Novel Mutations of the KIT (Mast/Stem Cell Growth Factor Receptor) Proto-Oncogene in Human Piebaldism

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Piebaldism is an autosomal dominant genetic disorder of pigmentation characterized by congenital patches of white skin and hair that lack melanocytes. Piebaldism results from mutations of the KIT proto-oncogene, which encodes the cellular receptor transmembrane tyrosine kinase for mast/stem cell growth factor. Here we describe two novel KIT mutations associated with human piebaldism. These amino acid substitutions, located in the most highly conserved section of the KIT kinase domain, would be expected to dominantly negatively inhibit KIT-dependent signal transduction, resulting in aberrant melanocyte proliferation or migration during embryologic development. J Invest Dermatol 101:22-25, 1993

Piebaldism is a rare autosomal dominant genetic disorder of melanocyte development characterized by white patches of skin and hair that completely lack pigment, located principally on the scalp, forehead, ventral chest and abdomen, and extremities [1–3]. Melanocytes are absent from the regions of leukoderma [4,5], and piebaldism is thought to result from defective melanocyte proliferation or migration from the neural crest during development. In contrast to vitiligo, with which it is often confused, piebaldism is both congenital and non-progressive. Because of its distinctive phenotype, piebaldism has been known since at least ancient Greek times [6]. Piebaldism was one of the first genetic disorders for which a pedigree was presented [7], and several families have been reported that trace inheritance of the disorder over hundreds of years (reviewed in [2]).

Etymologically, “pie” apparently refers to the variegated black-and-white plumage pattern characteristic of the magpie, and “bald” derives from the Greek phallos, “having a white spot.” In mice, a disorder similar to human piebaldism, “dominant white spotting” (W), characterized by defects of pigmentation, hematopoiesis, and germ-cell development (reviewed in [8,9]), results from deletions or point mutations of the KIT proto-oncogene [10–16]. KIT, originally identified as an oncogene in the HZ4-feline sarcoma virus [17], encodes the cell surface transmembrane receptor protein tyrosine kinase for the mast/stem cell growth factor [18–21]. The KIT receptor polypeptide consists of a penta-repetitive extracellular ligand-binding domain, a short transmembrane domain, and a bipartite intracellular tyrosine kinase domain [22]. On binding of growth factor ligand to the extracellular domain, the KIT receptor monomer undergoes dimerization within the cell membrane, activating the kinase and initiating the signal-transduction process (reviewed in [23,24]).

The human KIT gene consists of 21 exons spanning more than 34 kbp of DNA [25] at chromosome segment 4q11–q12 [22,26]. The observation of interstitial deletions of chromosome segment 4q11 in some patients with piebaldism [27–30] initially suggested that human piebaldism, like mouse dominant white spotting, might result from defects of the KIT gene. This hypothesis was supported by the demonstration of complete deletion of the KIT gene in two patients with piebaldism [31,32]. We [33–35] and others [36] have recently described a variety of point mutations of the KIT gene in patients with piebaldism. Here we report two novel missense mutations in the tyrosine kinase domain of the KIT gene in two additional families with piebaldism. The human KIT gene mutations closely parallel those of mouse and would be expected to dominantly negatively disrupt function of the KIT receptor, resulting ultimately in the aberrant embryologic distribution of melanocytes that is characteristic of piebaldism.

MATERIALS AND METHODS

Description of Probands Family 1 is a large kindred from Cape Verde Island (Africa) in which piebaldism can be traced for at least four generations. Proband 1 exhibited a large white forelock and extensive leukoderma of the forehead and abdomen, with numerous hyperpigmented macules and cafe-au-lait spots interspersed among and within the regions of hypopigmentation. A complete blood count was within normal limits. The proband’s only child, a daughter, also exhibited white forelock and extensive leukoderma of the abdomen, arms, and legs, with numerous interspersed hyperpigmented macules.

Family 2, in which piebaldism can be traced over three generations, has previously been described in detail in the literature [37]; proband 2 is individual III-5 of that kindred. The piebald phenotype in this family is also relatively severe, including white forelocks and extensive leukoderma of the forehead, chin, ventral trunk, and extremities. Numerous hyperpigmented macules and cafe-au-lait spots are interspersed within and among the regions of hypopigmentation.

Polymerase Chain Reaction Amplification and Analysis of the Human KIT Gene The 21 exons of the human KIT gene and their flanking sequences [25] were amplified from genomic DNA. This PCR product was analyzed by the single-strand confirmation polymorphism (SSCP) method. Amplified DNA was PCR-amplified, and single-strand conformation polymorphisms were detected by the SSCP method. The SSCP method was performed using 5% polyacrylamide gels containing 37.5% acrylamide and 0.5 M urea, and runs were performed at 12°C with a temperature ramp of 0.5°C per minute. The SSCP method was performed using 5% polyacrylamide gels containing 37.5% acrylamide and 0.5 M urea, and runs were performed at 12°C with a temperature ramp of 0.5°C per minute.
both the normally pigmented person demonstrated abnormalities of the intracellular tyrosine kinase domain. This substitution, AGA → GGA, results in the substitution of glycine for the normal arginine at this site.

Proband of Family 2 is Heterozygous for a Missense Substitution at Codon 812 Combined SSCP/heteroduplex analysis of the 21 KIT exons amplified from DNAs of proband 2 and an unrelated normally pigmented person also demonstrated an aberrant pattern for exon 17 of this proband. As shown in Fig 1, exon 17 of the proband exhibited abnormalities of both the SSCP and heteroduplex patterns. Analyses of the remainder of the KIT exons of the proband appeared normal (data not shown).

The exon 17 segment was independently reamplified from genomic DNA of proband 1 and cloned in M13mp18. As shown in Fig 1B, DNA sequence analyses demonstrated that she is heterozygous for a single-base substitution within exon 812, within the intracellular tyrosine kinase domain. This substitution, GGT → GTT, results in the substitution of valine for the normal glycine at this site.

SSCP/heteroduplex analysis of KIT exon 17 fragments polymerase chain reaction amplified from DNA of the proband’s similarly affected daughter and sister likewise demonstrated the same aberrant patterns as the proband (Fig 1). Thus, the codon 812 mutation appears to co-segregate with the piebald phenotype in this family.

DISCUSSION

We used the approach of polymerase chain reaction amplification, rapid screening by combined SSCP/heteroduplex analysis, and subsequent DNA sequence analysis to quickly and efficiently identify two novel mutations of the KIT gene in patients with piebaldism. Both are missense substitutions located within the intracellular tyrosine kinase domain of the 976-amino acid KIT polypeptide.

Missense substitutions in the KIT tyrosine kinase domain inhibit function of the KIT receptor in a so-called dominant-negative manner. Receptor homodimers composed of two mutant KIT polypeptides containing substitutions in the tyrosine kinase domain lack kinase activity. However, receptor heterodimers formed between a normal and a mutant KIT monomer also lack kinase activity [40–43]. Thus, KIT-dependent signal transduction is reduced by approximately 75% in persons heterozygous for KIT kinase domain substitutions, accounting for the autosomal dominant mode of inheritance associated with piebaldism.

The codon 791 mutation of proband 1 is almost certainly pathologic. It is not a common polymorphism. We have not observed it in similar analyses of 37 other unrelated people. Furthermore, codon 791 is located in the center of region VIIb of the highly conserved kinase domain, one of the two most highly conserved portions of the kinase domain. In fact, the amino acid sequence of codons 790–792, His-Arg-Asp, is absolutely invariant among all known protein tyrosine kinases (reviewed in [44]), and the substitution of neutral glycine for basic arginine at the central position of this triplet would almost certainly be deleterious. Of interest, this human KIT gene mutation is only two amino acids away from the mouse \( W^{14z} \) mutation, at codon 790 [14] (note that the codon numbering of the human and mouse KIT polypeptides are not precisely the same), an additional indication that this region is particularly important for function of the KIT receptor kinase.

Similarly, the codon 812 mutation of proband 2 is also almost certainly pathologic. It is also not a common polymorphism, not occurring among 37 other unrelated persons. This mutation co-segregates with the piebald phenotype in first-degree relatives of the proband. Codon 812 is located in the center of region VII of the

Figure 1. SSCP/heteroduplex (HDX) analyses of KIT exon 17. The SSCP and HDX patterns are indicated: lane 1, proband 1; lane 2, unrelated normal person; lane 3, proband 2; lane 4, affected sister of proband 2; lane 5, affected daughter of proband 2. For reasons that are not apparent, the SSCP pattern of proband 1 was consistently faint.
highly conserved kinase domain, the second of the two most highly conserved portions of the kinase domain. In fact, the amino acid sequence of codons 810–812, Asp-Phe-Gly, is likewise absolutely invariant among all known protein tyrosine kinases (reviewed in [44]), and the substitution of valine for the glycine normally at codon 812 would almost certainly be deleterious. This human KIT gene mutation is only six amino acids away from the mouse Wt mutation, at codon 816 [16] (as noted above, the numbering of the human and mouse KIT codons is not identical). This further indicates that this region is particularly important for function of the KIT receptor kinase.

In fact, all of the seven human KIT missense substitutions thus far observed in patients with piebaldism ([33,34,36]; this report; unpublished data) closely parallel the five known KIT missense substitutions of W mutant mice with "dominant white spotting." As noted above, the codon 791 and 812 substitutions described here correspond closely to the mouse Wα2 and Wβ substitutions, respectively. We previously reported human KIT substitutions at codons 584 [34] and 664 [33], which, respectively, correspond closely to the mouse Wβ7 [12,13] and Wα [12] substitutions. Furthermore, Fleischman [36] recently reported a human KIT substitution at codon 583 that precisely corresponds to the Wβ7 substitution of mouse. The reason for the apparent close parallelism between human and mouse mutations is not yet clear. It may be that only a relatively limited number of sites in the KIT kinase domain are critical for function or that only mutations at a limited number of sites result in the piebald phenotype, the criterion by which these patients (and mice) have been selected. Alternatively, this apparent parallelism may be merely a coincidence, the result of the relatively small number of mutations that have been identified to date.

Figure 2. KIT exon 17 sequences in probands 1 and 2. A) Proband 1; sequences in the region of codon 791. B) Proband 2; sequences in the region of codon 812. For both probands, both the normal and mutant alleles are shown. The sequences indicated are of the coding strand. The abnormal codon is indicated in each case.
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REFERENCES