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A Common and Recurrent 13-bp Deletion in the Autoimmune Regulator Gene in British Kindreds with Autoimmune Polyendocrinopathy Type 1

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Summary

Autoimmune polyendocrinopathy type 1 (APS1) is an autosomal recessive disorder characterized by autoimmune hypoparathyroidism, autoimmune adrenocortical failure, and mucocutaneous candidiasis. Recently, an autoimmune regulator gene (*AIRE-1*), which is located on chromosome 21q22.3, has been identified, and mutations in European kindreds with APS1 have been described. We used SSCP analysis and direct DNA sequencing to screen the entire 1,635-bp coding region of *AIRE-1* in 12 British families with APS1. A 13-bp deletion (964del13) was found to account for 17 of the 24 possible mutant *AIRE-1* alleles, in our kindreds. This mutation was found to occur de novo in one affected subject. A common haplotype spanning the AIRE-1 locus was found in chromosomes that carried the 964del13 mutation, suggesting a founder effect in our population. One of 576 normal subjects was also a heterozygous carrier of the 964del13 mutation. Six other point mutations were found in *AIRE-1*, including two 1-bp deletions, three missense mutations (R15L, L28P, and Y90C), and a nonsense mutation (R257*). The high frequency of the 964del13 allele and the clustering of the other *AIRE-1* mutations may allow rapid molecular screening for APS1 in British kindreds. Furthermore, the prevalence of the 964del13 *AIRE-1* mutation may have implications in the pathogenesis of the more common autoimmune endocrinopathies in our population.

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Introduction

Organ-specific autoimmune diseases affect up to 4% of the female population, over a lifetime, and are inherited as complex multigenic traits (Vyse and Todd 1996). Despite the prevalence of these disorders and their well-established genetic basis (Davies et al. 1994; Sawcer et al. 1996), we have little knowledge of the molecular pathways implicated in their pathogenesis. However, the study of rare monogenic autoimmune syndromes may provide insights for the investigation of the more common, genetically complex autoimmune disorders. One such disorder is autoimmune lymphoproliferation syndrome (Canale and Smith 1967), in which inactivating mutations of either the *Fas* or the *Fas-L* gene lead to a failure of apoptotic elimination of activated peripheral T lymphocytes (Fisher et al. 1995; Rieux-Laucat et al. 1995; Drappa et al. 1996; Wu et al. 1996). Another inherited autoimmune syndrome is autoimmune polyendocrinopathy syndrome type 1 (APS1; MIM 240300), which is also referred to as "autoimmune polyendocrinopathy with candidiasis and ectodermal dystrophy" (Neufeld et al. 1981; Ahonen et al. 1990). APS1 is characterized by the triad of autoimmune hypoparathyroidism, autoimmune primary adrenocortical failure (Addison disease), and chronic mucocutaneous candidiasis (Hung et al. 1963; Kenny and Holliday 1964; Arulanantham et al. 1979; Neufeld et al. 1981; Ahonen et al. 1990). Hypoparathyroidism and candidiasis generally are manifest in affected subjects during childhood, with the onset of adrenal failure typically in early adolescence. Other autoimmune complications include type 1 diabetes mellitus, primary gonadal failure, pernicious anemia, hepatitis, alopecia, vitiligo, and hypothyroidism (Hung et al. 1963; Kenny and Holliday 1964; Arulanantham et al. 1979; Neufeld et al. 1981; Ahonen et al. 1990). Intestinal malabsorption, keratopathy, pitted nail dystrophy, dental enamel hypoplasia, splenic atrophy, gallstones, and oral carcinoma also may be present

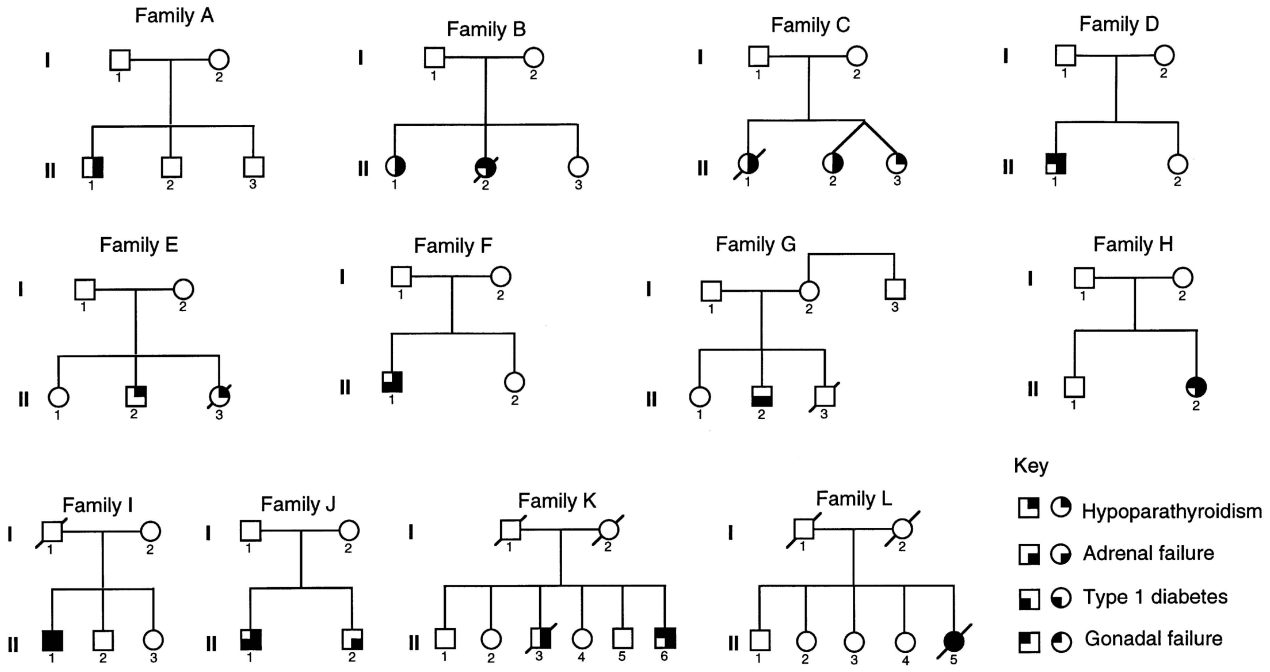

Figure 1 Pedigrees of the 12 kindreds with APS1. Males are represented by squares and females by circles. All affected subjects manifested chronic mucocutaneous candidiasis. The presence of hypoparathyroidism, adrenal failure, type 1 diabetes, and/or primary gonadal failure is indicated by different blackened quadrants in the subjects' symbols (see *key*). Pernicious anemia was present in subjects II-2, family B; II-1, family I; II-6, family K; and II-5, family L. Intestinal malabsorption was present in subjects II-2, family B; II-2, family G; II-1, family J; II-6, family K; and II-5, family L. Chronic autoimmune hepatitis was present in subjects II-2, family B, and II-1, family D. Alopecia totalis was present in subjects II-1, family F; II-1, family J; II-6, family K; and II-5, family L. Other autoimmune complications included hypothyroidism (subject II-1, family F), ankylosing spondylitis (subject II-1, family B), and red-cell aplasia (subject II-2, family G). Keratopathy was present in subjects II-1, family F, and II-6, family L. In addition, subject II-2, family G, manifested a squamous-cell carcinoma of his oral mucosa. Causes of death for affected family members were β-hemolytic streptococcal septicemia (subject II-2, family B), postanesthetic adrenal crisis with respiratory arrest (subject II-1, family C), fulminant hepatic failure (subject II-3, family E), systemic varicella infection (subject II-3, family K), and intracerebral hemorrhage (subject II-5, family L). In addition, subject II-3, family G, who had previously had oral candidiasis, died suddenly at age 6 years, during an episode of viral pneumonitis.

(Richman et al. 1975; Hong and Dibbell 1981; Neufeld et al. 1981; Ahonen et al. 1990; Friedman et al. 1991).

APS1 is inherited as an autosomal recessive trait (Ahonen 1985) and is a rare disorder in most populations. However, it has been estimated to occur in 1/25,000 people in Finland and in 1/9,000 Iranian Jews, because of founder effects (Ahonen 1985; Zlotogora and Shapiro 1992). The founder effect in Finnish APS1 kindreds has allowed the genetic mapping of APS1 to chromosome 21q22.3 and further refinement to a 350-kb region between markers D21S1912 and D21S1903, by linkage-disequilibrium mapping (Aaltonen et al. 1994; Björses et al. 1996; Aaltonen et al. 1997). Subsequently, the autoimmune regulator gene (*AIRE-1*) was identified in this region, and six different *AIRE-1* mutations have been found in European APS1 kindreds (Finnish-German APECED Consortium 1997; Nagamine et al. 1997). One particular nonsense mutation, R257*, was found to account for >80% of mutant Finnish *AIRE-1* alleles (Finnish-German APECED Consortium 1997; Nagamine et al. 1997). One in 250 healthy people in Finland were also found to be heterozygous carriers of this nonsense *AIRE-1* allele (Finnish-German APECED Consortium 1997). *AIRE-1* encodes a 545–amino acid protein that has a nuclear localization signal and two plant homeodomain (PHD)-type zinc-finger motifs (Aasland et al. 1995), suggesting a role as a transcription factor (Finnish-German APECED Consortium 1997; Nagamine et al. 1997). Five of the six *AIRE-1* mutations predicted a truncation of the protein, which is consistent with a loss of AIRE-1 function, leading to APS1 (Finnish-German APECED Consortium 1997; Nagamine et al. 1997). However, the exact role of AIRE-1 in regulating immune responses remains obscure. In this study, we performed a mutation analysis of *AIRE-1* in British APS1 kindreds, to define the spectrum of *AIRE-1* mu-

Table 1

Primer Sequences and Conditions for Amplification of *AIRE-1*

Exon	Primer Sequences[a]	Size of Product (bp)	Annealing Temperature (°C)
1	F: AAGCGAGGGGCTGCCAGTGTC	258	65
	R: GGGACTATCCCTGGCTCACAG		
2	F: TCCACCACAAGCCGAGGAGAT	389	62
	R: AGCTGGGCTGAGCAGGTGACA		
3	F: CTGAGGTTGGGACCCTGCTCC	231	63
	R: CTGGAGACCCTGGCTGGCTTC		
4	F: GGCACTCACCCCCACTGAGAG	202	63
	R: GCCCTGCTCTGACCCCTGAC		
5	F: GCCCAGTGCTGCCTGCTTCTG	256	63
	R: CCATCTTGGAGCCTGGGTCTC		
6	F: TGCAGGCTGTGGGAACTCCAC	303	59
	R: GGGGCATCAAGAGCCAGGCTC		
7	F: CATGTCCACCCTCGCTGCTGA	278	63
	R: AGAAAAAGAGCTGTACCCTGTGG		
8	F: CACCCCAGCCCAGTCTGCATG	229	63
	R: CTTCAGGGTCAGTGGGTGGAG		
9	F: CTGTCACCCGCTCTGTTGTTC	205	63
	R: GTGGCCATGTGGACAGGAGG		
10	F: CCCAGCAGTCACTGACTCCTG	311	62
	R: CGTAGGTCCTGGGCTCCTTGA		
11	F: CTCGGGTTCGGGTTCAGCTAC	233	63
	R: TGTGGGTGTGGGTTCAGGCCT		
12	F: CATACCCCGGAGGTGGCACTC	205	64
	R: CAGCACCGGCATGCATGGAGG		
13	F: CTGTGGGAGTTGGGCTGACCT	141	60
	R: AGTGGAGGAGCACCAGGAGG		
14	F: ATGGCCATGATTGTGTGGCTG	188	59
	R: CTCAGCACTCTCTCATCAGAG		

[a] F = forward, and R = reverse.

tations in our population and to allow a molecular genetic screening strategy for predictive use in apparently unaffected siblings from APS1 kindreds.

Patients and Methods

Patients

Twelve nonconsanguineous sibships with at least one affected APS1 member were identified, and paraffin-embedded material or blood samples were obtained from a total of 43 (16 affected and 27 unaffected) kindred members. All affected members had chronic mucocutaneous candidiasis, with an age at first onset between 18 mo and 21 years. Details of the other clinical manifestations are shown in figure 1. The clinical details for subject II-1 from family B have been reported elsewhere (Kendall-Taylor et al. 1988). Studies were performed with the informed consent of each subject and with the approval of the regional ethics committee.

DNA Extraction and SSCP Analysis

DNA from leukocytes and paraffin-embedded sections was prepared by SDS lysis, proteinase K digestion, and phenol-chloroform extraction, in accordance with standard methods. Genomic DNA from the proband of each family and from four unrelated normal individuals was used as a template for PCR with oligonucleotide primers (table 1) that encompassed each of the 14 exons and the 26 splice sites of *AIRE-1*. PCR was performed with 200 ng template DNA, 50 pmol of each primer, 200 μM dNTPs, 1 mM $MgCl_2$, 50 mM KCl,10 mM Tris-HCl (pH 8.3), and 1 U *Taq* DNA polymerase (Gibco BRL), in a final volume of 50 μl. After an initial denaturation at 94°C for 5 min, 35 cycles of PCR amplification were performed, with each cycle consisting of 30 s at 93°C, 30 s at a primer pair–dependent annealing temperature (see table 1), and 30 s at 72°C. After 35 cycles, a final DNA extension step at 72°C for 5 min was performed. PCR products were analyzed for SSCPs by use of precast 12.5% polyacrylamide gels run on the Phast minigel electrophoresis system (Pharmacia LKB). Gels were run

Table 2

***AIRE-1* Mutations in British APS1 Families**

Kindred	Exon	Position[a]	Nucleotide Change	Amino Acid Change	Confirmed by[b]
A	8	964	13-bp deletion	372-residue truncation	*Bsr*BI
	8	964	13-bp deletion	372-residue truncation	*Bsr*BI
B	10	1264	1-bp deletion	478-residue truncation	*Bsu36*I
	8	964	13-bp deletion	372-residue truncation	*Bsr*BI
C	8	964	13-bp deletion	372-residue truncation	*Bsr*BI
	8	964	13-bp deletion	372-residue truncation	*Bsr*BI
D	1	44	CGG→CTG	Arg15Leu	*Bcg*I
	8	964	13-bp deletion	372-residue truncation	*Bsr*BI
E	6	768	CGA→TGA	Arg257Stop	*Taq*I
	2	271	TAT→TGT	Tyr90Cys	ASO
F	8	964	13-bp deletion	372-residue truncation	*Bsr*BI
	8	964	13-bp deletion	372-residue truncation	*Bsr*BI
G	1	83	CTG→CCG	Leu28Pro	*Msp*AI
	10	1249	1-bp deletion	478-residue truncation	ASO
H	8	964	13-bp deletion	372-residue truncation	*Bsr*BI
	8	964	13-bp deletion	372-residue truncation	*Bsr*BI
I	8	964	13-bp deletion	372-residue truncation	*Bsr*BI
	8	964	13-bp deletion	372-residue truncation	*Bsr*BI
J	8	964	13-bp deletion	372-residue truncation	*Bsr*BI
	8	964	13-bp deletion	372-residue truncation	*Bsr*BI
K	8	964	13-bp deletion	372-residue truncation	*Bsr*BI
	8	964	13-bp deletion	372-residue truncation	*Bsr*BI
L	8	964	13-bp deletion	372-residue truncation	*Bsr*BI

NOTE.—Data for paternally inherited mutations are shown above those for the maternal mutation, for each proband from families A–H (fig. 1).

[a] Nucleotides are numbered from the first position of the initiator methionine codon.

[b] The restriction enzyme is given for those mutations confirmed by restriction-enzyme digestion.

for 150–185 V-h, at a temperature of 10°C. The gels then were fixed and stained with 0.4% silver nitrate, as described elsewhere (Pearce et al. 1995).

DNA Sequence Analysis

Twenty microliters of each of the PCR products that gave an abnormal SSCP pattern were gel purified in a 1.5% agarose gel, and the DNA sequence of both strands was determined by *Taq* polymerase cycle sequencing with fluorochrome-labeled dideoxy terminators resolved by a laser detection system (373A sequencer, Applied Biosystems). For the analysis of heterozygous deletions, the abnormal PCR product was cloned into the pGEMT-easy vector (Promega), and five clones were sequenced from M13 primers. Each DNA sequence abnormality was confirmed to be present either by restriction-enzyme analysis or by allele-specific oligonucleotide (ASO) hybridization to wild-type and mutant sequence, ^{32}P–end labeled oligonucleotides, as described elsewhere (Pearce et al. 1995). In addition, either restriction-enzyme analysis or ASO was used to screen DNA from other affected and unaffected family members and from 56 unrelated healthy subjects. All nucleotide sequences were numbered from the first position of the initiator methionine codon of the *AIRE-1* sequence (GenBank accession number z97990).

Microsatellite Polymorphism Analysis

Six microsatellite polymorphisms (D21S49, D21S1890, D21S1912, PFKL, D21S171, and D21S1903), which span a 2-cM region flanking *AIRE-1* (Dib et al. 1996; Aaltonen et al. 1997), were typed in the nine kindreds with available parents, by use of methods described elsewhere (Pearce et al. 1995). The analysis of allelic association at the PFKL locus was performed by use of the Yates-corrected χ^2 test. Ten microsatellite markers (D2S116, CTLA4[AT]$_n$, D2S2289, D14S267, D14S68, D14S74, D14S70, D14S72, D14S51, and D14S1054 [Dib et al. 1996; Nistico et al. 1996]), each with eight or more alleles and a heterozygosity >70%, were used to exclude nonpaternity, as described elsewhere (Pearce et al. 1995).

Results

SSCP analysis of the PCR products encompassing all 14 exons and 26 splice junctions of *AIRE-1* (table 1) was performed on each APS1 proband, followed by di-

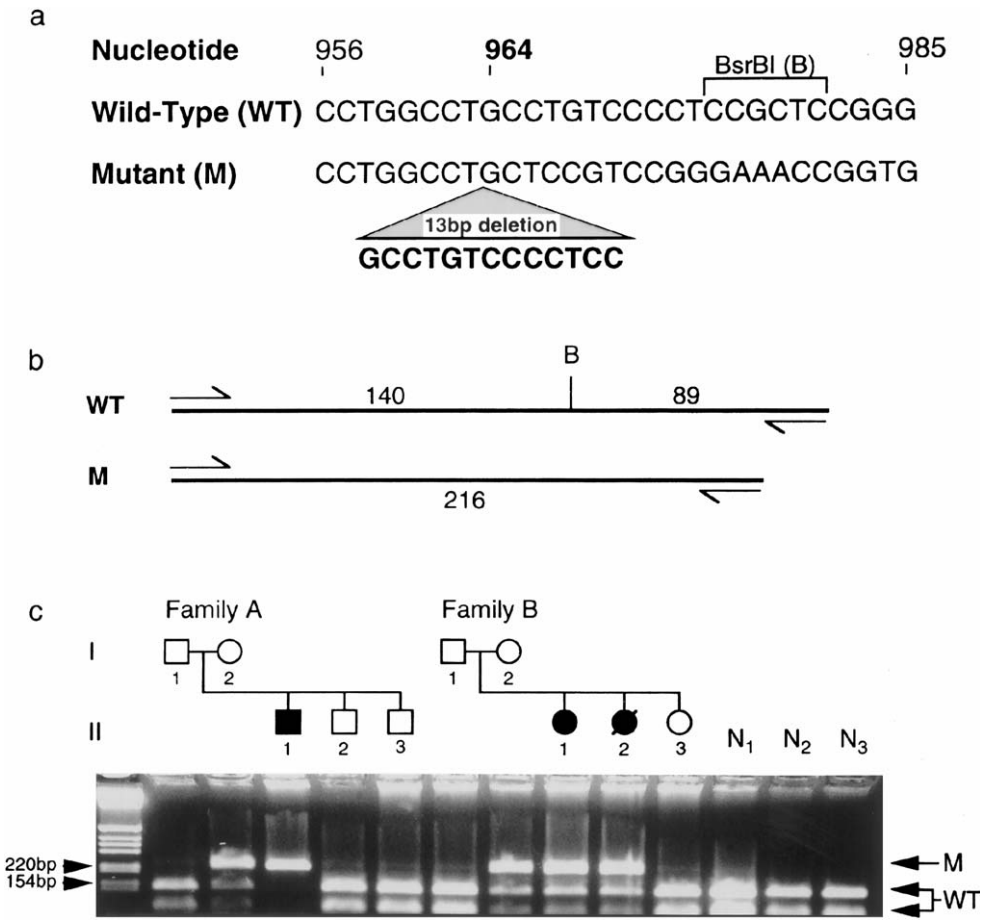

Figure 2 Demonstration of the 13-bp deletion (964del13) at nucleotide 964 in families A and B, by use of restriction enzyme *Bsr*BI. *a,* Wild-type and mutant (964del13) DNA sequences. The deletion abolishes the recognition site (CCG/CTC) for the restriction enzyme *Bsr*BI, shown above the wild-type sequence. *b,* Restriction map of the wild-type and mutant PCR products with restriction enzyme *Bsr*BI. *c,* Results of PCR amplification of exon 8 followed by *Bsr*BI digestion in families A and B. Subject II-1 from family A has the mutant 216-bp band and neither of the wild-type 140- or 89-bp products, demonstrating that he is homozygous for the 13-bp deletion. His mother, I-2, can be seen to be heterozygous for this deletion, with each of the 216-, 140-, and 89-bp products present. In contrast, his father, I-1, has only the wild-type 140- and 89-bp products. Because paternity could not be refuted by the analysis of 14 microsatellite polymorphisms, the mutation in the paternal allele in subject II-1 from family A was determined to be a de novo mutation. The 964del13 mutation also was determined to be heterozygous in subjects II-1 and II-2 from family B, who are affected with APS1, and in their mother, I-2. Their unaffected younger sister, II-3, and three unrelated normal subjects (N_1–N_3) have only the wild-type digestion products. This method also was used to confirm the presence of the 964del13 mutation in affected members of families C, D, F, and H–L (table 2).

rect DNA sequence analysis of any product with an abnormal SSCP pattern. This strategy allowed the identification of 23 *AIRE-1* mutations in the 12 APS1 probands (table 2).

Deletions

A 13-bp deletion at nucleotide 964 in exon 8 was found to be present in 10 of the APS1 probands. This deletion (964del13) abolished a *Bsr*BI restriction-enzyme site (fig. 2), and this was used to demonstrate that the deletion was homozygous in seven APS1 probands and heterozygous in three affected subjects (table 2). Thus, 964del13 accounted for 17 of the 24 possible mutant *AIRE-1* alleles in our families. Screening for 964del13 in DNA from a total of 576 unrelated control subjects with *Bsr*BI showed one heterozygous carrier of the deletion, which predicts a truncated AIRE-1 protein of only 372 amino acids.

The 964del13 mutation appeared to be homozygous in subject II-1 from family A, and his mother (I-2) was found to be a heterozygous carrier (fig. 2). However, his father (I-1) did not carry this mutation in his leukocyte DNA (fig. 2). Similarly, his youngest brother, II-3, who also had inherited the "affected" paternal haplotype

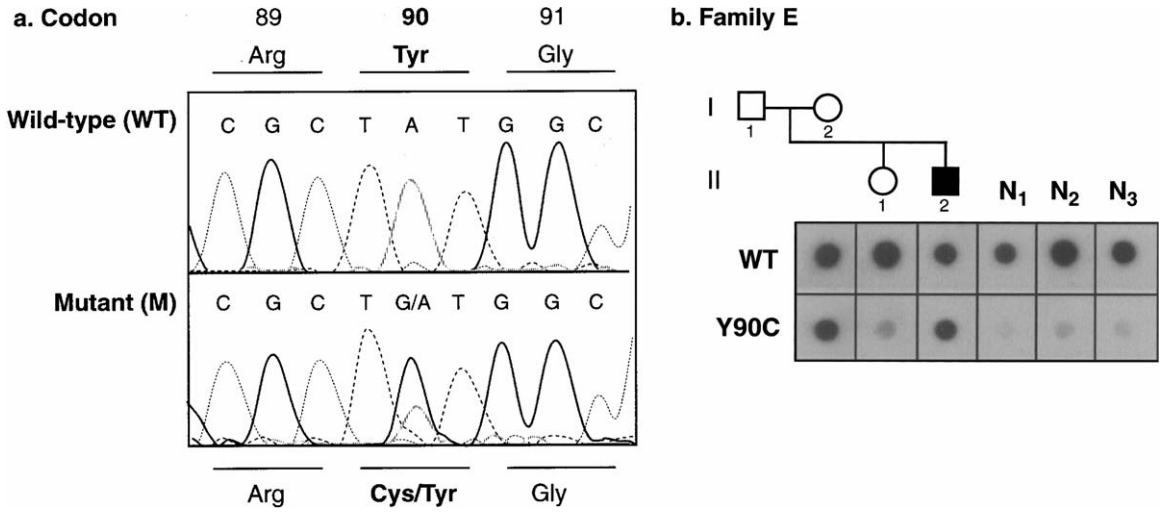

Figure 3 Tyrosine to cysteine missense mutation at codon 90 in family E. *a,* Wild-type DNA sequence chromatogram, from codons 89–91 in exon 2 of *AIRE-1,* with the mutant heterozygous A→G transition found in subject II-2 shown in the lower box. *b,* Hybridization of ^{32}P-labeled ASO to dot-blotted exon 2 PCR products from members of family E and from three unrelated normal controls (N_1–N_3). The hybridization signal is present in all samples with the wild-type oligonucleotide; however, in the samples with the mutant (Y90C) oligonucleotide, hybridization is seen only for subjects I-2 and II-2 from family E. This demonstrates that the Y90C mutation is heterozygous in subjects I-2 and II-2. The Y90C mutation was not found in the unaffected sister of the proband (II-1) or in 56 normal controls, of whom the results for N_1–N_3 are shown.

(data not shown), did not carry the 964del13 allele. Nonpaternity was excluded by the demonstration of allele sharing between father and son, at the six microsatellite polymorphisms flanking *AIRE-1* and at an additional 10 highly polymorphic markers. These findings demonstrate that, in family A, the 964del13 mutation had occurred de novo in the paternally inherited *AIRE-1* allele.

Two heterozygous 1-bp deletions were found in exon 10, at nucleotides 1249 (1249delC) and 1264 (1264delC), in the probands of families G and B, respectively (table 2). Neither of these 1-bp deletions, which both predicted premature AIRE-1 truncations, was found in 56 unrelated control subjects without autoimmune disease.

Nonsense Mutations

The nonsense mutation in exon 6, R257* (CGA→TGA), which is the founder Finnish *AIRE-1* mutation (Finnish-German APECED Consortium 1997; Nagamine et al. 1997), was found in the proband of family E (table 2). This family could not trace any Finnish ancestry. This nonsense mutation predicted a truncated AIRE-1 protein of 256 amino acids and eliminated a *Taq*I restriction-enzyme site, which was used to confirm its heterozygosity in the proband of this family. The *Taq*I digestion assay was used to demonstrate that the R257* mutation was not present in DNA from 56 control subjects.

Missense Mutations

The proband of family D (II-1) was found to be heterozygous for a G→T transversion at nucleotide 44. This base change predicted the missense mutation R15L (CGC→CTC) and abolished a *Bcg*I restriction-enzyme site in exon 1, which was used to demonstrate that his apparently unaffected sister (II-2) also carried this mutation. The proband of family G was found to be heterozygous for a T→C transition at nucleotide 83, predicting the missense mutation L28P (CTG→CCG) and creating an *Msp*AI restriction-enzyme site. Subject II-2 from family E was found to have an A→G transition at nucleotide 269, predicting a Y90C (TAT→TGT) missense mutation (fig. 3). ASO was used to confirm the presence of this mutation and to demonstrate its maternal inheritance (fig. 3). Each of these missense mutations predicted a significant change in the amino acid sequence and was demonstrated not to be present in DNA from 56 unrelated control subjects, by use of either restriction-enzyme digestion (R15L and L28P) or ASO (Y90C).

Noncoding Polymorphisms

Polymorphisms that did not code for an amino acid change were found at nucleotide 517 (codon 196) in exon 5 (TCC→TCT) in 40% of alleles and at the nucleotide position +5 from the donor splice site of exon 9 (GGtatggc→GGtatgac) in 8% of alleles.

Family/ Parent	Mutation	D21S49	D21S1890	D21S1912	PFKL	D21S171	D21S1903
F/P	964del13	1	3	4	3	3	8
A/M	964del13	5	1	4	3	3	3
B/M	964del13	3	6	4	3	3	6
D/M	964del13	3	1	4	3	3	9
H/M	964del13	3	4	4	3	3	4
H/P	964del13	1	4	4	3	3	7
I/P	964del13	2	4	4	3	3	7
C/P	964del13	3	1	5	3	3	7
F/M	964del13	2	1	6	3	3	7
C/M	964del13	4	2	2	3	3	9
I/M	964del13	2	1	2	3	3	8
A/P	964del13†	3	3	1	2	3	3
B/P	1264delC	4	5	3	3	3	9
D/P	R15L	1	5	1	3	3	5
E/P	R257*	2	3	3	1	2	9
E/M	Y90C	3	9	8	4	3	3
G/P	L28P	2	4	3	3	2	4
G/M	1249delC	2	3	8	3	3	9
Distances (cM)			0.95	0.6	0.15	0.2	0.1

Figure 4 Chromosome 21q22.3 haplotypes and corresponding *AIRE-1* mutation in the nine affected kindreds (A–I; fig. 1) for which at least one parent was available for study. The marker order is given on the top line, with sex-averaged genetic distances given on the bottom line (Bjorses et al. 1996; Dib et al. 1996; Aaltonen et al. 1997). *AIRE-1* is located between D21S1912 and PFKL. Haplotypes are designated by family and by the paternal (P) or maternal (M) transmission. The dagger symbol (†) denotes the de novo origin of the 964del13 mutation on the paternal haplotype of family A (A/P). The common haplotype (4-3-3-7) for markers D211912, PFKL, D21S171, and D21S1903 in the 964del13-carrying chromosomes is boxed.

Chromosome 21q22.3 Haplotype Analysis

The haplotypes over a 2-cM region of chromosome 21q22.3, encompassing *AIRE-1,* were determined in the nine families who had one or more parents available for study. The marker order and genetic distances were as follows: cen–D21S49–0.95 cM–D21S1890–0.6 cM–D21S1912–0.15 cM–(*AIRE-1*)–PFKL–0.2 cM–D21S171–0.1 cM–D21S1903–tel, with PFKL being within 20 kb of *AIRE-1* (Finnish-German APECED Consortium 1997; Nagamine et al. 1997). Eleven of these 18 affected haplotypes occurred in association with a transmitted 963del13 allele, and a partially conserved common haplotype, over a 0.45-cM region between markers D21S1912 and D21S1903, was observed in these affected chromosomes (fig. 4). In addition to these 11 964del13 haplotypes, which all carried allele 3 at the PFKL locus (fig. 4), the probands of families J, K, and L also were found to be homozygous for allele 3 at the PFKL locus. Thus, all 16 of the 964del13 mutations transmitted from the parental germ line were associated with allele 3 at PFKL, compared with 55 (56%) of 98 unrelated control alleles ($\chi^2 = 9.48$, $P = .002$).

Discussion

AIRE-1 *Mutation Spectrum*

Our study has identified *AIRE-1* mutations in each of 12 probands with APS1, which confirms the genetic homogeneity of this disorder in our population. Of the total of 23 mutant *AIRE-1* alleles found, there were seven different mutations, five of which are novel. Each mutation either predicts a premature truncation of the AIRE-1 protein or a significant amino acid change, and, with the exception of the 964del13 allele, none were found in >50 unrelated normal subjects. When considering together the mutations found in our population with the six *AIRE-1* mutations described elsewhere (Finnish-German APECED Consortium 1997; Nagamine et al. 1997), we noted that the four missense mutations (R15L, L28P, K83E, and Y90C) all cluster in the amino-terminal region of AIRE-1, encoded by exons 1 and 2 (fig. 5). The AIRE-1 protein is predicted to contain two PHD-type zinc-finger domains (Aasland et al. 1995), encoded by exons 8 and 9 and exons 11 and 12 (fig. 5); therefore, the AIRE-1 protein has been proposed to act

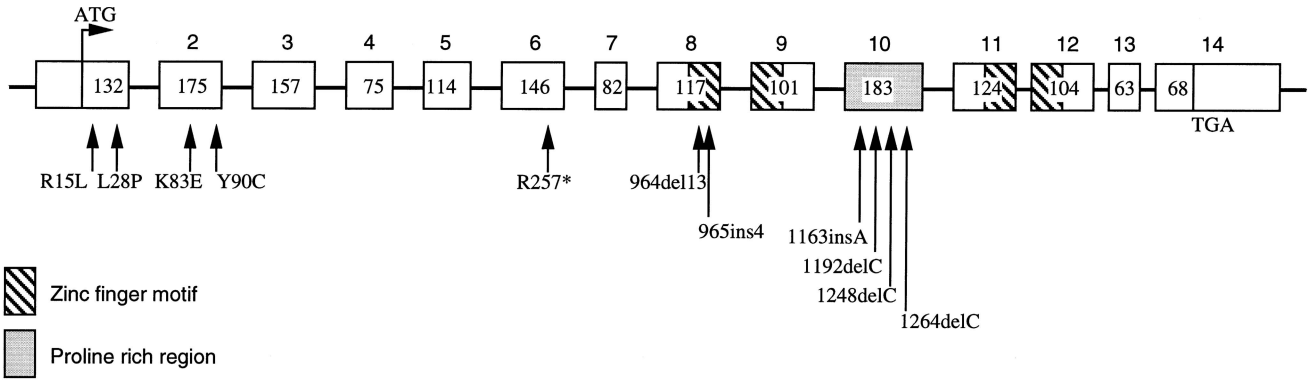

Figure 5 Schematic diagram of the genomic structure of *AIRE-1* and locations of the mutations found in the British APS1 subjects and of those reported previously (Finnish-German APECED Consortium 1997; Nagamine et al. 1997). The exon number is shown above each box, and the size of each exon is given inside each box. The regions encoding the two PHD-type zinc-finger domains are hatched, with the proline-rich region shaded. The missense mutations are shown to occur in exon 1 or 2. The common Finnish mutation R257*, which was also found in family E (table 2), is shown in exon 6. The common British mutation 964del13 is shown in exon 8, with the adjacent insertion at nucleotide 965 (965ins4). The remainder of the mutations, which are either 1-bp deletions or insertions, were all found in exon 10. The amino acids are designated by conventional single-letter codes.

as a transcription factor (Finnish-German APECED Consortium 1997; Nagamine et al. 1997). These clustered missense mutations in the amino terminus of the protein possibly act to disrupt binding of the currently undefined ligand. The remainder of the mutations are found in exon 6, 8, or 10, and each of these mutations, if translated, would predict a nonfunctional AIRE-1 protein that would lack either one or both of the putative zinc-finger domains. The observation that all of the 11 mutations thus far found in APS1 subjects cluster into 5 of the 14 exons of *AIRE-1* provides useful information for future molecular genetic screening of APS1 families (fig. 5).

Clinical Applications

We studied four apparently unaffected subjects who were <16 years of age and who were siblings of an APS1 proband (subjects II-2 and II-3 from family A, subject II-2 from family D, and subject II-2 from family F). These children had an ~25% risk of developing APS1 in the future (Ahonen 1985; Ahonen et al. 1990). Haplotype analysis, subsequently confirmed by mutation analysis, was able to demonstrate that the unaffected siblings in families A (fig. 2) and F did not carry either mutant *AIRE-1* allele, and we have stopped regular dynamic screening for primary adrenal failure in these children. The 13-year-old sister (II-2) of the proband (II-1) from family D, however, carried both affected chromosome 21q22.3 haplotypes and was found to carry the same *AIRE-1* mutations (R15L and 964del13) as her brother. We intensified her screening program accordingly, and she was found to have dental enamel hypoplasia and subsequently developed hypocalcemia, which is indicative of hypoparathyroidism. Thus, we were able to predict her high risk of developing APS1 (Ahonen 1985).

Twenty of the 23 mutations present in our APS1 subjects predict a nonfunctional AIRE-1 protein. However, three APS1 subjects (the probands of families D, E, and G) have one missense mutation that may lead to a diminution, rather than a total abrogation, of AIRE-1 function. Of these three subjects, two (II-2 from family E and II-2 from family G) have not manifested adrenal failure or hypoparathyroidism, at the ages of 30 years and 33 years, respectively. Although this is a small number of patients, it suggests that there may be a genotype-phenotype correlation for the spectrum of clinical manifestation of for the age at onset of the autoimmune manifestations of APS1.

Mutation Prevalence and Pathogenesis

The finding that the 13-bp deletion in exon 8 (964del13) accounts for 17 (71%) of a possible 24 mutant alleles in our population was initially unexpected. However, the demonstration that 964del13 had a population frequency of 1/576 healthy subjects shows that it is an infrequently carried heterozygous mutation in the British population. The novel observation of this mutation as a de novo event in the paternal allele of family A (fig. 2) shows that it is also recurrent in this case. The DNA sequence surrounding nucleotide 964 (CCTGGCCTGCCTG) contains two different repetitive sequences (fig. 2)—namely, three repeats of a CCTG sequence and two overlapping repeats of a GCCTG sequence, with the G at nucleotide 964 forming the overlap. These repetitive sequences may predispose to slipped-strand mispairing (Farabaugh et al. 1978; Fu-

kuhara et al. 1990), resulting in the high frequency of the 964del13 mutation that we observed and in the occurrence of the 4-bp insertion (CCTG) at nucleotide 965 that had been found previously (Finnish-German APECED Consortium 1997).

The common haplotype observed in chromosomes carrying the 964del13 mutation (fig. 4) and the strong association of allele 3 of the PFKL locus suggests that there is a founder effect for this mutation in our population. Linkage disequilibrium in ancient populations may be found over localized chromosomal regions of ⩽100 kb (de la Chapelle 1993; Hastabacka et al. 1994; Houwen et al. 1994; Jorde 1995), and the PFKL locus is within 20 kb of the 964del13 mutation (Finnish-German APECED Consortium 1997; Nagamine et al. 1997). Thus, the 964del13 *AIRE-1* allele appears to be present in our population because of a combination of recurrent mutation (as seen in family A) and an ancient founder effect. The finding of this common *AIRE-1* deletion in our APS1 patients has implications for populations such as Anglo-Americans, who have, in part, common ancestry with the British population. Furthermore, this deletion, which is carried infrequently as a heterozygous allele, may be important in the pathogenesis of the more common autoimmune endocrinopathies (Vyse and Todd 1996) or of chronic mucocutaneous candidiasis.

Note added in proof.—Scott et al. (1998) have recently made findings complementary to those of this study.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

GenBank, http://www.ncbi.nlm.nih.gov/Web/Genbank/ (for *AIRE-1* cDNA sequence [z97990])

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for APS1 [MIM 240300])

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