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Genetics

Mutation of the *KIT* (mast/stem cell growth factor receptor) protooncogene in human piebaldism

(pigmentation disorders/white spotting/oncogene/receptor/tyrosine kinase)

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ABSTRACT **Piebaldism is an autosomal dominant genetic disorder characterized by congenital patches of skin and hair from which melanocytes are completely absent. A similar disorder of mouse, dominant white spotting (*W*), results from mutations of the c-*Kit* protooncogene, which encodes the receptor for mast/stem cell growth factor. We identified a *KIT* gene mutation in a proband with classic autosomal dominant piebaldism. This mutation results in a Gly → Arg substitution at codon 664, within the tyrosine kinase domain. This substitution was not seen in any normal individuals and was completely linked to the piebald phenotype in the proband's family. Piebaldism in this family thus appears to be the human homologue to dominant white spotting (*W*) of the mouse.**

Piebaldism is an autosomal dominant genetic disorder characterized by congenital patches of skin and hair that completely lack pigment, principally on the forehead, ventral chest and abdomen, and extremities (1–3). Pie refers to the variegated black and white plumage pattern characteristic of the magpie, and bald apparently derives from the Greek phalios, "having a white spot." Known since at least ancient Roman times (4), piebaldism, sometimes referred to as partial albinism, was one of the first autosomal dominant disorders recognized (5). Piebaldism was also one of the first genetic disorders for which a pedigree was presented (6, 7), and several pedigrees have been reported in which the disorder has been traced over hundreds of years (for review, see ref. 3). A similar phenotype in the mouse, dominant white spotting (*W*), has recently been shown to result from deletions or point mutations within the *Kit* protooncogene (8–11), which encodes the tyrosine kinase transmembrane cellular receptor for mast/stem cell growth factor (12–15). The human piebaldism locus was provisionally mapped to chromosome segment 4q12 on the basis of interstitial chromosomal deletions (16–19), coincident with the location of the human *KIT* protooncogene in chromosome segment 4q11–q12 (20, 21). This result suggested that human piebaldism might, like mouse dominant white spotting, also result from mutations of the c-*kit* gene.

We have determined the DNA sequence of the normal human *KIT* gene to facilitate analyses of human *KIT* genomic DNA segments from probands with piebaldism using the PCR. Here, we report a missense mutation, resulting in a Gly → Arg substitution at codon 664, within the *KIT* gene of a proband with classic, autosomal dominant piebaldism. This mutation is not observed in normally pigmented individuals and is completely linked to the piebald trait in the proband's kindred. The human codon-664 substitution, within the tyrosine kinase domain of the KIT protein, is similar to several c-*Kit* substitutions in *W* mice with dominant white spotting. Piebaldism in this proband and his family thus appears to represent a human homologue to dominant white spotting (*W*) of the mouse.

MATERIALS AND METHODS

Description of the Proband. The proband† was an adult man with typical features of piebaldism, including nonpigmented patches on his central forehead, central chest and abdomen, and arms and legs. Pigmentation of his scalp hair and facial hair was normal, although several other family members had white forelocks in addition to nonpigmented skin patches. His irides and retinae were normally pigmented, and hearing and visual acuity were normal. There was no family history of dysmorphic facial features, deafness, or anemia, and complete blood count and red cell morphology, both of the proband and two other affected family members, were normal. The proband was a member of a very extensive Ashkenazi Jewish kindred that can trace the inheritance of piebaldism through at least 15 generations (Fig. 1).

PCR Amplification and Sequencing of Genomic DNA. Human *KIT* cDNA phckit-171 (20) was obtained from the American Tissue Culture Collection. This 1.25-kilobase (kb) cDNA includes only codons 1–413. Therefore, we isolated an additional human *KIT* cDNA containing codon 427 through part of the 3-untranslated region of KIT mRNA. Total cDNA was prepared (22) from 4 μg of total RNA from HEL cells (23), which express abundant KIT mRNA (24). One-twentieth of the reaction was then used as template to amplify a 1556-base-pair (bp) fragment of KIT cDNA by 40 cycles of PCR (25) by using *Taq* DNA polymerase (AmpliTaq; Perkin–Elmer/Cetus), an automated DNA thermal cycler (Coy Laboratory Products, Ann Arbor, MI), and two oligonucleotide primers, 5′-CAATGTGTGGCAGCAGGATTC-3′/5′-GAAGAGATCATTCCTGGAGG-3′, derived from the nucleotide sequence of human KIT cDNA (20). These human KIT cDNA segments were then used as probes to screen two normal human recombinant λ phage genomic libraries for human *KIT* genomic clones. Mapping and nucleotide sequence analyses of the normal human *KIT* gene showed that the coding portion of the human *KIT* gene is divided into 21 exons (unpublished data).

Based on the DNA sequence of the normal human *KIT* gene (unpublished data), we designed 42 oligonucleotide primers to amplify each of the 21 *KIT*-coding exons by PCR. The primers were derived from the intervening and flanking sequences and, therefore, amplified the complete exon and the adjacent splice-junction and noncoding regions. DNA segments containing the exons were amplified from 0.1 μg of genomic DNA of the proband by 35 cycles of PCR, as described above. Each cycle consisted of 30 sec at 94°C, 1 min at 50°C, and 1 min per kb at 72°C. The products of three

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†A lymphoblastoid cell line established from the proband has been deposited in the National Institute of General Medical Sciences Human Genetic Mutant Cell Repository.

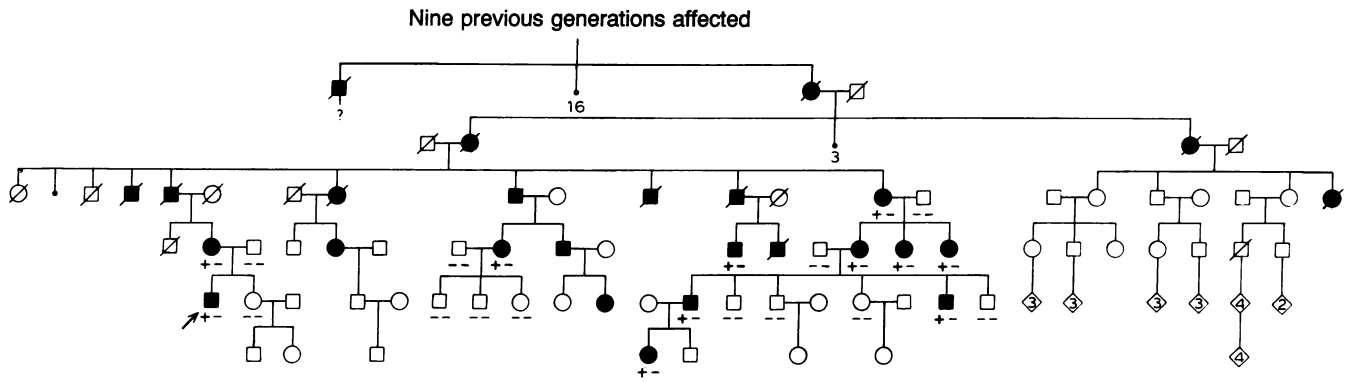

FIG. 1. Pedigree and linkage analysis of piebaldism and the *KIT* gene codon-664 mutation. Arrow, proband; solid symbols, individuals with piebaldism; open symbols, normal individuals; +, codon-664 mutation; −, absence of codon-664 mutation. Numbers indicate multiple individuals of unspecified gender.

replicate PCR amplifications were pooled and purified by centrifugation through Centricon 100 microconcentrators (Amicon). For coding exons 2, 4, 8, 11, 13, 14, and 21, the complete DNA sequences of the purified, double-stranded PCR products were determined directly, essentially by the method of Sanger *et al.* (26), using [α-^{35}S]dATP and *Taq* DNA polymerase. For coding exons 1, 3, 5, 6, 7, 9, 10, 12, and 15–20, the PCR products were cloned into M13mp19, and the nucleotide sequences were determined for at least six independent clones using single-stranded DNA templates.

***Mnl* I Cleavage and Genetic Linkage Analyses.** DNA was isolated from peripheral-blood leukocytes from 22 unrelated normal individuals of northern European Caucasian ethnic origin, 17 unrelated normal Ashkenazi Jewish individuals, and the family members indicated in Fig. 1. A 250-bp fragment of the *KIT* gene consisting of coding exon 13 (codons 627–664; unpublished data) plus portions of the adjacent intervening sequences was PCR-amplified from each as described above, by using a pair of 19-mer oligonucleotide primers, 5-CATCAGTTTGCCAGTTGTG-3′/5′-TTTATAATCTAGCATTGCC-3′, derived from the *KIT* genomic nucleotide sequence (unpublished data). The products of the amplification reactions were cleaved with *Mnl* I, electrophoresed through 6% polyacrylamide gels, and scored for the presence of the codon-664 mutation. Linkage was assessed by determination of logarithm of odds (lod) scores using version 3 (1987) of the LIPED program (26), assuming complete penetrance and an estimated frequency of 10^{-4} for piebaldism.

RESULTS

Nucleotide Sequence Analysis of the *KIT* Gene of the Proband. Southern blot analysis of the proband's DNA, using KIT cDNA and genomic fragments as probes, identified no *KIT* gene deletion or other rearrangement (data not shown). Therefore, all 21 KIT-coding exons, plus adjacent portions of the intervening and 5′ and 3′-untranslated and -flanking sequences, were amplified by PCR and sequenced from DNA of the proband. This analysis demonstrated only a single difference from the normal gene, a GGG → AGG substitution within codon 664, the last base of coding exon 13 (Fig. 2). This results in a Gly → Arg substitution in the tyrosine kinase domain of KIT. As expected for an autosomal dominant disorder, the proband was heterozygous for this change.

To determine whether the codon-664 substitution is a common nonpathologic polymorphism, we took advantage of the fact that this substitution eliminates a *Mnl* I restriction enzyme cleavage site (5′-GAGG-3′/5′-CCTC-3′). PCR fragments (250 bp) of KIT-coding exon 13 were amplified from DNA of 22 unrelated normal individuals of northern European Caucasian ethnic origin, cleaved with *Mnl* I, and analyzed by gel electrophoresis. All exhibited only the normal pattern (data not shown). To determine whether the codon-664 substitution is a nonpathologic polymorphism restricted to the Ashkenazi Jewish population, we also analyzed *KIT* exon 13 PCR fragments amplified from DNA of 17 unrelated normal individuals of Ashkenazi Jewish ethnic origin. As shown in Fig. 3, the proband exhibited an equal mixture of the normal pattern (99-bp, 89-bp, and 62-bp fragments) plus the abnormal pattern (161-bp and 89-bp fragments), consistent with his being heterozygous for the codon-664 substitution. In contrast, all of the normal individuals exhibit only the normal pattern. Together, these data indicate that the codon-664 substitution is not a common polymorphism, in either the general northern European Caucasian or Ashkenazi Jewish populations.

Genetic Linkage Analysis. To determine whether the codon-664 mutation is genetically linked to piebaldism in the proband's family, we amplified coding exon-13 fragments by PCR of DNA from both normal family members and those affected with piebaldism. The amplified exon-13 fragments were then analyzed by *Mnl* I cleavage, as described above (data not shown). As illustrated in Fig. 1, there was perfect concordance between heterozygosity for the codon-664 mutant allele and the piebald phenotype in this family; all affected family members exhibited an equal mixture of both the normal and abnormal fragments, and all normal individuals exhibited only the normal fragments. Two-point linkage analysis, performed with the LIPED program (27), yielded a maximal logarithm of odds (lod) score of 6.02 at $\theta = 0$. These data thus strongly support the hypothesis that piebaldism in this family is tightly linked to the *KIT* protooncogene.

DISCUSSION

Piebaldism is a rare autosomal dominant disorder characterized by congenital patches of skin and hair in which pigment is entirely lacking. The characteristic nonpigmented patches, most prominent over ventral surfaces of the face, chest, trunk, and extremities, and the white forelock are present at birth and do not change over time. We have shown that in the study family, piebaldism is linked to a missense substitution at codon 664 of the *KIT* protooncogene, which encodes the cell-surface receptor for mast/stem cell growth factor (12–15). This mutation, within the tyrosine kinase domain of *KIT*, is similar to those observed in *W* mutant mice (9–11) and exhibited complete genetic linkage to the piebald phenotype in this family. Furthermore, we did not observe this mutation among normal individuals, indicating that it is not a common polymorphism. Recently, we studied three additional families with piebaldism and identified three different *KIT* gene

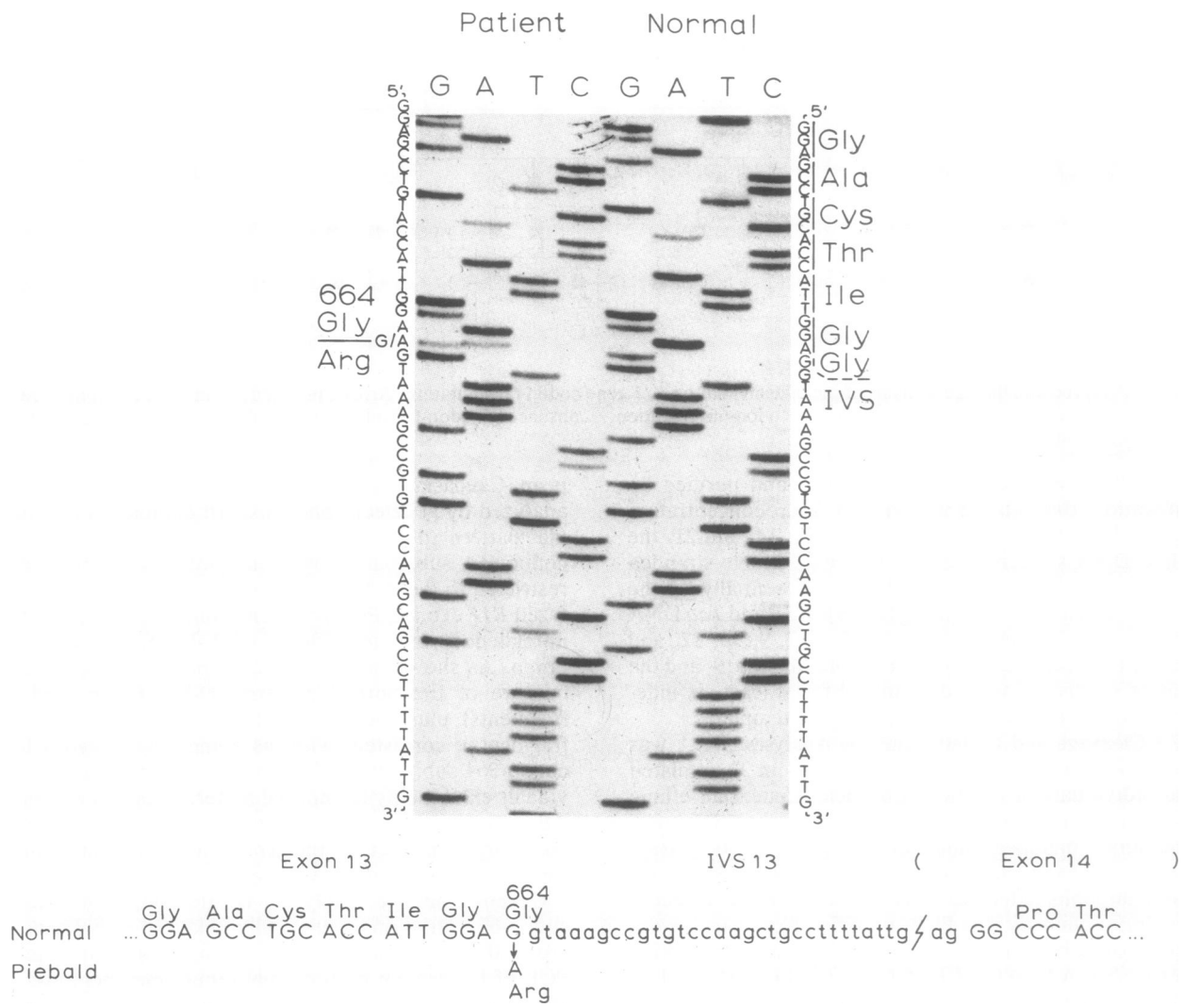

FIG. 2. Sequences in region of the codon-664 mutation. (*Upper*) DNA sequence of coding exon 13 was determined directly, without cloning; the sequence analysis thus sampled both *KIT* alleles simultaneously. (*Lower*) Sequence of coding strand. Lowercase letters indicate intervening sequence (IVS). Part of sequence of coding exon 14 is shown to aid interpretation.

mutations, a missense substitution and two frameshifts that are completely linked to piebaldism (unpublished data). It is thus apparent that in these families mutations of the *KIT* protooncogene constitute the molecular basis of piebaldism, which thus represents the human homologue of dominant white spotting (*W*) of mice.

The c-*kit* gene, originally identified in the genome of the HZ4-feline sarcoma virus isolated from a feline leukemia virus-associated feline fibrosarcoma (28), encodes a 976-amino acid polypeptide consisting of a hydrophobic leader, an extracellular ligand-binding domain, a hydrophobic transmembrane region, and an intracellular tyrosine kinase domain and hydrophilic carboxyl-terminal tail (20). The codon-664 Gly → Arg substitution, within the tyrosine kinase domain, changes a neutral hydrophobic amino acid to a charged hydrophilic residue and, thus, could be expected to have a very deleterious effect on function of the resultant KIT polypeptide. The biological action of the KIT receptor most likely requires dimerization in response to ligand binding (for review, see ref. 29); therefore, the cellular response to ligand binding may be decreased by ≈75% in individuals heterozygous for *KIT* missense substitutions in the tyrosine kinase domain.

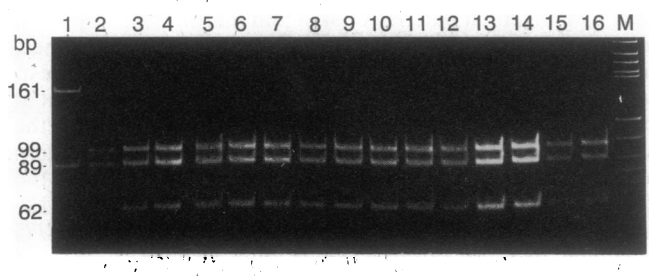

FIG. 3. *Mnl* I cleavage analysis of the *KIT* codon-664 mutation in normal Ashkenazi Jewish individuals. Exon 13 was amplified by PCR and cleaved with *Mnl* I. Lanes: 1, proband; 2–16, 15 unrelated normal Ashkenazi Jewish individuals; M, molecular-size standard (pBR322 digested with *Bst*NI plus *Hae* III).

KIT expression has been documented in cells of the melanocytic, mast-cell and erythroid, and germ-cell lineages and in the brain (20, 24, 30), and *W* mutant mice exhibit pleiotropic, yet specific, cell-autonomous defects of melanogenesis, hematopoiesis, and germ-cell development. For example, mice heterozygous for the W^v and W^{55} mutant alleles, which both involve Thr → Met substitutions at codon 660 of *Kit* (9, 10), only four amino acids away from the human codon-664 substitution reported here, exhibit a phenotype of dominant white spotting, mild anemia, mast-cell deficiency, and normal fertility. Affected members of this human kindred with the codon-664 mutant allele exhibit dominant white spotting, with no anemia and apparently normal fertility.

White spotting in human piebaldism results from the absence of melanocytes from the nonpigmented patches of skin (31) and from hairbulbs in the white patches of hair (32), apparently because of defective migration of melanoblasts from the neural crest to the epidermis during development. Humans (33, 34) and mice (35) with piebaldism and other white spotting disorders, such as Waardenburg syndrome, occasionally also lack ganglion cells of the intestinal enteric neural plexus, which, like melanoblasts, are derived embryologically from the neural crest. In this regard, it is perhaps noteworthy that several affected members of this study family reported lifelong severe constipation, possibly the result of defective migration of enteric-plexus ganglion cells from the neural crest to the gut.

The *KIT* gene product is one of a family of transmembrane tyrosine kinase receptors that also include the platelet-derived growth factor receptor α and β chains and the colony-stimulating factor 1 receptor (20). The genes encoding platelet-derived growth factor receptor β chain and colony-stimulating factor 1 receptor (c-*fms*) are closely linked in chromosome segment 5q31–q33 (36, 37). Similarly, the platelet-derived growth factor receptor α chain and *KIT* genes both map near each other in chromosome segment 4q11–q12 (20, 21, 38, 39). Deletion of the platelet-derived growth factor receptor α locus has recently been associated with another dominant white spotting disorder in mouse, called patch (*Ph*) (40), and a third dominant white spotting disorder of mouse, rump-white (*Rw*), is very closely linked genetically to both *W* and *Ph*. Accordingly, it is not unlikely that other human genetic disorders with phenotypes of piebaldism-like white spotting may also be associated with defects of these loci in humans.

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