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Novel Mutations and Deletions of the *KIT* (Steel Factor Receptor) Gene in Human Piebaldism

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Summary

Piebaldism is an autosomal dominant genetic disorder of pigmentation characterized by white patches of skin and hair. Melanocytes are lacking in these hypopigmented regions, the result of mutations of the *KIT* gene, which encodes the cell surface receptor for steel factor (SLF). We describe the analysis of 26 unrelated patients with piebaldism-like hypopigmentation—17 typical patients, 5 with atypical clinical features or family histories, and 4 with other disorders that involve white spotting. We identified novel pathologic mutations or deletions of the *KIT* gene in 10 (59%) of the typical patients, and in 2 (40%) of the atypical patients. Overall, we have identified pathologic *KIT* gene mutations in 21 (75%) of 28 unrelated patients with typical piebaldism we have studied. Of the patients without apparent *KIT* mutations, none have apparent abnormalities of the gene encoding SLF itself (*MGF*), and genetic linkage analyses in two of these families are suggestive of linkage of the piebald phenotype to *KIT*. Thus, most patients with typical piebaldism appear to have abnormalities of the *KIT* gene.

Introduction

Piebaldism is an autosomal dominant disorder of melanocyte development characterized by congenital patches of white skin and overlying hair, principally on the forehead, chest, and abdomen, and on the extremities. These hypopigmented regions are relatively lacking in melanocytes, most likely the result of defective proliferation of embryonic melanoblasts prior to migration into the dermis. We have previously shown that human piebaldism results from mutations in the *KIT* proto-oncogene (Giebel and Spritz 1991), and we and others have described a total of 2 deletions and 11 different point mutations of the *KIT* gene in 14 unrelated patients with piebaldism (Fleischman et al. 1991; Giebel and Spritz 1991; Fleischman 1992; Spritz et al. 1992*a*, 1992*b*, 1992*c*, 1993*a*, 1993*b*).

The *KIT* gene encodes the cell surface receptor tyrosine kinase for an embryonic growth factor variously referred to as "mast cell growth factor," "stem cell factor," "kit ligand," or "steel factor" (SLF). Mutations in *KIT* result in piebaldism in humans and in "dominant white spotting" in *W* mice (Chabot et al. 1988; Geissler et al. 1988; Nocka et al. 1990) and rats (Tsujimura et al. 1991). SLF is itself the product of the *MGF* gene, and *MGF* mutations in steel (*Sl*) mutant mice are likewise associated with *W*-like abnormalities of pigmentation. The KIT receptor polypeptide consists of an extracellular domain with five immunoglobulin-like repeats, a transmembrane domain, and a bipartite intracellular tyrosine kinase domain. Binding of SLF to the extracellular domain results in dimerization of the KIT receptor polypeptide, activating the intracellular tyrosine kinase and resulting in consequent phosphorylation of tyrosine residues both on KIT itself and on various distal proteins in the KIT-mediated pathway of signal transduction, culminating in cell proliferation. Although the precise relationship between reduced KIT function and the characteristic pattern of hypopigmentation remains uncertain, KIT function is required just prior to migration of melanoblasts into the dermis during embryogenesis (Nishikawa et al. 1991), and experimentally induced inhibition of KIT function results in reduced proliferation of melanocytes in culture (Spritz and Strunk 1994). Together, these findings suggest that reduced KIT-mediated signal transduction may result in insufficient proliferation of embryonic melanoblasts prior to migration during development.

Here we report the analyses of the *KIT* gene in 26 unrelated patients with piebald-like pigmentary anomalies: 17

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Table 1

Analysis of *KIT* and *MGF* Genes in 26 Patients with Piebald-Like White Spotting

Patient	Ethnic Group	Age	Sex	Phenotype in Proband	Phenotype in Family	Abnormality in *KIT* Gene[a]	Abnormality in *MGF* Gene[b]
1	Bangladeshi	Adult	F	Severe	Relatively severe in four generations	A621T	nt
2	Caucasian	17 years	M	Severe	Relatively severe in four generations	H650P	nt
3	Caucasian	27 years	F	Severe	Variable severity in four generations	630insA	nt
4	Caucasian	Child	F	Mild	Variable severity in three generations	630insA	nt
5	Caucasian	38 years	F	Mild	Relatively mild in four generations	W557X	nt
6	Caucasian	18 years	F	Mild	Relatively mild in two generations	Deletion	None[c]
7	Caucasian	Adult	F	Moderate	Variable severity in four generations	Deletion; (M318G)	None[c]
8	Caucasian	Adult	M	Mild	Relatively mild in three generations	None[d]	None[c]
9	Caucasian	6 years	F	Moderate	Sporadic	Deletion	None[c]
10	Caucasian	75 years	M	Moderate	Moderate severity in four generations	Deletion	None[c]
11	Caucasian	Adult	M	Mild	Variable severity in four generations	None[d]	nt
12	Caucasian	Adult	F	Mild	Relatively mild in four generations	Deletion	None[c]
13	Caucasian	25 years	M	Moderate	Moderate severity in siblings	None[d]	None[c]
14	Caucasian	23 years	F	Moderate	Variable severity in four generations	IVS1 + 4A	nt
15	Caucasian	Adult	M	Moderate	Variable severity in six generations	None[d]	None[c]
16	Caucasian	Adult	M	Moderate	Variable severity in five generations	None;[d] (G93G)	None[c]
17	Caucasian	Adult	F	Moderate	Variable severity in six generations	None[c]	None[c]
18	Pakistani	Adult	F	Mild	Variable severity in two generations	Deletion	nt
19	Caucasian	12 years	F	Severe	Sporadic	E861A	nt
20	Oriental	14 years	M	Severe	Sporadic	None[c]	None[c]
21	Caucasian	Adult	F	Mild	Sporadic	None[c]	None[c]
22	Caucasian	Adult	M	Mild	Sporadic	None[c]	None[c]
23	North Indian	35 years	M	Vitiligo	Sporadic	None[c]	None[c]
24	Caucasian	Adult	F	WS1?[e]		None[d]	nt
25	Caucasian	Adult	F	WS1?[e]		None[c]	None[c]
26	Inuit Indian	2 years	M	WS2[f]		None[c]	None[c]

[a] Parentheses indicate nonpathologic polymorphisms.
[b] nt = Not tested.
[c] No abnormalities detected by SSCP/HDX analysis.
[d] No abnormalities detected by SSCP/HDX and quantitative genomic Southern blot hybridization.
[e] Waardenburg syndrome type I.
[f] Waardenburg syndrome type II.

with typical autosomal dominant piebaldism; 5 with piebald-like phenotypes but atypical family histories; 1 with vitiligo; and 3 with unusual variants of Waardenburg syndrome. We identify novel pathologic mutations or deletions of the *KIT* gene in 10 (59%) of the patients with typical piebaldism, in 2 (40%) of the patients with atypical family histories, and in none of the other patients. No patients had abnormalities of the *MGF* gene. We discuss these findings in the context of our overall experience with molecular analyses of patients with piebaldism.

Subjects, Material, and Methods

Subjects

We studied a total of 26 unrelated patients with piebald-like pigmentary anomalies (table 1). Patients 1–8 and 10–18 had typical autosomal dominant piebaldism, with characteristic patches of hypopigmented skin and overlying hair, principally involving the forehead, chest, abdomen, and extremities, with or without white forelock. Patients 9 and 19–22 exhibited either typical or somewhat unusual piebald-like pigmentary anomalies but had negative or uncharacteristic family histories. Patient 23 was initially thought to have sporadic piebaldism but was subsequently diagnosed with vitiligo. Patients 24–26 had piebald-like pigmentary anomalies associated with clinical features suggestive of Waardenburg syndrome type I or II either in themselves or in other family members.

PCR Amplification and Analysis of Genomic DNA

Genomic DNA was isolated from peripheral blood leukocytes by standard procedures, except for patients 24 and 25, from whom DNA was isolated from cultured lymphoblastoid cells, and patient 26, from whom DNA was isolated from cultured skin fibroblasts. The 21 exons of the human *KIT* gene plus adjacent noncoding and flanking sequences (Giebel et al. 1992) were amplified in duplicate from 0.1 μg of DNA of each patient by 35 cycles of the

PCR (Saiki et al. 1988) as described elsewhere (Spritz et al. 1992*a*). The products of the duplicate PCRs for each exon were pooled and screened by combined SSCP/heteroduplex (HDX) analyses using MDE gels (AT Biochemical) as described elsewhere (Spritz et al. 1992*b*). *KIT* exon fragments that yielded aberrant SSCP/HDX patterns were independently reamplified in duplicate, were pooled, were purified in 4% polyacrylamide gels, were eluted, and were cloned in M13mp18. The nucleotide sequences of at least six independent clones of each were determined (Sanger et al. 1977).

For patients 6–10, 12, 13, 15–17, 20–23, 25, and 26, in whom we detected no apparent point mutations of the *KIT* gene, the nine exons (and adjacent intron and flanking sequences) of the human *MGF* gene (Martin et al. 1990) were also analyzed in the same manner. *MGF* primer pairs were 5′-CCTTGCCTGCTTCTCGCCTA-3′/5′-CACCGGGCGCGATTTTTCCT-3′ (exon 1); 5′-TTGAATGATTACAGATCTTA-3′/5′-ACAGGAAAAAGAGCCACAGC-3′ (exon 2); 5′-TGATTAATTGATGTATAAGA-3′/5′-AATGATCCCATTTCTTCTAT-3′ (exon 3); 5′-CCGAGTTTTATGGCACTTACT-3′/5′-TAGAGAAGCGTAATGAAAAAT-3′ (exon 4); 5′-ACCTCAGTTAAGTCTGAAGA-3′/5′-ATAGGGAAGATTTAAGTTTG-3′ (exon 5); 5′-CTCTATAACTCATACAAATC-3′/5′-ATACTCCTATAGGTGCTAAT-3′ (exon 6); 5′-ACTTCTAGAATGGATATGCT-3′/5′-ATGTAAACATAGCAATTTTT-3′ (exon 7); 5′-TATTAGACCATTCATTGATT-3′/5′-CAGTGTGTGAAATGGCAATG-3′ (exon 8); and 5′-GTGTACCTCAGAAT CTCTGA-3′/5′-CATGTACCCTAGAACTTAAAG-3′ (exon 9).

Restriction Cleavage Analyses

For patient 1, the *KIT* exon 12 PCR fragment was independently reamplified and, with the exon 12 fragment amplified from a normal control, was cleaved with *Mae*III and analyzed by 6% PAGE. For patient 19, the *KIT* exon 18 PCR product was similarly analyzed by cleavage with *Alu*I and with *Hha*I.

Genetic Linkage Analyses

Genetic linkage analyses of the families of patients 12, 15, and 16 were carried out using a single-base polymorphism in IVS1 (Spritz and Holmes 1993) and a *Hae*III RFLP within the 3′ UTR (Poduslo et al. 1991) of the *KIT* gene. The *KIT* IVS1 polymorphism was scored using a 187-bp PCR product containing exon 1 and part of IVS1, amplified and assayed by SSCP/HDX analysis as described elsewhere (Spritz and Holmes 1993). The *KIT* 3′ UTR RFLP was scored using a 296-bp PCR product amplified from genomic DNA using 20-mer primers 5′-TTGTGCACGACGATGTCTGA-3′ and 5′-AGTCCATACCTCCCTCTCTT-3′. The products were cleaved with *Hae*III and electrophoresed through 5% polyacrylamide. Linkage between the *KIT* polymorphisms and piebald phenotype was assessed by determination of logarithm of odds (lod) scores by using version 3 (1986) of the LIPED program (Ott 1974).

Quantitative Genomic Southern Blot Hybridization Analysis

Approximately 10 μg of genomic DNA from patients 6–16, 18, and 24, in whom we identified no abnormalities of the *KIT* gene by SSCP/HDX analysis, and from an unrelated normally pigmented individual was cleaved with *Eco*RI and was analyzed by Southern blot hybridization (Southern 1975). Two nonoverlapping partial human *KIT* cDNA fragments were purified from plasmids phKIT1-1250 (*KIT* cDNA nt 1–1250) and phKIT1300-2976 (*KIT* cDNA nt 1300–2976), both of which had previously been prepared (R. A. Spritz, unpublished data) by reverse-transcription PCR of mRNA from normal human melanocytes, using oligonucleotide primers derived from the human *KIT* cDNA sequence (Yarden et al. 1987). The two *KIT* cDNA fragments were radiolabeled and used simultaneously as probe. After autoradiography the filters were stripped and were rehybridized to a referent probe prepared using a 1,050-bp PCR product containing exon 1 of the human tyrosinase (*TYR*) gene (Spritz et al. 1992*a*), located within chromosome segment 11q14-q21 (Barton et al. 1988). Filters were autoradiographed and signals were quantitated by use of a scanning densitometer (Hoefer Scientific Instruments). Alternatively, an independently prepared blot was detected and signals were analyzed quantitatively by using a Molecular Dynamics 425e PhosphorImager.

Results

Novel Mutations of the KIT Gene in Patients with Piebaldism

SSCP/HDX screening and subsequent DNA sequence analyses resulted in our identifying novel point mutations in 8 of the 17 patients with typical piebaldism and in 1 of the 5 atypical patients (see table 1). Of these mutations, six (patients 1–6 and 19) appear to be pathologic, two (patients 7 and 16) are rare, nonpathologic polymorphisms, and the role of another (patient 14) is uncertain.

Patient 1 had typical severe piebaldism; at least four generations of her family were also relatively severely affected. SSCP/HDX and DNA sequence analyses of her *KIT* gene showed that she was heterozygous for a novel missense substitution in kinase subdomain 2, codon 621 GCT (Ala)→ACT (Thr) (i.e., A621T) (fig. 1*A*). This substitution creates a novel *Mae*III cleavage site, and *Mae*III digestion of the exon 12 PCR product independently amplified from genomic DNA of the patient permitted us to confirm the codon 621 mutation. Ala621 is one of two residues that

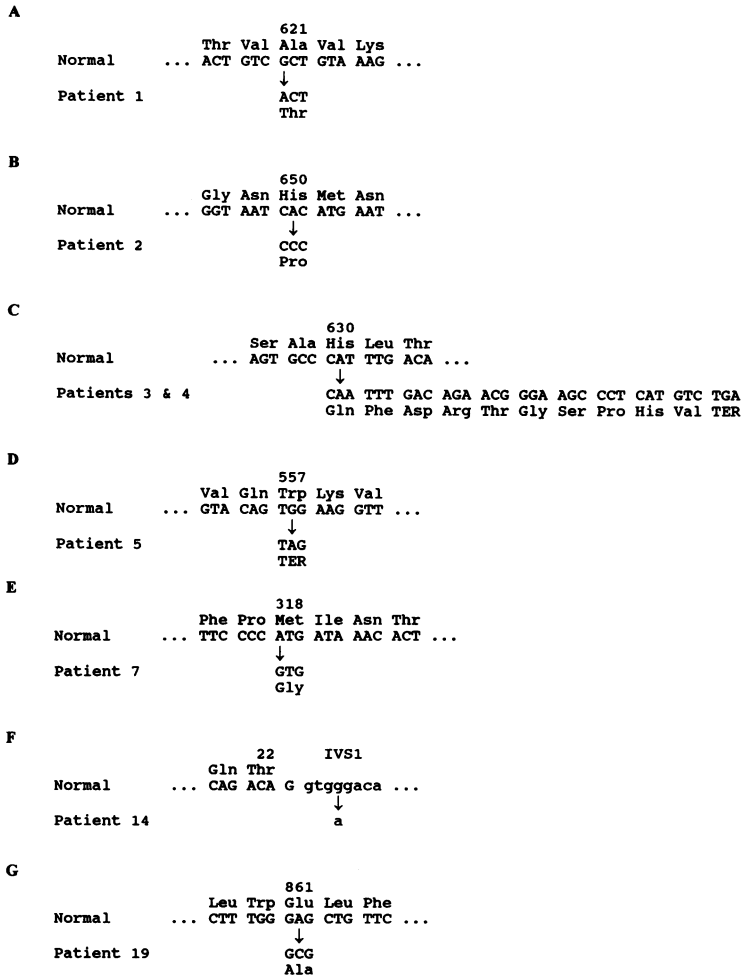

Figure 1 *A, KIT* gene sequences in region of the A621T mutation of patient 1. *B, KIT* gene sequences in region of the H650P mutation of patient 2. *C, KIT* gene sequences in region of codon 630insA mutation of patients 3 and 4. *D, KIT* gene sequences in region of codon W557X mutation of patient 5. *E, KIT* gene sequences in region of M318G polymorphism of patient 7. *F, KIT* gene sequences in region of IVS1 +4A mutation/polymorphism of patient 14. Uppercase indicates coding sequence and lowercase indicates intervening sequence (IVS1). *G, KIT* gene sequences in region of E861A mutation of patient 19.

define kinase subdomain II and is conserved among all known protein tyrosine kinase sequences.

Patient 2 also had typical severe piebaldism; at least four generations of his family were also relatively severely affected. Analyses of his *KIT* gene showed that he was heterozygous for a novel missense substitution in kinase subdomain 3, codon 650 CAC (His)→CCC (Pro) (i.e., H650P) (fig. 1*B*). His650 is conserved among about two-thirds of all known protein tyrosine kinases, including both the murine and feline *KIT* genes.

Patient 3 also had typical severe piebaldism; at least four generations of her family were affected, but with very variable phenotypes, ranging from severe to very mild (white forelock only). Patient 4 had typical relatively mild piebaldism, involving only the knees, as well as patent ductus arteriosus, strabismus, and developmental delay. Karyotype was normal. Three generations of her family were affected, but with a quite variable phenotype. Analyses of the *KIT* genes of patients 3 and 4 showed that both were heterozygous for the same novel frameshift in kinase subdomain 3, codon 630 CAT→CAAT (i.e., 630insA) (fig. 1*C*). The resultant distal nonsense polypeptide terminates 10 residues downstream at a novel in-frame TGA. These two families could identify no apparent relationship; however, both are from England, and although it is unlikely, their piebaldism thus might derive from a common ancestor.

Patient 5 had typical mild piebaldism; at least four generations of her family were relatively mildly affected. Analyses of her *KIT* gene showed that she is heterozygous for a novel nonsense mutation, codon 557 TGG (Trp)→TGA (Ter) (i.e., W557X) (fig. 1*D*), which would result in truncation of the KIT polypeptide just distal to the transmembrane domain and proximal to the kinase domain.

Patient 6 had typical mild piebaldism; her mother was mildly affected. SSCP/HDX screening of both her *KIT* and *MGF* genes detected no apparent abnormalities.

Patient 7 had typical moderately severe piebaldism; at least four generations of her family were affected, but with a quite variable phenotype. Analyses of her *KIT* gene showed that she is heterozygous for a novel missense substitution, codon 318 ATG (Met)→GTG (Gly) (i.e., M318G) (fig. 1*E*). However, SSCP/HDX analysis demonstrated that this mutation was not present in her similarly affected sister and thus is apparently a nonpathologic polymorphism. Analyses of the patient's *MGF* gene appeared normal.

Patient 8 had very mild piebaldism (white forelock only); at least three generations of his family were also mildly affected. SSCP/HDX screening of his *KIT* and *MGF* genes detected no apparent abnormalities.

Patient 9 had typical moderately severe piebaldism, mild delay of motor and speech development, and dyspraxia. The family history was negative for piebald-like pigmentary anomalies. SSCP/HDX screening of her *KIT* and *MGF* genes detected no apparent abnormalities.

Patient 10 had typical moderately severe piebaldism; at least four generations of his family were affected. SSCP/HDX screening of his *KIT* and *MGF* genes detected no apparent abnormalities.

Patient 11 had typical mild piebaldism; more than four generations of his family were relatively mildly affected, as are very many individuals in two adjacent counties of his state. SSCP/HDX screening of his *KIT* gene detected no apparent abnormalities.

Patient 12 had typical mild piebaldism; at least four generations of her family were relatively mildly affected. SSCP/HDX screening of her *KIT* and *MGF* genes detected no apparent abnormalities.

Patient 13 had typical moderately severe piebaldism. His parents were said to be normal, but two sibs were moderately severely affected. SSCP/HDX screening of his *KIT* and *MGF* genes detected no apparent abnormalities.

Patient 14 had typical moderately severe piebaldism; at least four generations of her family were affected, but with quite variable phenotypes. Analyses of her *KIT* gene showed that she is heterozygous for a novel base substitution within IVS1, g→a at position 4 (i.e., IVS1+4A) (fig. 1*F*). SSCP/HDX analysis of her *MGF* gene detected no abnormalities. This substitution within the IVS1 *5′* splice consensus sequence may be the cause of piebaldism in this patient; however, in the absence of functional studies of RNA splicing we cannot exclude the possibility that it is a rare nonpathologic polymorphism.

Patient 15 had typical moderately severe piebaldism; at least six generations of his family were affected, but with quite variable phenotypes. SSCP/HDX screening of his *KIT* and *MGF* genes detected no apparent abnormalities.

Patient 16 (patient 6d in Gatto et al. 1985) had typical moderately severe piebaldism. Numerous members in several generations of his extensive kindred were affected, many also with cancer. SSCP/HDX screening of his *KIT* and *MGF* genes detected no apparent pathologic abnormalities. However, he was heterozygous for a novel silent polymorphism of *KIT*, codon 93 GGC (Gly)→GGT (Gly) (i.e., G93G) (not shown), that did not segregate with the piebald phenotype in his family.

Patient 17 had typical moderately severe piebaldism; at least six generations of her family were affected, but with quite variable phenotypes. SSCP/HDX screening of her *KIT* and *MGF* genes detected no apparent abnormalities.

Patient 18 had typical mild piebaldism, and a daughter was also affected, with a moderately severe phenotype. SSCP/HDX screening of her *KIT* gene detected no apparent abnormalities.

Patient 19 had typical severe piebaldism, but no other family members were affected. Analyses of her *KIT* gene showed that she was heterozygous for a novel missense substitution within kinase subdomain 9, codon 861 GAG (Glu)→GCG (Ala) (i.e., E861A) (fig. 1*G*). This substitution both creates a novel *Hha*I cleavage site and abolishes an *Alu*I site. *Hha*I and *Alu*I digestion of the exon 18 PCR product independently amplified from genomic DNA of the patient permitted us to confirm the codon 861 mutation.

Patient 20 (patient 2 in Ishii et al. 1985) had typical severe piebaldism, but no other family members were affected. SSCP/HDX screening of her *KIT* and *MGF* genes detected only a novel T→C substitution at position −136 in the *5′* UTR of *MGF* (not shown), which most likely represents a nonpathologic polymorphism.

Patient 21 exhibited congenital mild piebald-like hypopigmented patches confined to just the left leg and pubic region. No other family members were affected. SSCP/HDX screening of her *KIT* and *MGF* genes detected no apparent abnormalities.

Patient 22 was an adult Caucasian male with mottled hypopigmented areas on the arms and a diffuse white forelock and early graying. No other family members were affected; however, the patient as well as numerous normally pigmented family members also had an unusual form of marrow hypoplasia (Aufderheide 1972). SSCP/HDX screening of his *KIT* and *MGF* genes detected no apparent abnormalities.

Patient 23 was an adult Indian male with severe cutaneous depigmented patches and early graying of his hair. Some other family members reportedly exhibited unknown forms of hypopigmentation. SSCP/HDX screening of his *KIT* and *MGF* genes detected no apparent abnormalities. Subsequent clinical reevaluation demonstrated significant repigmentation and a distribution of depigmented patches strongly suggestive of vitiligo, rather than piebaldism.

Patient 24 was an adult Caucasian female with piebald-

like pigmentary anomalies. Numerous other family members exhibited phenotypes characteristic of Waardenburg syndrome type 1. SSCP/HDX screening of her *KIT* and *MGF* genes detected no apparent abnormalities.

Patient 25 was an adult Caucasian female with piebald-like pigmentary anomalies. Numerous other members of her extensive eight-generation pedigree exhibited dystopia canthorum but not deafness. SSCP/HDX screening of her *KIT* and *MGF* genes detected no apparent abnormalities.

Patient 26 was an Inuit boy with piebald-like hypopigmented areas, heterochromia irides, deafness, and Hirschsprung disease. Previously described as "piebaldism-Waardenburg syndrome" (Kaplan and Chaderévian 1988), the clinical features are most suggestive of Waardenburg syndrome type 2. SSCP/HDX screening of his *KIT* and *MGF* genes detected no apparent abnormalities.

Genetic Linkage Analysis in Three Families with Typical Piebaldism

We were unable to identify pathologic *KIT* mutations in patients 6–13, 15–18, or 20–26. To determine whether the piebald phenotype is linked to the *KIT* gene in at least those cases in which sufficient family members were available, we performed genetic linkage analysis in the families of patients 12, 15, and 16 by using two common intragenic *KIT* polymorphisms, one within IVS1 and the other within the 3′ UTR.

In the family of patient 12 we analyzed 18 members in four generations. Two-point linkage analysis yielded a maximum lod score of 3.25 at $\theta = 0$, indicative of linkage between the piebald phenotype and the *KIT* gene in this kindred.

In the family of patient 15 we analyzed nine members in two generations. Two-point linkage analysis yielded a maximum lod score of 1.03 at $\theta = 0$. These data are suggestive of, although they do not prove, linkage between the piebald phenotype and *KIT* in this kindred.

In the family of patient 16 we analyzed seven members in two generations. Two-point linkage analysis yielded a maximum lod score of 0.51 at $\theta = 0$. These results are also suggestive of genetic linkage between the piebald phenotype and *KIT* in this kindred.

Quantitative Southern Blot Hybridization Analysis

To determine whether some of these patients without apparent *KIT* point mutations might have gene deletions not detected by assays dependent on the PCR, we carried out quantitative Southern blot hybridization analyses. Genomic DNAs from 11 patients with typical piebaldism (patients 6–8, 10–16, and 18), one atypical patient (patient 9), one Waardenburg syndrome variant (patient 24), and three normally pigmented individuals were cleaved with *Eco*RI, fractionated by gel electrophoresis, and hybridized simultaneously to two *KIT* cDNA probes spanning almost the complete coding region (nt 1–1250 and nt 1300–2976).

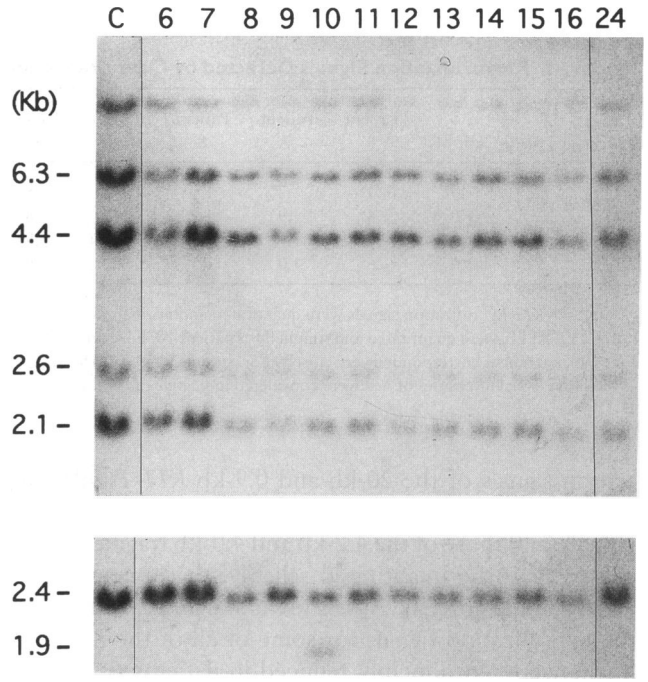

Figure 2 Quantitative Southern blot hybridization analysis of the *KIT* gene. Genomic DNA of the indicated patients and a normally pigmented control was digested with *Eco*RI and was hybridized to cDNA probes for the human *KIT* (*top panel*) and *TYR* (*bottom panel*) genes, and the filter was autoradiographed and signals were quantitated by scanning densitometry. In an independent experiment the signals were detected and quantitated by phosphoimaging. There was very good agreement between the two experiments, and bands that yielded average *KIT*/*TYR* ratios of ≤0.7 times that of the normal controls were scored as deleted. Lane C, Normal control. Lanes 6–24, Patients 6–24. The sizes of major bands for both the *KIT* gene and *TYR* exon 1 are indicated on the left. Two hybridizing signals for *TYR* exon 1 can be seen for patient 10; this was reproducible in four independent experiments and most likely indicates a novel rare *Eco*RI RFLP. These signals were summed for quantitative calculations involving patient 10.

The filters were then stripped and rehybridized to a probe for exon 1 of the *TYR* gene, located on chromosome 11. Hybridization signals were detected either autoradiographically or by phosphoimaging. Although the *KIT* gene consists of 21 exons, most are relatively small; therefore, the *KIT* probe detected only six major *Eco*RI fragments, ~20, ~6.3, ~4.4, ~2.6, ~2.1 and ~0.9 kb in size, as well as two weakly hybridizing fragments, sized 4.2 kb and 4.0 kb (fig. 2). The 20-kb, 2.6-kb, 2.1-kb, and 0.9-kb fragments were detected by a probe consisting of only nt 1300–2976 of *KIT* cDNA (not shown); these therefore correspond to exons located in the 3′ half of the gene. The 4.4-kb *Eco*RI fragment was detected by a probe consisting of only *KIT* exon 1 (Spritz et al. 1992*a*).

The signal intensities of the 6.3-kb, 4.4-kb, 2.6-kb, and 2.1-kb *KIT Eco*RI fragments were measured by scanning densitometry or phosphoimaging and were normalized to the signals for the *TYR* gene segment. Because the hybrid-

Table 2

Relative *KIT* Hybridization Signals Detected by Quantitative Southern Blot Hybridization Analysis

KIT Fragment	Controls	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10	Patient 11	Patient 12	Patient 13	Patient 14	Patient 15	Patient 16	Patient 18	Patient 24
6.3 kb	1.0	.4[a]	.7[a]	1.0	.5[a]	.5	.9	.9	1.0	1.3	.8	.9	.6	.8
4.4 kb	1.0	.4[a]	.9	1.2	.6[a]	.8	.9	1.2	1.3	1.4	1.1	1.0	.6	.9
2.6 kb	1.0	.6[a]	1.1	.9	.7[a]	.8	1.0	.5[a]	.9	1.1	1.0	.9	1.1	.8
2.1 kb	1.0	.6[a]	1.0	1.1	.5[a]	.9	1.0	.6[a]	1.0	1.1	1.0	.9	1.0	.8

NOTE.—*KIT* hybridization signals were quantitated and normalized to the corresponding *TYR* signal for each sample. Two tyrosinase signals, the apparent result of a novel *TYR* RFLP, were quantitated and summed for patient 10. For patients 6–8, 10–12, 15, and 16 the values represent the mean of two experiments; the individual values for each experiment corresponded very closely. For patients 9, 13, 14, 18, and 24 the values are derived from only one experiment.

[a] Relative signal intensities of ≤.7 were considered indicative of deletion.

ization signals of the 20-kb and 0.9-kb *KIT* *Eco*RI fragments appear somewhat variable even among normal individuals, and those of the 4.2-kb and 4.0-kb fragments were too weak to be quantitated reliably, these were not included in the analysis. As is shown in figure 2 and table 2, the hybridization signals for some or all of the *KIT* fragments were reproducibly reduced in patients 6, 7, 9, 10, 12, and 18, indicative of gene deletions. In patients 6 and 9 the signal intensities of all four *Eco*RI fragments were reduced, indicating that the entire gene may be deleted. In patients 7 and 10 only the signal of the 6.3-kb fragment was reduced, and in patient 18 the signal intensities of the 6.3-kb and 4.4-kb fragments were reduced (not shown), indicative of partial gene deletions involving the 5′ portion of *KIT*. In patient 12 the signals of the 2.6-kb and 2.1-kb fragments were reduced, indicative of partial deletions involving the 3′ portion of *KIT*. Patients 6–8, 9–16, and 18 had typical piebald phenotypes and positive family histories, whereas patient 9 was an atypical patient, with sporadic piebaldism associated with developmental delay.

Discussion

Here we describe analyses of the *KIT* gene in 26 unrelated patients with piebald-like pigmentary abnormalities. Seventeen of these patients had typical piebaldism, with characteristic clinical features and apparent autosomal dominant familial inheritance. Five others had either atypical piebald-like clinical features, atypical family histories, or both. Among the 17 typical patients, we identified pathologic abnormalities of *KIT* in 10 (59%): five point mutations and five gene deletions (the IVS1 +4 mutation in patient 14 may, in fact, be a rare nonpathologic polymorphism and has not been counted as pathologic). In general, the phenotypes associated with the *KIT* point mutations conformed to the paradigm we elaborated elsewhere (Spritz et al. 1992*b*, 1992*c*). That is, dominant-negative missense substitutions within the KIT intracellular tyrosine kinase domain (patients 1, 2, and 19) were associated with relatively severe piebald phenotypes; mutations that would truncate the KIT polypeptide within the intracellular tyrosine kinase domain (patients 3 and 4) were associated with variably severe phenotypes; and a mutation that would truncate the KIT polypeptide proximal to the tyrosine kinase domain (patient 5) was associated with a relatively mild phenotype, the result of haploinsufficiency of KIT-dependent signal transduction. We also detected one missense substitution within the extracellular ligand-binding domain (patient 7). This substitution appears to be a rare nonpathologic polymorphism, as we elsewhere suggested might be the case with most missense substitutions in this region (Spritz et al. 1992*c*).

Most patients with partial or complete *KIT* gene deletions did not exhibit relatively mild piebald phenotypes characteristic of haploinsufficiency, as might be predicted by the above paradigm. We have recently shown that *KIT* is located in the middle of a cluster of genes encoding type III receptor tyrosine kinases, organized *PDGFRA-KIT-KDR* (Spritz et al. 1994). Therefore, in addition to *KIT*, some of these deletions might involve multiple genes in this cluster. In particular, deletion of *Pdgfra* in patch (*Ph*) mutant mice is itself associated with piebald-like pigmentary abnormalities (Smith et al. 1991; Stephenson et al. 1991), and an inversion involving this region is associated with piebald-like features in rump-white (*Rw*) mice (Stephenson et al., in press). Interestingly, we detected apparent complete deletion of the *KIT* gene in one of the atypical patients (patient 9), who exhibited sporadic piebaldism in association with developmental delay. We speculate that this patient may have a de novo microdeletion that involves a more extensive segment of 4q12, including *KIT* and also other neighboring genes, although none of these patients have been investigated cytogenetically. Fleischman et al. (1991) previously reported an apparently de novo microdeletion including both *KIT* and *PDGFRA* in a patient with sporadic piebaldism, and we have described deletion of both *KIT* and *PDGFRA* in a patient with sporadic piebaldism and mental retardation associated with de novo cytogenetic deletion of 4q12-q21.1 (Spritz et al. 1992*a*).

Overall, we have detected clearly pathologic *KIT* gene

mutations or gene deletions in 21 (75%) of the 28 unrelated patients with typical piebaldism we have studied to date. We may speculate on the possible molecular defects in the patients in whom we did not detect abnormalities. In the families of patients 15 and 16 (present report) genetic linkage analyses were at least consistent with linkage of the piebald trait to *KIT* intragenic polymorphisms. It thus seems likely that many such patients have occult *KIT* mutations either not detected by SSCP/HDX screening or located in parts of the gene not analyzed, or they may have partial *KIT* gene deletions that were not detected by quantitative Southern blot hybridization. However, it remains formally possible that at least some such patients might have defects in other genes, perhaps located near *KIT*, that are also required for normal pigmentation. It is surprising that we have observed no patients with piebaldism resulting from abnormalities of the *MGF* gene, which encodes SLF, the ligand for the KIT receptor. It may be that, in contrast to steel mutant mice, in humans *MGF* mutations either do not result in pigmentary abnormalities or are lethal.

We can be even less certain about the basis of piebald-like features among patients with atypical white-spotting phenotypes. As noted above, patient 9 had a deletion of *KIT*, and patient 19 had a missense mutation. None of the other atypical patients had abnormalities of either *KIT* or *MGF*. Patients 20–22, all of whom exhibited sporadic piebald-like phenotypes, might have de novo mutations of *KIT* that were not detected by SSCP/HDX screening. Moreover, patient 22 had a quite unusual asymmetric phenotype, and she might even be mosaic for a somatic *KIT* gene mutation not present in leukocyte DNA. We also found no abnormalities of the *KIT* or *MGF* genes in the patient with vitiligo (patient 23) or in any of the three patients with Waardenburg syndrome (patients 24–26), all of whom also lack apparent abnormalities of the *PAX3* gene (J. Asher and C. Baldwin, personal communications; S. A. Holmes and R. A. Spritz, unpublished data). These patients may well have novel disorders that affect melanocyte development associated with abnormalities of genes that have yet to be identified.

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