
<td>Absent</td>
</tr>
<tr>
<td>VLDL-C</td>
<td>2.9</td>
</tr>
<tr>
<td>apo A-I</td>
<td>Absent</td>
</tr>
<tr>
<td>apo B</td>
<td>113</td>
</tr>
</tbody>
</table>

Absent = not measurable

**Nerve biopsy findings**

Sural nerve biopsies of the index patient at the age of 45 and 54 years revealed subtotal and finally complete loss of nerve fibres and Schwann cells in the endoneurium (Fig. 4B and C). The former biopsy showed only rare myelinated nerve fibres surrounded by an increased amount of endoneurial collagen (Fig. 4B). There were no onion bulb formations. No clusters of regenerated nerve fibres were detectable. Rare lipid-laden macrophages were related to endoneurial blood vessels. The nerve fascicles were 200–300 μm in diameter (Fig. 4A). These were filled with collagen showing only 1–3 capillaries per fascicle. One fascicle contained an extraordinary fat cell.

![Fig. 3 Sequencing analysis of exon 18 in the Tangier disease family. (A) A single nucleotide substitution (T→C) at nt2665 leads to exchange of arginine to a stop-codon at position 909. This mutation predicts preterminal truncation of the ABC1 gene. Patient V:2 was homozygous for this mutation; her sister (V:6) and one of her nieces (VI:4) showed a heterozygous state. (B) The presence of the mutation was assayed by restriction digestion with DdeI, which cuts only the mutant allele. Lanes: M = molecular weight marker (Biomarker Low, BioVentures, Murfreesboro, USA); C = healthy control; V:2 = homozygous index patient; V:6, VI:1, VI:2, and VI:4 = heterozygous family members; VI:3 = healthy family member.](http://example.com/fig3.png)
in the endoneurium (Fig. 4A). The perineurium was increased in width. In the later biopsy, neither nerve fibres nor Schwann cells had survived (Fig. 4C). Endoneurial cells with thin but long cytoplasmic extensions (Fig. 5B) were identified as fibroblasts dividing the endoneurium into incomplete compartments. The walls of the epineurial vessels contained occasional vacuoles, but no more severe signs of atherosclerosis were detectable. Electron microscopy of the second biopsy revealed rare persisting fibroblasts and macrophages with vacuoles containing lipids, pleomorphic granules and osmiophilic components (Fig. 5A). Nerve fibres or Schwann cells were no longer detectable. The endoneurium was filled with collagen fibres and rare oxtalan fibres (Fig. 5B). The perineurium was rather well preserved. However there was an increased amount of collagen fibres between the perineurial cells, indicating some degree of fibrosis. The basal lamina of occasional inner perineurial cells was patchy rather then continuous (Fig. 5C and D). Immunohistochemically, the perineural cells expressed epithelial membrane antigen, but no such cells were seen in the endoneurium (data not shown).

Discussion

The present study revealed a novel nonsense mutation in the \textit{ABC1} gene in a family with Tangier disease. The index patient presented the rare syringomyelia-like phenotype of Tangier disease and no significant signs of cardiovascular disease. The transition of C\textsuperscript{c}T implemented a new stop codon instead of arginine in codon 909 of the ABC1 protein. Therefore, a premature termination of the mRNA-translation was predicted. The termination occurred in the highly hydrophobic linker region connecting the two functional parts of the symmetrically structured protein.

The clinical history and neurological examinations revealed a progressive syringomyelia-like syndrome and were compatible with predominant loss of smaller nerve fibres, thus explaining the early decrease of pain and temperature sensation. Electrodiagnostic investigations at the present stage of the disease detected, with the exception of only two peripheral nerves, no compound motor or sensory action potentials. These data documented the final stage of progression of the polyneuropathy.

Neuropathological examination of two sural nerve biopsies at the age of 45 and 54 years showed an unusually extensive degree of endoneurial sclerosis and a complete loss of nerve fibres and Schwann cells at the time of the second biopsy (Fig. 4C). A progression of the pathological process within 10 years was apparent. Former studies of Tangier disease with the syringomyelia-like phenotype showed marked reduction of myelinated and unmyelinated nerve fibres with some, but not such an extensive endoneurial collagen replacement of the parenchyma (Gibbels \textit{et al.}, 1985). Previous ultrastructural findings comprised membrane-bound vacuoles and non-membrane-bound lipid droplets in Schwann cells, in perivascular macrophages and in fibroblasts (Pollock \textit{et al.}, 1983). Even though some of these features were still present in the first biopsy of our case, the complete endoneurial sclerosis in the latter specimen is a unique finding. It has not been seen in a large series of more than 7000 sural nerve biopsies (Schröder, 2001) and has to the best of our knowledge not been reported before.

An additional observation was some degree of fibrosis of the perineurium of the nerve fascicles with an increased amount of collagen fibrils between the perineurial cell layers (Fig. 5C). Although the perineurial cells expressed epithelial membrane antigen, the patchy basal lamina at some sites was considered as a sign of a minor degree of dedifferentiation of these cells (Fig. 5D). The development and integrity of the perineurium is normally mediated by the signalling protein, Desert hedgehog, which is expressed by Schwann cells (Parmantier \textit{et al.}, 1999). Obviously, the absence of Schwann...
cells in the endoneurium of the presented case had caused only minor perineurial alterations.

As expected, the daughters of the index patient were heterozygous for the mutation. At the age of 15 and 27 years, respectively, they complained of no symptoms of neuropathy. HDL-C, apo-AI, and VLDL levels were moderately reduced in one daughter (V:2), indicating a heterozygous state. Interestingly, only the VLDL level in her half-sister was decreased (Table 1). Taking into account the fact that both sisters had different fathers, the deviant plasma levels were probably due to the respective genetic backgrounds.

Almost complete absence of HDL and accumulation of cholesteryl esters in various organs including blood vessels, liver, tonsils, and peripheral nerves characterize Tangier disease. The reason for decreased LDL-C levels is not fully understood. However, in conjunction with former studies, which showed that low LDL-C levels were an important factor for reduced risk of cardiovascular disease (Schaefer et al., 1994), the presented index patient showed absence of LDL-C in the serum and no signs of atherosclerosis. In the peripheral nerves, the accumulation of lipids in Schwann cells, which maintain large amounts of membranes, probably leads to a primary degradation of this cell type accompanied by loss of axons. From other hereditary sensorimotor neuropathies, it is known that defective membrane-bound proteins such as connexin32 and myelin protein zero may cause axonal degeneration in addition to demyelination (Marrosu et al., 1998; Senderek et al., 1998, 2000; Hanemann et al., 2001). On the other hand, lack of regenerating axons in Tangier disease suggests a neuronal type of neuropathy. In a single case of Tangier disease, lipid accumulation was shown to occur in small dorsal root ganglion cells (Schmalbruch et al., 1987). However, the cause of the premature loss of small nerve fibres in the syringomyelia-like phenotype remains to be explained. Since polyneuropathy in Tangier disease occurs more often than the syringomyelia-like phenotype, it will be of interest to determine whether different genetic defects in the ABC1 gene may lead to either of these mutually exclusive subtypes.

According to the Online Mendelian Inheritance in Man database (OMIM, http://www.ncbi.nlm.nih.gov/omim), sequencing analysis has revealed a mutation in the ABC1 gene in 13 pedigrees so far. Except for the present patient, only one further case with a syringomyelia-like syndrome has been characterized genetically (Serfaty-Lacrosniere et al., 1994; Brousseau et al., 2000). This case developed syringomyelic symptoms at 25 years of age and premature cardiovascular disease. Genetic analysis revealed a point mutation in the highly conserved region of the second transmembrane domain of ABC1. It was supposed that this mutation would induce a conformational change of the protein. However, our case was predicted to have a disrupted protein with complete loss of the second half of the ABC1 protein. This supports the former suggestion of Brousseau and colleagues that the second transmembrane domain may be crucial for the reverse cholesterol transport (Brousseau et al., 2000). A nerve biopsy was not available in this case compared with the present case.

ABC1 belongs to a family of evolutionary conserved ATP-binding cassette transporters, which are responsible for the ATP-dependent membrane transport of amino acids, ions, peptides and vitamins. It is also involved in the phenomenon of multi-drug resistance. A functional ABC transporter requires the symmetrical association of six membrane-spanning helices and two nucleotide-binding folds responsible for the interaction with ATP. Mutational analysis of the P-glycoprotein, another member of this family with similar structure, suggests that the two halves of the human ATP-transporter interact to form a single transporter, and that the major drug binding domains reside in or near transmembrane domains 5, 6, 11, and 12 (Ambudkar et al., 1999). Yet, the full complement of substrates transported by ABC1 has not been understood.
identified. It is possible that variability in the phenotype is due to mutation-specific differences in substrate specificity.

In conclusion, a novel point mutation in the ABC1 gene was detected leading to a premature stop of translation, which results in a shortened and presumably non-functional ABC1 protein. The presented index patient showed the most severe form of the rare syringomyelia-like phenotype in Tangier disease reported thus far, but no signs of cardiovascular disease. In conjunction with the second genetically characterized case with a syringomyelia-like syndrome (Brousseau et al., 2000), the novel mutation suggests that the C-terminal part of the symmetrical protein is crucial for the development of this rare phenotype.

Acknowledgements

This study was supported by the Deutsche Forschungsgemeinschaft (Schr195/17–2). Professor Dr Werner Paulus, Director of the Department of Neuropathology, University of Münster, Münster, Germany, kindly provided the sural nerve biopsy of the year 1992. We are particularly grateful to the family members who have supplied information and samples for this study.

References


