GENE POLIMORPHIMS AND MUTATIONS OF HEARING IMPAIRMENT IN ROMA AND HUNGARIAN POPULATIONS

PhD Thesis

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1. Introduction

Genetics of hearing impairment

Hearing loss is the most common sensory disorder, influences the normal communication and affects more than 350 million people worldwide. Hearing loss has 3 main types. Conductive hearing loss occurs in the ear canal, the middle ear, the ear drum, or the bones in the middle ear or in the membranous labyrinth. Sensorineural hearing loss develops in the inner ear, and affected the auditory nerve, the auditory pathways, or the auditory cortex. The third type is the mixed hearing loss. This is the combination of the conductive and sensorineural hearing loss. In most cases hearing loss is a multifactorial disorder, caused by genetic and environmental factors or a combination of these. However, one gene mutation also can cause the disease. Approximately, 200 genes are responsible for hearing, of which 30 are known.

One of the best known genes, whose mutation causes hearing loss, is the *GJB2* gene. *GJB2* has one coding exon, therefore it belongs to the group of small genes. Nowadays, more than 6 autosomal dominants, and 70 autosomal recessive inherited genetic mutations have been reported in the *GJB2* gene. The W24X is a nonsense mutation causing recessive deafness phenotype, and detected only in Indian populations.

NAT2 gene plays an important role in the metabolism of reactive oxygen species (ROS) and there is a correlation between *NAT2* rs1799930 and the age-related hearing impairment. *NAT2* gene is highly polymorphic and there are 3 types of NAT2 enzyme metabolism: fast, intermediate, and slow acetylator phenotypes. These phenotypes show Mendelien inheritance and all of them cause hearing loss.

GRM7 has a central role in glutamate synaptic transmission and homeostasis in the cochlea at the synapses between the dendrites and hair cells of afferent auditory nerve fibers. Glutamate toxicity plays a role in a formation of noise induced and age-related hearing loss in many ways. The presence of glutamate in large quantity causes neurotoxicity in auditory neuron, because of its stimulating property. A false allele of *GRM7* changes the synaptic autoregulation of glutamate in the synaptic gap of hearing neurons and hair cells, which leads to glutamate accumulation, resulting cell death.

GRHL2 is a transcription factor which is expressed in different epithelial cells, and responsible for the maintenance of these cells. *GRHL2* gene contributes to epithelial barrier formation and wound healing, closes neutral tube, maintains mucociliary respiratory epithelium and tumor suppression. In the *GRHL2* gene several SNPs have been associated with autosomal dominant, noise induced and age-related hearing loss.

MARVELD2 gene associated DFNB49 locus and its mutations causes non-syndromic bilateral, prelingual, moderate or severe deafness. This transmembrane protein is mainly concentrated in tricellular tight junctions (tTJ) in the cochlear cells and plays an important role in forming an epithelial barrier against paracellular flux of ions and solutes, which is essential to maintain the ion composition of inner ear fluids and proper hearing function. Up to now, six deafness causing recessive mutations in this gene have been identified in 15 families worldwide.

Several mitochondrial DNA mutations have been associated with hearing loss. Some mutations in the 12S rRNA and tRNA Ser genes of the mitochondrial DNA cause non-syndromic hearing loss, which may be induced by aminoglycoside exposure or develop independent of it. Aminoglycoside-containing antibiotics such as gentamicin, streptomycin and tobramycin are clinically important drugs. The use of these drugs often leads to toxicity, affecting the kidney or the auditory and balancing system. Damage of the kidney is usually reversible, but injury of the auditory and balance organs is irreversible. The m.1555A>G and m.1494 T>C mutations in 12SrRNA were identified in patients with aminoglycoside-induced hearing loss. Seven additional mutations in 12S rRNA (m.827 A>G, m.961delTinsC, m.961 T>C, m.961 T>G, m.1005 T>C and m.1095 T>C, m.1116 A>G) have been reported as mitochondrial non-syndromic hearing loss mutations. Although their pathogenic significance remains contested. A large phenotypic variation is detected in mtDNA-caused hearing loss. Hearing loss may only occur after exposure to aminoglycoside or without it. Furthermore, the degree of hearing loss alters, even within families. Some mtDNA mutations may not trigger hearing loss on their own, but only in the presence of other environmental or genetic elements that modify the variability and penetrance of the hearing loss associated with these mtDNA variations. These genetic variants can be identified as essential risk factors rather than pathogenic mutations.

2. Aims of the study

The aim of our experiments was to investigate the frequency and distribution of susceptible gene polymorphisms and mutations of hearing impairment in Roma and Hungarian populations. Furthermore, we would have liked to examine, that the significant genetic differences between the investigated groups may make the populations more susceptible, or even more vulnerable to hearing loss.

1. Our aim was to characterize the frequency of *NAT2* rs1799930, *GRM7* rs11928865, *GRHL2* rs10955255, rs13263539 and rs198161 and *GJB2* rs104894396 variants in Roma and Hungarian populations, and to establish the haplotypes profile of *GRHL2* gene polymorphisms in both examined populations.

2. Further aim was to examine the prevalent and clinical effect of *MARVELD2* c.1331 + 2 T>C mutation in deaf Hungarian and Slovak Roma patients, and to analyze the common ancestral origin of this mutation in the affected Roma and Pakistani patients.

3. Our aim was also to investigate the distribution of the susceptible mitochondrial DNA polymorphisms (m.827 A>G, m.961 T>C, m.961 T>G, m.1005 T>C, m.1095 T>C, m.1116 A>G, m.1494 T>C and m.1555G>A) in Roma and Hungarian population samples.

3. Material and methods

Studied populations

The DNA samples of the Roma and the Hungarian population originated from the central Biobank governed by the University of Pecs, as part of the National Biobank Network of Hungary (www.biobanks.hu), which belongs also to the pan-European Biobanking and Biomolecular Resources Research Infrastructure project (http://bbmri.eu/bbmri/). The maintenance and governance principles of the Biobank have been approved by the National Scientific Research Ethics Committee (ETT TUKEB, Budapest, Hungary). The collection and usage of DNA samples and management of data followed the Helsinki Declaration of 1975. DNA samples for the *MARVELD2* investigation came from DIABGENE Laboratory, IEE SAS., at the ORL department of the University Hospital, and from Department of Molecular Biology, Faculty of Natural Sciences, Bratislava. A total of 298 healthy Roma (118 males, 180 females; mean age 42,33 ± 15,51 years) and 298 healthy Hungarian subjects (168 males, 130 females; mean age 37,43 ± 12,53 years) were investigated in case of *NAT2* rs1799930, *GRM7* rs11928865, *GRHL2* rs10955255, rs13263539 and rs1981631 SNPs. Furthermore, 113 deaf Roma people (57 males and 56 females) were characterized for the *NAT2* rs1799930, *GRHL2* rs13263539, and rs1981361. 493 healthy Roma samples (250 males and 243 females, mean age 50 ± 19 years) and 498 healthy Hungarian (268 males and 230 females, mean age 36 ± 12 years) were used for *GJB2* rs104894396. 85 deaf Hungarian Roma, 502 healthy Hungarian Roma, 143 deaf Slovak Roma, 200 healthy Slovak Roma and 375 deaf Slovak people were investigated for the c.1331+2 T>C mutation in *MARVELD2* gene. Furthermore, 21 polymorphisms (rs542778, rs4699896, rs4976108, rs67911569, rs10059317, rs56103849, rs4252228, rs1168405, rs1168402, rs299086, rs299093, rs2434507, rs299075, rs299078, rs28652974, rs28409706, rs468467, rs188123810, rs467880, rs466930, and rs2133729) spanning 5,34 megabasis around the c.313 + 2T> C mutation were genotyped, in case of 5 Slovak, 7 Hungarian probands, 5 Czech and 4 Pakistani patients. Polymorphisms were selected from the dbSNP database based on their chromosomal position and minor allele frequency (MAF) value. A control group of 20 unrelated hearing impaired Roma patients and 36 unrelated normal hearing Roma individuals without the c.1331+2T>C mutation was genotyped along to determine genetic variability of the selected SNP markers. Total of 200 healthy Roma (72 males, 123 females; mean age 43,65 ± 16,21 years) and 200 healthy Hungarian (106 males, 94 females; mean age 37,15 ± 11,93 years) subjects were used in case of mitochondrial DNA polymorphisms.

Molecular biology methods

Genomic DNA was isolated from peripheral EDTA-anticoagulated blood samples using a standard desalting method. The first step of the genotyping was the polymerase chain reaction (PCR) using specific oligonucleotide primers, dNTP, Taq polymerase, buffer and DNA template. It followed by restriction endonuclease digestion (RFLP). The amplicon was digested by allele-specific restriction endonuclease, and contained an obligatory cleavage site to enable us to control the efficacy of the digestion. The digested PCR products were separated by electrophoresis using a 3% agarose gel, ethidium-bromide dye and standard DNA ladder.
Direct sequencing was performed by ABI 3500 Genetic Analyzer (Applied Biosystems [CA, USA]) on random samples to confirm our results using BigDye Terminator v.1.1 cycle sequencing kit.

Statistical evaluations

Statistical analyses were performed using SPSS Statistics 20.0 package for Windows. We applied Chi-square test to compare the differences between studied groups, $p \leq 0.05$ value was considered as statistically significant. For haplotype analyses we used Phase 2.1. software and for the investigation of linkage equilibrum were used Haploview 3.3 software.

4. Results

NAT2 rs1799930

We found significant differences in the investigated polymorphism of the *NAT2* gene, the AA homozygous genotype had an almost two-fold higher prevalence in the Roma population (14,1%) compared to the Hungarians (7,7%). Furthermore, the minor allele frequency was shown to be significantly higher in the Roma samples than in Hungarian population (38,0% and 26,7%, $p < 0,05$) (table 1.). However, we did not find significant differences between the healthy and deaf Roma populations.

1. Table: Genotypes and minor allele frequencies of *NAT2* rs1799930 polymorphism in case of Roma and Hungarian populations

***NAT2* rs1799930**	**Roma (n=298)**	**Hungarian (n=298)**
GG	114 (38,20%)	162 (54,30%)
GA	142 (47,60%)	113 (38,00%)
AA	42 (14,00%)*	23 (7,70%)
A allele frequency	38,00%*	26,70%

* $p<0,05$

***GRM7* rs11928865**

A *GRM7* rs11928865 TT genotype was not shown significant differences between the Roma and Hungarian populations (6,7% vs.7,0%), and we did not find significant differences in case of the minor allele frequency too (25,6% vs. 25,8%). The prevalence of TT genotype and the allele frequency is almost the same in the two populations.

***GRHL2* rs10955255 rs13263539 and rs1981361**

We did not find significant differences neither in the homozygous genotype (23,7% vs. 21,0%) nor in the minor allele frequency (50,8% vs. 47,0%) in case of *GRHL2* rs10955255 between Roma and Hungarian populations. However, we found significant differences for rs13263539 and rs1981361 of *GRHL2* in the minor allele frequency and the homozygous genotype too with a lower prevalence in the Roma population (table 2.). We examined rs13263539 and rs1981361 polymorphisms in healthy and deaf Roma populations, but we did not find significant differences. Haplotype analysis of the 3 SNPs determined 8 haplotypes and the occurrence of the GGT, GAC haplotypes were two-fold higher and GAT haplotypes was five-fold higher in the Hungarians than in Roma population (table 3.). Linkage between the 3 polymorphisms was stronger in Roma populations than in Hungarian samples. Comparing these data with European and Asian linkage maps, we can see that the values slightly differ in the Roma and Asian, and in the Hungarian and European linkage maps. However, the linkage of the rs10955255 and rs13263539 polymorphisms in European populations is stronger than in Hungarian population (figure 1.). Furthermore, the polymorphisms of *NAT2, GRM7* and *GRHL2* were compared to other European and Asian populations. We could observed, that the allele frequencies of *GRHL2* rs10955255 and *GRM7* rs11928865 SPNs in the Roma population

are similar to the values in the European populations. However, the allele frequencies of *NAT2* rs1799930, *GRHL2* rs13263569 and rs1981361 in Roma population are similar to Asian populations. From these observations we can conclude that the Roma population - which derived from India - has been mixed to some extent with the Hungarian population over the centuries (table 4.).

2. Table: Genotypes and minor allele frequencies of *GRHL2* rs13263539 and rs1981361 polymorphisms in case of Roma and Hungarian populations

GRHL2	**Roma (n=298)**	**Hungarian (n=298)**
rs13263539		
GG	102 (34,20%)	69 (23,20%)
GA	157 (52,30%)	153 (51,30%)
AA	39 (13,00%)	76 (25,30%)*
A allele frequency	37,90%	51,00%*
rs1981361		
GG	87 (29,00%)	59 (19,80%)
GA	162 (54,40%)	143 (48,00%)
AA	49 (16,50%)	96 (32,30%)*
A allele frequency	43,60%	56,20%*

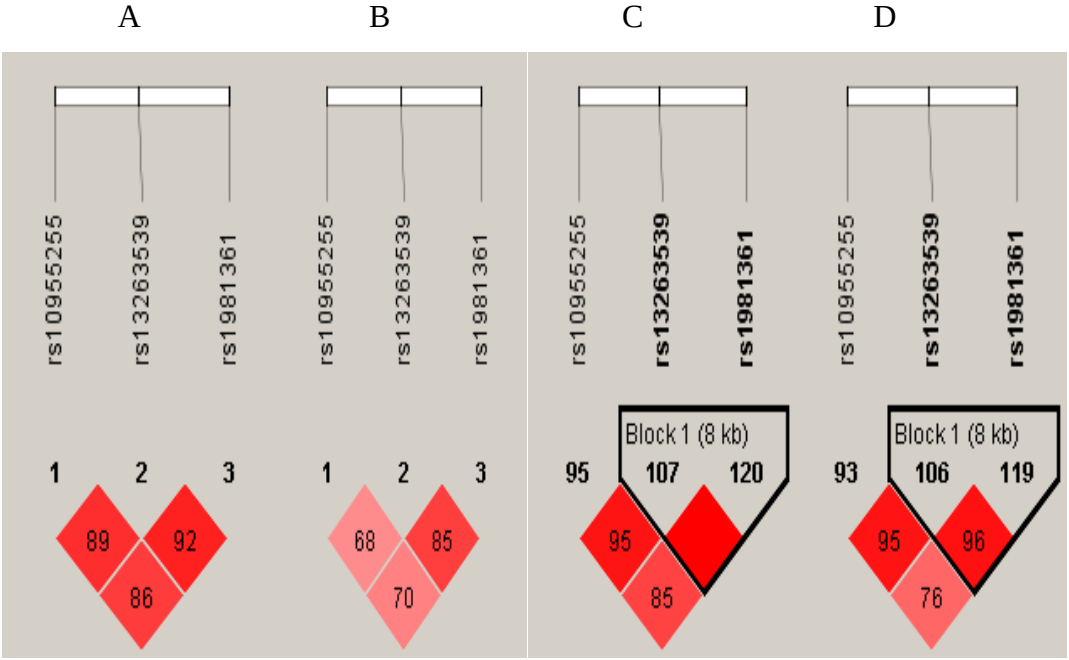
* p<0,05

3. Table: Major haplotypes (ht) and observed frequencies of the detected haplotypes in Roma and Hungarian populations

rs10955255	**rs13263539**	**rs1981361**	**Haplogroups**	**Roma (%)**	**Hungarian (%)**
A	G	C	ht1	7,66	5,80
A	G	T	ht2	3,17	3,97
A	A	C	ht3	0,69	0,99
A	A	T	ht4	37,60	44,20
G	G	C	ht5	46,90	34,90
G	G	T	ht6	1,87	4,47*
G	A	C	ht7	0,91	2,07*
G	A	T	ht8	1,15	5,51*

* p<0,05

1. **Figure:** Linkage disequilibrium analysis for the *GRHL2* rs10955255 (1), rs13263539 (2) and rs1981361 (3) polymorphisms in Roma (A), Hungarian (B), Asian (C) and European (D) populations.



4. **Table**: Major and minor allele frequencies (af) of *NAT2* rs1799930; *GRM7* rs11928865; *GRHL2* rs10955255, rs13263539 and rs1981361 polymorphisms in case of different populations, based on www.ensembl.org.

Populations	n	*NAT2* rs1799930		*GRM7* rs11928865		*GRHL2* rs10955255		*GRHL2* rs13263539		*GRHL2* rs1981361	
		G af (%)	A af (%)	A af (%)	T af (%)	A af (%)	G af (%)	A af (%)	G af (%)	C af (%)	T af (%)
EUR	1006	71,80	28,20	72,20	27,80	47,10	53,00	41,20	58,80	40,30	59,70
HUN	**298**	**73,30**	**26,70**	**74,20**	**25,80**	**49,00**	**51,00**	**53,00**	**47,00**	**43,80**	**56,20**
Roma	**298**	**62,00**	**38,00**	**74,40**	**25,60**	**62,10**	**37,90**	**49,20**	**50,80**	**56,40**	**43,60**
CEU	198	70,20	29,80	70,70	29,30	49,00	51,00	42,90	57,10	43,90	56,10
FIN	198	73,70	26,30	65,70	34,30	37,40	62,20	31,80	68,20	34,80	65,20
GBR	182	72,50	27,50	70,90	29,10	47,80	52,20	42,30	57,70	39,00	61,00
IBS	214	70,60	29,40	73,80	26,20	51,90	48,10	64,30	53,70	43,50	56,50
TSI	214	72,00	28,00	79,00	21,00	49,10	50,90	73,00	57,90	39,70	60,30
SAS	978	64,00	36,00	78,00	22,00	74,90	25,10	73,00	27,00	74,10	25,90
BEB	172	73,30	26,70	76,70	23,30	72,10	27,90	72,10	27,90	69,80	30,20
GIH	176	61,20	38,80	74,80	25,20	71,80	28,20	69,90	30,10	72,30	27,70
ITU	204	65,70	34,30	80,90	19,10	82,80	17,20	79,40	20,60	82,40	17,60
PJL	192	63,50	36,50	80,20	19,80	70,30	29,70	69,30	30,70	69,30	30,70
STU	204	57,80	42,20	77,50	22,50	77,00	23,00	74,00	26,00	76,00	24,00

n= number of samples, af=allele frequency

***GJB2* W24X *(*rs104894396)**

None of the subjects in Roma and Hungarian samples were find to carry the *GJB2* rs104894396 AA homozygous genotype. However, significant differences were find comparing the minor allele frequencies between the Roma (1,62 %) and the Hungarian (0,20%) populations (table 5.).

5. Table: Genotypes and minor allele frequencies of *GJB2* rs104894396 polymorphism in case of Roma and Hungarian populations

***GJB2* rs104894396**	**Roma (n=493)**	**Hungarian (n=498)**
GG	485 (98,40%)	497 (99,80%)
GA	8 (1,62%)	1 (0,20%)
AA	0 (0,00%)	0 (0,00%)
A allele frequency	0,008%*	0,001%

* $p<0,05$

***MARVELD2* c.1331+2 T>C**

Investigation of 143 unrelated hearing impaired Roma patients from Slovakia detected the c.1331+2T>C mutation in a homozygous state in five, and in a heterozygous state in one affected individual. Analysis of 200 unrelated normal hearing Romas revealed nine individuals heterozygous for the c.1331+2T>C mutation, but homozygous state was not observed. The C allele frequency of the deaf Slovak Roma patients was 3,85%, while in case of normal hearing Slovak Roma patients was 2.25%. The c.1331+2T>C mutation was not find in the group of 375 hearing impaired non-Roma patients. Screening of the c.1331+2T>C mutation in the group of 85 unrelated deaf Hungarian Roma individuals identified seven homozygous and three heterozygous patients. In the control group of 502 normal hearing Hungarian Roma person, the c.1331+2T>C mutation was find in 5 heterozygous individuals. In case of C allele frequency, we find significant differences between the deaf Hungarian Roma (10,0%) and deaf Slovak Roma (3,85%) groups. Furthermore, the C allele frequency in the healthy Hungarian Roma (0,50%) group was 4 times lower than in the healthy Slovak Roma (2,25%) group (table 6-8). To confirm the common ancestry of the c.1331+2T>C mutation in all patients from our study, (as well as among the Pakistani subjects where the mutation was first detected), we analyzed 21 SNPs. The identified common haplotype defined by 18 SNP markers was shared by all

Hungarian and Slovak Roma patients in a homozygous state, suggesting a common ancestor for this mutation in Central European Roma patients. Furthermore, these 18 SNPs were genotyped in 56 unrelated Roma individuals without the mutation. The haplotype covering 18 SNPs was not detected in a homozygous form in any of the analyzed control samples, supporting the hypothesis of a common ancestry of this mutation among these individuals.

6. **Table:** Genotypes and minor allele frequencies of *MARVELD2* c.1331+2 T>C in case of deaf Hungarian Roma and healthy Hungarian Roma populations

***MARVELD2* c.1331+2 T>C**	**Deaf Hungarian Roma (n=85)**	**Healthy Hungarian Roma (n=502)**
TT	75 (88,23%)	497 (99,00%)
TC	3 (3,53%)	5 (0,99%)
CC	7 (8,23%)	0 (0,00%)
C allele frequency	10,00%*	0,50%

* $p<0.05$

7. **Table:** Genotypes and minor allele frequencies of *MARVELD2* c.1331+2 T>C in case of deaf Hungarian Roma and deaf Slovak Roma populations

***MARVELD2* c.1331+2 T>C**	**Deaf Hungarian Roma (n=85)**	**Deaf Slovak Roma (n=143)**
TT	75 (88,23%)	137 (95,80%)
TC	3 (3,53%)	1 (0,69%)
CC	7 (8,23%)*	5 (3,49%)*
C allele frequency	10,00%*	3,85%*

* $p<0.05$

8. Table: Genotypes and minor allele frequencies of *MARVELD2* c.1331+2 T>C in case of heathy Hungarian Roma and healthy Slovak Roma populations

***MARVELD2* c.1331+2 T>C**	**Healthy Hungarian Roma (n=502)**	**Healthy Slovak Roma (n=200)**
TT	497 (99,0%)	191 (95,5%)
TC	5 (0,99%)	9 (4,5%)
CC	0 (0,00%)	0 (0,00%)
C allele frequency	0,50%*	2,25%

* $p<0,05$

Mitochondrial polymorphisms

We did not find significant differences in the investigation of mitochondrial DNA polymorphisms between the two populations. The 200 Roma DNA samples had normal genotypes. However, we found in the Hungarian population homoplasmic polymorphisms, 1 in case of m.961 T>G, 5 of m.961 T>C and 2 of m.1555 A>G. Heteroplasmy was not observed in any case.

5. Discussion

Polymorphisms of the *NAT2, GRM7* and *GRHL2* genes are involve in the development of age-related hearing impairment. Many factors can contribute to aging, including genetic mutations associated with environmental interactions, or large accumulation of reactive oxygen species. The blood flow decreased in the circulatory system of the inner ear within the cochlea during the aging. Our aim was to reveal the interethnic differences in the genotype and allele frequency of *NAT2*, *GRM7* and *GRHL2* polymorphisms between Roma and Hungarian populations. We found significant differences between the two populations in case of *NAT2* rs1799930 and *GRHL2* rs13263539 and rs1981361 polymorphisms. The presence of these polymorphisms is associated with an increased risk of developing age-related hearing impairment. Carrying of the mutant allele of *NAT2* rs1799930 polymorphism showed a significantly higher value in the Roma population than in the Hungarians. In contrast, the presence of mutant allele in case of *GRHL2* rs13263539 and rs1981361 polymorphisms was higher in the Hungarian population. Furthermore, the linkage between the examined SNPs of *GRHL2* gene was stronger in Roma populations than in Hungarian samples. Over and above, we identified 8 haplotypes, whereby the frequency of GGT, GAC and GAT haplotypes was significantly higher in the Hungarian population than in Romas. We did not find significant differences between the two population in case of *GRM7* rs11928865 and *GRHL2* rs10955255 polymorphisms. In conclusion, ethnic differences in the allele frequencies of the susceptible polymorphisms have important implications for the preventive and therapeutic treatments in different populations in the future. However, to determine the exact role of this susceptibility SNPs more studies are needed on larger population samples.

In most cases, the mutations of the *GJB2* gene show ethnic differences. In most Caucasian populations, the 35delG mutation in *GJB2* gene is the most common variant which causes genetic non-syndromic deafness. However, in populations of non-European ethnic background, other *GJB2* gene mutations dominate in patients with hearing loss, like W24X in India. The 71G>A (W24X) mutation has been detected in Europeans, but its presence is about three times higher in Pakistanis and at least 20 times higher in Indians. This variant is relatively common in affected populations with deafness from India and in people to be originated from the Indian subcontinent, such as the Roma. The mutant allele frequency of *GJB2* W24X found in the Hungarian (0,10 %) and the Roma populations (0,81 %) is significantly differ from each other and the allele frequency of the Roma population is similar to the measured data in the Indian populations.

The most frequent *MARVELD2* mutation is the c.1331+2T>C, identified in twelve Slovak and Hungarian Roma family in homozygous form. The currency of *MARVELD2* related hearing loss was 3.5% in the group of deaf Slovak Roma individuals and 8,23% in deaf Hungarian Roma patients. We can find a similar contrast in case of C allele frequency between the groups of Slovak (3,85%) and Hungarian (10,0%) deaf Romas. In addition to, a 4,5 times higher difference in opposite direction was identify in the carrier rate of the *MARVELD2* mutation between control groups of healthy Slovak and Hungarian Roma patients. By analyzing this mutation in a control group of 375 hearing impaired Slovak Caucasians, we have confirmed previous results that presence of this mutation in Europe is probably limited to the Roma population, and is still not known in Slavic Caucasian ethnicity. In our study, we tested patients with the c.1331+2T>C mutation whether have a common ancestral haplotype. In seventeen Roma patients (5 Slovak, 5 Czech and 7 Hungarian), we analyzed 21 biallelic polymorphisms spanning 5,34 Mb around the mutation. Haplotype analysis showed a common haplotype of 18 SNPs, which was present in the homozygous form in all Roma c.1331+2T>C individuals, but none of the 56 control Romas. These data support the hypothesis of common ancestor for the c.1331+2T>C variant in all analyzed Slovak, Czech and Hungarian Roma individuals.

200 healthy Hungarian and 200 healthy Roma individuals were tested by Sanger sequencing method. We did not find significant differences between the Roma and the Hungarian populations. A larger sample number should be used to reveal significant differences between the two populations.

6. Summary

1. *NAT2* rs1799930 polymorphism showed significant differences, the AA homozygous genotype and the minor allele frequency had a higher prevalence in the Roma population compared to the Hungarian cohorts.

2. We did not find significant differences between the two populations in case of *GRM7* rs11928865 *GRHL2* rs10955255 polymorphisms.

3. We found significant differences for *GRHL2* rs13263539 and rs1981361 polymorphisms in the minor allele frequency and the homozygous genotype too, with a lower prevalence in the Roma population than in the Hungarians.

4. The linkage between the *GRHL2* rs132635393, rs1981361 and rs10955255 polymorphisms was stronger in Roma population than in Hungarian samples.

5. Haplotype analysis of the *GRHL2* polymorphisms determined 8 haplotypes. The occurrence of GGT and GAC haplotypes were two-fold higher, and GAT haplotype was almost five-fold higher in Hungarian, than in Roma populations.

6. We found significant differences for *GJB2* rs104894396 polymorphism in the A allele frequency between the two populations.

7. Analysis of the *MARVELD2* c.1331+2T>C mutation in the group of deaf Hungarian Romas showed higher C allele frequency value, than in control group of normal hearing Hungarian Romas. We found significant differences for this mutation in the minor allele frequency and the homozygous genotype with a higher prevalence in deaf Hungarian Romas compared with the deaf Slovak Roma group. However, the C allele frequency was higher in the healthy Slovak Romas, than in the healthy Hungarian Roma group. Furthermore, our results provide support for the hypothesis of a possible common ancestor of the Slovak, Hungarian and Czech Roma, as well as Pakistani deaf patients.

8. We did not significant differences between the two populations in case of mitochondrial DNA polymorphisms.

7. Acknowledgements

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8. Publications

Publications supporting the dissertation

Matyas P, Postyeni E, Komlosi K, Szalai R, Bene J, Magyari L, Melegh B, Hadzsiev K. 2018. **Age-related hearing impairment associated *NAT2, GRM7* and *GRHL2* susceptibility gene polymorphisms and haplotypes in Roma and Hungarian populations.** *Pathology & Oncology Research.* ***IF: 1.736***

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