SHORT COMMUNICATION

Testicular Cancer in Monozygotic Twin Brothers with Urticaria Pigmentosa

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Accepted Dec 6, 2017; Epub ahead of print Dec 12, 2017

Urticaria pigmentosa (UP) is the most common variant of cutaneous mastocytosis. The occurrence of UP in twins is rare. We have described a case of monozygotic twin brothers, with the first signs of UP at the age of 4 months (1) and now present the development of their disease until 43 years of age, with the additional appearence of testicular cancer at age 30.

CASE REPORT

The UP lesions in both twins – diffuse macules affecting the whole body (**Fig. 1**), except the face – remained clinically unchanged during the 43 years of life apart from the short period of chemotherapy in one of the twins. In 2003, left orchiectomy was performed on one twin due to palpable testicular tumour, and in 2004, right orchiectomy was performed on the other due to the same reason. In both brothers the histology confirmed mixed germ cell tumour with the dominant component of embryonal carcinoma (more than 90% of the tumour) with a minor level of a yolk sac component and choriocarcinoma. Both twins were treated with 4 cycles of chemotherapy in monthly intervals (bleomycin, etoposide, cisplatine), and so far no recurrence of the tumour has been observed. In one twin, the UP lesions temporarily disappeared after the first cycle of chemotherapy, and reappeared after the last session, whereas in the second twin the UP lesions remained stable during the chemotherapy.

In both brothers, all routine biochemical parameters were within normal levels. The serum tryptase levels, 13.1 and 16.8 ng/ml, were both within reference values (below 20 ng/ml for adults – ImmunoCAP Tryptase test). The abdominal ultrasonography and chest X-ray were without any pathology.

The skin biopsy from both brothers had identical histological features of UP with an intact epidermis and a pleomorphic, mainly granulated mast cells with positive chloroacetate esterase, and CD117 – proto-oncogene *c-kit* in the upper corium. The bone marrow trepanobiopsy in both brothers showed proportional representation of precursors of all 3 lines of hematopoiesis, the presence of trilinear maturation without proliferation of blasts with the presence of rare, dispersedly situated granulated mast cells ($CD117^+$, $CD25^-$), without infiltration of mast cell populations, whereas the skeletal scintigraphy was negative (with Tc-MDP methylene diphosphonate as imaging agent).

Whole blood samples and saliva were collected from the twins. DNA was isolated by using DNeasy Blood and Tissue Kit (Qiagen). First we worked with DNA isolated from the whole blood, where Sanger sequencing of the entire coding region of the proto-oncogene *KIT* was performed (2). Since Sanger sequencing of c-kit gene did not reveal any pathogenic mutation, we decided to analyse the whole exome, where we found *D816V* of the *KIT* gene in both samples. However, the mutation rate was very low, which is why Sanger sequencing did not reveal any mutation. Assuming that the twins originate from a mosaic embryo, we isolated DNA from the saliva for further examination. Allele-specific PCR (AS-PCR) for the *KIT D816V* were performed in patients' saliva samples (3). We found *D816V* mutation in both twins (Fig. S1[1]).

Sequencing libraries were prepared from DNA using Agilent SureSelectXT HumanAllExon V5 kit according to the manufacturer's instructions (Agilent, Technologies, USA). High throughput sequencing (2x75 cycles) was performed on NextSeq500 using High Output Kit (Illumina, USA). Read pairs were aligned to the human genome reference (hg19) using Burrows-Wheeler aligner and futher processed by Picard tools http://broadinstitute.github.io/picard/) (4). Variant calling was performed using VarScan (5). Regions of interest were visually inspected in Integrative Genomics Viewer (IGV) (6). Part of the exone 17 (Chr4: 55599271-55599370; hg19) of the *KIT* gene (NM_000222) surrounding codon D816 was amplified by single-round PCR using primer pairs listed in Table SI[1].

[1]https://doi.org/10.2340/00015555-2861

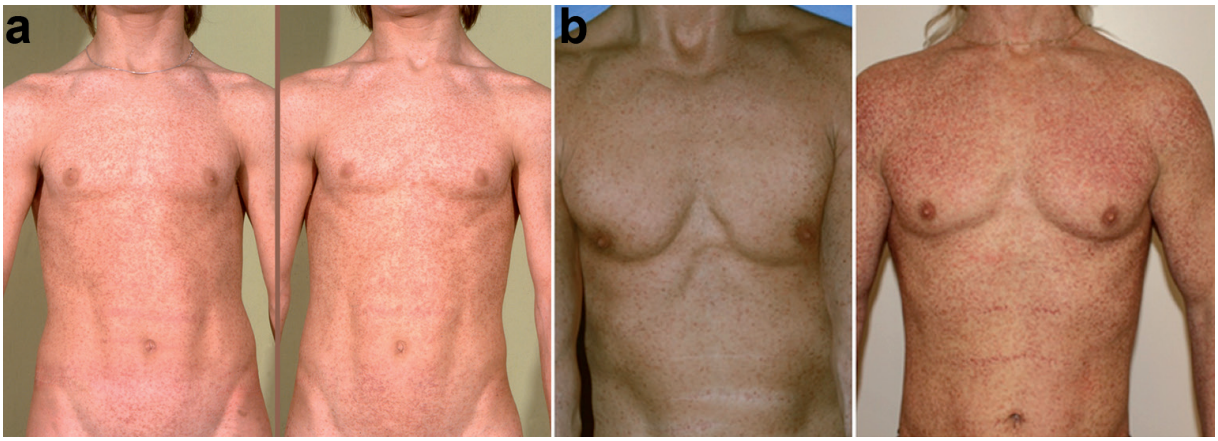

Fig. 1. The brothers' skin phenotypes at the age of 16 years (a) and 43 years (b), respectively.

doi: 10.2340/00015555-2861
Acta Derm Venereol 2018; 98: 528–529

The resulting indexed libraries were sequenced on Ion Torrent PGM using 400bp chemistry according to the manufacturer's instructions (Life Technologies, USA). Fastq files were processed from raw data and reads were mapped to hg19 using Torrent Suite software (Life Technologies). Variant calling was performed using Variant Caller plugin in Torrent Suite software (parameter settings: somatic variant frequency, low stringency). Mapped reads were visually inspected in IGV. Codon D816 was covered by >25,000 reads in both patient samples peripheral blood samples and by >10,000 reads in saliva samples. To distinguish mutations from the potential PCR and/or sequencing errors, error rate at the respective position was analyzed in two buffy coat samples from healthy individuals. The frequency of A>T error at chr4: 55599321 was 0.04% and 0.05%, respectively (position coverage >22,000 reads).

Using whole exome sequencing (WES), the KIT gene mutation D816V was identified at a relatively low frequency in peripheral blood of first twin (variant allele frequency 9%; 5 mutated out of total 56 reads). Although no KIT mutation was found in the second twin, a visual inspection of mapped reads in IGV revealed KIT gene mutation identical to that found in first twin in one out of total 47 reads. To validate these findings deep amplicon sequencing of the respective KIT gene region was performed using the same DNA samples. In agreement with WES results, D816V mutation was detected at low level in peripheral blood of both brothers with variant allele frequency 9% in first twin and 3% in second twin. Subsequent deep sequencing analysis revealed D816V mutation also in saliva samples: 5% and 2% in first and second twin, respectively.

DISCUSSION

Familial cutaneous mastocytosis – especially in monozygotic twins – occurs very rarely (7) and is considered as genetically determined, perhaps autosomal dominant disease (8). However, Sato-Matsumura et al. (9) did not find any gene mutations at exon 11 or 17 *c-kit* in monozygotic twins with UP. Mastocytosis in childhood is defined as disease starting before the age of 15 years (8), usually affecting only the skin, perhaps as a clonal proliferation of benign prognosis (10), in contrast to the beginning of the disease after age 15 with chronic progression, development of haematological malignancies (11), as well as induction of potentially oncogenic somatic *c-KIT* mutations (12, 13). The serum tryptase levels generally correlate with the proliferation and bone marrow infiltration of mast cells (10, 11). Elevation in serum tryptase above 20 ng/dl is an indication for bone marrow trepanobiopsy.

In the described monozygotic twins, the skin lesions were *KIT D816V* negative, there were no mast cell infiltration of the bone marrow, and serum tryptase was below the reference level. However, the *D816V* mutation was detected in low levels in peripheral blood as well as saliva samples. The *KIT D816V* mutation does not necessarily correlate with clinical manifestations, as investigated in indolent systemic mastocytosis (14). However, its detection may prove very useful in patients with systemic mastocytosis but very low mast cell burden, and who do not meet WHO 2008 histological criteria for bone marrow involvement (15). Serum tryptase levels, on the other hand, correlate with the burden of *D816V* mutation, making it a disease marker in systemic mastocytosis (16).

The twins we describe presumably originated from a mosaic embryo and as such presented no systemic signs of mastocytosis. The clinical picture correlates to low level *KIT D816V* mutation in peripheral blood and saliva. This probably represent a mild systemic form of mastocytosis with some predisposition to future systemic complications Whether or not testicular cancer in the twins is part of this predisposition or represents an unrelated genetic trait remains to be elucidated.

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