ONLINE MUTATION REPORT

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 years[2] 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.

Key points

- Five cases of cutaneous mastocytosis occurring in three generations of a family are described.
- A germline mutation was identified in the transmembrane domain of KIT (A533D). This amino acid change is predicted to induce ligand-independent dimerisation and activation of KIT.

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 had dermographism on the trunk (fig 2) and erosions and crusting of the scalp. He intermittently developed groups of 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

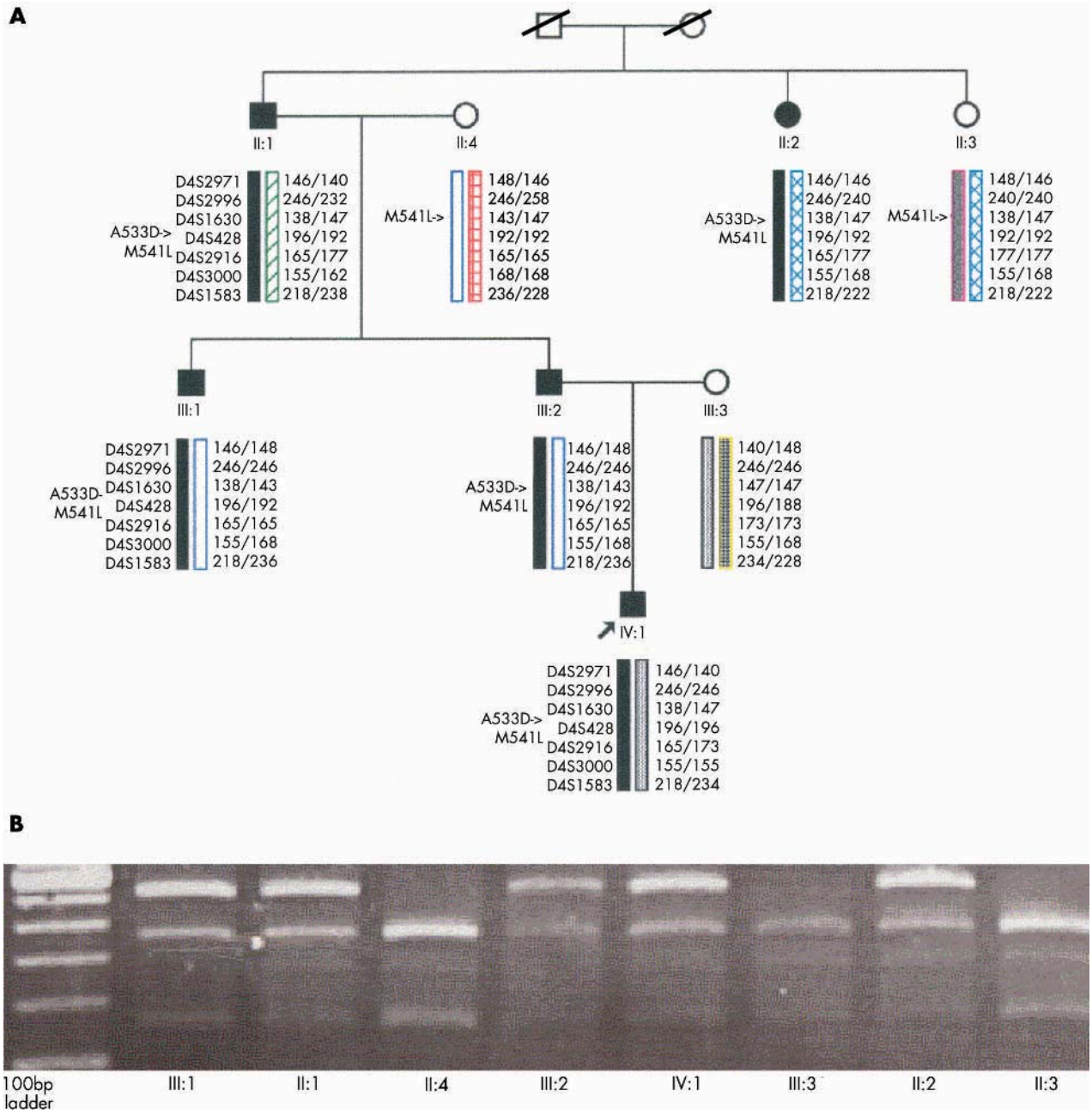

Figure 1 (A) Illustrated is the pedigree of the family, with filled squares (male) and circles (female) indicating individuals with DCM and open squares and circles indicating unaffected individuals. The subject number is indicated below each symbol. Listed to the left of subjects II:1 and III:1 are the microsatellite markers used to genotype the family. Below each subject are indicated the allele numbers for each microsatellite marker observed for the two chromosome 4q12 haplotypes inherited by each individual. The disease haplotype is blackened. A533D is the mutation identified in this family. M541L 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 obliterates that AluI site so that the heterozygotes also have an undigested 510 bp product.

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 transversion was found in all affected patients but not in unaffected individuals. The germline nature of the mutation was confirmed by the presence of A533D in DNA extracted from buccal washings of the proband and his affected father. The presence of the mutation was confirmed in all affected family members by restriction enzyme digestion and gel electrophoresis (fig 1B).

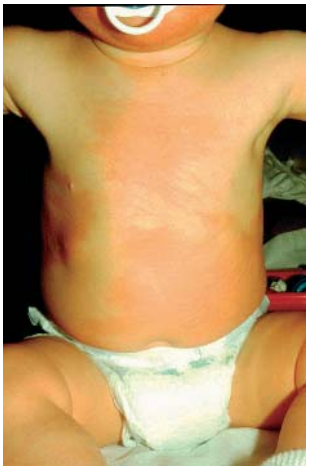

Figure 2 Marked dermographism on the proband at the age of 10 months.

To exclude the possibility that the 1619 C>A transversion was a common polymorphism, the genomic DNA from 56 consented unrelated healthy controls was analysed. The mutation was not found in any of the 112 chromosomes.

A previously reported polymorphism (M541L) found in the DNA encoding the KIT transmembrane domain was detected during the analysis.[4] This mutation was present in all affected family members but also in two unaffected individuals (II:4 and II:3). It is therefore unlikely to contribute to the disease in this family.

A Q576R gain-of-function change in the IL-4R α-chain that is associated with atopy has been suggested to modify the severity of cutaneous mastocytosis by making mast cells relatively resistant to the effects of activating mutations in *KIT*.[5] Sequence analysis was used to identify whether Q576R was present in family members. Of the five affected individuals analysed, Q576R was present in II:1 and II:2 but not in the other three affected individuals. There was no clear difference in disease severity between those with and without Q576R although the only individual with known systemic disease (III:2) lacked the polymorphism.

DISCUSSION

The proto-oncogene *KIT* encodes a class III receptor tyrosine kinase. In common with other receptors of this class, it is characterised by an extracellular ligand binding domain, a single transmembrane domain, a juxtamembrane domain, and two intracellular kinase domains. Binding of its ligand leads to dimerisation of the receptor and activation of 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.[6 7] 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.[8] 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.[9] 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.[2] However these mutations have been found only rarely in children with progressive mastocytosis and not in familial mastocytosis.

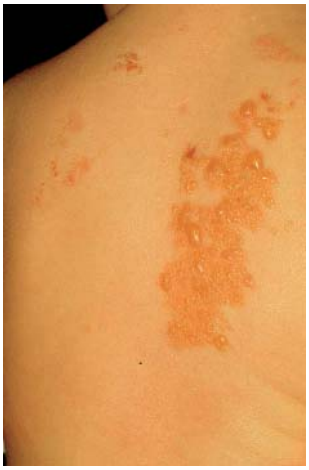

Figure 3 Grouped subepidermal blisters on the trunk of the proband at the age of 10 months.

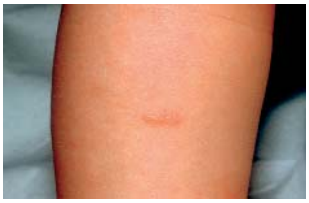

Figure 4 Single yellow-brown oval papule on forearm of proband. The appearance suggests a solitary mastocytoma.

We have now identified a new germline mutation in the transmembrane domain of *KIT* (KIT^{A533D}) 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.[10] 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 transmembrane domains of receptor tyrosine kinases have been shown to play a critical role in the regulation of receptor dimerisation and activation.[11] The mutation G380R in human *FGFR3* is found in individuals with achondroplasia and results in constitutive FGFR3 activation.[12 13] 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,[13] 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

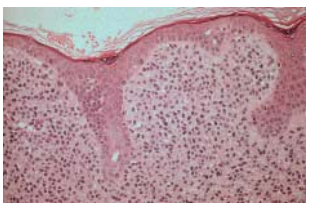

Figure 5 Histology of skin biopsy from clinically unaffected skin of upper back from the proband. Throughout the dermis there is a dense infiltrate of mast cells. (haematoxylin and eosin, original magnification ×200).

Table Primers used for DNA sequencing of *KIT* and *IL4-RA*

Primer name	Oligonucleotide	Position	Exon position	Exon number
KIT exon1F	GCATTAACACGTCGAAAGAGC	6033–6053	6247–6334	1
KIT exon1R	GTCCTCTCTCCGGATGCAC	6411–6430		
KIT exon2F	GTGCTTTATTTCGCCAAGGA	43708–43727	43768–44037	2
KIT exon2R	CCTTCTAGACCCAGCCAGAA	44137–44156		
KIT exon3F	GGGCCACTAGTCATGAAAGG	46435–46454	46537–46818	3
KIT exon3R	GGTGGATCAACGAGAAGAGAA	46877–46897		
KIT exon4F	TTGCTGGTACCTTCAGATATG	47714–47734	47883–48019	4
KIT exon4R	AGTCGAGGCAGTTTCAGGAC	48094–48113		
KIT exon5F	TGGAGAAGTTAATTGCTGCTA	51872–51892	51976–52144	5
KIT exon5R	CATTGCAAGAGGCTACAAGGA	52279–52299		
KIT exon6F	GGAAATCAACCAATTGTTTTTG	55263–55284	55349–55538	6
KIT exon6R	CGTGGATTTACGGGTTACAGA	55609–55629		
KIT exon7F	CGTTGGTCCCAGATGGAATA	57582–57601	57673–57788	7
KIT exon7R	TGCAGCTGTTACAAACACGA	57839–57858		
KIT exon8F	CCTTTGAACTTGCTCCCTCA	71696–71715	71830–71944	8
KIT exon8R	AAAGCCACATGGCTAGAAAAA	72061–72081		
KIT exon9F	AAGTATGCCACATCCCAAGTG	74025–74045	74103–74284	9
KIT exon9R	TGACATGGTCAATGTTGGAA	74355–74374		
KIT exon10,11F	GGCTGTGAGTTGGGAGGTG	75363–75381	75464–75570	10
KIT exon10,11R	AACAAAGGAAGCCACTGGAG	75852–75871	75662–75788	11
KIT exon12,13F	ATGGTCCTTCAATTCCACCA	76006–76025	76069–76173	12
KIT exon12,13R	AATCTAGCATTGCCAAAATC	76430–76450	76257–76367	13
KIT exon14F	TGACCACCCTTGGGTATTTT	77465–77484	77581–77731	14
KIT exon14R	GCCTTGATTGCAAACCCTTA	77785–77804		
KIT exon15F	AGGGGATGAGGAGGTAGAGC	79496–79515	79574–79665	15
KIT exon15R	CCATTGGCACTGCTACCATA	79778–79797		
KIT exon16F	GATCTGCCTGCAAGTTCACA	79996–80015	80117–80244	16
KIT exon16R	TGGCTCTAAAATGCTCTGTTC	80320–80340		
KIT exon17F	TGAACATCATTCAAGGCGTA	81142–81161	81317–81439	17
KITexon17R	TGTTCAGCATACCATGCAAA	81587–81606		
KITexon18,19F	CCACATTTCAGCAACAGCAG	84594–85092	84687–84798	18
KITexon18,19R	ACCCTCAACATCTGGGTTTC	85073–85092	84910–85009	19
KITexon20F	TCCATATGTCCAGTTGCATAGC	85249–85270	85364–85469	20
KITexon20R	GCCCAATTTGCAACCTAAGA	85579–85598		
KITexon21F	GGCCACAAAGTTCTTGGAAA	86479–86498	86617–86745	21
KITexon21R	AGAAAGACAGGATTGCAGTGG	86856–86876		
KIT816F	GCAGCCAGAAATATCCTCCTTA			
KIT816R	GCAGGACTGTCAAGCAGAGAATG			
KIT560F	ATCGTAGCTGGCATGATGTG			
KIT560R	GCCACTGGAGTTCCTTAAAGTC			
IL-4RAF	GAAACCTGGGAGCAGATCCT			
IL-4RAR	GCCTTGTAACCAGCCTCTCC			

hydrophobic amino acid side chain in the transmembrane domain is substituted by an acidic side chain. It has been shown that an apparently complex transmembrane domain can be substituted by simple sequences of repeated residues that will dimerise, provided that amino acids capable of hydrogen bonding are included at appropriate positions.[14] Zhou *et al* investigated the ability of polar side chains (N, D, Q, E, H, S, Y, and T) to mediate transmembrane helix association and demonstrated that D, Q, and E induce strong helix association.[15] Further studies have shown that D induces the strongest homo-oligomeric association.[16 17] 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* 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.[18 19] 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*.[20] The therapeutic efficacy of the tyrosine kinase inhibitor, Imatinib, in GIST[21] raises the possibility of developing selective treatment using tyrosine kinase antagonists in some forms of cutaneous mastocytosis.

Authors' affiliations

X Tang, M Boxer, Department of Medical Genetics, University of Glasgow, Glasgow G11 6NT, Scotland, UK
A Drummond, M Hodgins, A D Burden, Department of Dermatology, University of Glasgow, Glasgow G11 6NT, Scotland, UK
P Ogston, Department of Pathology, University of Glasgow, Glasgow G11 6NT, Scotland, UK

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Correspondence to: Dr A D Burden, University Department of Dermatology, Western Infirmary, Glasgow G11 6NT, Scotland, UK; david.burden@northglasgow.scot.nhs.uk

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