Genetic polymorphism- and haplotype-based methods in different human model systems

Ph.D thesis

Petra Kiszel

Semmelweis University
Doctoral School of Molecular Medical Sciences

Supervisor: Prof. Dr. George Füst
Opponents: Dr. József Prechl
Dr. Barna Vásárhelyi

President of Comprehensive Examination Committee:
Prof. Dr. László Kalabay
Members of Comprehensive Examination Committee:
Prof. Dr. Gabriella Sármay
Dr. András Treszl

Budapest
2007

Introduction

Single nucleotid polymorphism (SNP)- and haplotype-based methods offer a powerful approach to catch candidate genes for multifactorial diseases. Haplotypes are the particular combinations of different SNP alleles observed in a population. SNP is the most common type of variants in human genom and in one haplotype block several SNP-s can be found. Furthermore, only the most informative SNP-s are genotyped from one haplotype block in disease association studies. These tagSNP-s are capable to distinct the all observed haplotypes. After the selection of disease associated SNP-s, *in vitro* model systems are used to examine their functionality.

In our studies, we showed the practical applications and difficulties of these haplotype-based methods in three different human model systems.

The major histocompatibility complex (MHC) on human chromosome 6 is a special region, whereas the linkage disequilibrium is very high between locuses. The LD and hot-spot structure of the MHC are still incomplete. Identification of hot-spot regions is more informative to select candidate genes for diseases. The frequency of 8.1 ancestral haplotypes

and its haplotype variants can be useful to find hot-spot regions.

In next studies we examined the genetic background of a multifactorial disease, the autoimmune reaction of atherosclerosis. Several research groups showed high serum levels of soluble Hsp60 heat-shock proteins and anti-Hsp60 autoantibody in coronary heart and artery disease. Hsp60 molecule is phylogenetically highly conserved protein. After infections specific antibodies against microbial Hsp60 can crossreact with human Hsp60. Furthermore atherosclerosis risk factors alter the physiological appearance of Hsp60 and it becomes autoantigen. The IL-6 can play an important role in the development of autoantigen reactive plasma cells. IL-6 is a B-cell growth factor that induces the maturation of B-cells. Elevated IL-6 plasma levels were shown in atherosclerotic and autoimmune diseases. The effect of IL-6 -174 G/C polymorphism was investigated most often, because this SNP is close to an important transcription factor binding site.

Aims

I. Model system

To determine and compare the frequency of 8.1 ancestral haplotype and its haplotype variants in two different Caucasian populations and in one family study

II. Model system

To examine the associations between *IL-6* -174 G/C polymorphism and anti-Hsp60 autoantibody levels in one independent, healthy Hungarian population. To expand the analysis with several new SNP-s in IL-6 promoter and coding region. To determine the relationship between IL-6 haplotypes and autoantibody levels.

III. Model system

To examine the functionality of *IL-6* -174 G/C polymorphism in a direct biological model system. To compare the LPS or IL-1β induced IL-6 production of HUVECs with different genotypes at *IL-6* -174 position.

Materials and methods

In the I. model system 127 healthy Hungarian individuals, 101 healthy Ohioan female and 9 Hungarian families affected with type I diabetes mellitus were recruited. HLA-DQ, HLA-DR, *RAGE* -429 T/C, *C4A/B* (L/S), *HSP70-2* +1267 A/G, *TNFA* -308 G/A polymorphisms of MHC region on chromosome 6 were genotyped by PCR-RFLP and Southern blot methods.

In the II. model system 313 healthy Hungarian individuals and 399 healthy Finnish subjects were recruited. The level of anti-Hsp60 autoantibodies was detected by indirect ELISA. The *IL-6* -174 G/C polymorphism was genotyped by PCR-RFLP. The other SNP-s in IL-6 promoter and coding region (*IL-6* -9316 T/C, -7164 C/A, -1363 G/T, -597 G/A, +1753 C/G, +2954 G/C) were genotyped using a 5' nuclease (TaqMan) assay for allelic discrimination.

In the III. model system 33 umbilical cords were collected in Budapest. The *IL-6* -174 G/C polymorphism was genotyped by PCR-RFLP in HUVECs. The ICAM-1 expression of IL-1β and LPS stimulated HUVECs was measured by cellular ELISA to detect the appropriate activation of these cells. IL-6 concentration of supernatants was detected by sandwich ELISA.

Results

The frequency of 8.1 ancestral haplotype (AH8.1) and its haplotype variants were determined in parents of 8 healthy children and 8 children affected with type I diabetes mellitus.

There were remarkable differences in the frequency of AH8.1 markers in the Hungarian and Ohioan Caucasian population.

The frequency of 8.1 ancestral haplotype was the same in the two Caucasian healthy populations.

The *HSP70-2* +1267 G allele occurred the most frequently in an 8.1 haplotype variant with one 8.1 AH marker.

The linkage disequilibrium was relatively low when linkage of one AH8.1 marker to the *HSP70-2* G allele was tested. The linkage of two-three AH8.1 marker fragments to the *HSP70-2* G allele was high and significant D' values were obtained.

Our original observation on the association between *IL-6* -174 G allele carriers and high autoantibody levels was repeated in an independent Hungarian population.

In the *IL-6* promoter and coding region six new SNP-s were genotyped in a smaller Hungarian group. The individuals were chosen randomly. Two 2000 bp long haplotype blocks were found between -9316 and -7164 positions and among -597, -174 and +1754 positions.

Five SNP-s (-9316T/C, -1363G/T, -174G/C, +1753C/G, +2954G/C) in *IL-6* promoter and coding region were genotyped in the whole Hungarian population. No significant association was observed between four new SNP-s and anti-Hsp60 autoantibody levels.

Significantly lower autoantibody levels were observed in homozygote carriers of the most common halotype (-9316C/-1363G/-**174C**/+1753C/+2954G), than the non-carriers.

The maximal IL-6 response of HUVECs was significantly higher after IL-1β than after LPS stimulation.

The IL-6 productions did not differ among the different genotyped HUVECs at IL-6 -174 G/C polymorphism after IL-1β and LPS stimulation.

Conclusions

Out of the examined 8.1 AH markers, *HSP70-2* +1267 G allele occurred most frequently in a recombinant 8.1 AH variant, which carried only one 8.1 AH marker. Therefore, we can conclude that hot-spot regions may be located around *HSP70-2* gene. In the next studies the *IL-6* -174 G allele carriers have significantly higher autoantibody levels than subjects with CC genotype in healthy Hungarian population. Two 2000 bp long haplotype blocks were found. The most frequent haplotype, which carried C allele at -174 position, had low autoantibody levels. These results can be explained in several ways. High IL-6 levels can cause high autoantibody levels or other genes, which are in linkage disequilibrium with IL-6 gene on the chromosome 7, may have indirect effect on the production of autoantibodies. In our third study we examined the functional effect of *IL-6* -174 G/C polymorphism in *in vitro* model system. No significant differences were observed between IL-6 expression of HUVEC cells with different *IL-6* -174 G/C genotypes. IL-6 production of HUVECs did not depend on different genotypes at IL-6 -174 position. However, we can not exclude that the IL-6 production can be influenced by IL-6 -174 SNP in endothelial cells with different origins.

Publications

Publications with relevance to the current work

I. *Kiszel P, Fust G, Pessi T, Hurme M, Prohaszka Z.* Associations between Interleukin-6 genetic polymorphisms and levels of autoantibodies to 60-kDa heat-shock proteins. ***Human Heredity*** 2006; 62:77-83; **IF: 2.051**

II. *Kiszel P , Mako V, Prohaszka Z, Cervenak L.* Interleukin-6 -174 promoter polymorphism does not influence IL-6 production after LPS and IL-1beta stimulation in human umbilical cord vein endothelial cells. ***Cytokine*** 2007 Oct; 40 (1): 17-22; **IF: 2.355**

III. *Kiszel P, Kovacs M, Szalai C, Yang Y, Pozsonyi E, Blasko B, Laki J, Prohaszka Z, Fazakas A, Panczel P, Hosszufalusi N, Rajczy K, Wu YL, Chung EK, Zhou B, Blanchong CA, Vatay A, Yu CY, Fust G.* Frequency of carriers of 8.1 ancestral haplotype and its fragments in two Caucasian populations. ***Immunol Invest.*** 2007; 36 (3): 307-19; **IF: 1.276**

Publications in the topic of the current work

IV. *Harcos P, Laki J, Kiszel P, Szeplaki Z, Szolnoki Z, Kovacs M, Melegh B, Szeplaki G, Fust G, Blasko B.* Decreased frequency of the TNF2 allele of TNF-alpha -308 promoter polymorphism is associated with lacunar infarction. ***Cytokine*** 2006 Jan; 33(2):100-5; **IF: 2.355**

V. *Laki J, Kiszel P, Vatay A, Blasko B, Kovacs M, Korner A, Madacsy L, Blatniczky L, Almassy Z, Szalai C, Rajczy K, Pozsonyi E, Karadi I, Fazakas A, Hosszufalusi N, Panczel P, Arason GJ, Wu YL, Zhou B, Yang Y, Yu CY, Fust G.* The HLA 8.1 ancestral haplotype is strongly linked to the C allele of -429T>C promoter polymorphism of receptor of the advanced glycation endproduct (RAGE) gene. Haplotype-independent association of the -429C allele with high hemoglobin(A1C) levels in diabetic patients. ***Mol. Immunol.*** 2007 Jan; 44 (4): 648-55; **IF: 4.768**

Other publications

VI. *Fust A, Veres A, Kiszel P, Nagy ZZ, Cervenak L, Csakany B, Maka E, Suveges I, Grus FH.* Changes in tear protein pattern after photorefractive keratectomy. ***Eur J Ophthalmol.*** 2003 Jul; 13(6): 525-31; **IF: 0.519**