Carcinogenesis related allelic polymorphisms in the Hungarian Roma population

PhD Thesis

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I.Introduction

I.1. The Roma people, main groups, ethnography, demography

The Roma/Gipsy population (approximately 10-12 million people) is the largest European minority (Kalaydieva, 2001). The “Gipsy” word is often used in pejorative sense in several languages, and the first Roma World Congress in London decided to use the “Roma” (Romani) name for the population (http://romediafoundation.worldpress.com). The origin of the Roma people is Northern and Southern-Northern India, they arrived to Europe approximately 1000 years ago after a long wandering (Gresham, 2001). Irrespectively from the fact that the Roma minority means one single ethnic group to the majority, it should be noted that the Roma population does not form a homogeneous group. The most pregnant characteristic of the Roma people is their language. Based on this criterion, in Hungary there are three major Roma community. The largest group (70%) is the so called Hungarian Roma (“Romungro” or “musician Gipsy”), they speak only the Hungarian language. Approximately 20% of the Roma belongs to the Vlax Roma (“oláh cigányok”, “kolompárok”). Besides the Hungarian they typically speak the “lovári” variant of the Vlax language. The lovári language, as official Roma languge is widely and actively used in this ethnic group. A smaller group of the Roma is the Bayash (“beás”) Roma, they speak an archaic version of the Roman language. Particulary in the Transdanubian region the new generations of the Bayash Roma do not use the traditional language, which may slowly die out.

At the 2011 census 315,583 persons declared themselves to belong to this ethnic group, this was 3.2% of the total Hungarian population. It is not easy to define who belongs to the Roma minority. Legally those persons can be considered as Roma who declares themselves as Roma, but because of the numerous prejudices concerning these ethnic groups, much less people do that (Ladányi, 2004, Tomka, 1991). Social researchers and sociologists agree that the true number of Roma people is much higher than it is shown by the self-declaration based data of censuses (Kemény, 2004, Puporka, 1999). Based on a series of special estimations, the real number of Roma was approximately 640,000 in 2009 (6-7% of the total population, Hablicsek, 2007). The demographic characteristics of Roma communities significantly differ from those of the majority, their natural demographic indicators (age distribution shifted to the young, high fertility, high mortality, low life expectancy at birth) are similar to populations in the stage of demographic transition (Hablicsek, 2007). In the next period significant increase can be expected in the Roma populations, in all age groups.

I.2. Health status of the Roma populations

While the Roma groups do not form one single population, all the published results show a uniform and significant difference between the health indicators of the Roma and non Roma people. The life expectancy at birth is with approximately 10-15 years below the average of the majority population (Bogdanović, 2007; Kósa, 2002; Koupilova, 2001). In the industrial countries the major causes of deaths are given by chronic non communicable diseases. In relation to malignant

tumors, according to the study of Delphoi Consulting in 2004, the Roma mortalities were 1.8 times higher than that of the non Roma average (Delphoi Consulting, 2004; Babusik, 2005).

In the etiology of cancers both environmental and genetic factors play an important role. In relation to the Roma population, there is a lack of studies in this field concerning the genetic factors of public health importance. The population attributable risk of genetic factors causing hereditary diseases is relatively low, due to the low occurrence of these factors. If we would like to study the factors with a high impact on incidence and mortality. then we should study the so called "individual susceptibility factors". These are genetic polymorphisms with minor to moderate effect on the risk of cancer, but occurring with a much higher frequency. Based on the above described assumptions, in the present study we investigated allelic polymorphisms with high population attributable risk on cancer. We tried to model the early carcinogenesis as accurately as possible, by the inclusion of genes in the biotransformation of xenobiotics, DNA repair processes, regulation of the cell proliferation. The selected allelic polymorphisms are proved to have an influence on cancer susceptibility, but, according to our best knowledge, they have not been studied in relation to Roma populations yet. As subjects of our investigation, the Vlax Roma were selected, since this is a relatively large, but closed enough community which strongly keeps its traditions. The following figure illustrates the processes of early carcinogenesis and their key genes, and subsequently the selected genes will be shortly described.

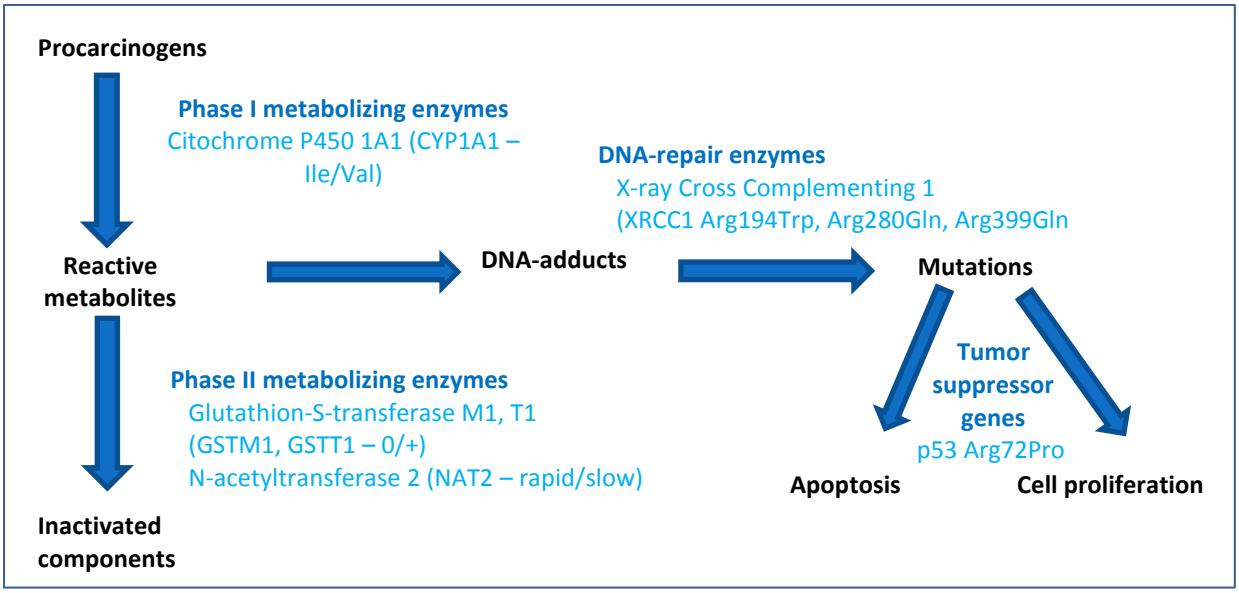


Figure 1.: Early stages of carcinogenesis, with the genes and polymorphisms studied in our investigation

I.3. The studied genetic factors

I.3.1. Cytochrome P450 1A1 (CYP1A1 – Ile/Val polymorphism)

The CYP 1A1 – as a Phase I metabolizing enzyme – often forms more toxic metabolites than that of the original compound (Nelson, 1996). It has several external – potentially carcinogenic – chemical substances (e.g. benzpyrene and other polycyclic aromatic hydrocarbons) and endogenous (e.g. steroid hormones, fatty acids) substrates. The functional Ile/Val polymorphism of the CYP 1A1 gene is caused by an A→G substitution on the area of exon 7, causing an isoleucin→valin change. The protein encoded by the Val allele has a higher enzymatic activity, and thus activates the externally derived, potentially carcinogenic substances faster and more efficiently (Kawajiri, 1993; Hayashi, 1991).

I.3.2. Glutathione-S-transferase M1 (GSTM1), Glutathione-S-transferase T1 (GSTT1) – Ins/Del polymorphism

During biotransformation the intracellular level of reactive metabolites of xenobiotic substances and molecules formed during oxidative stress decreases due to the effect of phase II metabolizing enzymes. The most known representatives of these detoxifying processes belong to the glutathione-S-transferase superfamily. The external chemical carcinogenic molecules, e. g. polycyclic aromatic hydrocarbons, 4-(methylnitrosamino)-1-(3-pyridil)-1-butanone, dimethylbenzanthracene, methylcolantrene, benzpyrene, and majority of epoxides are detoxified by the mju (GSTM) and theta (GSTT) GST families (Hayes, 2005; Ketterer, 1988). Both the GSTT1 and GSTM1 enzymes a section of the gene may be missing, leading to a truncated protein which is not able to perform the conjugation with glutathione. The so called null genotype indicates the lack of a functional enzymes (both parental alleles encode the truncated enzyme).

I.3.3. N-acetyltransferase 2 (NAT2) – slow and rapid acetylator polymorphism

The NAT2 enzyme belong to the family of N-acetyltransferases, and depending on its substrates, it can catalyze both phase I and phase II reactions. The NAT2 takes part in the biotransformation of aromatic amines and hydrazides. Due to NAT2 polymorphisms the transcribed enzymes have different activities (Borlak, 2006). The wild type (NAT2*4) allele encodes an enzyme with rapid acetylating ability. The several polymorphic alleles can be divided into 3 categories, based on the phenotype (enzymatic activity). Rapid acetylators are homogeneous for the wild type allele, while slow acetylators carry two copies of variant alleles. The third phenotype is the intermediate acetylator, these people have a wild type and a variant allele. There is a simplified categorization with only two categories as well: rapid acetylators carry the wild type allele while slow acetylators do not (Seow, 1999).

I.3.4. X-ray repair cross complementing 1 (XRCC1) DNA repair gene – Arg194Trp, Arg280His, Arg 399Gln polymorphisms

The intracellular concentration of carcinogenic substances is determined by the activity or inactivity of our metabolizing enzymes. However, even in spite of an effective detoxification, reactive metabolites can always be found in our cells, which form adducts with macromolecules. Ionizing

radiations, smoking, alcohol, oxidative stress typically lead to single strand breaks of DNA and base defects. These DNA lesions are primarily repaired by base excision repair mechanisms. The XRCC1 gene is a key protein in the BER. Allelic polymorphisms of genes participating in the DNA repair are proved to influence the susceptibility to cancer (Wu, 2011; Engin, 2011).

I.3.4. TP53 tumor suppressor gene – Arg72Pro polymorphism

The p53 protein (containing 393 amino acids) takes part in several regulational processes trying to preserve the integrity of the cell. The p53 protein stops the cell cycle in the G1 phase, thus preventing the proliferation of potentially damaged cells. This gives time to the DNA repair enzymes to correct the DNA damage. If the DNA damage cannot be repaired, the p53 protein induces apoptosis, and thus protects the whole organism. Mutations of the TP53 gene have been described in approximately 50% of the human tumors (Bennett, 1999). The gene, however, possesses allelic polymorphisms as well, with the codon 72 Arg/Pro polymorphism being the most important one. This is caused by a G→C substitution leading to an arginine-proline change. This amino acid replacement is on the transactivational domain of the protein; the apoptotic and transcriptional activity of the protein differs from that of the wild type allele (Dumont, 2003). The Arg/Pro allelic frequencies show a wide distribution in different races/ethnic groups, and are also responsible for the individual/interethnic differences in cancer susceptibility (Wu, 2002).

I.3.6. MicroRNAs – micrRNA-146a (miR-146a) polymorphism

The majority of the human genome is under microRNA regulation (Friedman, 2009), including cancer susceptibility genes. Similarly to the functional genes, the microRNA-coding sequences may also possess polymorphisms which may affect the process of maturation or the function of the mature microRNA. These allelic polymorphisms can affect the maturation and/or stability of the microRNA, even if they are on areas which are cleaved during microRNA processing. Among the targets of miR-146a there are several genes which play an important role in the human carcinogenesis, so through the regulation of these genes the miR-146a can also have an influence on the risk of human cancers. In the mentioned own pilot study we analyzed the effect of miR-146a rs 2910164 polymorphism on the risk of head and neck cancer in a case-control study.

I.4. Head and neck cancer

Among the European countries Hungary had the highest mortality of head and neck cancer in 2012. The strongest known risk factors of head and neck tumors are smoking and consumption of alcoholic beverages. Their effect is significant separately, but in case of their simultaneous presence the risk of lip and mouth cancers is multiplied. Bad or inadequate oral hygiene is also a prominent risk factor. The role of high-risk HPV types (e.g. 16, 18) in the etiology of squamous head and neck cancers is also well known (Kreimer, 2005). Among people in low socioeconomic classes the incidence of head and neck cancers is much higher than in higher socioeconomic groups, since these risk factors have much higher prevalence among the poor. Because of the high Hungarian head and neck cancer mortality and the significantly elevated risk of the Roma population a separate study was included (independently from the main train of thought of the PhD work) to describe the effect of pre-miR-146a allelic polymorphism on the risk of head and neck tumors. This

study was not performed on an ethnic bases, the subjects was a group from the “general Hungarian population”. The advantage of this strategy is to supply results applicable to the total Hungarian population.

II. Objectives

- Description of the allelic distributions of certain genes encoding metabolizing enzymes (CYP1A1, GSTM1, GSTT1, NAT2) in the Hungarian Vlax Roma population.
- Description of the allelic frequencies of XRCC1, a key DNA repair enzyme, in the Hungarian Vlax Roma population.
- Description of the allelic frequencies of TP53 tumor suppressor gene, in the Hungarian Vlax Roma population.
- Studying and describing the effect of pre-miR-146a rs2910164 allelic polymorphism on the risk of head and neck cancer.
- Description of the allelic frequencies of pre-miR-146a rs2910164, in the Hungarian Vlax Roma population.
- Description of the above allelic polymorphisms in a Hungarian, non Roma population, comparison between Roma and non Roma allelic frequencies. Based on the Indian origin of the Roma, comparison with Indian allelic distributions, taken from the literature.

III. Materials and methods

III.1. Participants, study design

III.1.1. Comparison between Roma and non Roma allelic distributions

In the present PhD work 195 Vlax Roma persons have been genotypes. The Roma participants were identified, selected, contacted in the framework of a Roma project of the Johan Béla National Center for Epidemiology, including the collection of blood samples. The samples were kindly provided to us by dr. Judit Béres. The sampling and the examinations were carried out in the possession of the necessary ethical permissions and informed consent of the participants; the participation was voluntary. Five hundred forty seven Hungarian non Roma persons served as controls (peripheral blood was taken from them, and the allelic distributions were compared with those of the Roma participants). These allelic frequencies were also compared with distributions

from the Indian literature, based on the Indian origin of the Roma (Mittal, 2011; Majumder, 2005; Buch, 2002; Tandle, 2001; Zhao, 1995). Within our limitations, we tried to use Northern Indian populations for these comparisons.

III.1.2. Association between the risk of head and neck tumors and the pre-miR-146a rs 2910164 polymorphism

This case control study was performed independently from the Roma/non Roma comparisnos, its goal was to describe the effect of pre-miR-146a rs2910164 allelic polymorphism on the risk of head and neck cancer in the Hungarian population. The ethnic identity of the participants was unknown (not asked/registered), such selection was not applied. Both the case and control groups consisted of 468 participants. Members of the control group were individually adjusted to the patients, based on age (±5 years), gender and smoking habits.

III.2. Genotyping

The peripheral blood was repeatedly centrifuged by 0,84% ammonium-chloride solution to remove red blood cells, and DNA was isolated (GenomicPrep, Pharmacia, Uppsala, Sweden, according the manufacturer's instructions) from the remaining white blood cells. The genotyping methods were as follows:

III.2.1. CYP1A1 Ile/Val polymorphism

The genotype for exon 7 Ile/Val polymorphism can be determined by an allele specific polymerase chain reaction (PCR) (Hirvonen, 1992). The reaction is performed in parallel, in two tubes, with the same ipstrea, but with different downstream primers. The sequence of the upstream primer was GAAAGGCTGGGTCCACCCTCT, and the downstream primers were AAGACCTCCCAGCGGGCAAT and AAGACCTCCCAGCGGGCAAC. The amplification occurred in the tube with the fully complementary downstream primer.

III.2.2. GSTM1 – GSTT1 simultaneous genotyping

The reaction mix contained GSTM1 and GSTT1 primers, and additionally (for control purposes) primers for a segment of the ß-globin gene (Pool-Zobel, 1998). GSTM1-F: GAACTCCCTGAAAAGCTAAAGC, GSTM1-R: GTTGGGCTCAAATATACGGTGG, GSTT1-F: TTCCTTACTGGTCCTCACATCTC, GSTT1-R: TCACCGGATCATGGCCAGCA, ß-globin-F: CAACTTCATCCACGTTCACC, ß-globin-R: GAAGAGCCAAGGACAGGTAC. In case of the + genotype an amplification occurred, while there was no amplification at the 0 genotype.

III.2.3. NAT2 rapid/slow acetylator polymorphism

The products after PCR amplification were divided into 3 parts and digested with 3 different restriction endonucleases (KpnI, TaqI, and BamHI), in order to identify the most frequent slow acetylator genotypes. In 95% of the cases these alleles /M1 (KpnI), M2 (TaqI), M3 (BamHI)/ are responsible for the slow NAT2 acetylating phenotype. The participant was considered as slow acetylator if the wild type allele was not present, so both alleles were variant alleles (Okkels, 1997). Primers: GGAACAAATTGCACTTGG, TCTAGCATGAATCACTCTGC.

III.2.4. XRCC1 – DNA repair enzyme polymorphisms

The variants of the XRCC1 enzyme were studied with restriction fragment length polymorphism (Xing, 2002; Lee, 2001; Lunn, 1999). Codon 194 primers: 5'-GCC AGG GCC CCT CCT TCA A -3', 3'-TAC CCT CAG ACC CAC GAG T -5', digestion: PvuII, codon 280 primers: 5'-TTG ACC CCC AGT GGT GCT AA -3', 3'-GGC TGG GAC CAC CTG TGT T -5', digestion: RsaI, codon 399 primers:5'- TTG TGC TTT CTC TGT GTC CA -3', 3'- TCC TCC AGC CTT TTC TGA TA -5', digestion: MspI.

III.2.5. TP53 Arg/Pro polymorphism

The allele specific amplification was performed parallel in two tubes, with the same 3' primer, but with primers differing in their last 5' base (Murata, 1996). 3' primer:GCAACTGACCGTGCAAGTCA, 5' primers: ATGCCAGAGGCTGCTCCCC**G**, ATGCCAGAGGCTGCTCCCC**C**.

III.2.6. Pre-miR-146a rs2910164 polymorphism

The miR-146a genotyping was performed with the method of the so called confronting primer pairs (Hishida, 2011; Hamajima, 2000). The two primer pairs were designed so that in case of pair 1 the last base of the reverse primer, while at pair 2 the forward primer fell to the SNP site. The first primer was fully complementary with one, the second primer with the other allele, and thus the following reults are achieved: A 261 bp fragment is produced always (1. pair forward and 2. pair reverse primers), carriers of the C allele will give an additional 128 bp fragment (1. primer pair), and the G allele will generate a 182 bp fragment (2. primer pair). Primers: F1: AAGCAGCTGCATTGGATT, R1: CAGCTGAAGAACTGAATTTCAC, F2: GTTGTGTCAGTGTCAGACCTC, and R2: CAAGCTCTTCAGCAGACTGA.

III.3. Statistical methods

Comparison of the allelic frequencies was made by computing odds ratios (OR) and 95% confidence intervals (95% CI), along with p-values of the Pearson's chi-square test. Fitting to the Hardy-Weinberg equilibrium was tested with chi-square test. In the case-control study the age was compared with Student's t-test, while for the frequency based variables chi-square test was used. Connection between risk factors and head and neck cancer was analyzed by logistic regression analysis, adjusted according age, educational status and presence of chronic oral disorders. IBM SPSS v19 software was used for the statistical analysis.

IV. Results

The allelic distributions of the Hungarian non Roma participants were compared to the results of similar studies on other European or American populations, and our results fitted to the line of those studies (Piacentini, 2011; Borlak, 2006; Matullo, 2001; Matthias, 1998; Själander, 1995). Concerning the pre-miR-146a polymorphism, our own results somewhat differed from those of a US study, but showed no statistically significant difference from the results of a Turkish publication (Permuth-Wey, 2011; Akkiz, 2011). All of the studied allelic distributions fitted to the Hardy-Weinberg equilibrium.

IV.1. Metabolizing enzymes

The allelic distributions of CYP1A1 and the GSTT1 genotypes were similar for all the three studied populations. Concerning the prevalence of GSTM1 0 genotype, there was a statistically significant difference between the Hungarian Roma and non Roma population (OR: 0,49, 95% CI: 0.34-0.70, p<0,001), (Figure 2A). The Roma population showed a distribution similar to the Indian one, without a statistically significant difference. With respect to the frequency of NAT2 rapid acetylators, the Hungarian Roma population was between the Hungarian non Roma and the Indian distributions – the latter two differed significantly from each other – but it was a little closer to the Indian one (Figure 2B). The Roma genotype-frequencies (XXXittamagyarbanelütéstjavítani) differed statistically significantly from those of the non Roma (OR: 1,42, 95% CI: 1,00-2,00, p=0,039), while there was a borderline significant difference from the Indian distribution (OR: 0,64, 95% CI: 0,41-1,02, p=0,048).

IV.2. XRCC1 DNA repair enzyme

The Hungarian Roma and non Roma distributions showed a significant difference for the Arg194Trp polymorphism, the minor (Trp) allele was more frequent among Roma than in the majority population (OR: 1,75, 95% CI:1,19-2,57, p=0,003). The Hungarian non Roma distributions, however, did not differ statistically significantly from those of the Indian population, which means that the Hungarian Roma population showed a somewhat longer distance from the Hungarian non Roma than the Indian population did. At the Arg280Gln polymorphism the minor allele (Gln) was also more frequent among Roma than in the non Roma participants the difference proved to be statistically significant (OR: 1,68, 95% CI:1,15-2,46, p=0,003). In case of the Arg399Gln polymorphism the minor allele (Gln) was less frequent in the Vlax Roma population, with a borderline significance (OR: 0,78, 95% CI:0,60-1,01, p=0,048), (Figure 2 C,D,E).

IV.3. TP53 tumor suppressor gene

The largest difference between the Indian and Hungarian non Roma populations was found here: While the proportion of Arg/Arg homozygous participants was 64,9% in the Hungarian sample, in India this was only 14.4%. The Hungarian Roma minority displayed more or less the Indian distributions, with a strong and statistically difference from the Hungarian non Roma population (OR: 4,36, 95% CI: 3,38-5,63, p<0,001) (Figure 2F).

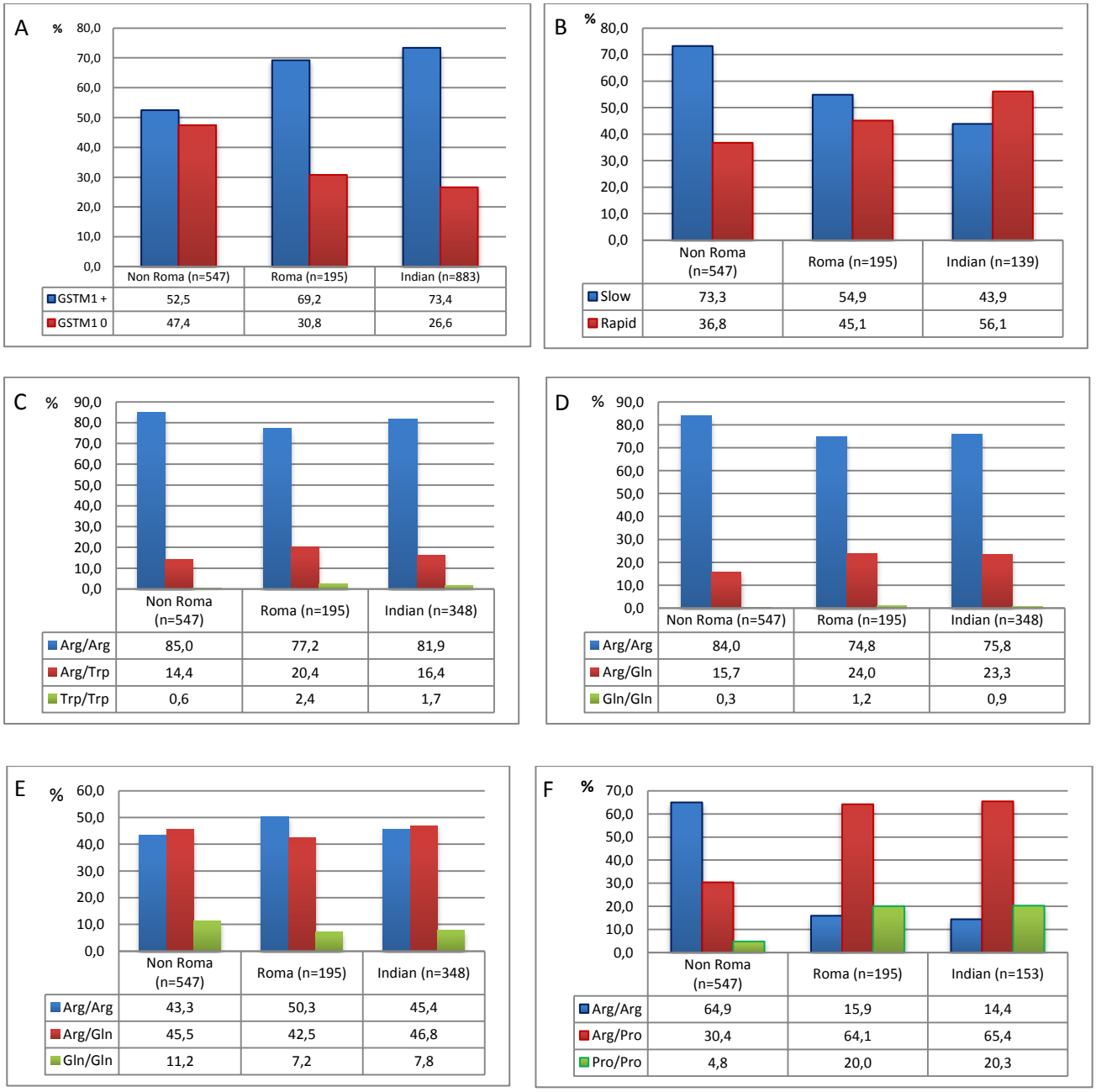


Figure 2.: Occurrence of GSTM1, NAT2 genotypes, XRCC1 codon 194, 280 and 399 alleles and TP53 codon 72 alleles in the Hungarian Roma, non Roma and Indian populations (%).

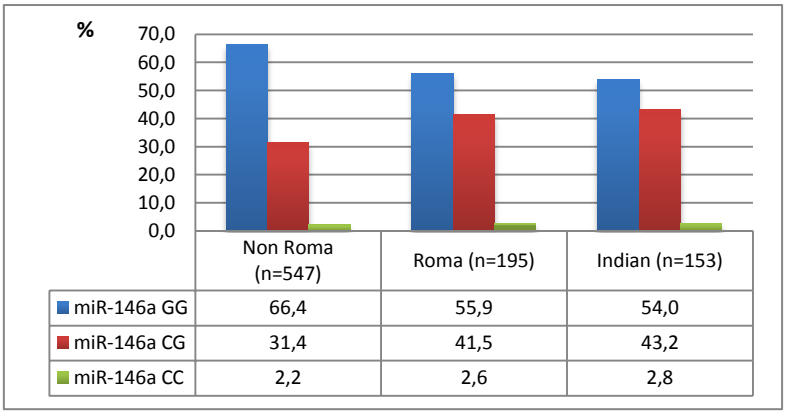
IV.4. Association between pre-miR-146a polymorphism and head and neck cancers

The following distribution was found for the pre-miR-146a rs2910164 genotypes (case – control): GG 60.7% vs. 69.0%, GC 35.9% vs. 29.1%, CC 3.4% vs. 1.9%. The studied distributions in both groups fitted to the Hardy-Weinberg equilibrium. The multiple logistic regression analysis showed an association between pre-miR-146a polymorphism and the formation of head and neck cancers.

Compared to the GG homozygotes proportion of both the heterozygotes (OR: 1,46, 95% CI: 1,10-1,95, p=0,009) and CC homozygotes (OR: 2,37, 95% CI: 1,01-5,60, p=0,048) was higher among cases than in controls. The connection was also statistically significant when heterozygotes and CC homozygotes were handled together as one single group (OR: 1,52, 95% CI: 1,15-2,01, p=0,004). Although it was not the main goal of our study to analyze the association between alcohol consumption and head and neck cancer, we found a statistically significant, dose dependent connection between these two factors (OR: 3,35, 95% CI: 2,18-5,17, p<0,001). Occurrence of chronic oral conditions proved also to be a statistically significant risk factor (OR: 1,87, 95% CI: 1,43-2.45, p<0,001), while no connection was found with the level of education. In our stratified analysis gender proved to be an effect modifier: Alcohol consumption (high intake: OR: 4,26 vs. OR: 5,60), oral disorders (OR: 1,75 vs. OR: 2,52) and presence of pre-miR-146a C allele (OR: 1,44 vs. OR: 1,84) showed a stronger connection with cancer risk in females than in males. The stratified analysis also found an interaction between the pre-miR-146a polymorphism and smoking habits. Smoking increased the risk-increasing effect of the pre-miR-146a C allele, either in relation to the number of cigarettes smoked or to the duration of smoking: This risk-increasing effect was strong or statistically significant in participants smoking at least 20 cigarettes per day or in long-time smokers.

IV.5. Pre-miR146a allelic distributions in the Roma population

The allelic distribution in Hungarian majority differed statistically significantly from both the Roma (OR: 1,39, 95% CI: 1,04-1,86, p=0,025) and the Indian (OR: 1,48, 95% CI: 1,14-1,93, p=0,003) distributions. The C allele occurred more frequently in Roma and Indians than in the Hungarian non Roma population. The Roma and Indian frequencies did not differ from each other significantly (Figure 3).



	Non Roma (n=547)	Roma (n=195)	Indian (n=153)
miR-146a GG	66,4	55,9	54,0
miR-146a CG	31,4	41,5	43,2
miR-146a CC	2,2	2,6	2,8

Figure 3: Distribution of miR-146a genotypes in the Hungarian Roma, non Roma and Indian populations (%).

V. Discussion

Cancer is the second leading cause of deaths in Hungary. The main goal of the present PhD work was to address the question of whether genetic factors can be find in the background of the high Hungarian Roma cancer mortalities (and if yes, then to assess the extent of their contribution). We tried to cover the broadest possible steps of the early carcinogenic processes. All the selected allelic polymorphisms have been already studied in our Department in relation to the risk of tumors in the Hungarian population.

Due to the lack of Hungarian miR-146a data, we planned an own study to describe the Hungarian allelic frequencies, and, on the other side, to assess the effect of this polymorphism on the risk of head and neck cancer. The effect of miR-146a rs2910164 polymorphism on the risk of head and neck cancer has been studied in one single study yet (Liu, 2010). This study did not find an association between this polymorphism and the risk of head and neck tumors, but somewhat controversially, the same polymorphism seemed to have an influence on this risk in an interaction with other polymorphisms. In our study we tried to eliminate the possible sources of error in the study of Liu et al. This is why we chose the matched case-control design (according to gender, age, smoking habits), which could eliminate the effects of possible important confounders (Orsós, 2013). There was a difference between the proportions of smokers as well, in the study of Liu it was lower than the usually reported proportions in developed countries. Based on the above considerations we believe that our results are more accurate and applicable to the Hungarian population than those of the US study.

The possible risk modifying effect of the rs2910164 polymorphism may be based on the fact that it modifies the amount of the mature miR-146a in the cell. The miR-146 has an influence on several cell differentiation processes, thus it is potentially connected the cancer formation (Rusca, 2011). The only study in relation to miR-146a expression in oral cancer found that an increased miR-146a expression is associated with a worse prognosis (Hung, 2012). While this is not an etiological, but a prognostic study, to a certain extent it still supports our results by confirming the connection between miR-146a and head and neck cancers. Due to a G:U→C:U base change the rs2910164 polymorphism leads to a base mismatch and reduced amount of the mature miRNA, as it was demonstrated by Jazdzewski with the help of expression vectors (Jazdzewski, 2008). The supposed tumor suppressor function of the miR-146a is further supported by the fact that the majority of related studies found its lower expression in human tumors than in healthy tissues. The rarely found overexpression (Hung, 2012; Lavon, 2012) could be explained by a possible feed-back mechanism, as a reaction to the disturbed regulatory mechanisms.

The essential part of our study was the comparison between Roma and non Roma allelic distributions. From the 9 studied polymorphisms only the CYP1A1 did not show a difference in this respect, the allelic distributions were similar in the studied populations. Except from that, no difference was found between the Hungarian Roma and non Roma populations for the GSTT1 polymorphism. Here, however, there was a statistically significant difference between the Hungarian non Roma and the Indian populations, and the Roma distributions were approximately “halfway” between them (showing no significant distance from either population).

There was a statistically significant difference between the allelic distributions of the Hungarian Roma and non Roma populations at the other 7 studied allelic polymorphisms (TP53, GSTM1, NAT2, pre-miR-146a, XRCC1 codon 194, 280, 399).

In case of NAT2 the situation was similar to that of the GSTT1, the Roma population was halfway between the non Roma and Indian populations (the latter two shoed a statistically significant difference) (OR: 2.20, 95% CI: 1.48-3.27, p<0.001). The distance between the two "base populations" was very large, while the Hungarian non Roma populations consisted of 73.3% rapid acetylators, this genotype was found in only 56.1% of the Indians. A similar phenomenon was published by Sipeky for the C1326T polymorphism of the MDR1 gene (Sipeky, 2011). This polymorphism was the only one with a statistically significant difference between the Roma and Indian populations, which might be explained by the high number – above 60 - of allelic variants of the NAT2 gene (indicating a stronger variability of this region).

The allelic distributions of the TP53 tumor suppressor gene, similarly to the NAT2, displayed a significant difference between Hungarian Roma and Indian populations. The allelic frequencies show an opposite tendency in these two populations as well: in Hungary the predominant allele was the Arg allele, while in India the Pro allele. The Roma frequencies were very similar to the Indian distribution.

The pre-miR-146a distributions also showed a significant difference between non Roma and Indians, and the Roma distribution was also similar to the Indian one. The same tendency was seen at the GSTM1 polymorphism as well.

All the three XRCC1 polymorphism showed the "usual" difference between the two base populations. Interestingly the Roma distributions were more distant from the Hungarian non Roma than the Indian distribution itself. This is, however, not a unique phenomenon in the literature (Sipeky, 2011).

In spite of the existing statistically significant differences for almost all the studied allelic distributions, our results indicate that these genetic factors are not responsible for the high cancer mortality in the Hungarian Roma population. Namely, the allelic distributions seem to balance the risk increasing / risk decreasing effects of each other (at certain polymorphisms the high-risk alleles are more frequent among Roma, while at other polymorphisms the low-risk alleles). Naturally, this complex question cannot be answered by studying only 9 polymorphisms of 7 genes, so our present study can be considered as a pilot study, which will hopefully be followed by numerous such investigations, covering the broadest possible range of genetic factors. Improvement of the Roma health indicators and reduction in their mortality can be reached by exerting an influence on the external factors, so the primary goal is to eliminate the socioeconomic inequalities. However, an influence on the health behavior can only be reached by programs which are based on the understanding and accepting the cultural and traditional characteristics of the Roma population.

VI. Summary of the new findings

- The following allelic distributions have been found in the Hungarian Vlax Roma population:

    - CYP1A1: Ile/Ile 75.9%, Ile/Val: 23.1%, Val/Val: 1.0%
    - GSTM1: + genotype 69.2%, 0 genotype 30.8%
    - GSTT1: + genotype 82.1%, 0 genotype 18.0%
    - NAT2: slow acetylators 54.9% rapid acetylators 45.1%

- XRCC1:
  - Arg194Trp: Arg/Arg 77,2%, Arg/Trp 20.4%, Trp/Trp 2.4%
  - Arg280Gln: Arg/Arg 74.8%, Arg/Gln 24.0%, Gln/Gln 1.2%
  - Arg399Gln: Arg/Arg 50.3%, Arg/Gln 42.5%, Gln/Gln 7.2%
- TP53: Arg/Arg 15.9%, Arg/Pro 64.1%, 20.0%
- pre-miR-146a rs2910164: G/G 55.9%, G/C 41.5%, C/C 2.6%

- Eight of the above allelic distributions did not exhibit a statistically significant difference from the Indian allelic frequencies. Our results suggest that the Roma population conserved its ancient genetic characteristics.
- There was a statistically significant difference between the Hungarian Roma and non Roma populations with respect to GSTM1, NAT2, TP53, XRCC1 Arg194Trp, Arg280Gln, Arg399Gln (borderline) and pre-miR-146a rs2910164 polymorphisms.
- In a separate study we demonstrated that the pre-miR-146a rs2910164 C allele statistically significantly increased the risk of head and neck cancer in the Hungarian population.

VII. References

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VIII. Publications

VIII.1. The thesis is based on the following publications

1. **Zs. Orsós**, I. Szanyi, A. Csejtei, I. Gerlinger, I. Ember, I. Kiss: Association of pre-miR-146a rs2910164 polymorphism with the risk of head and neck cancer. Anticancer Res. 2013;33(1):341-6. imp.f.: 1.7
2. **Zs. Orsós**, J. Béres, E. Marek, I. Ember, I. Kiss: Allelic polymorphisms in the Hungarian Roma population. Ethnicity & Health, közlésre elküldve

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VIII.2. Other publications

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Impact factor (papers published and accepted for publication): 15,9

VIII.3. Book chapters

1. **Orsós Zs.**: Daganatok epidemiológiája. (XI/3. fejezet) In: Népegészségügyi Orvostan (szerk: Ember István, Kiss István, Cseh Károly). Dialóg Campus , 2013.
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VIII.4. Citeable abstracts:

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20. J. Cseh, **Zs. Orsós**, E. Pázsit, Z. Ozsváth, I. Ember, I. Kiss: Allelic polymorphisms as risk/prognostic factors in cervical cancer. International Conference of Preventive Medicine and Public Health. Magyar epidemiológia. VII. 4:

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