Heritability of myopia and its relation with 
GJD2 and RASGRF1 genes in Lithuania

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Abstract

Background: This study aimed to assess heritability of myopia in Lithuania and evaluate both genes GJD2 (Gap Junction Protein, Delta 2) and RASGRF1 (RAS protein-specific guanine nucleotide-releasing factor 1) relation with myopia.

Methods: In this study Lithuanian twin population aged between 18 and 40 (n = 460) were examined. Single-nucleotide polymorphisms of the RASGRF1 (rs8027411) and GJD2 (rs634990) genes were assessed by real-time polymerase chain reaction method.

Results: Intrapair correlations for spherical equivalent in all twin pairs were significantly higher in MZ twin pairs r = 0.539 (p < 0.001, 95% CI 0.353–0.684) than in DZ twin pairs r = 0.203 (p < 0.01, 95% CI 0.063–0.442) in myopia group. Correlations for spherical equivalent in emmetropia group were not significant in MZ twin pairs r = 0.091 (p > 0.05, 95% CI -0.215–0.381) and in DZ twin pairs r = −0.220 (p > 0.05, 95% CI -0.587–0.222). The odds ratio (95% CI) were 2.7 (1.018–7.460) for combinations of genotypes of rs634990 CC and rs8027411 GT (p = 0.046).

Conclusions: Our studies have shown that the heritability of myopia makes 67.2% in Lithuania. Persons with combinations of genotypes rs634990 CC and rs8027411 GT have 2.7 times higher odds to have myopia.

Keywords: RASGRF1, GJD2, Twin, Myopia
Genome-wide association studies (GWAS) for refractive error showed that single nucleotide polymorphisms (SNPs) in 15q25 and 15q14 were associated with refractive error and myopia [9]. RASGRF1 is a gene made of 28 exons. This gene has a significant influence on development of myopia. RASGRF1 gene encodes Ras protein-specific guanine nucleotide-releasing factor-1, which is highly expressed in the retina and neurons. Then RASGRF1 gene proceeds to activate Ras [10, 11]. Also, RASGRF1 is a nuclear exchange factor that promotes GDP/GTP exchange on the Ras family GTPases and is related to synaptic transmission of the photoreceptor responses [12]. Muscarinic receptors and retinoic acid can regulate RASGRF1 expression as well [11]. Some animal and human studies showed that muscarinic inhibitors prevented the development of myopia [13]. In animal models of myopia there was detected reduced synthesis of choroidal retinoic acid [14]. To date, studies of SNP rs8027411 of RASGRF1 gene associations with high myopia in different populations have provided controversial results [6, 15–17].

The GJD2 gene at 15q14 encodes a neuron-specific protein connexin 36 (CX36), a 36 kDa protein, which is a neuron-specific protein of a family of integral membrane proteins [18]. CX36 forms gap junction channels between adjacent membranes of neuronal cells. It is present in photoreceptors, bipolar and amacrine cells, and, by enabling intercellular transport of small molecules and ions, plays an essential role in the transmission process of the retinal electric circuitry [18, 19].

The aim of our research was to find associations between the GJD2, RASGRF1 genes and myopia development and to assess the heritability of myopia in Lithuania.

**Methods**

**Ethics statement**

Permission (Number P1–52/2005) to undertake the study was obtained from the Kaunas Regional Biomedical Research Ethics Committee. Before the study, the
procedure and purpose of the study was explained, and an informed consent was obtained from all participants.

**Study samples**
The twins participating in this study were from the Twin Centre of Lithuanian University of Health Sciences. The Twin Centre has registered more than 600 twin pairs who agreed to participate in various medical and genetic studies. The study was conducted in the Institute of Biological Systems and Genetics Research, Lithuanian University of Health Sciences.

**Refractive error measurement**
Refractive error was measured with Sol. Cyclopentolate 1% using an autorefractor (Accuref-K9001, Shin-Nippon, Japan) and calculated by the mean spherical equivalent for each of the two eyes of every individual. The mean spherical equivalent was calculated using the standard formula: spherical equivalent = sphere + (cylinder/2).

MZ and DZ twins with spherical equivalent of at least one eye ≥ −0.5 D were assigned to the myopia group. Twins whose spherical equivalent was between 0.49 and −0.49 D were included in the emmetropia group. The myopia degree was determined by the strength or optical power of a corrective lens that focuses distant images on the retina: from −0.5 D to −3 D mild-degree myopia; from −3 D to −6.0 D medium-degree myopia; and −6.0 D and over high-degree myopia [20, 21].

The exclusion criteria were as follows: 1) cataract, refractive surgery or other previous interventions that might have affected refractions; 2) other refractive errors; 3) refusal to participate in the research.

The inclusion criteria were as follows: 1) no ophthalmological eye disorders were found on detailed ophthalmological evaluation; 2) participation consent.

Lenses were evaluated by a slim-lamp biomicroscopy with the illumination source at a 45 degree angle and the light beam set being set to 2 mm width.

**Verification of zygosity**
Zygosity was determined using a DNA test. The polymerase chain reaction set (AmpFISTR® Identifiler®, Applied Biosystems, Foster City, CA, USA) was used to amplify short tandem repeats. 15 specific DNA markers were used for comparison of genetic profiles: D8S1179, D21S11, D7S820, CSF180, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TROX, D18S51, D5S818, and Amelogenin. The sample’s gender, age, zygosity characteristics and spherical equivalents are shown in Table 1.

**Table 2** Frequency of GJD2 and RASGRF1 genotypes and allele (%)

| SNP ID   | Gene   | N   | Genotype Frequency | Minor allele frequency | P value |
|----------|--------|-----|--------------------|------------------------|---------|
| rs634990 | GJD2   | 272 | CC, CT, TT         | C                      | 0.1692  |
|          |        |     | 56 (20.6%), 147 (54%) | 259 (47.6%)          |         |
| rs8027411| RASGRF1| 285 | GG, GT, TT         | G                      | 0.0913  |
|          |        |     | 60 (21.1%), 157 (55.1%) | 277 (48.57%)         |         |
DNA extraction
Peripheral blood samples were collected from each individual in ethylenediaminetetraacetic (EDTA) tubes for DNA extraction. DNA was extracted from leukocytes using a reagent kit (NucleoSpin Blood L Kit; Macherey & Nagel, Düren, Germany). DNA samples from one member of each MZ pair were used for genotyping.

Genotyping
SNP of the GJD2 gene (rs634990) were assessed using a commercial genotyping kit C_2088259_10. SNP of the RASGRF1 gene (rs8027411) was assessed using a commercial genotyping kit C_185318_10 (Applied Biosystems, Foster City, CA, USA). The Applied Biosystems 7900HT Real-Time Polymerase Chain Reaction System was used for detecting the SNPs. The cycling program started with heating for 10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C. Allelic discrimination was carried out using the software of Applied Biosystems. Both SNPs (rs8027411, rs634990) were present in two previous GWASs.

Statistical analysis
The data was analysed with the statistical software package SPSS version 19.0 for Windows. Odds ratios and 95% confidence intervals were computed to assess the association between two SNPs and myopia multivariate logistic regression (Tables 3). Statistical significance was determined at a two-tailed p = 0.05 level. Estimate of heritability (h²) was obtained Pearson’s correlations (r) for MZ and DZ twin pairs: h² = 2 × (rMZ - rDZ) [22].

Results
230 pairs of twins (135 MZ and 95 DZ) aged between 18 and 40 participated in the study, their mean age being 25.08 years (SE 0.7 years) (Table 1). The mean spherical equivalent was −1.324 ± 0.150, with a range from 0.49 D to −7.375 D. There were no significant differences between MZ and DZ twins in age and spherical equivalent of the left and right eyes.

Refractive errors for twin 1 versus twin 2 for mean of spherical equivalent are shown in figs. 1 and 2. Intrapair correlations for spherical equivalent in twin pairs were significantly higher in MZ twin pairs r = 0.539 (p < 0.001, 95% CI 0.353–0.684) than in DZ twin pairs r = 0.203 (p < 0.01, 95% CI 0.063–0.442) in myopia group. Correlations for spherical equivalent in emmetropia group were not significant: r = 0.091 (p > 0.05,99% CI -0.215–0.381) in MZ twin pairs and r = −0.220 (p > 0.05, 95% CI -0.587–0.222) in DZ twin pairs. The correlations of MZ were clearly higher compared to DZ pairs, indicating genetic effects on myopia.

We genotyped two SNPs (rs634990 and rs8027411) in 189 myopia (83 MZ and 106 DZ) and 96 emmetropia (25 MZ and 71 DZ) subjects. The results are shown in Table 2. The distribution of the two SNP genotypes matched the Hardy-Weinberg equilibrium (p ≥ 0.05).

High-degree myopia was present in 11 cases, 33 twins had medium-degree 10 and 145 twins mild-degree myopia. But we didn’t find significant correlations between myopia degree and genotypes.
5 models were used to calculate the odds ratios to have myopia separately with each gene (GJD2 or RASGRF1), results were not significant (Table 3, Table 4).

But we found significant association between the combinations of GJD2 CC and RASGRF1 GT and myopia (Table 5). The odds ratio of myopia compared to emmetropia (95% confidence intervals [CIs]) was 2.7 (1.018–7.460) for GJD2 CC and RASGRF1 GT genotypes.

The number with combinations of genotypes rs634990 CC and rs8027411 GT and myopia degrees are shown in Table 6.

Discussion

We estimated heritability of myopia according to correlations for MZ and DZ twin pairs and our study showed 67.2% heritability of myopia. Three published twin studies of refractive error have found heritability from 84 to 86% [23], 89 to 94% [24] and 75 to 88% [25]. It is indicate that heritability in Lithuania is lower than in other European populations. Dirani et al. have reported that different populations have shown a wide range of heritability estimates ranging from 50 to 90% [25]. Results shown that the samples of the population and different methods may affect the estimates of heritability [25]. In our study 77% twins had mild-degree myopia, 17% - medium-degree and 6% - high-degree myopia. Meanwhile, the medium-degree myopia accounted for the largest portion in the mentioned studies of heritability in Europe.

Study showed that the gene GJD2, located nearest to the locus 15q14, and RASGRF1 15q25 are important for the transmission and processing of visual signals [23, 26]. The studies of genetic associations in some European and Japanese populations showed that common genetic variations located in GJD2 and RASGRF1 were associated with common myopia and refractive error [5, 11, 15].

A study of genome-wide associations (GWASs) showed associations of SNP with refractive error in 5328 individuals of the Dutch population which were not related. They found that carriers of the C allele of rs634990 have a higher risk of myopia [5]. Qiang et al. found that RASGRF1 gene was significantly associated with high-degree myopia (risk allele T) but GJD2 gene was not [15]. Also, results of meta-analysis, which included 2529 individuals with high-degree myopia and 3127 controls, showed that RASGRF1 was significantly associated with high-degree myopia in Chinese and Japanese populations. However, carriers of the RASGRF1 G allele had a lower risk of high-degree myopia compared to carriers of the T allele (G versus T) [27]. Also, Hysi et al. found that individuals carrying TT alleles on the RASGRF1 were significantly more likely to have myopia than those homozygous for the non-susceptibility GG alleles. We found a significant association between combinations of GJD2 and RASGRF1 genotypes and myopia. Our study showed that individuals with combinations of GJD2 CC and RASGRF1 GT genotypes were 2.7 times more likely to have myopia (p = 0.046). This indicates that some of our results are consistent with the previous reports. Individuals carrying CC alleles on the GJD2 were significantly more likely to have myopia than carriers of TT alleles. But carriers of GT allele on the RASGRF1 gene had more risk to have myopia than carriers of wild type alleles.

Conclusion

Our studies have shown that the heritability of myopia makes 66.4% in Lithuania. We detected significant associations between the combinations of GJD2 CC and RASGRF1 GT and odds ratio of developing myopia.

Abbreviations

D: Diopters; DZ: Dizygotic twins; GJD2: Gap Junction Protein Delta 2; MZ: Monozygotic twins; RASGRF1: RAS protein-specific guanine nucleotide-releasing factor 1; SE: Standard error

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Availability of data and materials

The datasets during and/or analyzed during the current study available from the corresponding author on reasonable request.

Authors’ contributions

EK, RL, MS analyzed the data. EK, RL wrote the manuscript. EK, AS developed the structure for the paper. AS, RL, MS made critical revisions. EK, AS, ITM approved final version. All authors approved of the final manuscript.
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