Report

Mutations of the RNA-Specific Adenosine Deaminase Gene (*DSRAD*) Are Involved in Dyschromatosis Symmetrica Hereditaria

Yoshinori Miyamura,[*] Tamio Suzuki,[*] Michihiro Kono, Katsuhiko Inagaki, Shiro Ito, Noriyuki Suzuki, and Yasushi Tomita

Department of Dermatology, Nagoya University Graduate School of Medicine, Nagoya, Japan

Dyschromatosis symmetrica hereditaria (DSH) (also called "reticulate acropigmentation of Dohi") is a pigmentary genodermatosis of autosomal dominant inheritance characterized by a mixture of hyperpigmented and hypopigmented macules distributed on the dorsal aspects of the hands and feet. To determine the gene responsible for this disease, we performed a genomewide search in three families with DSH and mapped the DSH locus to chromosome 1q21.3. The mutations involved in causing DSH have been identified in the gene that encodes double-stranded RNA-specific adenosine deaminase (*DSRAD*) as the disease gene.

Dyschromatosis symmetrica hereditaria (DSH [MIM 127400]) (also called "reticulate acropigmentation of Dohi") was first described by Toyama (1910, 1929) and has been reported mainly in Japan, although it occurs in families of every ethnic origin all over the world (Oyama et al. 1999). Patients with DSH have pinpoint, pea-sized hyperpigmented and hypopigmented macules on the back of their hands and the tops of their feet (fig. 1). The face is spared, apart from a few scattered small discrete, pigmented macules. These abnormalities are otherwise asymptomatic and do not affect the general health of the patient. The prevalence of DSH in the population of Japan is estimated to be ~1.5 per 100,000. DSH generally shows an autosomal dominant pattern of inheritance, with high penetrance, but some patients with sporadic DSH have been reported. When similar skin lesions occur all over the body, the disease is discriminated from DSH and is named "dyschromatosis universalis hereditaria" (DUH [MIM 127500]). The clinical features of DSH and DUH are quite distinct and have not been found in the same pedigrees. Although clinical and morphological investigations of DSH and DUH have been reported, the cause

and the pathogenesis of the diseases, as well as the pathological gene(s), have not yet been clarified. Until now, the DSH gene was hypothesized to be located on chromosome 9, because of another family with DSH and idiopathic torsion dystonia (the gene responsible for the latter disease is localized on chromosome 9) (Patrizi et al. 1994), but our early analyses of three Japanese families with DSH (as shown in fig. 2*A*–*C*) excluded chromosome 9 as a possibility (Kono et al. 2000).

We then tried an entire genomewide scan, using 343 microsatellite markers for linkage analysis in the three families with DSH (41 affected and 47 unaffected individuals, as shown in fig. 2*A*–*C*), using the method detailed elsewhere (Kono et al. 2000). In brief, genotyping errors or misspecified relationships within the pedigrees were checked by the PedCheck software package. DNA fragment–length analysis on PCR products of microsatellite markers from the Linkage Mapping Set (Perkin Elmer) was performed, using a Macintosh Centris 650 (Apple Japan) personal computer, with 672 Genescan software and Genotype, version 1.1 (both Perkin Elmer products), as described in the manufacturer's manuals. The allele size of each marker was rounded, using the GAS package, version 2.0 (as found on the Oxford University Web site). Calculations for linkage analysis were performed with the FASTLINK software package, version 4.0. (Lathrop et al. 1984; Cottingham et al. 1993; Schaffer et al. 1994), under an assumed genetic model as follows: autosomal dominant, gene frequency of 0.0001, marker-allele frequency set to be equal, complete pene-

Figure 1   Small hyperpigmented and hypopigmented macules on the dorsal aspects of the hands and feet. *Upper,* Sixty-year-old female. *Lower,* Forty-year-old male.

trance, zero phenocopy rate, and no sex difference. Two-point LOD scores were calculated with the MLINK module in the FASTLINK software package. Kosambi map function was used to convert the recombination fractions to map distances. Haplotype analyses were performed using the SimWalk2 software. The study was approved by the ethics committee of Nagoya University School of Medicine.

In a genomewide scan for linkage analysis of three pedigrees, we obtained cumulative LOD scores >3.0 with satellite markers D1S502, D1S252, D1S498, and D1S484 in chromosome 1q (table 1; fig. 3). To determine
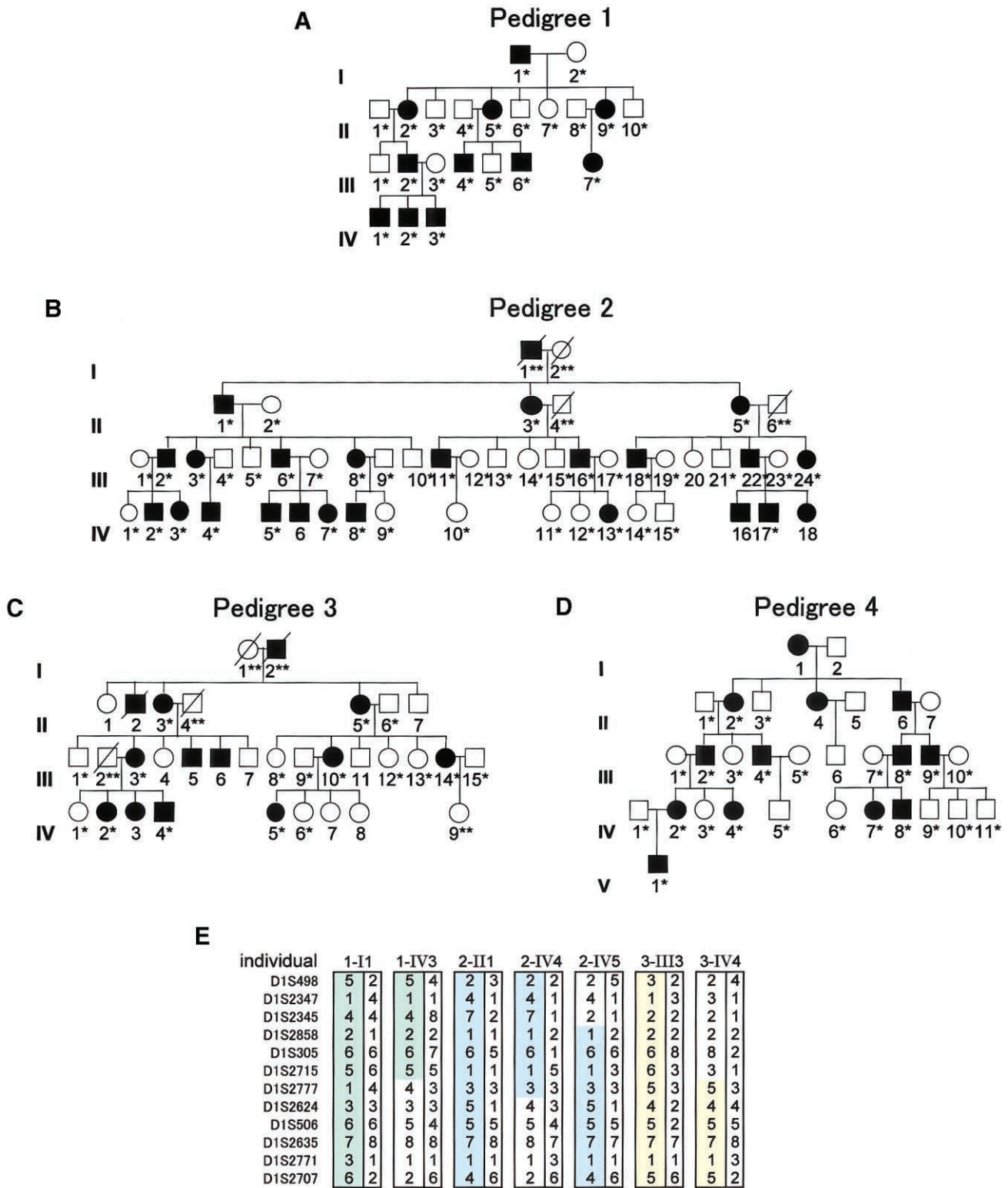
Figure 2 Pedigrees and haplotypes. *A,* Pedigree 1. *B,* Pedigree 2. *C,* Pedigree 3. *D,* Pedigree 4. Affected and unaffected individuals are represented by the blackened and unblackened symbols, respectively. An asterisk (*) indicates individuals who provided blood. Single asterisk (*) and double asterisk (**) indicate members in pedigrees 1, 2, and 3 who were utilized for linkage analysis. *E,* Haplotype analysis. The shared disease-associated haplotype is colored.

Table 1

Cumulative Two-Point LOD Scores

|					LOD at θ =								
Marker	0	.05	.1	.15	.2	.25	.3	.35	.4	.45	.5	Z_{max}	θ_{max}
D1S424	$-\infty$	.17	1.15	1.51	1.59	1.51	1.30	1.01	.66	.29	0	1.59	.20
D1S206	$-\infty$	−1.21	.56	1.29	1.59	1.64	1.51	1.25	.90	.48	0	1.64	.25
D1S502	$-\infty$	3.81	4.88	5.02	4.75	4.23	3.51	2.65	1.68	.71	0	5.02	.15
D1S252	$-\infty$	5.46	5.96	5.78	5.29	4.61	3.77	2.82	1.79	.78	0	5.96	.10
D1S498	$-\infty$	4.49	4.43	4.09	3.62	3.05	2.42	1.75	1.06	.45	0	4.49	.05
D1S484	$-\infty$	1.80	2.99	3.34	3.31	3.05	2.62	2.07	1.42	.71	0	3.34	.15
D1S196	$-\infty$	−.76	.46	.99	1.21	1.25	1.17	.98	.72	.40	0	1.25	.25
D1S218	$-\infty$	−7.17	−4.03	−2.40	−1.41	−.77	−.36	−.11	.02	.05	0	.05	.45
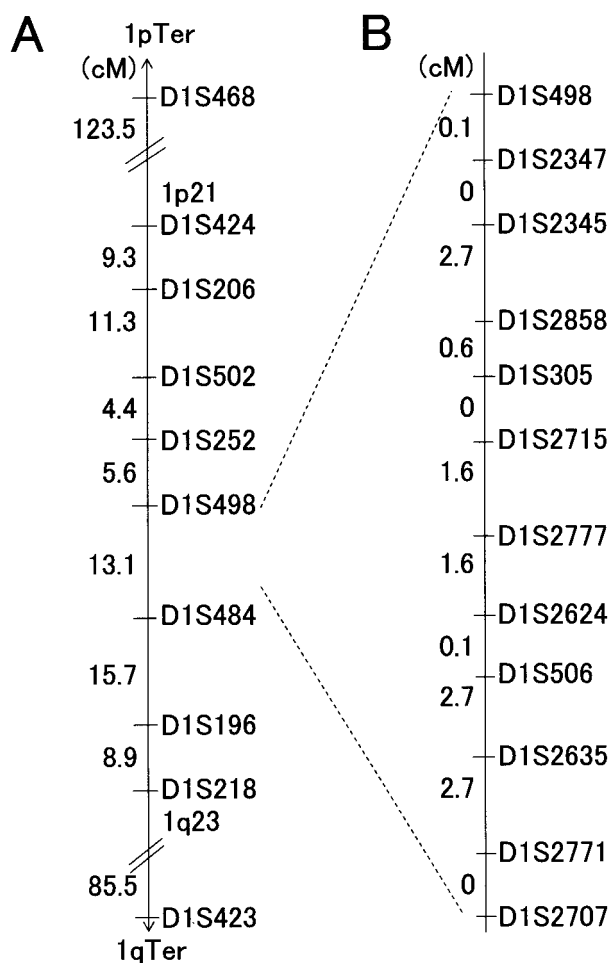
Figure 3 Genetic map position for microsatellite markers. *A,* Marker used in the linkage analyses, as shown in table 1. *B,* Markers used in the haplotype analyses, as shown in figure 2*E.* cM = Genetic distance. D1S468 and D1S423 are the nearest markers to the telomeres of the p and the q arms, respectively.

a smaller interval containing the DSH locus, haplotype analysis was performed, and the results suggested that the gene responsible for DSH lies between D1S2715 and D1S2777 (figs. 2*E* and 3). To further refine the localization, we identified novel SNPs and integrated the genetic and physical maps of the region. The final DSH genetic interval, of ~500 kb, was bound proximally by the *IL6R* gene and distally by the *KCNN3* gene, at chromosome 1q21.3. Between those two genes, seven genes are mapped on the Entrez Genome Map View, NCBI Web site: namely, *NICE-5*, cholinergic receptor, double-strand RNA-specific adenosine deaminase (*DSRAD*), and four hypothetical proteins (LOC126669, LOC126668, LOC343053, and LOC343054).

To detect pathological mutations in the seven candidate genes, four affected individuals from each of pedigrees 1–3 and pedigree 4 (a fourth family with DSH added to the study, with 10 affected and 14 unaffected individuals) were screened by single-strand conformational polymorphism analysis (SSCP), which revealed mutant heteroduplexes made by hybridizing PCR fragments of the *DSRAD* gene. Every patient in each pedigree showed the mutant SSCP pattern of exons 2, 10, 10, and 15 in pedigrees 1, 2, 3, and 4, respectively. Direct sequence analysis of the PCR products showed that they were heterozygous for mutations of Arg474STOP (CGA→TGA), Leu923Pro (CTC→CCC), Lys952STOP (AAA→TAA), and Phe1165Ser (TTT→TCT) in pedigrees 1, 2, 3, and 4, respectively (fig. 4). None of those four mutations were found in the blood samples of any of the 55 unaffected individuals in any of the four pedigrees or in the 116 unrelated, normally pigmented Japanese adults who were surveyed. Thus, we concluded that those four mutations are not polymorphic but are the pathologic ones that cause the disease.

DSRAD is composed of 1,226 amino acid residues,
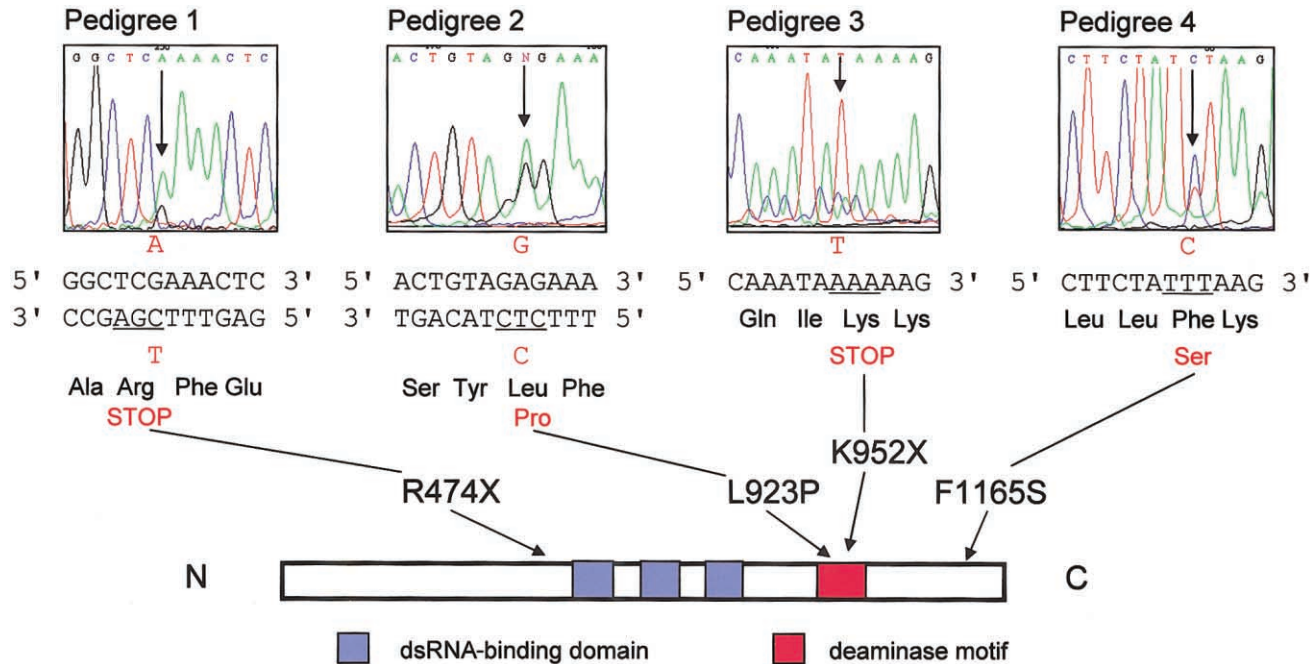
Figure 4 Mutations of the *DSRAD* gene found in patients with DSH. Antisense sequences are shown for pedigrees 1 and 2. L923P and K952X are located in the deaminase domain (*red box*). N = N-terminal; C = C-terminal.

with a calculated molecular mass of 139 kDa (O'Connell et al. 1995). It catalyzes the deamination of adenosine to inosine in double-stranded RNA substrates (Bass and Weintraub 1988; Wagner et al. 1989), which results in the creation of alternative splicing sites (Rueter et al. 1999) or alterations of codons and thus leads to functional changes in proteins. *DSRAD* is expressed ubiquitously (O'Connell et al. 1995), but only three target genes for DSRAD—including ionotropic glutamate receptor (Higuchi et al. 1993; Lomeli et al. 1994), the serotonin receptor 2C subtype in the brain (Burns et al. 1997), and hepatitis delta virus antigen in the liver (Polson et al. 1996)—are known. Recently, it was reported that *DSRAD* expression was increased in the spleen, thymus, and peripheral lymphocytes of endotoxin-treated mice (Yang et al. 2003). The target genes and the induction of the enzyme in lymphocytes seem not to be involved in the pathogenesis of DSH.

Since the *DSRAD* gene has 15 exons, and since three copies of the dsRNA-binding domains and the putative deaminase domain are reported to be located in exons 2–7 and 9–14, respectively (Wang et al. 1995), it is understandable that truncated proteins with no functional activity would be synthesized from genes with a nonsense mutation in exon 2 or in exon 10, as found in pedigrees 1 and 3, respectively.

Since the amino acid residue at 923 in exon 10 is also located in the putative deaminase domain, and since the

4–amino acid sequence containing it is absolutely conserved among all 11 species, as shown in figure 5A, the leucine at 923 is suspected to play an important role in the conformation of the catalytic site of the enzyme, and the mutation of Leu923Pro probably compromises enzyme activity. On the other hand, the missense mutation at 1165 in exon 15 is a little bit difficult to explain, because exon 15 is not involved in the catalytic domain. Phe1165 is supposed to contribute to the structure of the enzyme, an expectation supported by evidence that Phe1165 is conserved among all available sequences (fig. 5B); therefore, its mutation may induce an unstable tertiary structure of the protein that results in defective activity.

Heterozygosity for the *Dsrad* knockout causes embryonic lethality in mice (Wang et al. 2000). In contrast, patients with DSH have a good prognosis, with only the dyschromatosis. The prognostic difference between the two species may depend on differences between them. The reason the skin lesions are localized specifically on the backs of the hands and on the tops of the feet is difficult to explain pathogenetically, since *DSRAD* is ubiquitously expressed all over the skin. In the case of piebaldism—an autosomal dominant disease resulting from mutations in the c-*KIT* proto-oncogene (Fleischman et al. 1991; Giebel and Spritz 1991)—white patches on the forehead, chest, abdomen, and extremities are induced by the reduced activity of the c-kit receptor,
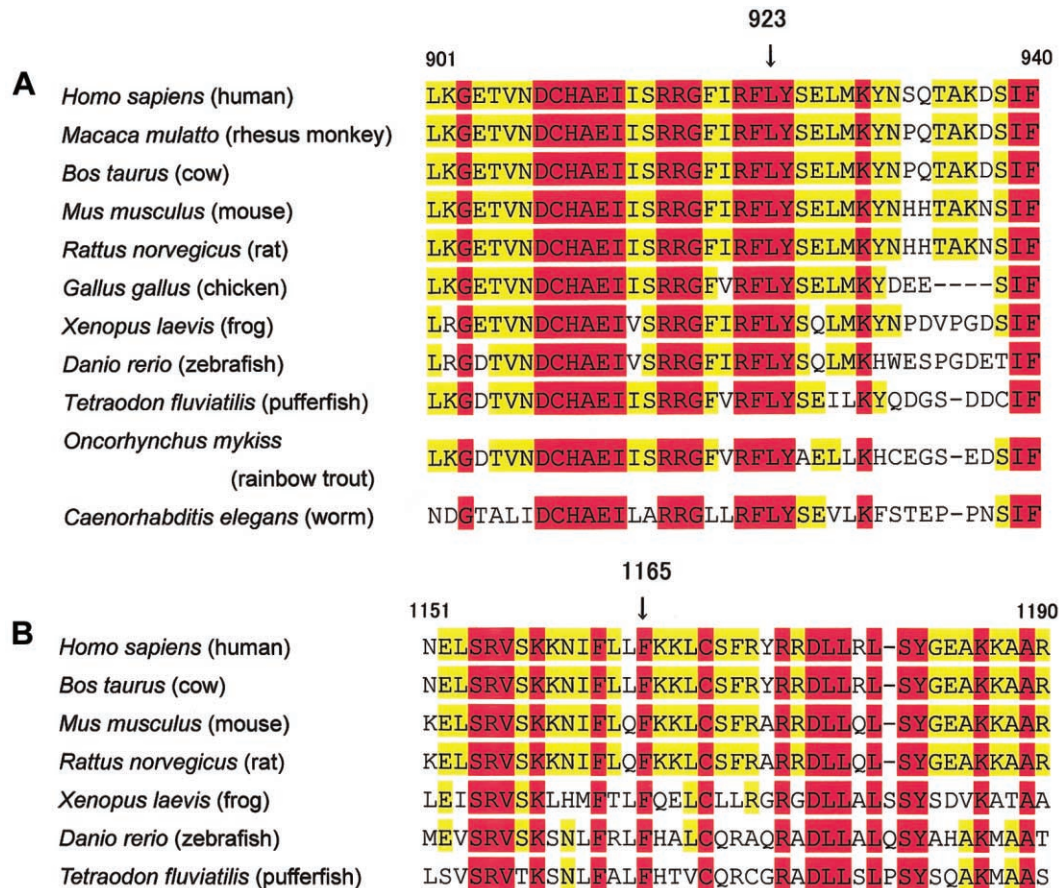
Figure 5 Amino acid alignment of various species around the two missense mutations. Human DSRAD amino acid residues 901–940 (*A*) and 1151–1190 (*B*) are shown aligned to known *DSRAD* orthologues. Residues L, at 923, and F, at 1165, are conserved in all species compared in the figure. Residues conserved among all available sequences and among all mammalian orthologues are shown with red or yellow background, respectively. All data were cited from GenBank.

which is required for the migration of melanocyte precursors throughout the skin during the early fetal period (Nishikawa et al. 1991). Similarly, we speculate that, when melanoblasts migrate from the neural crest to the skin during development, a greater reduction in DSRAD activity might occur at anatomic sites most distant from the neural crest. The failure of correct RNA editing may induce the differentiation of melanoblasts to hyperactive or hypoactive melanocytes, then colonizing in an irregular distribution in the skin lesions.

During the preparation of this manuscript, the locus for DSH was reported, by Zhang et al. (2003), to be at chromosome 1q11-1q21 and, by Xing et al. (2003), to be at chromosome 6q24.2-q25.2. The patients of Xing et al. (2003) showed dyschromatosis over almost their entire bodies, which suggests that they have DUH. If so, there is no contradiction between the two studies; that is, the *DSH* gene is located on chromosome 1, whereas the *DUH* gene is located on chromosome 6.

Acknowledgments

Electronic-Database Information

Accession numbers and URLs for data presented herein are as follows:

Entrez Genome Map Viewer, NCBI, http://www.ncbi.nlm.nih .gov/mapview/maps.cgi (for physical map of chromosome 1q)

GenBank, http://www.ncbi.nlm.nih.gov/Entrez/ (for the cDNA of human *DSRAD* [accession number NM_001111], the genome of human *DSRAD* [accession number NT_004668], and the DSRAD-related amino acid sequences or EST of rhesus monkey [accession number CB553335], cow [accession number CB449783], mouse [accession number BAC40888], rat [accession number NP_112268], chicken [accession num-

ber BU437798], frog [accession number T30340], zebrafish [accession number NP_571671], pufferfish [accession number AAF69674], rainbow trout [accession number CA359632], and worm [accession number T16913])

Genetic Analysis System (GAS), http://users.ox.ac.uk/~ayoung/gas.html (for allele size of microsatellite markers [package 2.0, Oxford University, 1993–1995])

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for DSRAD and DSH)

PedCheck, http://watson.hgen.pitt.edu/register/docs/pedcheck.html (for checking genotyping errors and misspecified relationships within pedigrees)

SimWalk2, http://watson.hgen.pitt.edu/docs/SW2_Overview.html (for haplotype analyses)

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