Carbamazepine-induced thrombocytopenic purpura in a child: insights from a genomic analysis

To the Editor,

Carbamazepine is an effective anticonvulsant and has a relatively low incidence of adverse effects, although it occasionally causes hematologic disorders. We herein describe a patient with carbamazepine-induced thrombocytopenic purpura that was investigated by pharmacological, immunological and genomic assays.

A 9 years old Caucasian girl was referred to emergency room because of a skin rash to the lower limbs of three days' duration. There was no associated fever, sore throat, abdominal pain or other systemic manifestations. Two weeks before, a diagnosis of symptomatic epilepsy, due to cortical dysplasia, with partial seizures, was made and she was treated with carbamazepine 20 mg/kg in 2 daily doses. After seven days of treatment carbamazepine plasma level was in the therapeutic range (8.2 μg/ l).

There was no previous history of drug allergy, bleeding diathesis or family history of coagulopathy. Physical examination was unremarkable, except for extensive purpura and petechiae mainly over lower limbs and few elements on upper trunk (Figure 1). The child was in good general condition, with no pallor, abdominal examination was normal without splenomegaly, blood pressure was normal. Haematological examination revealed only a platelet count of 43.000/mm^3; coagulation studies were normal. Liver and kidney function tests were within the normal limits. Urine test did not show red cells or proteins.

Carbamazepine-induced thrombocytopenia was suspected and therapy was switched to a non-aromatic anticonvulsant (levetiracetam). On the fifth day from drug discontinuation a rapid rise in platelet count, up to 375.000/mm^3 was detected; at this time platelet associated antibodies were negative and purpura had almost disappeared.

To investigate the molecular mechanism of carbamazepine-induced thrombocytopenia, pharmacological and genomic assays were performed.

Written informed consent was obtained from the patient and parents. The presence of carbamazepine-dependent IgG antibodies in serum, reactive with platelets, was tested by cytofluorimetry [1]. Proliferation of PBMC from patients and control volunteers was measured using lymphocyte transformation test, using H^3-thymidine [2]. HLA genotyping was performed by sequence-specific oligonucleotide primed PCR and whole genome genotyping by Illumina Infinium HumanOmniExpressExome BeadChip. Selection of candidate genes and variants involved in carbamazepine pharmacokinetics and pharmacodynamics was done using the Pharmacogenomics

Knowledge Base (PharmGKB): in particular, 52 SNPs in 22 genes are listed in this pathway and, of these, our platform allowed to genotype 29 SNPs in 19 genes. Genotyping of CNVs was done by the software PennCNV [3].

Two months after the thrombocytopenic event, the presence of drug-dependent antibodies, able to bind platelets in the presence of carbamazepine or epoxy-carbamazepine, was tested in patient's serum; the antibodies test was performed using normal platelets and incubating them in the absence or presence of carbamazepine or epoxy-carbamazepine and resulted negative for patient's serum. At the same time, no proliferation could be observed on patient's PBMC after *in vitro* treatment with increasing concentrations of carbamazepine.

HLA genotyping identified HLA-A*02:05/*24:02, HLA-B*18:01/*50:01 and HLA-C*06:02/*07:01 alleles. Genotyping of SNPs in carbamazepine pharmacokinetic and pharmacodynamic pathways did not display any homozygous variant for candidate causative polymorphisms (Supplementary Table 1). Three chromosome regions with CNVs were identified (Table 1). Four genes are located in these regions: CSMD1 (chromosome 8: 4480667 – 4484362), HSD17B2 (chromosome 16: 82027399 – 82169385), MPHOSPH6 (chromosome 16: 82027399 – 82169385) and FOXC2-AS1 (chromosome 16: 86583894 – 86566042).

Carbamazepine occasionally causes hematologic disorders such as aplastic anaemia, thrombocytopenia and leukopenia. Thrombocytopenia is distinctly uncommon and most often develops 2 weeks after initiation of treatment and recovers within 1 week after drug discontinuation [4]. In our patient, thrombocytopenia started within 14 days of carbamazepine initiation and recovery in platelets counts occurred within 96 hours since drug withdrawal. The pathophysiologic mechanism remains unknown and is believed to be immune related, with the development of drug-dependent antibodies to platelets and secondary platelet destruction. The most commonly targeted platelet membrane epitopes are glycoproteins complexes on platelet surface [5]. Once established, drug sensitivity probably persists indefinitely and patients should be advised to avoid permanently the medication and other aromatic anti-epileptic drug because of cross-sensitivity.

Our patient did not display drug-dependent antibodies nor an activation of PBMCs in the presence of carbamazepine, therefore a direct immunological mechanism could not be recapitulated *in vitro*. Genetic analysis could not identify in this patient an HLA allele or variant in the pharmacokinetic and pharmacodynamic pathways that could predispose to the adverse drug reaction observed, especially for important polymorphisms such as those of epoxide hydroxylases [2]. Interesting insights were however obtained by analysis of CNVs in patient's DNA. The largest and most significant CNV was a 142 kDa fragment elided heterozygously: this alteration affected two genes encoding for *HSD17B2* and *MPHOSPH6*, leading to the full heterozygous loss of both genes.

HSD17B2 is an enzyme capable of catalysing the interconversion of testosterone and androstenedione, as well as estradiol and estrone. Interestingly CYP3A4- and CYP3A7-mediated carbamazepine 10,11-epoxidation is activated by differential endogenous steroids [6] and therefore a reduction in the activity of HSD17B2 could modify the patterns of carbamazepine biotransformation, predisposing the patient to the adverse effect observed. MPHOSPH6 is an RNA-binding protein that associates with the RNA-exosome complex and is also involved in the response to chemicals, steroids and oxidative stress [7]. A 2 kDa heterozygous deletion was present in chromosome 16 and should lead to the reduction of *FOXC2-AS1*, an RNA gene that is affiliated with the non-coding RNA class. Another CNV (3 kDa) was detected in an intron of *CSMD1*: the large membrane protein encoded by this gene is known to be modulated by PACSIN2, a protein important for the demarcation membrane system in megakaryocytes and platelet production [8]. Therefore, a contribution of the CNV in CSMD1 to the pathogenesis of carbamazepine-induced thrombocytopenia observed in our patient could be considered.

Previous studies demonstrated that the incidence of drug-induced thrombocytopenia is affected by genetic variants of various genes, such as ITPA for ribavirin [9] or TDAG8 and HLA-DRA for heparin [10], however these are likely not involved in the pathogenesis of carbamazepine-induced thrombocytopenia.

In summary, although carbamazepine-induced thrombocytopenic purpura appears to be a rare phenomenon, clinicians should be aware of this potential serious adverse effect and consider regular complete blood cell counts, especially in the first few weeks following treatment initiation. Moreover, this study demonstrates that carbamazepine thrombocytopenia can occur even in the absence of detectable drug-dependent antibodies and with no causative genetic variant in the pharmacokinetic and pharmacodynamic pathways. Finally, this report illustrates that CNVs analysis provides interesting insights on patient-specific genetic features that might be involved in the molecular mechanism predisposing to carbamazepine-induced thrombocytopenia, confirming that CNVs effect on drug response is relevant but still overlooked.

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Figure 1: evidence of thrombocytopenic purpura on patients' skin. A. few petechial elements on upper trunk; B. petechial on volar surface of the foot; C. petechiae on lower limbs