MUTATION IN BRIEF

Human Piebaldism: Six Novel Mutations of the Proto-oncogene KIT

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Communicated by Mark H. Paalman

Human piebaldism is a rare autosomal dominant disorder that comprises congenital patchy depigmentation of the scalp, forehead, trunk and limbs. It is caused by mutations in the cell-surface receptor tyrosine kinase gene (KIT, also c-kit). We screened three families and three isolated cases of piebaldism from different countries for mutations in the KIT gene using automated sequencing methods. We report six novel KIT point mutations: three missense (C788R, W835R, P869S) at highly conserved amino acid sites; one nonsense (Q347X) that results in termination of translation of the KIT gene in exon 6; and two splice site nucleotide substitutions (IVS13+2T>G, IVS17–1G>A) that are predicted to impair normal splicing. These mutations were not detected in over 100 normal individuals and are likely to be the cause of piebaldism in our subjects. © 2002 Wiley-Liss, Inc.

KEY WORDS: Piebaldism; proto-oncogene; KIT; mutation screen

INTRODUCTION

Piebaldism (MIM# 172800) is a rare genetic disorder of the development of melanocytes. In humans piebaldism is inherited in an autosomal dominant mode and is characterised by congenital white patches of skin and hair which entirely lack pigment (Spritz, 1994). Depigmented areas are mainly found on the scalp, forehead, trunk and limbs. Melanocytes are not present in such areas of leukoderma and therefore it is thought that piebaldism is due to defective melanoblast proliferation or migration from the neural crest during early development. Mutations in the KIT proto-oncogene (MIM# 164920) have been identified as the cause of human piebaldism (Spritz, 1994).

KIT encodes the cell-surface receptor tyrosine kinase for the mast/stem cell growth factor (Giebel et al., 1992; Vandenbark et al., 1992). The KIT receptor consists of three main domains: extracellular, transmembrane and cytoplasmic, with the latter containing the highly conserved tyrosine-specific kinase activity and ATP binding sites (Yarden et al,. 1987). A variety of studies have reported KIT point mutations and deletions in piebald individuals (Giebel and Spritz, 1991; Fleischman et al., 1991; Fleischman, 1992; Spritz et al., 1992a; Spritz et al., 1992b;

Received 29 April 2002; accepted revised manuscript 25 June 2002.

DOI: 10.1002/humu.9057

Spritz et al., 1993; Ezoe et al., 1995; Riva et al., 1995; Fleischman et al., 1996; Nomura et al., 1998; Richards et al., 2001). Furthermore, we have recently reported three novel mutations in two families and one isolated case (Syrris et al., 2000). The severity of phenotype depends on the location of the causative mutation with those in the intracellular tyrosine kinase domain having a "dominant-negative" effect resulting in severe manifestation of the disease (Spritz, 1994).

In this study, we investigated three familial and three isolated cases of piebaldism from different countries and have identified six novel mutations in the KIT gene.

MATERIALS AND METHODS

Study subjects

Patient 1 was the proband of a large Italian pedigree with multiple affected individuals. They presented with cutaneous areas of hypopigmentation with interspersed spots of hyperpigmentation, mainly involving the limbs, and white hair since birth.

Patient 2 was a 3-year old female with typical piebaldism. She had a white forelock, a V-shaped hypopigmented patch on her forehead and hypopigmented skin on her trunk and knees. Hearing tests were normal. Both her parents were unaffected.

Patient 3 had typical pigmentary changes of severe piebaldism. She had generalised pigmentary disturbances on her trunk and on her lower limbs. There was a central white forelock above a V-shaped area of hypopigmentation on her forehead whilst the medial aspects of her eyebrows are white. Both her parents were unaffected.

Patient 4 was the proband of a British family with piebaldism. Several family members showed a white forelock and characteristic depigmentation of lower limbs.

Patient 5 was an 11-year old girl from Turkey referred with typical manifestations of severe piebaldism. She was the third child from healthy non-consanguineous parents. Her two older brothers were also unaffected. There was no family history of heterochromia, dystopia canthorum or abnormal skin pigmentation. She had a white forelock, poliosis of eyebrows and eyelashes and extensive areas of depigmentation on the trunk and limbs with small islands of hyperpigmentation which superficially resembled café-au-lait patches. Extensive tests showed normal EEG, cranial MRI, acoustic canals and cranial nerves morphology with a 15% hearing loss in her right ear.

Patient 6 was a male adult from a Belgian 4-generation family with typical manifestations of piebaldism. Affected members had delineated areas of dipigmentation, mostly on the trunk whilst a white forelock was present only in some of them. There was no associated deafness.

DNA Sequencing analysis

All exons of the *KIT* gene were amplified by PCR using primer pairs detailed previously (Spritz et al., 1992a). PCR fragments were subjected to direct DNA sequencing as described before (Syrris et al., 2000). Sequence variants were independently re-amplified and sequenced twice in both directions (sequencing data reviewed but not shown).

RESULTS AND DISCUSSION

Direct sequencing of the proto-oncogene KIT in piebald patients revealed six novel point mutations (Table 1). In particular, Patient 1 was found to be heterozygous for a novel missense mutation 2362T>C in exon 17. This mutation leads to the substitution of a highly conserved cysteine by an arginine at codon 788 (C788R). The presence of the mutation was confirmed in other affected individuals of her family whilst it was absent in normal members. Patient 2 harbored a de novo mutation in exon 18 (2503T>C) which results in a tryptophan/arginine change at codon 835 (W835R). Both her parents were homozygous for the wild type sequence. Similarly, Patient 3 was heterozygous for a de novo mutation in exon 19 (P869S) whilst both her parents were homozygous for the wild type allele. All three missense mutations affect amino acids in the intracellular tyrosine kinase domain of KIT at highly conserved positions amongst protein-tyrosine kinase family members (Yarden et al., 1987).

Patient 4 had a nonsense mutation in exon 6 that leads to premature end of translation of the KIT receptor at codon 347 (Q347X). His extended family comprised several affected relatives including his brother and son but

none were available for genetic screening. Patients 5 and 6 were heterozygous for two novel mutations affecting splice site junctions of the KIT gene. In more detail, Patient 5 had a de novo transversion in intron 13 (donor site, IVS13+2T>G). Both her parents were normal. Patient 6 had a transition in intron 17 (acceptor site, IVS17-1G>A) which was confirmed in other affected family members. Both splice site mutations were evaluated with SpliceView, a freely available computer program that predicts and analyses the exon/intron structure of protein-coding genes (http://125.itba.mi.cnr.it/~webgene/wwwspliceview.html). This program scores potential splice sites by using weight matrix as described by Shapiro and Senapathy (1987), with high scores generally reflecting a greater potential for a particular sequence to act as a splice site. The scores for the wild type sequence for the splice junctions for exon 13/ intron 13 and intron 17/ exon 18 were 83 and 87 respectively. In contrast, both mutant forms completely abolish the potential of the sequence to act as a splice signal (no scores generated). This strongly suggests that some kind of abnormal splicing (for example exon skipping) may take place resulting in a non-functional form of the KIT receptor.

In summary, we have shown the presence of six novel KIT mutations which were not found in 100 healthy individuals screened by the same methods. The pathogenicity of mutations C788R, W835R, P869S can be explained by the fact that they are located at conserved parts of the cytoplasmic domain where a number of mutations have been detected before (Spritz, 1994). Mutation Q347X is clearly pathogenic as it causes truncation of KIT translation. Finally, the two splice junction mutations are predicted to drastically reduce the efficiency of production of the KIT protein. Furthermore, the deleterious nature of mutations W835R, P869S and IVS13+2T>G is supported by their de novo origin in Patients 2, 3 and 5 respectively. These findings confirm the involvement of KIT in human piebaldism and contribute to the diversity of mutations in this gene responsible for the condition.

Table 1. Novel KIT Mutations in Patients with Piebaldism

Patient	Exon/Intron	Nucleotide change (Codon change)	Mutation Result	Position in protein	Inheritance
1	Exon 17	2362 T>C (TGT788CGT)	C788R	Cytoplasmic domain	Familial
2	Exon 18	2503T>C (TGG835CGG)	W835R	Cytoplasmic domain	De novo
3	Exon 19	2605C>T (CCC869TCC)	P869S	Cytoplasmic domain	De novo
4	Exon 6	1039C>T (CAG347TAG)	Q347X	Extracellular domain	Familial
5	Intron 13	IVS13+2T>G	Abnormal splicing		De novo
6	Intron 17	IVS17-1G>A	Abnormal splicing		Familial

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