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Phenotypic expression and founder effect of *PANK2* c.1583C>T (p.T528M) mutation in Serbian pantothenate kinase-associated neurodegeneration patients

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Abstract: Pantothenate kinase-associated neurodegeneration (PKAN) is an autosomal recessive disorder characterized by dystonia, parkinsonism, cognitive and visual impairment, and iron accumulation in the brain. Many cases of PKAN result from mutations in the *PANK2* gene that encodes pantothenate kinase 2, a key regulatory enzyme in the biosynthesis of coenzyme A. We previously detected six Serbian patients with clinically suggestive PKAN, all of whom had *PANK2* c.1583C>T (p.T528M) mutation either in the homozygous or in the heterozygous state. In this study we explored the phenotypic expression and a possible founder effect of this substitution. We performed the analysis of linkage disequilibrium (LD) and organization in haplotypes of 23 single nucleotide polymorphisms (SNPs) adjacent to the *PANK2* gene in all of the six patients and their parents, as well as in control healthy child-parents trios. The age of *PANK2* c.1583C>T mutation was determined using the r^2 degeneration method. Clinical findings in our patients were markedly similar. Different LD structures between patients and controls is revealed, and *PANK2* c.1583T allele was significantly associated with a particular haplotype. The age of *PANK2* c.1583C>T mutation was estimated to be about 15 generations. Our results suggest that *PANK2* c.1583C>T in Serbian PKAN patients represents a founder mutation descended from one common ancestor.

Keywords: founder effect; *PANK2* mutation; phenotype; PKAN

INTRODUCTION

Neurodegeneration with brain iron accumulation (NBIA) incorporates a group of progressive extrapyramidal disorders characterized by iron accumulation in the brain [1]. Pantothenate kinase-associated neurodegeneration (PKAN; previously Hallervorden-Spatz syndrome), the major form of NBIA that accounts for approximately 50% of cases [2], is an autosomal recessive disorder characterized by dystonia, parkinsonism, cognitive and visual impairment and iron accumulation in the brain.

Many cases of PKAN result from mutations in a gene located on chromosome 20p13 [3]. The offender gene (*PANK2*) encodes pantothenate kinase 2, a key regulatory enzyme in the biosynthesis of coenzyme A (CoA). Altered mitochondrial CoA synthesis is predicted to cause mitochondrial dysfunction with subsequent degeneration of susceptible brain tissues [4]. The neurodegeneration is accompanied by iron accumulation, which can be verified radiologically as "the eye-of-the-tiger sign" on MRI [1,4]. Recent data suggest that mutations in the *PANK2* gene are found

in about 97% of all clinically diagnosed PKAN cases with "the eye-of-the-tiger" sign [2]. The estimated prevalence of these rare disorders is 1-3 per million, with carrier frequency of one in 275-500 [5]. According to the Human Mutation Database, more than 100 *PANK2* mutations have been identified to date.

We detected six Serbian patients with clinically suggestive PKAN, all of whom had *PANK2* c.1583C>T mutation (p.T528M) either in the homozygous or in the compound heterozygous state (five of them have already been reported in a large European NBIA study) [6]. As they all shared the same mutation, in this study we examined a possible founder effect of this gene change.

MATERIALS AND METHODS

Ethics statement

The study was approved by the Ethical Committee of the Faculty of Medicine University of Belgrade. After written informed consent was obtained from the patients, detailed clinical data and DNA samples were collected.

Clinical data

We identified 6 patients from 6 unrelated, non-consanguineous families with clinical presentation of extrapyramidal symptoms and signs, and neuroimaging evidence of iron deposition in the basal ganglia, at the Institute of Neurology (Belgrade, Serbia). Patients were clinically examined by two independent neurologists (MS, VSK), and MRI scans were analyzed by the same neuroradiologist (Fig. 1).

Genetic data

PANK2 gene analysis in our patients was performed at the Institute of Human Genetics, GSF Research Centre for Environment and Health in Neuherberg, Germany, as described in Hartig et al. [6].

Analysis of the founder effect

We analyzed linkage disequilibrium (LD) and organization in haplotypes of single nucleotide polymorphisms (SNPs) adjacent to the *PANK2* gene on chromosome 20p13 in 6 patients previously identified as having *PANK2* c.1583C>T mutation and their parents. The analysis was also performed in 30 healthy child-parents trios originating from the same geographical region. Twenty-three SNPs spanning 86.3 kb of the *PANK2* locus were analyzed by sequence-based genotyping using the ABI 3730 Genetic Analyzer (Applied Biosystems, USA). SNPs were derived from HapMAP and selected according to the confidence interval. Haploview [7] was used to estimate LD and infer haplotypes. The haplotype difference between cases and controls was assessed by the chi-square test. The age the *PANK2* c.1583C>T mutation was determined by the r2 degeneration method [8]. Using formula $1\text{-}r2/\theta$, the value was calculated for SNPs flanking the haplotype linked to the mutation.

RESULTS

Genotype phenotype analysis in Serbian patients with the *PANK2* c.1583C>T mutation

PANK2 mutations were previously identified in all our patients who were ethnic Serbs [6]. Family history was negative in all of them and there was no consanguinity between their parents. A missense mutation (c.1583C>T; p.T528M) was found in 9, and a null mutation (c.1418del7) in 3 out of 12 alleles. Three patients were homozygous for c.1583C>T substitution, and 3 were compound heterozygotes (1418del7/C1528>T). Although our patients were born from non-consanguineous parents, they shared the same mutation in homozygous (3 patients) or compound heterozygous status (3 patients) (Table 1).

Table 1. PANK2 mutations in Serbian PKAN patients. Position on cDNA and type of detected PANK2 mutations in six Serbian patients, assigned P1 to P6; consequence of the mutation at protein level is in brackets.

patient	Mutation
P1	c.1583C>T (p.T528M); homozygous
P2	c.1583C>T (p.T528M); homozygous
P3	c.1583C>T (p.T528M) / c.1418del7; frameshift
P4	c.1583C>T (p.T528M); homozygous
P5	c.1583C>T (p.T528M) / c.1418del7; frameshift
P6	c.1583C>T (p.T528M) / c.1418del7; frameshift

Clinical findings were markedly similar, presenting before the age of 25 (Supplementary Table S1). However, patients homozygous for missense mutation c.1583C>T showed later onset of disease than compound heterozygotes with frameshift mutation on the other allele (21+/-0 years and 13+/-0 years, respectively). All of our patients fulfilled the criteria for clinical diagnosis of PKAN, with atypical age of onset in 5 of them [5]. The most prominent clinical sign was dystonia (lingual dystonia, jaw closing or opening dystonia, axial dystonia). Anarthria was also an outstanding feature, caused by lingual and oromandibular dystonia. Two patients had palilalia (P1, P4), and during the course of disease they also developed freezing. Postural instability due to axial dystonia mainly resulted in an inclination for backward falls. Imaging data revealed a bilateral "eye-of-the-tiger" sign in all 6 patients. Cerebellar atrophy was a prominent feature in one patient.

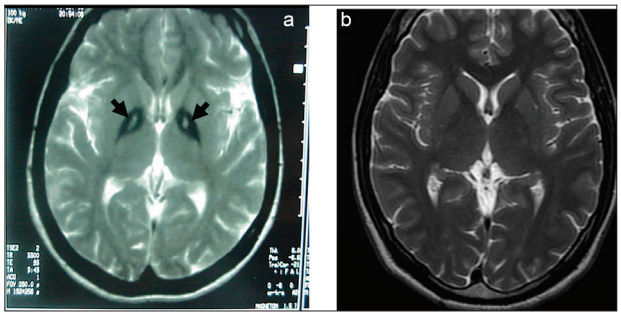

Fig. 1. Brain MRI illustrating "the-eye-of-the-tiger" sign; **a** – patient P1's MRI with "the-eye-of-the-tiger" sign showing a hyperintense signal (arrows) surrounded by a hypointense area at the medial aspect of the globus pallidus; **b** – normal MRI.

Founder effect study of *PANK2* c.1583C>T (p.T528M) mutation

LD display with indicated D' in controls and patients is given in Fig. 2a and b, respectively. Different LD structures between patients and controls are revealed, with 3 major blocks being distinguished in controls and two LD blocks in patients.

Overlapping segments between patients and controls can be observed in block 2. It spans the region from SNP rs4815627 to rs14047 and has a particularly strong LD in patients. The *PANK2* mutation resides within this segment and this region was subsequently analyzed in more detail. LD display with pairwise R^2, inferred haplotypes and corresponding frequencies are given in controls and patients in Fig. 2c and d, respectively. Different haplotype distribution is seen between patients and controls, with one haplotype (TCCCTTCG) being absent in patients. Particularly striking is the difference in frequency for haplotype TCCGTGTG, which was underrepresented in patients as compared to controls (5.6% vs 34%) and for haplotype TCCGCGTA which, in contrast, occurred with a higher frequency in patients than in controls (50% vs 18%; Fig. 1c and d). Interestingly, *PANK2* mutation arises at the background of TCCGCGTA haplotype. Fig. 2e presents the same analysis in patients, as given in Fig. 1d, but with the inclusion of the *PANK2* c.1583C>T mutations. The majority of TCCGCGTA carriers had the T1583 allele (39% vs 11% TCCGCGTA with the C allele), resulting in a significant association (p=0.0001). The age of *PANK2* c.1583C>T mutation was estimated at about 15 generations.

DISCUSSION

The presence of the same mutation (*PANK2* c.1583C>T) in all examined patients points to a possible founder effect. Our results confirmed the strong association of *PANK2* T1583 allele and a particular 8 SNP haplotype (TCCGCGTA) covering 22.37 kb around the mutation point, with p=0.001. Estimation of the mutation's age showed that the most recent common ancestor in Serbia lived about 15 generations ago (i.e. 600 years ago). Because c.1583C>T is one of the most common mutations in European NBIA patients, we speculate that it was introduced in the Serbian population during the 14-15th century. A founder effect was already shown for at least three other *PANK2* mutations. It was observed that the c.G411R mutation shares a common 1cM haplotype in 27 families, predominantly of European descent [1]. A novel c.1142_1144delGAG mutation in four Dutch unrelated NBIA families and in one German patient of unknown descent has been reported [9]. A conserved haplotype of 1.5 cM was found for all mutation carriers. In addition, all the Dutch families originated from the same geographical region in the Netherlands. It was estimated that this mutation arose at the beginning of the 9th century, about 38 generations ago. In the recent Mexican familial clinical-genetic PKAN-disease study, the observed clustering of patients

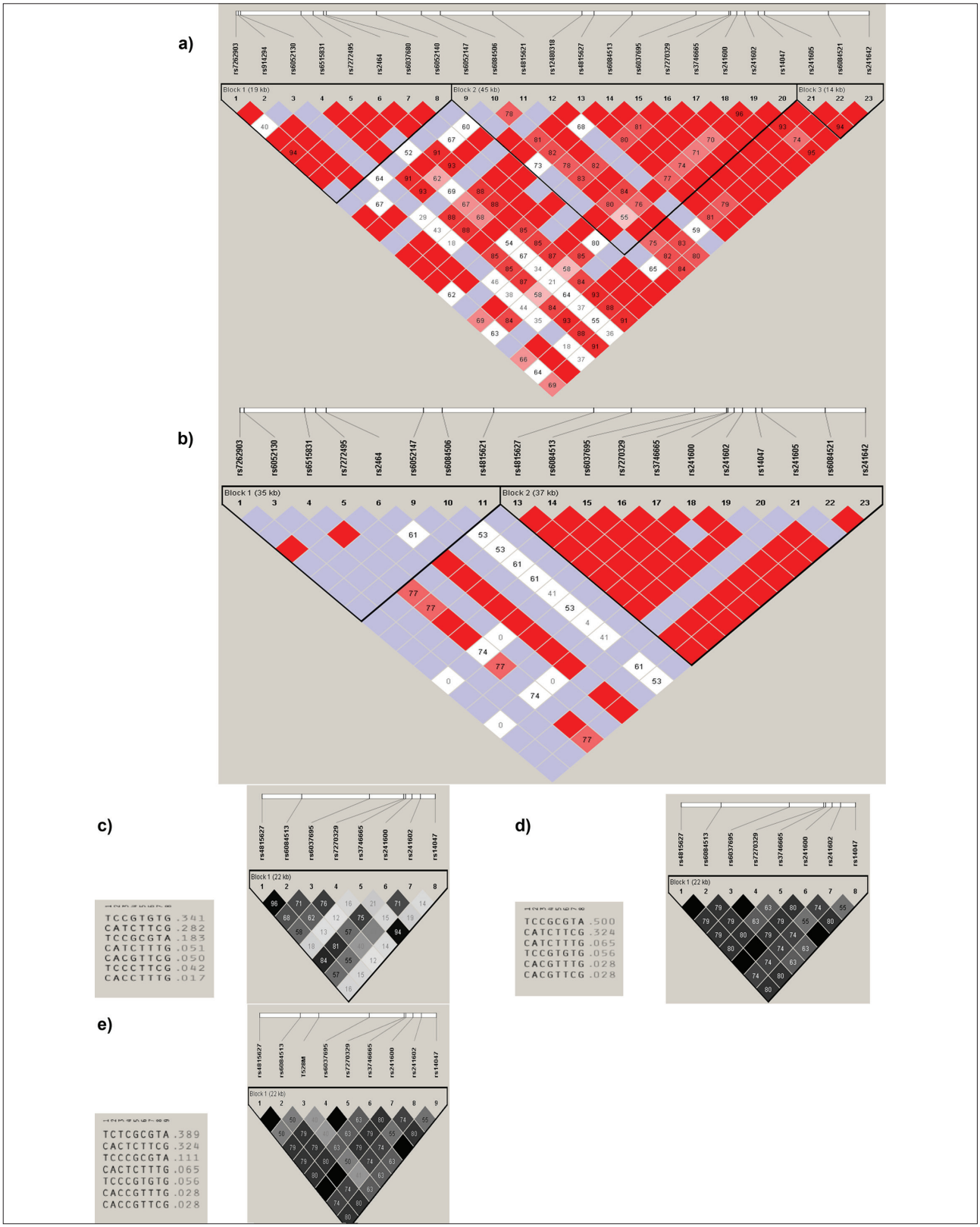

Fig. 2. Haploview LD display. Haploview LD display for all 23 analyzed SNPs based on D', in controls (**a**) and patients (**b**). Haploview LD display from rs4815627 to rs14047 with pairwise r^2, inferred haplotypes and corresponding frequencies in controls (**c**) and patients (**d**); **e** – same analysis as in Fig.2d but with inclusion of the *PANK2* c.1583C>T mutation.

harboring the p.N404I mutation strongly suggested a founder effect for this mutation [10].

In the large European study of NBIA syndrome, *PANK2* c.1583C>T was identified as the most frequent mutation (11 out of 96 alleles), followed by c.573delC and c.1561G>A (10 out of 96 alleles each) [6]. Substitution c.1583C>T in exon six of the *PANK2* gene affects the catalytic domain of the enzyme with replacement of threonine to methionine at position 528 [1]. All of our cases have this missense mutation on at least one allele: three patients were homozygous and three compound heterozygous for c.1583C>T. The fact that almost all patients with atypical disease and PKAN have missense mutations indicates that many of them may have residual PANK2 activity [1,6]. This could explain the delayed disease onset in our patients, who otherwise had typical clinical presentation [5]. In our group, homozygotes for missense mutation c.1583C>T showed a markedly later onset of disease than compound heterozygotes with frameshift deletion on the other allele. As expected, the *PANK2* mutation spectrum differs in other world populations [10-14]. A genotype-phenotype correlation is not always clear because of the number of *PANK2* rare variants in homozygous or compound heterozygous states. The majority of *PANK2* mutations reduces or abolishes the activity of the enzyme, and recent therapeutic options are oriented toward treatment with pantothenate, the PANK2 enzyme substrate [15,16]. Results of *in vitro* studies suggest that such treatment can stabilize the expression levels of PANK2 only in selected mutation, underlining the importance of precise *PANK2* genotypization in each PKAN case [16].

Family history was negative in all our patients. They were not consanguineous, although about 23% of previously published families had known or suspected consanguinity, and 33% of the families demonstrated homozygous *PANK2* mutations [5] (50% in our study). Also, the one-to-one correlation between the "eye-of-the-tiger sign" and mutations in *PANK2* was confirmed in our six patients [6].

In conclusion, our results suggest that the presence of the same mutation (*PANK2* c.1583C>T) in Serbian PKAN patients, either in the homozygous or heterozygous state, represents a founder mutation descended from one common ancestor.

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Conflict of interest disclosure: There is nothing to disclose.

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Supplementary Data

Supplementary Table S1.
Available at: http://serbiosoc.org.rs/NewUploads/Uploads/Svetel%20et%20al_3802_Supplementary%20Table%20S1.pdf