Compound heterozygous *AIRE-1* mutations causing autoimmune polyendocrinopathy syndrome type 1

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Presentation of clinical case

The patient is at present a 9 year old boy who is the second child of non-consanguineous parents who are of British and Romany origin. He was born at full-term by a normal delivery after an uncomplicated pregnancy, with a birth weight of 3.5 kg. At 2 months of age he had dyspnoea and stridor, but no seizures and at 12 months of age he sustained a right forearm fracture following minimal trauma. He did not suffer from repeated infections, and did not have facial dysmorphology, deafness or cardiac lesions. He was not mentally retarded and attained developmental milestones without delay. His growth continued on the 50th centile. At the age of 3.5 years he developed carpo-pedal spasms, and investigations revealed hypocalcaemia [serum corrected calcium concentration = 1.44 mmol/L (normal 2.20-2.65 mmol/L)], hyperphosphataemia [phosphate = 3.21 mmol/L (normal 1.29-1.78 mmol/L)], and an undetectable circulating PTH concentration (<10 ng/L). Other serum measurements, which included sodium, potassium, urea, creatinine, liver function tests, magnesium and fasting glucose were all normal. He was noted to have dystrophic nails at this time, and subsequently developed alopecia areata, which progressed to alopecia totalis by the age of six years (Figure 1). Thyroid function and synacthen tests have been normal, and an assessment for autoantibodies that included those for anti-nuclear, anti-parietal, anti-smooth muscle, anti-mitochondrial, anti-reticulin, anti-adrenal, anti-insulin, anti-thyroglobulin, anti-peroxidase and anti-parathyroid antibodies was negative.
Haemoglobin and MCV were also normal. There was no history of neck surgery or trauma and there was no family history of autoimmune related diseases, and in particular hypoparathyroidism, alopecia, vitiligo, or Addison's disease. Examination revealed him to have dystrophic nails, consistent with moniliasis, and alopecia (Figure 1). There were no other abnormalities and in particular there was an absence of vitiligo, cataracts, ectopic calcification, dental caries, enamel hypoplasia, and brachydactyly. Renal ultrasonography revealed that both kidneys were of normal size and architecture.
Treatment with 1α-hydroxycholecalciferol (alfacalcidol) restored normocalcaemia and he has not suffered further from carpo-pedal spasms or seizures. The clinical diagnosis was therefore of hypoparathyroidism due to autoimmune polyendocrinopathy syndrome (APS) type 1, and to further confirm this, mutational analysis of the autoimmune regulator 1 (*AIRE-1*) gene was undertaken.

Methods

Venous blood was obtained after informed consent, from the proband and his parents, using guidelines approved by the local ethical committee, and used to extract leukocyte DNA (1). Fourteen pairs of gene-specific oligonucleotide primers were employed for the PCR amplification of the 14 coding exons and adjoining splice junctions of the *AIRE-1* gene using methods previously described (2). The DNA sequences of the resultant PCR products were determined by the use of Thermo Seque-

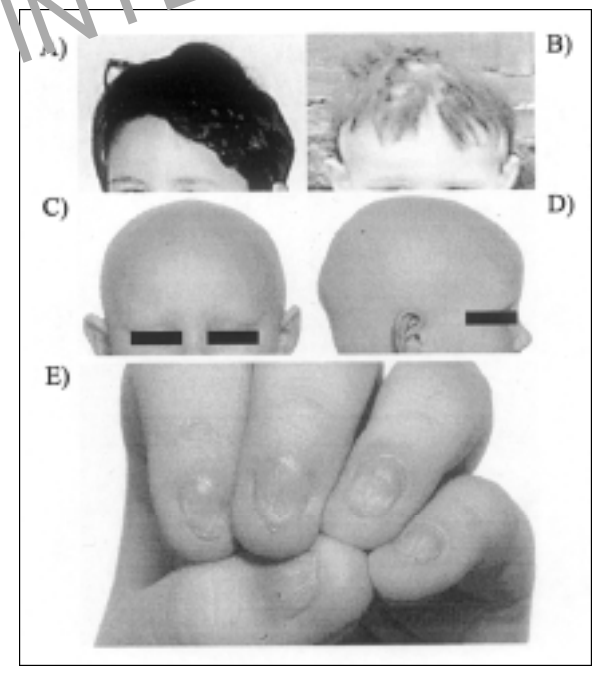

Figure 1 - Signs of APS1 in the patient.Panels A-D showing progression of hair loss from normal hair distribution at age3 years (panelA) to alopecia areata (panel B) at age 5 years and ultimately to alopecia totalis (panels C andD) by age 6 years.Note also the progressive loss of eyebrows over this time period. Panel E shows dystrophic and hypoplastic finger nails, consistent with moniliasis.

nase II DNA polymerase dye terminator cycle sequencing (Amersham Pharmacia Biotech Inc, Little Chalfont, Bucks, UK) and a semi-automated detection system (ABI 373 sequencer, Applied Biosystems, Foster City, CA, USA) as previously described (3). DNA sequence abnormalities were confirmed either by restriction enzyme analysis or by agarose gel electrophoresis as previously reported (2, 4).

Results

DNA sequence analysis of the entire 1635 bp coding region and 26 exon/intron boundaries of the *AIRE-1* gene in the patient revealed the presence of two mutations.These consisted of a maternally inherited nonsense mutation (Arg257Stop) in exon 6 (Figure 2) and a paternally inherited 13 bp deletion in exon 8 (Figure 3).Thus, the patient with APS1 has 2 different *AIRE-1* mutations and hence is a compound heterozygote.The sole occurrences of the Arg257Stop mutation in the unaffected mother (Figure 2) who is of Romany origin, and the 13 bp deletional frameshift in the unaffected father, who is of British origin, are consistent with the autosomal recessive inheritance of APS1.

Discussion

Our studies of a patient with APS1 have identified two *AIRE-1* mutations, which consisted of a maternally inherited nonsense mutation (Arg257Stop), and a paternally inherited 13 bp deletional frameshift.The *AIRE-1* gene encodes a 545 amino acid protein (4), that contains:2 plant homeodomain (PHD) zinc finger motifs (codons 298-343, and codons 433-478); 4 LXXLL motifs (codons 7-11, 63-67, 414-418, and 516-520); and a proline rich domain in exon 10 (codons 366 to 420) (1-6). The 2 *AIRE-1* mutations (Figures 2 and 3) identified in the patient with APS1 (Figure 1) are predicted, if translated, to result in truncated forms of the protein. Thus, the Arg257Stop mutation would result in a 256 amino acid peptide that lacks the 2 PHD zinc finger domains, 2 of the 4 LXXLL domains, and the proline rich domain. The 13 bp deletional-frameshift would result in a 372 amino acid peptide that contained 50 missense C-terminal amino acids, a disrupted first PHD zinc finger domain, and a lack of the second PHD zinc finger domain together with 2 of the 4 LXXLL domains and the proline rich region.These truncated *AIRE-1* proteins are likely to be inactive, and it has been shown with the use of transcriptional reporter assays, that the Arg257Stop mutation leads to a loss of transcriptional transactivation activity (7).In addition, deletion constructs lacking the PHD zinc finger domains have been shown to have grossly disordered sub-cellular localization (8). Thus, the 2 mutations identified in this patient withAPS1 are likely to result in a loss of transcriptional activity.

The 13 bp deletional framshift and the Arg257Stop mutations are the most frequently occurring mutations in the British, and the Central and Eastern European populations, respectively (2, 9, 10), and this is consistent with the British and Romany parentage of the patient with APS1. The 13 bp deletional frameshift has increased prevalences of 70% and 53% in the genetically outbred British and NorthAmerican populations, respectively, when compared to the world prevalence of 23% (2, 10-12). This would seem to suggest that there is not only a founder effect but also a recurrence of the same mutation and it is important to note that the region of the mutations is flanked by repetitive DNA sequences. The heterozygous carrier rate of this mutation in the British population has been estimated to be 1/576 (2), and several mechanisms, that include slipped-strand mispairing and formation of hairpin structures from the imperfect inverted repetitive sequence, have been proposed for the increased susceptibility for mutagenicity (2, 5).

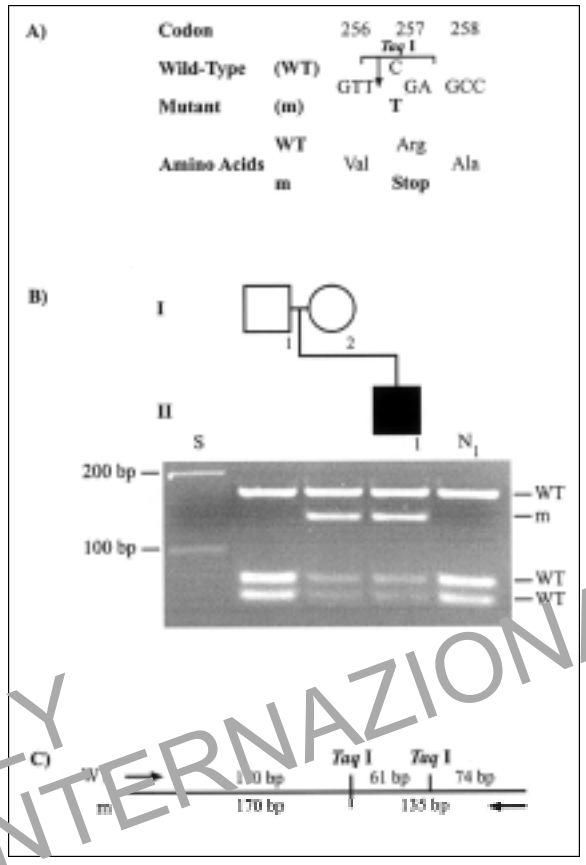

Figure 2 - Maternally inherited *AIRE-1* nonsense mutation inAPS1 patient, detected by restriction enzyme analysis.DNA sequence analysis of the patient (II-1) revealed a C→T transition at codon 257, in exon 6, thereby altering the wild-type (WT) sequence CGA encoding an arginine (Arg) to the mutant (m) sequence, TGA, encoding a termination signal (Stop) (A).This nonsense mutation,Arg257Stop, also resulted in a loss of a *Taq* I restriction enzyme site (T/CGA) and this facilitated the detection of the mutation (B).Following PCR amplification and *Taq* 1 digestion one product of135 bp is obtained from the m sequence, but two products of 61 bp and 74 bp are obtained from theWT (normal) sequence (C).The patient and his unaffected mother (I-2) are heterozygous (WT-m), for the wild-type and mutant sequences, whilst the unaffected father (I-1) is homozygous for the wild-type sequence.The mutation was absent from110 alleles of 55 unrelated normals (N_1 shown), consistent with it not being a common DNA sequence polymorphism. Individuals are represented; males as squares, females as circles, unaffected as open symbols and affected as filled in symbols.A standard-size marker (S), in the form of a 1 Kb ladder, is shown.

It is interesting to note that APS1 is unusual among diseases caused by transcription factor mutations in that it requires both alleles to be inactivated in order to produce a disease phenotype. This is in contrast to the situation seen with the development of the hypoparathyroidism, deafness and renal dysplasia (HDR) syndrome which is due to haploinsufficiency of the transcription factor *GATA3* (13, 14).This may be related to a gene dosage effect, whereby the wild-type allele is able to compensate for the loss of function due to the mutant in the case of *AIRE-1* but not in the case of *GATA3.*

Finally, the monogenic aetiology of APS1 makes it a unique

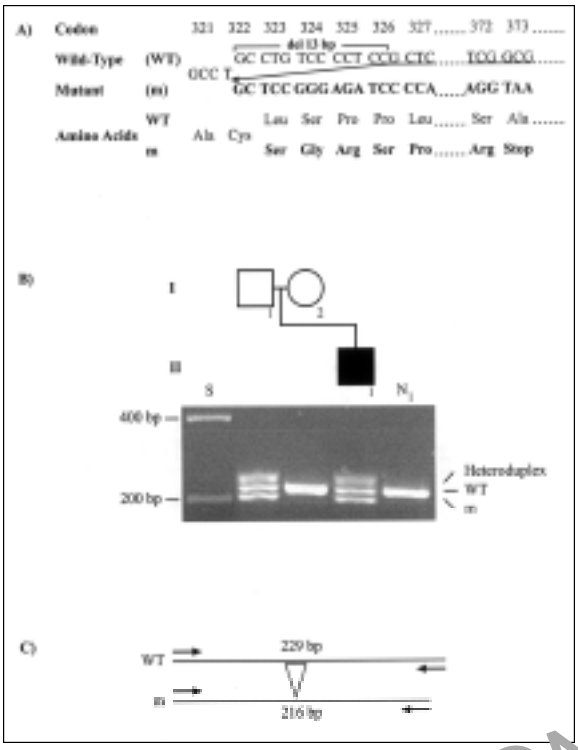

Figure 3 - Paternally inherited *AIRE-1* deletional mutation in APS1 patient, detected by agarose gel electrophoresis.DNA sequence analysis of the patient (II-1) revealed a 13 bp deletion (del 13 bp) at nucleotide (nt) 967-979 (codon 322 - 326), in exon 8 (A). The deletion results in a frameshift (indicated by underlined sequence and an arrow) that leads to the incorporation of 50 missense amino acids after which a premature termination signal (Stop) (TAA) is encountered. The 13 bp deletion results in the formation of heteroduplex (WT/m), and homoduplexes (WT/WT and m/m) that can be detected by agarose gel electrophoresis (B). Thus, the mutant (m) PCR product is 216 bp, whereas the WT product is 229 bp.(C) and a heteroduplex is observed at approximately 250 bp.The patient (II-1) and his unaffected father (I-1) are heterozygous (WT/m) for the wild-type and mutant sequence, whilst the unaffected mother (I-2) is homozygous for the wild-type sequence.The 13 bp deletion was absent from 110 alleles of 55 unrelated normals (N_1 shown), consistent with it not being a commonDNA sequence polymorphism. The symbols representing the individuals are as described in Figure 2.A standard-size marker (S), in the form of a 1 Kb ladder, is shown.

model for studying the biological events leading to autoimmunity and autoimmune diseases (15). Several major autoantigens, in APS1, have been identified, and these include the P450 enzymes 17α-hydroxylase, 21-hydroxylase and side chain cleavage enzyme (16-18). Tissue-specific autoimmune targets have also been reported, including thyroid peroxidase, thyroglobulin, insulin, liver specific P450 enzymes and melanocyte transcription factors (SOX9 and SOX10) (19). The *in vivo* effects of *AIRE-1* loss have been studied in mice deleted for *AIRE-1*, and homozygous (-/-) null mice were found to develop normally, but to exhibit features of APS1 including multiorgan lymphocytic infiltration, circulating autoantibodies and infertility (20). Further studies in such mice have demonstrated that *AIRE* deficiency causes an almost complete failure to delete organ-specific cells in the thymus, and thus APS1 is likely to be caused, at least in part, by a failure of the negative selection of forbidden organ-specific T cells in the thymus (21).

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