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Research Article

Human VKORC1 2255T>C polymorphism: distribution analysis in a Romanian population

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Abstract. The present paper analyses for the first time the distribution of the 2255T>C vitamin K epoxide reductase complex subunit 1 (VKORC1) gene polymorphism in a population of Romanian descent. A cohort of 120 healthy subjects has been genotyped using a PCR-RFLP protocol. Within the studied population, 48 individuals were found to be CC homozygotes (40%), 16 were TT homozygotes (13.33%), while 56 subjects were heterozygous (46.67%); the genotype distribution being in accordance with Hardy-Weinberg equilibrium (χ^2=0.0001; df=1). The 0.37 frequency of the minor allele is consistent with the generally described frequencies of other VKORC1 polymorphisms in Caucasian populations, providing further evidence for strong linkage disequilibrium between this gene's polymorphisms.

Key Words: Vitamin K epoxide reductase complex subunit 1, gene polymorphism, 2255T>C, Romanian

Rezumat: Studiul clinic investighează pentru prima dată distribuția polimorfismului 2255T>C al genei subunității 1 a complexului epoxid-reductazei vitaminei K (VKORC1) la o populație din România, prin genotiparea unui grup de 120 subiecți aparent sănătoși prin tehnica PCR-RFLP. În cadrul lotului examinat, 48 de indivizi au fost homozigoți pentru alela CC (40,6%), 16 au fost homozigoți pentru alela TT (13,33%), în timp ce 56 dintre subiecți au prezentat genotip heterozigot (46,67%), distribuția genotipurilor fiind în concordanță cu echilibrul Hardy-Weinberg (χ^2=0,0001; df=1). Frecvența de 0,37 a alelei minore se află în acord cu distribuția general descrisă in cazul altor polimorfisme ale genei VKORC1 si raportată pentru populații caucaziene, sugerând existența unui puternic dezechilibru de lincaj între polimorfismele acestei gene.

Cuvinte cheie: subunitatea 1 a complexului epoxid-reductazei vitaminei K, polimorfism genic, 2255T>C, români.

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Introduction

The Vitamin K epoxide reductase complex subunit 1 (VKORC1) gene, located on chromosome 16p11.2, encodes an 18 kDa transmembrane protein consisting of 163 amino acids, involved in vitamin K metabolism activation. Vitamin K represents one major covariate in the synthesis of proteins that depend on gamma-carboxylation such as bone matrix proteins osteocalcin (OC) and matrix gla protein (MGP).

The VKORC1 gene (GenBank Acc No AY587020) is highly polymorphic, and several frequent polymorphisms have been identified. To date, the most commonly investigated polymorphisms are: Rs9923231 (-1639G>A; 3673G>A), Rs2359612 (2255T>C) and Rs9934438 (1173C>T). Pharmacogenetic studies revealed strong associations between several VKORC1 gene polymorphisms and rare blood clotting diseases, and warfarin dosing requirements, respectively (D'Andrea 2005; Yuan *et al* 2005; Dumas *et al* 2007; Militaru *et al* 2012). Based on the key role of vitamin K in bone metabolism, VKORC1 polymorphisms have been investigated in association with bone mineral density and the susceptibility to osteoporosis (Crawford *et al* 2010; Holzer *et al* 2010).

Hypothesizing that there is a correlation between the "coagulation cascade" and atherosclerosis, Wang *et al* (2006) reported in Chinese individuals that the 2255C VKORC1 gene allele conferred almost twice the risk of vascular diseases (stroke, coronary heart disease, and aortic dissection). Nevertheless, no confirmatory results of an association between 2255T>C VKORC1 gene polymorphism and stroke were obtained by another study that enrolled a Caucasian population of German descent (Arnold *et al* 2008).

The present paper aims to assess for the first time the distribution of 2255T>C VKORC1 gene polymorphism in a population of Caucasians of Romanian descent.

Materials and methods

After providing informed consent, one-hundred twenty healthy subjects of Romanian descent were sampled for blood. DNA isolation was performed using the GeneJET™ Whole Blood Genomic DNA Purification Mini Kit (Fermentas). The protocol recommended by the producer was followed, with two mentions: the starting material was represented by 500 µL whole blood and the final elution was performed in 150 µL

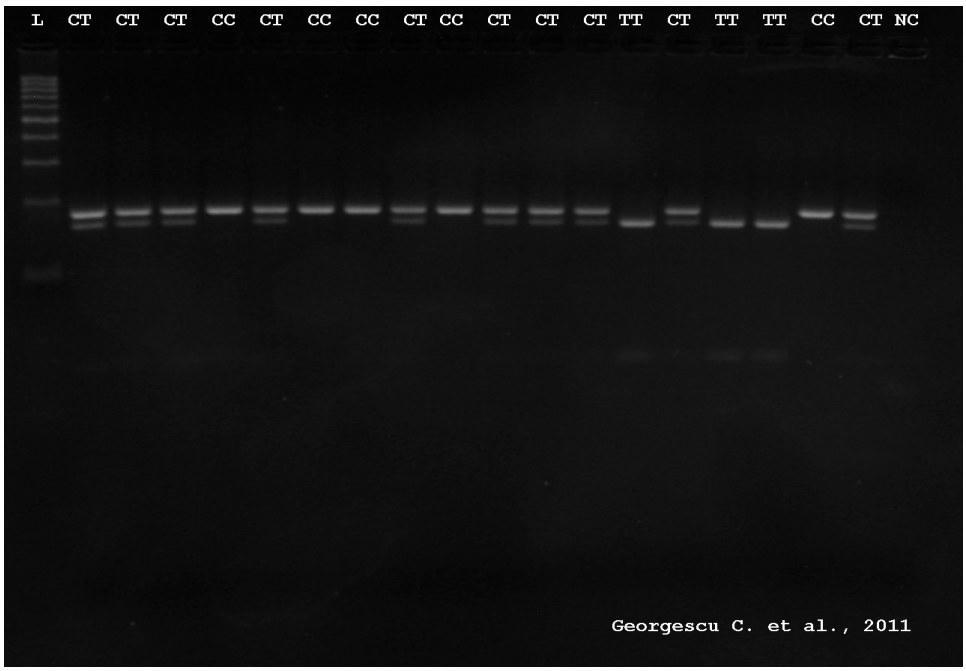

Figure 1. Observed VKORC1 genotypes, concerning 2255T>C. L – 100bp DNA Ladder (Bioline). NC – negative control

elution buffer (provided). Genotyping was conducted using the PCR-RFLP/NcoI protocol proposed by Wang *et al* (2006). A 198bp amplicon, harboring the SNP (2255T>C), was obtained, following the amplification of DNA samples using GoTaq Flexi DNA Polymerase (Promega) using following primers: 5'-TCTGAACCATGTGTCAGCCAGGACC-3' and 5'-GAACAGAGAGAGGAACCAAGGGAGTGGA-3', respectively. A 3 min denaturation period at 95˚C was followed by 39 cycles at 95˚C for 45 s, 56˚C for 45 s, and 72˚C for 30 s. A final extension step of 2 min at 72˚C completed the reaction. Once amplified, restriction of the amplification products, using NcoI endonuclease (Fermentas FastDigest), was performed by incubating the samples for 1 h at 37˚C. For optimal results, the restriction was performed in 200 µL PCR tubes, using a thermocycler. The cytosine from the +2255 position abolishes the NcoI restriction site, thus the C-allele remains uncut (189bp), while the T-allele yields two fragments (26 + 172bp). The restricted DNA amplification products were loaded onto 3.5% agarose gels (w/v), pre-stained with 1X Midori Green Advanced DNA Stain (Nippon Genetics Europe GmbH). Electrophoresis was carried out in 1X TBE buffer, at 100V, for approximately 1 hour. Image aquisition was performed using an UVP imaging system (UVP, LLC, Cambridge, UK) as shown in figure 1. Genotype and allele frequencies were computed using the consecrated formulae, while the population indexes (Effective number of alleles, Fixation index, χ^2) were computed using POPGENE, v1.32 (Yeh *et al* 2000).

Results and Discussion

The electrophoretic profiles of 18 subjects (comprising all three possible genotypes) are shown in Figure 1.

In the studied population, of Romanian descent, 48 individuals have been homozygous for the 2255C allele, 16 were homozygous for the 2255T allele, and 56 individuals were found to be heterozygous. The genotype frequencies are: CC – 40%, CT – 56 % and TT – 13.33%, while the allelic frequencies are pC = 0.63, qT = 0.37 (Table 1).

In the investigated population, the genotype distribution is in accordance with Hardy-Weinberg equilibrium, although the negative FIS value shows a slight heterozygote excess compared to Hardy-Weinberg equilibrium expectations.

In several studies concerning VKORC1 polymorphisms in Caucasians, the minor allele was reported to have a frequency close to 0.4 (Table 2), consistent with the 0.37 frequency of 2255T allele in Romanians. The fact that various VKORC1 polymorphisms have similar distributions suggests that these polymorphisms co-segregate (they are linked).

A recent study (Buzoianu *et al* 2012), investigating -1639G>A VKORC1 polymorphism in a Romanian population of 332 individuals, revealed a minor allele frequency of 0.42. The allelic frequencies concerning 2255T>C reported in our study are similar to those of Buzoianu *et al* (2012), this resemblance raising the hypothesis of the two polymorphisms being in linkage disequilibrium (C-G).

Table 1. Polymorphism distribution within the studied population

Genotype	Number of individuals	Genotype frequency (%)	Allele frequency		Effective number of alleles (Ne)	Fixation index (FIS)	Hardy-Weinberg equilibrium (χ^2)
			pC	qT			
CC	48	40					
CT	56	46.67	0.6333	0.3667	1.8672	-0.0048	0.0001
TT	16	13.33					

Table 2. Various VKORC1 polymorphisms and their frequencies in Caucasian populations

Polymorphism	Minor allele frequency	N	Stated ethnicity	Authors
-1639G>A	0.38	92	Caucasians	Yuan et al 2005
1173C>T	0.4	294	Caucasians	D'Andrea 2005
3730G>A	0.35	294	Caucasians	D'Andrea 2005
-1693G>A	0.43	297	Caucasians	Sconce et al 2005
2255T>C	0.44	93	European-American	Li et al 2006
3673G>A	0.36	167	Caucasians	Holzer et al 2008
3673G>A	0.38	2631	Non-Hispanic white	Crawford et al 2010
2255T>C	0.37	120	Romanians	present study

Conclusions

The 2255T>C polymorphism, investigated for the first time in 120 Romanian subjects, was found to be in accordance with the Hardy-Weinberg equilibrium.

The 0.37 frequency of the minor allele is similar with minor allele frequencies previously described for specific other VKORC1 gene polymorphisms in Caucasians (3673A>G, 3730G>A, 1173T>C). This comes to support the statements of other authors that a group of major VKORC1 gene polymorphisms are in strong linkage disequilibrium.

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