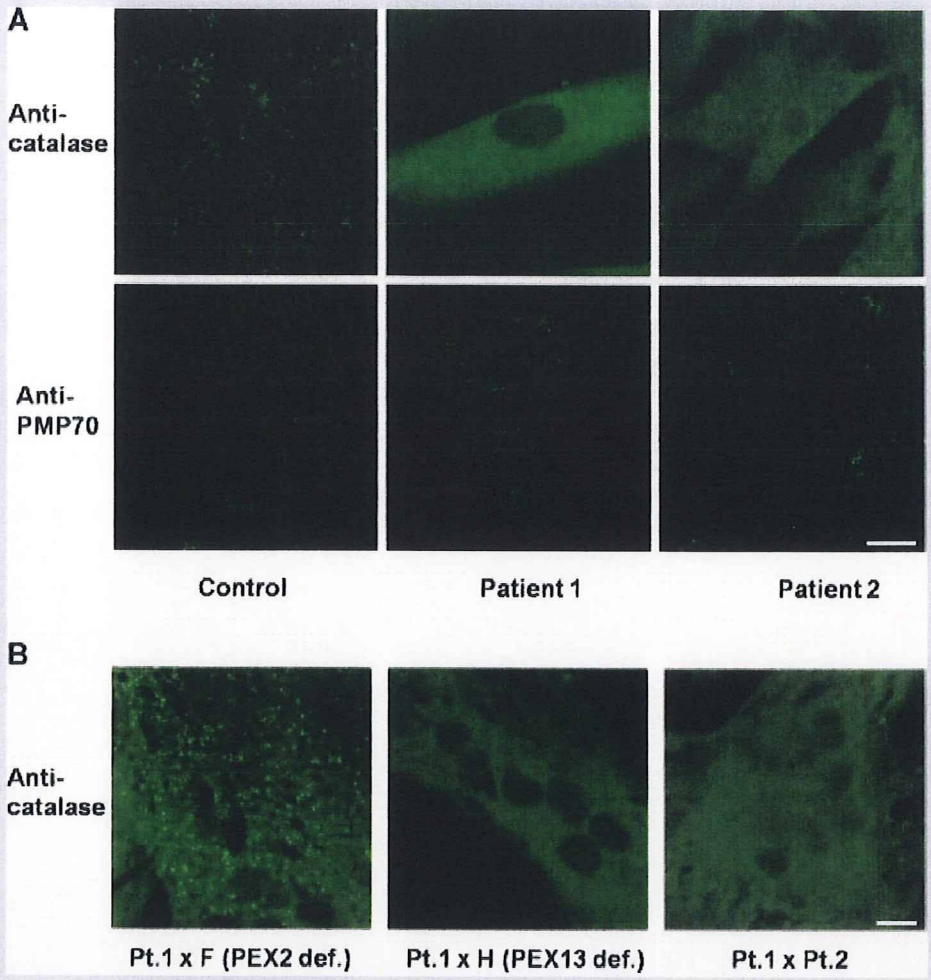

FIG. 1. A: Immunofluorescence microscopy analysis in fibroblasts from patients. Cells were stained using antibodies to human catalase and PMP70. No catalase-containing particle and a few PMP70-containing particles were seen in fibroblasts from both patients 1 and 2, as compared with findings in controls. B: Complementation of peroxisomes in fibroblasts from patients. Cells were stained using antibody to human catalase. Numerous peroxisomes were seen in the fused cells between fibroblasts from Patient 1 and fibroblasts from PEX2-defective patient (group F of PBDs), whereas no peroxisomes were seen in cells hybrid of the Patient 1 fibroblasts to those from both PEX13-defective patient (group H of PBDs) and Patient 2, which means Patient 1 and 2 belonged to group H of PBDs. Bar = 20 μm. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

both patients belonged to complementation group H of PBDs (equivalent to complementation group 13 at Kennedy Krieger Institute), caused by mutated PEX13 gene (Fig. 1B). The consanguineous nature of their parents suggested homozygosity at the disease locus. Therefore, we first confirmed that both patients were homozygous at the PEX13 locus using polymorphic microsatellite markers (data not shown). We then attempted to determine the mutation by amplifying the four exons of PEX13, but were unsuccessful in Patient 1 (Fig. 2A, lanes 2–6) whereas the control showed a normal amplification pattern. We concluded that there was homozygous deletion of PEX13 in Patient 1. To determine the extent of the deletion, we amplified STS markers and other short fragments from genes upstream and downstream (FLJ32312 and KIAA1841 respectively) of PEX13 and then designed a long range PCR to span the breakpoint. Sequencing of the resulting amplicon confirmed a total deletion size of 147,308 bp that included PEX13 in addition to 70,094 bp upstream and 45,692 bp downstream regions relative to the gene (Fig. 2A). Both deletion breakpoints fall within Alu repeats, raising the possibility it may have been mediated by those repeat elements [Kolomietz et al., 2002]. In Patient 2, direct sequencing revealed a 14 bp deletion (Fig. 2B) in exon 2 resulting in a frameshift and premature truncation (c.107_120del, p.G36DfsX61).

DISCUSSION

Peroxisomes are uniquely eukaryotic, membrane bound organelles that perform essential metabolic functions deficiency of which has

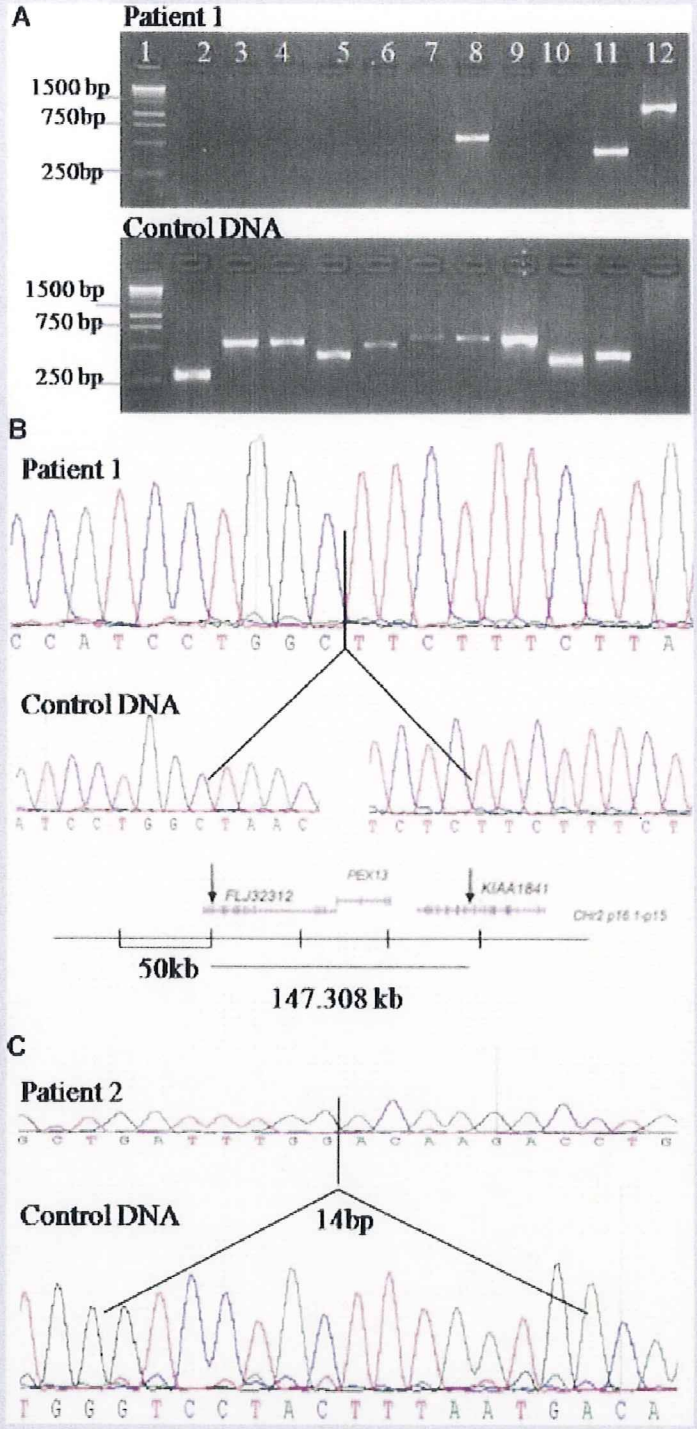

FIG. 2. A: Detection of *PEX13* deletion in Patient 1 by PCR. PCR analysis of DNA samples from Patient 1 reveals total deletion of all *PEX13* exons (lanes 2–7) in addition to upstream (lane 9) and downstream regions (lane 10). PCR amplification of normal *PEX13* gene detects six bands corresponding to all four exons, as shown in normal DNA in lanes 2–7. PCR that spans the deletion point gave band of ≈1.4 kb from Patient 1 while no band was detected from the DNA control (Lane 12). Lane 1: 1 kb marker. B: Chromatogram of the amplicon obtained from Patient 1 using forward primer located 70.334 kb upstream of *PEX13* gene and reverse primer 46.775 kb downstream *PEX13* gene. The line indicates the breakpoint and the DNA map below shows the coordinates of the deletion. The total deletion in Patient 1 is 147.308 kb starting 70.094 kb upstream (including 13 coding exons of *FLJ32312*) and ending 45.692 kb downstream of *PEX13* (including eight coding exons of *KIAA1841* gene). C: Sequence from exon 2 in Patient 2 shows out-of-frame 14 bp deletion. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

major clinical consequences as seen in patients with PD [Alberts et al., 2007]. For proper peroxisomal biogenesis, special matrix proteins are made in the cytoplasm and are targeted to the peroxisomes by virtue of an uptake-targeting signal found on their C-terminus (PTS1) or, less commonly, N-terminus (PTS2). These signals bind soluble receptor proteins, which escort the matrix proteins to a peroxisomal membrane receptor (PEX14), the major docking factor that facilitates their import inside peroxisomes [Gould and Valle, 2000]. PEX13 was originally cloned and described by Gould et al. [1992] as a novel peroxisomal membrane protein that acts as an essential docking factor for the import of PTS1-containing matrix proteins. PEX13 was also shown to be important for the import of PTS2-containing matrix proteins and for the function of PEX14 and [Girzalsky et al., 1999; Schell-Steven et al., 2005]. Not surprisingly, therefore, import of both PTS1- and PTS2-containing matrix proteins is disturbed in Pex13 knockout mouse [Maxwell et al., 2003].

Consistent with the essential role played by PEX13, deficiency of this protein in humans results in the clinical picture of PBD (recognized as complementation group H) [Liu et al., 1999; Shimozawa et al., 1999; Krause et al., 2006]. Given the rarity of this group, it came as a surprise to us that the first two patients enrolled in our study would turn out to belong to group H. Our analysis revealed loss of function of PEX13 since no import of catalase could be demonstrated on the fibroblasts from either of the two patients. This is consistent with the nature of the two mutations identified. The first mutation is clearly predicted to result in no PEX13 function since the entire gene is deleted. The relatively large genomic deletion additionally involved 13 coding exons of FLJ32312 and 8 coding exons of KIAA1841 thus rendering them both inactive. Both are hypothetical genes and neither is known to be related to any human disease so while the typical course of Patient 1 suggests that deficiency of the product of these two genes did not contribute significantly to his phenotype, we cannot rule out the possibility that a subtle clinical effect may have been overlooked. The second mutation identified in this study creates a frameshift and the resulting premature termination predicts either nonsense mediated decay [Wilusz et al., 2001] or a PEX13 that lacks both its membrane-spanning as well as the SH3 domains [Gould et al., 1992], either possibility would qualify the mutation as null and render the cell null for PEX13 function. This probably explains the highly similar biochemical as well as the functional perturbation observed in the two patients which is also similar to the severe clinical profile of the only reported patient with nonsense mutation [Krause et al., 2006].

In summary, our findings increase to five the number of mutant alleles reported so far in PEX13 and suggest that PEX13-related PBDs may not be rare among Arabs. Our ongoing molecular analyses of Arab patients with PBDs will shed more light on this hypothesis.

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