A germline mutation in KIT in familial diffuse cutaneous mastocytosis

X Tang, M Boxer, A Drummond, P Ogston, M Hodgins, A D Burden

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mastocytosis (MIM 154800) is a group of rare disorders which have in common an abnormal accumulation of mast cells in specific organs, including gastrointestinal tract, bone marrow, liver, spleen, lymph nodes, and skin, which is the most frequently affected organ.1 Based on the pattern of involvement, cutaneous mastocytosis (CM) is classified into solitary mastocytoma, urticaria pigmentosa, telangiectasia macularis eruptiva perstans, and diffuse cutaneous mastocytosis (DCM). DCM is the least common form of CM and usually develops early in life. It is characterised by an extensive erythroderma as a result of diffuse infiltration of the dermis by mast cells. CM usually occurs as a sporadic disease. It has become apparent over the past few years2 that the majority of adults with CM have a somatic mutation (D816V or V560G) in the receptor tyrosine kinase, KIT (MIM 164920). These somatic mutations have not however been detected in typical childhood CM or in the germline in the rare instances of familial CM reported.2 3

We describe a family in which DCM affects three generations. We found linkage to chromosome 4 and a novel germline mutation in KIT (A533D) in affected family members.

METHODS
Family
The family pedigree is illustrated in figure 1. The proband (IV:1) was born at term after an uneventful pregnancy. At 4 months of age he developed generalised pruritus and intermittent blistering on his scalp. He gave no history of wheezing, flushing, or diarrhoea. At the age of 9 months he intermittently developed groups of intermittent blistering on his scalp. He gave no history of thickening and pigmentation especially of flexural sites. He had dermographism on the trunk (fig 2) and erosions and wheezing, flushing, or diarrhoea. At the age of 25 years he presented with appendicitis and was found to have mast cell infiltration of the appendix and also the bone marrow. He has since remained well with no further haematological or gastrointestinal symptoms.

The father of the proband (III:2) developed blisters on his scalp and posterior trunk (fig 3). A solitary yellow-brown papule, similar in appearance to a solitary mastocytoma, was present on his arm and remained unchanged over 2 years (fig 4). Physical examination was otherwise unremarkable. In particular there were no cutaneous pigmentary abnormalities and no organomegally. Full blood profile, serum biochemical profile, immunoglobulins, and ultrasound scan of the abdomen were normal. An examination of the bone marrow was not undertaken. Biopsy of both clinically normal and involved skin revealed a dense infiltrate of mature mast cells in the upper dermis (fig 5). Treatment has included topical steroids and oral antihistamines with partial control of his symptoms.

The father of the proband (III:2) developed blisters on his trunk and scalp at the age of 4 months that resolved by the age of 2 years. His skin remains itchy, dry, and diffusely thickening with ill-defined flexural pigmentation. At the age of 25 years he presented with appendicitis and was found to have mast cell infiltration of the appendix and also the bone marrow. He has since remained well with no further haematological or gastrointestinal symptoms.

The paternal uncle (III:1), paternal grandfather (II:1), and paternal great aunt (II:2) were also diagnosed as having cutaneous mastocytosis in infancy, presenting with blistering of the trunk and scalp. They developed diffuse cutaneous thickening and pigmentation especially of flexural sites associated with pruritus and dermographism. None have evidence of systemic involvement and no family member was known to have a gastrointestinal stromal tumour (GIST).

DNA extraction
Genetic studies were approved by the local ethics committee and all subjects gave informed consent. DNA was extracted from peripheral blood mononuclear cells of affected family members and three unaffected relatives. To exclude contamination with DNA from circulating mast cells, DNA was also extracted from buccal smears, which do not contain mast cells. DNA was extracted from tissue mast cells of the proband by laser capture microdissection (LCM).

Linkage study
All available family members were genotyped using seven tandem repeat markers spanning 8 cM of chromosome 4q12 around KIT. The markers and their approximate genetic distance from the P terminus were D4S2971 (61.42 cM), D4S2996 (63.58 cM), D4S1630 (63.58 cM), D4S428 (64.28 cM), D4S2916 (65.81 cM), D4S3000 (68.94 cM), and D4S1583 (68.94 cM).

DNA sequencing
A total of 18 sets of primers (see table) were used to amplify the entire coding region of KIT and exon—intron boundaries. A 173 bp fragment flanking codon 576 in the interleukin-4 receptor α-chain was amplified with IL-4RA primer pair (see table).

RESULTS
Sequencing of KIT codons 816 and 560
Sequencing of the KIT targeted areas within the region encoding codon 816 and codon 560 excluded the presence of the common mutations D816V and V560G in the genomic DNA of the proband. DNA from the cutaneous mast cells

Abbreviations: CM, cutaneous mastocytosis; DCM, diffuse cutaneous mastocytosis; GIST, gastrointestinal stromal tumour; LCM, laser capture microdissection
isolated by laser microdissection similarly revealed wild-type sequence in the DNA encoding these two codons.

**Linkage analysis**

Using the GENEHUNTER program, the maximal non-parametric LOD score around the KIT locus was 2.3 (p = 0.03). The shared haplotype identified in this family segregated with the disease (fig 1A).

**Mutation analysis**

The entire coding region, plus flanking sequences, of KIT was sequenced in extracted genomic DNA of the proband. A cytosine to adenine transversion was identified in exon 10 at position 1619 resulting in an alanine to aspartic acid substitution at codon 533 of the transmembrane domain of KIT. Genomic DNA of the KIT exon 10 was sequenced from the other seven available family members (four affected and three unaffected). The 1619 C>A change is a known polymorphism. (B) A single AluI site is present in the product amplified from the wild type resulting in 157 and 353 bp products. The A1619C change abolishes that AluI site so that the heterozygotes also have an undigested 510 bp product.
tyrosine kinase activity. Various somatic mutations causing constitutive activation of KIT have been associated with disease, most notably acute myeloid leukaemia, myelodysplastic syndrome, sino-nasal lymphoma, seminomas, and GIST. The mutations tend to cluster in two regions. Those in exon 11 contained in the juxtamembrane region are associated with GIST, whereas exon 17 mutations of D816 to either V or H in the second half of the kinase domain are associated with mast-cell/myeloid leukaemias and seminomas, respectively. In 1993 two heterozygous activating mutations in the cytoplasmic domain of KIT (D816V or V560G) were first identified in a human mast cell line derived from a patient with mast cell leukaemia. Subsequently, all sporadic adult mastocytosis patients examined have been found to carry this somatic mutation regardless of the classification or the prognosis of their disease. However these mutations have been found only rarely in children with progressive mastocytosis and not in familial mastocytosis.

We have now identified a new germline mutation in the transmembrane domain of KIT (KIT-R53D) in members of a family with DCM. The transmembrane domain consists of 23 amino acids and is highly conserved across species. The A533D substitution is not conservative, in that a hydrophilic amino acid is substituted for a hydrophobic amino acid and we postulate that this will alter the properties of the transmembrane alpha helix. Only one point mutation in the transmembrane domain of KIT (I530V) has previously been identified, in a patient with acute myeloid leukaemia. However, point mutations in the transmembrane domains of two related receptor tyrosine kinases, neu and fibroblast growth factor receptor 3 (FGFR3), have been identified and lead to constitutive activation in the absence of ligand. The FGFR3 substitution is not conservative, in that a hydrophilic amino acid is substituted for a hydrophobic amino acid and we postulate that this will alter the properties of the transmembrane helix. Only one point mutation in the transmembrane domain of neu (G380R) in human FGFR3 has been found in individuals with achondroplasia and results in constitutive FGFR3 activation. Importantly, it has been reported that substitution of the transmembrane domain of neu with the mutant human FGFR3 (G380R) transmembrane domain results in ligand-independent activation of neu, suggesting a common mechanism for receptor activation. Furthermore, the constitutive activation of neu by another mutation in the transmembrane domain (V664E) has also been postulated to facilitate dimerisation. The interaction between the two alpha helical transmembrane domains is stabilised by intermolecular hydrogen bonding between the side chain of E664 in one receptor and the carbonyl oxygen of A661 in another receptor. The V664E mutation in neu is similar to A533D in KIT, in that a
Online mutation report raises the possibility of developing selective treatment using KIT mutation we have reported in the

These findings support our hypothesis that the A533D hydrogen bonding are included at appropriate positions. Zhou et al investigated the ability of polar side chains (N, D, and T) to mediate transmembrane helix association. Further studies have shown that D induces the strongest homo-oligomeric association. These findings support our hypothesis that the A533D mutation we have reported in the KIT transmembrane domain induces ligand-independent dimerisation and activation of KIT by forming hydrogen bonds between receptor monomers.

In summary we have demonstrated a novel germline mutation in KIT by forming hydrogen bonds between receptor monomers.

**Table**

<table>
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<tr>
<th>Primer name</th>
<th>Oligonucleotide</th>
<th>Position</th>
<th>Exon position</th>
<th>Exon number</th>
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<tr>
<td>KIT exon1F</td>
<td>TCCATATGTCCAGTTGCATAGC</td>
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<td>85364–85469</td>
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<td>KIT exon1R</td>
<td>CCACATTTCAGCAACAGCAG</td>
<td>84594–85092</td>
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<td>KIT exon1F</td>
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<tr>
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<td>6033–6053</td>
<td>6247–6334</td>
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</table>

In our study we have demonstrated a novel germline mutation in KIT in DCM. To our knowledge, this is the first report of familial DCM affecting three generations and the first report of a germline KIT mutation in this condition. There have been two reports of germline KIT mutations in families in which GISTs were inherited. An Italian family has been described in which the mother had multiple GIST and her son had urticaria pigmentosa. Both were found to have a germline V559A mutation in KIT. The therapeutic efficacy of the tyrosine kinase inhibitor, Imatinib, in GIST19 raises the possibility of developing selective treatment using tyrosine kinase antagonists in some forms of cutaneous mastocytosis.

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Conflict of interest: none declared.

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