

Candidate Genes of Juvenile Hypertension in the Region of the Major Histocompatibility System

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INTRODUCTION

Primary hypertension (PH), one of the most frequent risk factors of the cardiovascular disease (CVD), is a multifactorial disease, affecting 10-25% of adult population in economically developed countries. Although CVD manifests itself mostly only during adulthood, its initial stage has an onset in early childhood. Epidemiological studies demarcate subpopulations of families with marked accumulation of risk factors of CVD (hypertension, obesity, diabetes mellitus, myocardial infarction, cerebrovascular accident, etc.) among members of several generations of a studied pedigree. Due to the heterogeneous character of the CVD itself, however, a simple mode of its inheritance is unlikely to emerge. Thus, predicting a future manifestation of CVD in as yet healthy individuals is very difficult.

Etiopathogenesis of PH is affected by a combination of genetic and environmental factors. A complex mechanism regulating blood pressure (BP) is under a control of a number of genes.

Existing molecular genetic studies in patients with PH concern mostly genes encoding proteins of humoral and hormonal systems directly involved in regulation of BP. Clinical and experimental investigations involve mainly the gene polymorphism of the rennin-angiotensin system (gene for angiotensin II, angiotensinogen, angiotensin converting enzyme) (Celentano et al., 1999; Sagnella et al., 1999; Redon et al., 2000). Some recent studies have dealt with mutations of genes for selected receptors, e.g., the gene for insulin-like growth factor 1 (Morris et al., 1997), beta 2 (Katanko et al., 1997) and beta 3 adrenergic receptor (Kadowaki et al., 1995; Bendlová et al., 1996; Tonolo et al., 1999) or alpha 2 adrenergic receptor (Michel et al., 1999; Baldwin et al., 1999). The spectrum of molecular analysis also includes studies of genes for cytoskeletal protein - adducin (Tamaki, 1998), aldosterone

synthase (Brand, 1999), glucocorticoid receptor (LIN, 1999), bradykinin (Mukae, 1999) and receptors of the ion channels of epithelial cells or mutations of gene for nitric oxide synthase (Lacolley, 1998; Yasujima, 1998; Friend, 1996; Stepanov, 1998). No general agreement has been reached as yet, however, concerning the effect of the mutations of the above mentioned genes in regulation of BP in patients with PH. A number of studies analyzing gene maps of human chromosomes 1 (rennin, angiotensin), 7 (11-beta hydroxylase, glucocorticoid metabolism), short arm of chromosome 8 (lipoprotein lipase), chromosome 12 (pancreatic phospholipase A2) and chromosome 17 (gene for angiotensin I converting enzyme) similarly failed to contribute significantly to the discovery of specific candidate genes of PH. Considering that the past studies have been carried out in ethnically different populations, any generalization of the obtained results is difficult. Rather, the results of individual observations document the characteristics of the genetic composition of various ethnic groups. The rat model of spontaneous hypertension, used in studies of PH, is also not fully analogous to the human genome. Therefore, not even the animal experimental studies have contributed appreciably to the understanding of the effects of gene mutations in the development of PH. Some studies now in progress seem to be more likely to contribute to the clarification of the mechanisms of the etiopathology of PH. According to recent information, the candidate genes seem to be the genes for the main histocompatibility system (Sun et al., 1998), genes for alpha adducin (Melandar et al., 2000), genes for rennin-angiotensin-aldosterone system (Thomas et al., 2000), or a set of adrenergic receptors (Michel et al., 1999; Kotchen et al., 2000).

The present report involves an analysis of genes for the main histocompatibility system (HLA), carried out as part of our studies of the genetic markers of PH in children, adolescents

and young adults. Specific attention was given to antigens HLA-DR3, HLA-DQA1 and HLA-DQB1 (mutation ARG52/non ASP 57 of the human genome, Tao et al., 1995).

MATERIALS AND METHODS

Materials

The study involves 43 individuals with juvenile hypertension and 21 normotonic. Only asymptomatic hypertonic with an accidentally discovered hypertension were selected for the study. A necessary condition for their inclusion involved repeated abnormal BP levels, i.e., a minimum of two of three consecutive measurements taken within one to three weeks. The interpretations of the actual BP values were based on developmental distribution tables and curves (Update on the 1987 Task Force Report, 1996), according to sex, age and body height. In adults, the WHO criteria of the BP values were employed. The following series of examinations were directed toward the elimination of secondary type of hypertension in all probands. The control sample of normotonic uses individuals with repeatedly measured physiological levels of BP and negative history of specific diseases known to adversely affect the BP regulation.

The DNA bank, established with the permission of the involved probands or their legal guardians, contains samples of DNA isolated from the non-coagulating blood (from the peripheral leukocytes), using classical phenol-chloroform-isoamylalcohol extraction. A water solution of proteinase K at a concentration of 2 mg/ml was used for the deproteinisation of the nucleic acid. The precipitation was done in 96% ethanol. The isolated nucleic DNA was then dissolved in 100 – 300 µl TE-buffer (Tris-EDTA, pH = 8.0). Each DNA sample was adjusted to a working concentration of 100 ng/µl and stored in aliquot volumes in deep freezer at -70°C. The quantification of the DNA samples was done by spectrophotometric determination of the amounts of DNA and of contaminating proteins (Maniatis et al., 1989).

Methods

The polymerase chain reaction (PCR) is a technique routinely used to amplify a specific

section of DNA. In our study, the concentrations of the individual reagents used in the PCR system, and the temperature and timing regime of the amplification were identical to Maniatis et al., 1989. The proposed primers for a given DNA section are original. A horizontal electrophoresis in agarose gel was used to separate the DNA fragments cleaved by the restriction by restriction endonucleases exposing specific, one-point mutations of the appropriate gene. An agarose electrophoresis with concentrations 1.5 - 2.5 % of agarose was used for DNA separation (USB, USA). The control marker of the size of the DNA fragments used in most cases was the 100 bp ladder of the firm USB, USA. The technique of coloring with ethidiumbromide UV-ray wavelenght (300 nm) on a transilluminator (Appligene, USA) was used for the visualization of the fractioned DNA fragments. The vertical electrophoresis in polyacrylamid gel was used to separate the smaller fragments (up to 50 bp). The concentrations of the polyacrylamid gel were selected according to the need, depending on the expected size of the DNA fragments, between 6 and 15 %. The vertical electrophoresis was performed on the instruments of the firm Shelton, USA. Allelic ladders of 10 and 100 bp (USB, USA) were used for the exact determination of the DNA fragment sizes. Individual determinations were made in parallel reactions (3 independent measurements) independent of each other in time to minimize errors by individuals making the measurements.

The photo documentation of the genetic analyses was made in several ways. The routine agarose gels were documented using Polaroid (USA) photographic equipment. The polyacrylamid gels were identified and documented using computer sequencing equipment of Beckman Inc., USA and the documentation equipment of Appligene, USA.

RESULTS

Following are the results of a genetic analysis in three HLA loci: HLA-DRB3, HLA-DQA1 and HLA-DQB1. All probands, both the normotonic and hypertonic were tested for the presence of the variant alleles in the above mentioned genetic loci.

Table 1 summarizes the allelic variation in locus HLA-DRB3. In both studied groups, i.e., controls and individuals with juvenile (primary) hypertension, the ratio of alleles *-0101 and *-0202 is similar, approximately 3 : 1, favoring the *-0101 allele. The frequency of possible genotypes is significantly shifted toward the homozygous combination *-0101/*-0101, even more so in the juvenile hypertension group than in the controls. In general, the hypertonics were found to have fewer heterozygous combinations (*-0101/*-0202) than the controls.

Table 1: HLA-DRB3 typing in controls and individuals with primary hypertension

Alleles	Controls (n=21 individuals)		Primary hypertension (n=43 individuals)	
	n	%	n	%
*-0101	31	71.4	63	73.2
*-0202	13	28.6	23	26.8
<i>Genotypes</i>				
3*0101/0101	12	57.1	28	65.1
3*0101/0202	6	28.6	7	16.3
3*0202/0202	3	14.3	8	18.6

The allele distribution in Czech controls and primary hypertension individuals. The genotype frequencies are shown.

The results of the HLA-DQB1 typing are presented in table 2, listing the frequencies of the detected alleles signifying the presence or absence of the aminoacid asparagine in position 57 HLA-DQB1. In some diseases, e.g., Graves disease, the absence of asparagine in this position is considered as a possible mutation linked to the disease. No such association has emerged in our sample of patients with juvenile hypertension. Rather, the majority of the hypertonics were found to possess the Asp allele, even more frequently than the controls. This was also true for the homozygous state Asp/Asp where the difference between the two groups was even larger. The most frequent genotype in both studied groups, however, was the heterozygous NonAsp/Asp.

Table 3 illustrates a similar polymorphism in the HLA-DQA1 locus with arginine in position 52. The presence of arginine could be considered as a mutation with a possible genetic linkage to the disease. Among our controls, a higher percentage of individuals lacked the Arg allele than those possessing it, unlike in the individu-

Table 2: HLA-DQB1 typing (nonAsp57 sensitivity) in controls and individuals with primary hypertension

	Controls (n=21 individuals)		Primary hypertension (n=43 individuals)	
	n	%	n	%
<i>Genotypes</i>				
NonAsp57/ nonAsp57	6	28.6	10	23.3
NonAsp57/ Asp57	11	52.4	21	48.8
Asp57/Asp57	4	19.0	12	27.9
<i>Alleles</i>				
NonAsp57	23	54.8	41	47.7
Asp57	19	45.2	45	52.3

Note: nonAsp57(DQB1) - 0201 - 0501 - 0606

- 0202 - 0502 - 0608

- 0302 - 0504 - 0609

- 0304 - 0604

- 0305 - 0605

Asp57 - 0301 - 0503 - 0607

- 0302 - 0601

- 0401 - 0602

- 0402 - 0603

The nonAsp57(DQB1) is composed of 13 different alleles. The Asp57 restriction site is defined by 9 alleles.

Table 3: HLA-DQA1 typing (Arg52 sensitivity) in controls and individuals with primary hypertension

	Controls (n=21 individuals)		Primary hypertension (n=43 individuals)	
	n	%	n	%
<i>Genotypes</i>				
NonArg52/ nonArg52	4	19.1	3	7.0
NonArg52/ Arg52	16	76.2	33	76.7
Arg52/Arg52	1	4.7	7	16.3
<i>Alleles</i>				
NonArg52	24	57.1	39	45.3
Arg52	18	42.9	47	54.7

Note: nonArg52 - 0101 - 0103 - 0201

- 0102 - 0104

Arg(DQA1) - 0501 - 03011

- 0502 - 0302

- 0503 - 0401

- 0601

The nonArg52 is composed of 5 different alleles. The Arg52(DQA1) restriction site is defined by 7 alleles.

als with juvenile hypertension, the majority of whom had the allele. The difference was especially marked on the genotypic level where the homozygous Arg52/Arg52 combination was considerably more prevalent among the hypertonics than among the controls. The reverse

was found true for the homozygous nonArg/nonArg genotype. The frequency of the heterozygous combination was equivalent in both groups of tested individuals.

DISCUSSION

Existing epidemiological studies and statistical evaluations confirm the significant effect of CVD on morbidity and mortality in adult populations of economically developed countries. While all causes of CVD have not been well defined as yet, it is generally accepted that a combination of genetic and environmental factors is to blame. The current estimate of the ratio of these two main components assumes that primary hypertension is approximately 20-40% genetically determined. A possible intrauterine onset of CVD has been proposed. Even in such cases, other than genetic factors can play a role, including mother's malnutrition with following reduction of the fetal growth or an intrauterine exposure of the fetus to maternal glucocorticoids (Barker, 2000; Benediktsson, 1993). The most recent research implicates especially the interaction between genes and the environmental factors, capable of influencing the expression of the appropriate genes. Apart from the rennin-angiotensin prohypertensive system, genes of insulin resistance and the ensuing hyperinsulinism or genes associated with the angiotensin-converting enzyme, the current genetic research focuses on studies of the genes encoding the HLA antigen complex.

The number of epidemiological studies devoted to the relationship between CVD or primary hypertension and HLA is limited. Considering that the frequencies of individual HLA antigens of the first and second class show ethnic differences, no general agreement has been reached on the linkage between selected HLA antigens and PH. A significant relationship between the HLA genes and PH has been discovered in a Brazilian "mulatto" (dark) population (antigen HLA-DRB3, Gerbase DeLima, 1998) and Brazilian "Caucasian" (light) population (antigen HLA-DR4, Gerbase DeLima, 1998). A Chinese study demonstrated a linkage in genes HLA-DR2 (higher frequency in hypertonics) and HLA-DR7 (higher frequency in healthy indi-

viduals) (Tao, 1995). In the area of HLA-DQA1, allele *0302 has been proposed as being correlated to a gene for hypertension, while the allele HLA-DQA1*0103 as having a protective character (Sun, 1998). Alleles *0101 and *0102 are considered to be protective in individuals with the diagnosis of primary hypertension (Vidan-Jeras et al., 2000). In our pilot epidemiological study, alleles HLA-DQA1 *0101 and *0102 also seem to have a protective effect. In the area of the HLA-DQB1 locus, the protective alleles are marked in the footnote to Table 2 as DQB1. Correlating alleles are marked Asp57. Protective effects were described also in other studies, especially in connection with combination of alleles HLA-DRB1 *0601/2, DQB1 *0502, DQA1 *0102 or DRB3 (Vidan-Jeras et al., 2000). Certain genetic parallels are emerging also in the Czech hypertensive population, both in the area of the locus HLA-DQA1 and HLA-DRB3. In contrast, allele *0302 seems to be of no phenotypic significance among Czech patients with primary hypertension. Allele *0103, too, is of no predetermining value, as the present or absent mutations in HLA-DQA1 and HLA-DQB1 loci include several allelic variants. HLA-DQB1 typing in the present study does not allow differentiation of individual alleles with the nonAsp57 vs. Asp 57 set. Similar situation exists also in the HLA-DQA1 locus, where alleles *0302 and *0103 are present in nonAsp57 vs. Asp 57 sets. The relationship between allele *0302 and primary hypertension (Sun et al., 1998) has been confirmed in our study where the frequencies of homozygotes Arg52/Arg52 and heterozygotes nonArg52/Arg52 are considerably higher than in controls. It seems that allele *0103 may have a protective effect. Future studies of the Czech population should determine the variants in nonAsp57 and Asp57 (HLA-DQB1 locus), and nonArg52 and Arg52 (HLA-DQA1 locus) alleles. Comparisons between our and a recent Slovenian study (Vidan-Jeras et al., 2000) do not show any parallels in either the *102 - HLA-DQA1 or *0502/1 - HLA-DQB1, consistent with the incongruous molecular epidemiologic results. The homozygous genotypes (*0101 and *0202) of the DR-B3 locus, however, were increased among the patients with hypertension.

A larger population study will be required for a definitive determination of the role of the HLA-DQA1 and HLA-DQB1 loci in risk determination for primary hypertension in Czech population. Observed ethnic differences (Hamet, 1999; Kotchen et al., 2000) attest to considerable heterogeneity of the HLA-DQA1 and HLA-DQB1 loci. This heterogeneity may lead to a variable response under the influence of different environmental factors, i.e., to forming protective allelic variants as well as gene mutations that cancel such protection in affected individuals. The existing studies of the involvement of the HLA system in primary hypertension demonstrate the need for further intensive research in the areas of normal HLA functions and their pathogenesis in various ethnic populations.

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KEY WORDS Juvenile Hypertension. HLA-locuses. Czech Population.

ABSTRACTS The present paper informs about allele frequencies in three HLA locuses (HLA-DRB3, HLA-DQA1 and HLA-DQB1). The Czech population results are compared with data of different European and South American populations. The most important locus is locus HLA-DQA1, where alleles *0302 and *0103 are present in non Asp57/Asp57 set.

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