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A c-kit Mutation in Exon 18 in Familial Mastocytosis

Journal of Investigative Dermatology (2013) **133,** 839–841; doi:10.1038/jid.2012.394; published online 29 November 2012

TO THE EDITOR

Mastocytosis is a benign tumor with tumor cells usually located in the skin and/or bone marrow. It is often caused by a spontaneous mutation in the exon 17 (D816V) of the *c-kit* gene, which has been included in the 2007 WHO classification system of mastocytosis (Valent *et al.*, 2007 #5458). Inherited forms of mastocytosis are rare.

Eight members of a single family presented to a private allergy outpatient clinic with a history of flushing and vertigo owing to unspecific triggers, such as physical exercise or showering. Four of them (Figure 1) had brown-yellowish papules that responded with a wheal-and-flare reaction to mechanical triggers (positive Darier's sign). The serum tryptase levels in all family members were far in the normal range ($<<11.4\,\mu g\,dl^{-1}$, compared in the Supplementary Table S1 online). The presenting family members were two sisters and one brother (Figure 1: individuals III_2, III_6, and III_4). All of them had symptomatic children as well (IV_1, IV_2, IV_5, and IV_6). Patient IV_1 was the most severely affected individual, with attacks of severe genital angioedema up to twice a week. Skin punch biopsies from patient III_6 and her niece IV_1 affirmed the clinical diagnosis cutaneous mastocytosis (CM) (Figure 2a) with a condition suspicious of mast cell activation syndrome (sMCAS, (Hamilton *et al.*, 2011 #5461; Valent *et al.*, 2012 #9305)) consisting in episodes of severe hypotension, flushes, and angioedema. The patients were diagnosed as suffering from CM with sMCAS.

A more detailed analysis of the family's pedigree revealed that more ancestors also suffered from a sMCAS with episodes of flushing, fainting, and/or diarrhea (I_1 and II_2 in Figure 1), all of whom responded well to symptomatic therapy with H1-antihistamines. The mother (II_2) and the grandfather (I_1) both reported having suffered from brownish papules in their youth that had spontaneously dissolved when they had reached their 30s. Nevertheless, they occasionally still suffered from sMCAS owing to unspecific triggers such as cold water. Examinations in all patients, including bone marrow examinations in patient III_6 and her brother III_4, did not reveal any organ infestation.

Familial mastocytosis is a rare condition and only a few families have been described so far (Valent *et al.*, 2007 #5458). Multiple mutations in the *c-KIT* gene associated with mast cell disorders have been described so far (Bodemer *et al.*, 2010 #8513; Molderings *et al.*, 2010 #5411; Wasag *et al.*, 2011 #8310). The most prominent is the D816V mutation (Valent *et al.*, 2007 #5458). Sequencing the *c-KIT* gene by PCR in the skin biopsies from patients III_6 and her niece IV_1 (Figures 1 and 2b) showed a mutation in exon 18 at position 849 (S849I), which, to our knowledge, is previously unreported. A second mutation in exon 18 at position 835 (c-Kit M835K) was found exclusively in the most severely affected patient IV_1. The mutations could be identified in material derived from skin biopsies but not in blood samples or buccal mouth swabs from our patients

Abbreviations: CM, cutaneous mastocytosis; FFPE, formalin-fixed paraffin-embedded; sMCAS, suspected mast cell activation syndrome

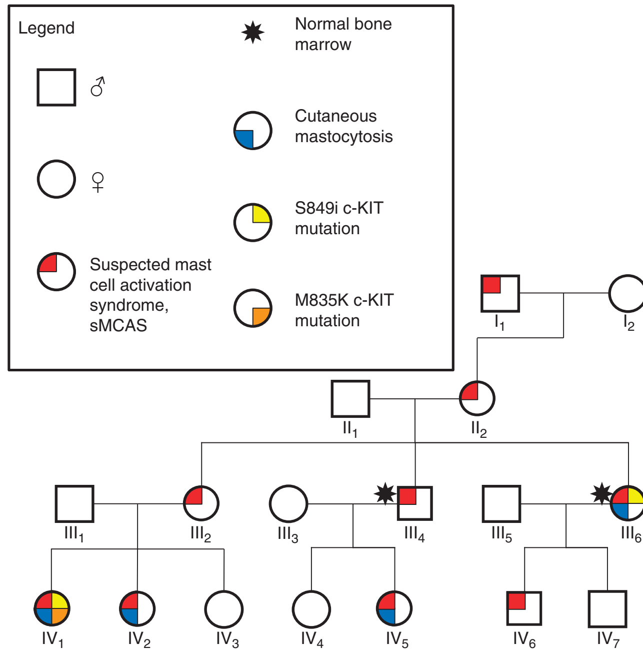

Figure 1. The family's pedigree. Sequencing demonstrated the novel c-KIT mutation in patient III_6 and IV_1.

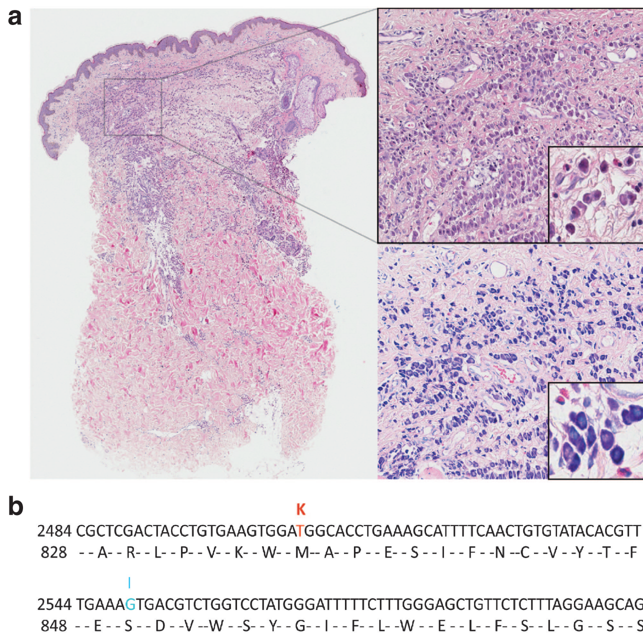

Figure 2. Histology and mutations in c-Kit exon 18. (**a**) Clinical picture and histology of the skin biopsy of patient IV_1 suffering from cutaneous mastocytosis (CM). The overview and close-up depict numerous mast cells (H/E and Giemsa stains), consistent with the diagnosis of CM. The magnified inserts show mast cells. (**b**) Sequence of c-kit exon 18 identified two mutations. Mutation 1: AAG instead of ATG leading to an exchange from methionine (M) to lysine (K) at amino-acid position 835 (c-Kit M835K). Mutation 2: ATT instead of AGT leading to a change from serine (S) to isoleucine (I) at position 849 (c-Kit S849I).

and all other family members. This could be explained by somatic mutations of the *c-Kit* gene, indicating mosaicism. An elevated frequency of somatic mutations in other hematological malignancies has already been observed in the Jak 2 locus (Campbell, 2009 #8516). However, technical insufficiencies might have resulted in an insufficient sensitivity of the 11 PCRs from the peripheral blood and the 18 PCRs from the mucosal mouth swabs. Taking into account this shortcoming, we might have overlooked a true, inherited germ-line mutation at position S849i.

The *c-kit* gene is located on chromosome 4 q11–12. By analyzing the clinical symptoms of members of the four generations of the concerned family, we assume that the c-KIT S849i mutation contributes to a rather benign phenotype of CM gradually, nevertheless incompletely resolving by age. The severe, relapsing genital angioedema of patient IV_1 is a rather untypical feature of the mast cell activation syndrome (Hamilton *et al.*, 2011 #5461). Hereditary angioedema as a differential diagnosis was ruled out by repeated, normal C4, C3c, and C1 esterase inhibitor measurements and by the good clinical response to the quadruple dose of nonsedating oral antihistamine used as a standard treatment of chronic, spontaneous urticaria/angioedema (Zuberbier *et al.*, 2009 #1110; Valent *et al.*, 2012 #9305). Nevertheless, we think that the angioedema in this patient more probably was a grade I anaphylaxis and, consequently, part of the sMCAS than a presentation of a second disease, chronic spontaneous angioedema. Overall, this family with a non-D816V mutation suffered from an early onset and a rather benign further course of the disease.

c-kit mutations can be detected in up to 90% of patients with mastocytosis (Teodosio *et al.*, 2010 #8510), about 50% are various mutations in c-kit exon 17 in adults (Lanternier *et al.*, 2008 #8511) and around 40% in children (Bodemer *et al.*, 2010 #8513). The S849i mutation in the family described herein lies on exon 18 but is situated in proximity to the most commonly identified mutation in CM at codon 816 (D816V). The localization in the tyrosine kinase domain region 2 (similar to the most common mutations) in the 11th β sheet structure (Laine *et al.*, 2011 #5410) suggests that this mutation may lead to functional alterations in the tyrosine receptor kinase activity of the c-kit molecule. Interestingly, in patients with CM, a mutation at position 839 (E839K, exon 18) has also been described (Laine *et al.*, 2011 #5410).

Summing up, we present a previously unreported mutation in exon 18 of the *c-KIT* gene, contributing to a phenotype of CM with sMCAS and a tendency to incomplete resolution in adulthood. We also identified an additional mutation in exon 18 of the *c-KIT* gene possibly associated with a more severe phenotype.

The study has been approved by the institutional ethics committee (Ethics committee of the Medical University of Vienna, approval number 901/2009). Informed written consent was given by all patients and their parents, respectively. The study adhered to the Declaration of Helsinki principles.

DNA from formalin-fixed paraffin-embedded (FFPE) skin biopsies were extracted using the DNAeasy Blood and Tissue Kit (Qiagen, Valencia, CA) after deparaffinization. For DNA extraction from blood, 200 μl of blood was used with the Qiagen DNA Blood and Tissue Kit according to the manufacturer's instructions. To examine c-Kit exons 8, 9, 11, 13, 17, and 18 for mutations in CM samples, FFPE-derived DNA was further amplified using nested PCR with a multiplex preamplification step to compensate for limited DNA yields from FFPE extraction. Preamplification was performed in a 25-μl volume with the Ampli Taq Gold 360 DNA Polymerase Kit (Applied Biosystems, Vienna, Austria) and at least 25 ng of DNA template. As a second step, amplification using nested primer pairs (see online repository for table) was performed with the Ampli Taq Gold 360 DNA Polymerase Kit and 1 μl of the preamplification product. For amplification steps for blood-derived DNA, the same protocol and primers were used as for FFPE material. After PCR amplification, appropriate exon lengths were controlled on a 1.5% agarose gel, and PCR products were subsequently sequenced using an ABI 3000 capillary sequencer (Applied Biosystems).

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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A Method for Intravital Monitoring of Human Cells Using a Far-Red Luminescent Probe in Graft-Versus-Host Disease Model Mice

Journal of Investigative Dermatology (2013) **133,** 841–843; doi:10.1038/jid.2012.345; published online 27 September 2012

TO THE EDITOR

In the past few years, intravital imaging has been developing, especially that using a multiphoton confocal technique (Li *et al.*, 2012). Such a technique is quite a useful tool for understanding cellular or molecular dynamic events *in vivo*. However, this technique requires labeling of fluorescence, and the fluorescence is usually labeled genetically, by transfection, or by direct linking. In the case of human cells, there is the concern that these treatments affect the functional capability of the treated cells. A method of intravital imaging that does not have biological influences is needed.

Recently, we developed a far-red luminescent probe as a convenient