Original Paper

ATG5 rs2245214 C/G Polymorphism Frequency in a Romanian Population

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ABSTRACT: Purpose. The aim of this study was to assess the frequency of a key autophagy gene ATG5 rs2245214 C/G polymorphism in a Romanian volunteer cohort, as there are no data regarding an Eastern European population. Material/Methods. DNA was extracted from peripheral blood of 105 Romanian unrelated volunteers. The ATG5 rs2245214 C/G polymorphism was genotyped by Real-Time PCR using allelic discrimination TaqMan assay. RealTime PCR was performed on a ViiA™ 7 Real Time PCR System. Hardy–Weinberg equilibrium of allele frequencies at individual loci was assessed using the Chi-squared test. Results. The genotype frequencies in controls were distributed in accordance with Hardy–Weinberg equilibrium ($\chi^2 = 1.07$; $p = 0.3$). We found CC genotype in 53 subjects (50.48 %), CG genotype in 40 (38.10 %) and GG genotype in 12 subjects (11.42 %).The G risk allele was found in 52 individuals, and the frequency of the minor G allele was 0.3. Conclusion. This is the first report on a Romanian population regarding the frequency of the ATG5 gene rs2245214 polymorphism. Our results are slightly different to the distribution pattern from other Caucasian populations and larger studies including various ethnic groups are required.

KEYWORDS: *autophagy, gene, ATG5 polymorphism, genotype*

Introduction

Autophagy is an ancient process of recycling cellular components, such as cytosolic organelles and protein aggregates, enabling cells to digest their own cytosol or invasive bacteria. Autophagy is activated in conditions of cell stress, hypoxia, starvation, or growth factor deprivation; it promotes cell survival by generating free metabolites and energy through degradation mediated by lysosomes [1,2]. Autophagy plays an important role in the pathophysiology of cancer, neurodegenerative diseases or aging [3].

During autophagy, a portion of cytosol is sequestered within a double-membrane vesicle, called an autophagosome. The building of autophagosomes occur in four stages: initiation, elongation, closure and maturation. Firstly, Atg14-Beclin1-hVPS34 complex acts to form an isolation membrane. The phagophore elongation and closure steps involve conjugation of the Atg5–ATG12–Atg16L1 complex and LC3, resulting in a double membrane organelle called autophagosome. Further, dynein and microtubules are required for the LC3 mediated movement of autophagosome to the lysosomes. The autolysosome results by fusing of the autophagosome with lysosome organells and the delivered content is degraded by lysosomal enzymes and acidic pH [1,4,5].

At the molecular level, autophagy involves multiple genes and ATG proteins are required for autophagosome formation and maturation [1]. ATG5 gene is a key gene in the autophagy process, being involved in autophagic vesicle formation. Moreover, Atg5 is necessary for antigen presentation [6] and can lead to increased viral clearance. Also, Atg5 is involved in apoptosis and regulation of interferon responses against viral infections, and Atg12-Atg5 conjugate has been shown to negatively regulate the type I interferon modulating pathway [7].

The aim of this study was to assess the frequency of ATG5 rs2245214 G/C (c.574-12777G>C) polymorphism in a Romanian volunteer cohort, as there are no data regarding an Eastern European population. Further we compared the frequency of genotypes with other ethnic control groups, as the ethnicity has been identified as an independent risk factor for the development of more diseases in multiracial polymorphism studies.

Material and Methods

A total number of 105 Romanian unrelated volunteers were enrolled in the study. Blood (2.5 ml) was collected in an EDTA tube and stored frozen until DNA extraction. Genomic DNA was extracted from peripheral blood using Wizard Genomic DNA Purification Kit (Promega), following the manufacturer protocol. The ATG5 rs2245214 C/G polymorphism was genotyped by Real-Time PCR using allelic discrimination TaqMan assay. Primers and allele specific dual fluorogenic probes labeled with Fam and Vic as a reporter and Tamra as a quencher were used to determine the allelic variants.

The RealTime PCR reactions were carried out in a 7.625 µl reaction volume containing 5 µl of sample DNA, 2.5 µl of the Universal Master Mix (Applied Biosystems, Foster City, CA), 0.125 µl TaqMan SNP Genotyping Assay 40x (C_3001905_20, Applied Biosystems, Foster City, CA) specific for each allele and 1.75 µl DNase-free, sterile-filtered water per reaction. RealTime PCR was performed on a ViiA™ 7 Real Time PCR System (Life Technologies, Carlsbad, USA) and cycling conditions were as follows: 95ºC for 10 min, followed by 50 cycles of 95º C for 15s and 60ºC for 1 min annealing temperature.

Hardy–Weinberg equilibrium of allele frequencies at individual loci was assessed by comparing the observed and expected genotype frequencies using the Chi-squared test.

The Ethics Committee of University of Medicine and Pharmacy of Craiova, Romania approved this study and a written informed consent has been obtained from all participating subjects

Results

A total number of 105 Romanian unrelated volunteers were enrolled in the study; including 49 men (46,67%) and 56 women (53,33%); the age of the participants ranged from 17 to 75 years (median 41 years).

Genotype frequencies of the ATG5 single nucleotide polymorphisms rs2245214 did not deviate significantly (χ^2 = 1.07; p = 0.3, MAF= 0.3) from those expected under the Hardy–Weinberg equilibrium (Table 1). We found a CC genotype (Fig. 1) in 53 subjects, CG genotype (Fig. 2) in 40 and GG genotype in 12 subjects (Fig. 3). The genotype frequencies are shown in Table 2. The risk G allele was found in 52 individuals, and the frequency of this minor allele was 0.3.

Table 1. Hardy–Weinberg equilibrium values

ATG5 rs2245214	Observed	Expected	χ^2; p
CC	53	51	1.07; 0.3
CG	12	44	
GG	40	10	

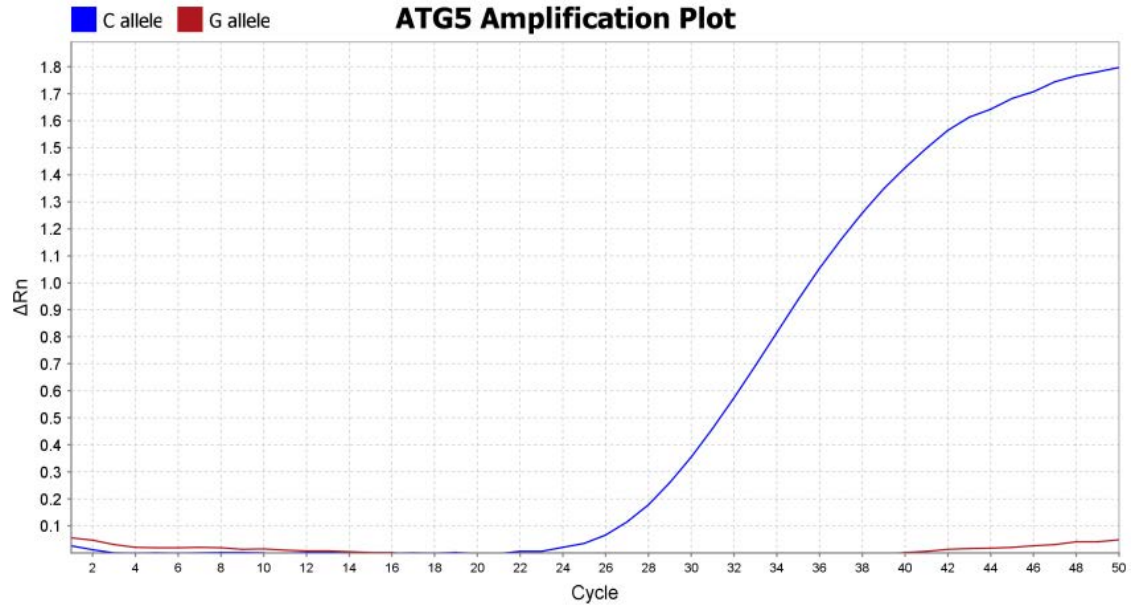

Fig.1. Allelic discrimination according to Fam and Vic – CC genotype

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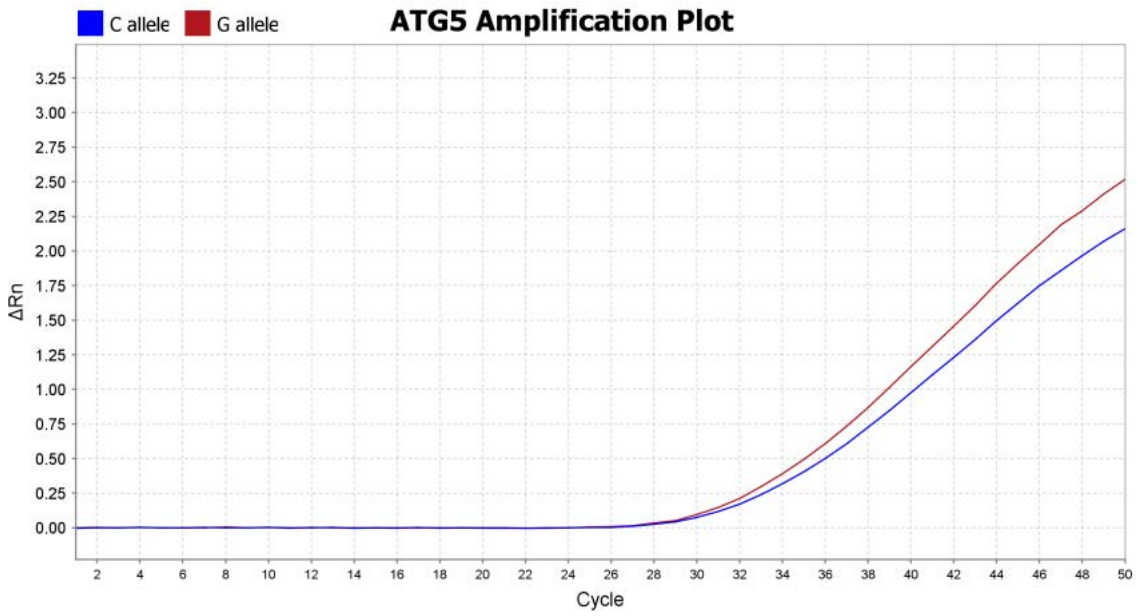

Fig.2. Allelic discrimination according to Fam and Vic – CG genotype

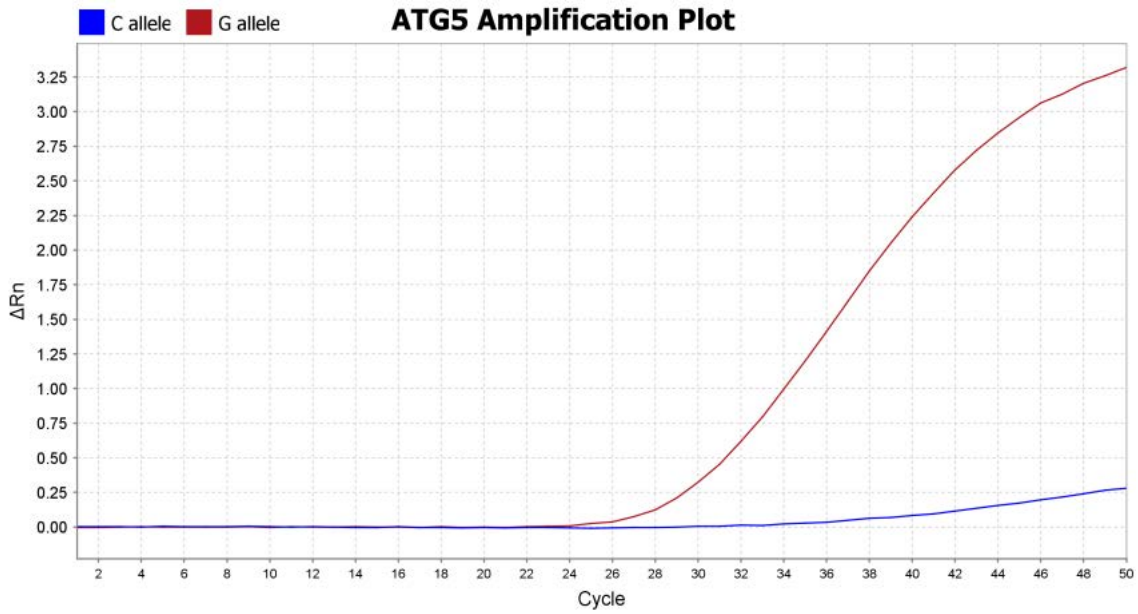

Fig.3. Allelic discrimination according to Fam and Vic – GG genotype

Table 2. Genotype and allele frequencies of ATG rs2245214 polymorphism

ATG5 rs2245214	Subjects n=105
CC	50.48 %
CG	38.10 %
GG	11.42 %
C allele	69.52 %
G allele	30.48 %

Discussion

We firstly evaluated the frequency of ATG5 rs2245214 G/C polymorphism in an Eastern European population. ATG5 gene is located on the long arm of chromosome 6q21. Several polymorphisms were described in the promoter and open reading frame. The rs2245214 polymorphism (c.574-12777G>C) consists of a G instead of C substitution in the intron 6.

Our allele frequency results are slightly different when we compared with other Caucasian cohorts, yet the deviation was higher for genotypes. In a Dutch case-control study, including 189 healthy controls, the genotypes were 35% CC, 52% CG and 13% GG [8]. This research showed that patients carrying the ATG5 rs2245214 polymorphism have a higher probability to develop thyroid carcinoma. In a replication study of previously reported systemic lupus erythematosus risk loci, including ATG5 rs2245214 polymorphism, the risk G allele frequency was 0.37 in 12188 controls. A separate comparation including only American and Swedish controls show a 0.353 respectively 0.407 of G allele [9]. In another case-control study including 952 Javanese controls (Indonesia) no association was observed between this polymorphism and susceptibility to pulmonary tuberculosis [10]. The frequency of ATG5 genotypes in control group were 35.8% CC, 48,5% GC and 15,7% GG, and minor G allele frequency was 39.9. The minor C allele was found in 47.4% controls in a Han Chinese population case-control study, including 807 systemic lupus erythematosus patients and 938 healthy controls, and no correlation with systemic lupus erythematosus was found [11]. Minor allele frequency in our study was lower than those found in HapMap public database: 0.30 (G) in our study versus 0.43 (G) in Caucasians, 0.45 (G) in African Americans, 0.47 (G) in Han Chineses and 0.49 in Japanases (National Center for Biotechnology Information dbSNP). In addition to the small number of samples, an explanation for our results could be the ethnicity of subjects.

Other ATG5 variants were investigated in several case-control studies. No association was found between Atg5 M129V (rs34793250) and age at onset of Huntington disease [12]. Polymorphism rs6568431 in ATG5 was initial associated with rheumatoid arthritis in a UK cohort, but the association did not remain statistically significant after Bonferroni correction [13]. Harley et al., identified ATG5 rs573775 (G/A) as a susceptibility locus for systemic lupus erythematosus [14], but their results were not replicated in a Finnish study [15]. Two other ATG5 polymorphisms (rs12201458 and rs510432) were associated with asthma. The minor allele (A) of ATG5 rs12201458 was associated with a decreased risk of asthma, while the minor allele (G) of ATG5 rs510432 was associated with increased asthma risk [16]. Alonso-Perez et al. showed that Atg5 rs573775 was more associated with systemic lupus erythematosus susceptibility in Central than in Southern Europeans [17], and T allele was a risk factor for systemic lupus erythematosus in carriers of the high IL-10 producer genotype [18].

Conclusion

This is the first report on a Romanian population regarding the frequency of the ATG5 gene rs2245214 polymorphism. Our results are slightly different to the distribution pattern from other Caucasian populations and larger studies including various ethnic groups are required.

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Conflict of interest: No conflict of interest to declare.

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