A novel mutation found in an adrenoleukodystrophy patient who underwent bone marrow transplantation


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Summary: We identified a novel mutation, L322P, in a patient with X-linked adrenoleukodystrophy (ALD) who underwent bone marrow transplantation (BMT). An identification of the ALD gene mutation enabled us to employ an approach not dependent on the use of radioisotopes for detecting mixed chimerism. This assay could show more than 99.0% of the patient’s peripheral white blood cells were replaced by the donor’s cells.

X-linked adrenoleukodystrophy (McKusick 300100) is an inherited peroxisomal disorder affecting 1/15,000–1/100,000 males as childhood cerebral adrenoleukodystrophy (ALD), adult adrenomyeloneuropathy or adrenocortical insufficiency. Diagnoses have been made on the basis of an elevated level of very long-chain fatty acids (VLCFA) in the serum or red blood cells together with the appropriate clinical presentation (Moser et al 1989). The gene responsible for this disease has been isolated by positional cloning (Mosser et al 1993; Sarde et al 1994). It encodes a member of the ATP-binding cassette (ABC) transporter superfamily of membrane proteins. So far, over a hundred mutations in the gene have been reported in ALD patients (Fanen et al 1994; Braun et al 1995; Kok et al 1995; Ligtenberg et al 1995; Watkins et al 1995; Feigenbaum et al 1996; Krasemann et al 1996).

There is no clear evidence that dietary treatment with oils containing monounsaturated fatty acids (oleic and erucic) and the restricted intake of VLCFA has any beneficial effect in halting progression of the disease (see Aubourg and Mandel 1996). Bone marrow transplantation (BMT) has reportedly been performed in over 50 patients, and in some cases has resulted in neurological improvement (Moser et al 1992; Aubourg and Mandel 1996; Malm et al 1997).
We found a novel point mutation in exon 2 of the ALD gene in a patient treated with BMT. We also show the replacement of the peripheral white blood cells carrying this mutated ALD gene with those harbouring the normal gene after BMT.

**MATERIAL AND METHODS**

**Patient:** The patient was a 12-year-old boy who developed normally until 5 years of age when his skin colour darkened. He was diagnosed with Addison disease and corticosteroid therapy was initiated. Involuntary movements and dizziness were noted at 10 years of age. He was then diagnosed with ALD on the basis of VLCFA level and CNS magnetic resonance imaging findings. Despite dietary therapy with oleic and erucic acid, he had developed difficulty hearing and swallowing by 12 years of age. He was subsequently admitted to our hospital. Standard chemotherapy and BMT from HLA-matched donor was undertaken.

**Methods:** Eight sets of oligonucleotide primers that encompassed all 10 exons of ALD gene were synthesized. A polymerase chain reaction (PCR) was performed to obtain a DNA concentration of 30–100 ng/ml. A standard protocol was used and is summarized as follows: after denaturing for 5 min at 94°C, 30–35 cycles of a 1 min denaturation at 94°C, a 1 min annealing at 55°C and a 1 min extension at 72°C were performed. The length of the final extension was increased to 7 min. The cycle number was adjusted to permit the synthesis of enough amplified DNA for heteroduplex analysis. PCR products from the patient (150–250 ng) were mixed with the same amount of control DNA. After inactivation of the DNA polymerase by adding EDTA to a final concentration of 5 mmol/L, the mixture was heated for 3 min at 95°C and cooled slowly (20–30 min) to 37°C. This mixture was added to the loading buffer and electrophoresed on a 1.0 mm-thick Hydrolink-MDE gel (AT Biochem, Malvern, PA, USA) for 15–30 h at 800 V. The gel was stained with ethidium bromide. The PCR products forming a heteroduplex band were subcloned using a TA Cloning Kit (Invitrogen, San Diego, CA, USA) and sequenced with an automatic DNA sequencer (Model 373A; Applied Biosystems).

**RESULTS**

Only the PCR products from exon 2 formed a heteroduplex band (Figure 1). Sequencing from four clones all revealed a T-to-C mutation at nucleotide number 1351 (see Mosser et al 1993). This mutation, CTG → CCG, converts the amino acid of codon 322 from leucine into proline and generates a MspI restriction site. We confirmed the absence of this L322P mutation in 75 unrelated Japanese individuals by restriction analysis.

To monitor the remaining host cells after BMT, we devised the following assay. Patient DNA was mixed with normal DNA in various concentration ratios. Using the same amount of this mixture as templates, PCR and digestion with the enzyme MspI were performed. Each sample was electrophoresed in a 12% acrylamide gel and stained with ethidium bromide. The bands from the mutated gene were detect-