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A SEX – SPECIFIC DIFFERENCE IN HOMOZYGOSITY FOR HLA – DR53 IN NEWBORNS*

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Homozygosity for HLA-DR53 confers increased susceptibility to major forms of leukemia. In childhood leukemia, this influence is male-specific. Two separate studies have shown a male-specific increase in the hemozoygosity rate for HLA-DR53 in healthy adults. This finding was attributed to possible preferential transmission of HLA-DR53 towards male offspring. If this is the case, the consequences of such a prenatal event should be evident in the newborn population. The present study investigated HLA-B and -DQA1 genotype frequencies in a sample of 134 newborns (73 boys, 61 girls) in Turkey. Restriction fragment length polymorphism (RFLP) analysis showed a homozygosity rate of 8.2 percent for HLA-DR53. Nine of 11 homozygotes were boys and the sex-specific rates were 12.3 percent vs 3.3 percent in boys and girls, respectively (p = 0.05). The DR53 homozygosity rate in males was higher than the expected rate (p = 0.02). These findigns suggested a prenatal mechanism behind the excess of DR53 homozygotes in the male population. To maintain equilibrium, this excess seems to be eliminated postnatally. This model also explains how a deleterious genotype escapes natural selection. *Key words: HLA-DR53 homozygosity, sex factors, genetics, newborn.*

The major histocompatibility complex (MHC) encodes for polymorphic transplantation antigens (H-2 in mice, HLA in humans) and a number of other genes with immunological or non-immunological functions[1]. Among those, there seem to be genes influencing the development of malignancies. The first diseases associated with the MHC were leukemia in mice[2] and Hodgkin's disease in humans[3]. The mouse-leukemia association is with homozygosity for the H-2^k haplotype, whereas several molecular studies have shown that its counterpart in humans is HLA-DR53[4-7]. This is not surprising, as these two speificities are serologically cross-reactive[8].

It was originally postulated that an HLA specificity conferring increased susceptibility to a lethal disease should have a portective mechanism to propagate itself over many generations, i.e., escape natural selection[9]. It appears

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that homozygosity for HLA-DR53 is increased in healthy males[10, 11]. This finding suggests an increased transmission ratio of HLA-DR53 from parents to offspring as the protective mechanism[10]. This model fits in with the original postulate that the susceptibility haplotype should behave like a mouse t-haplotype, which is a recessive lethal and protected against natural selection by its preferential transmission from heterozygotes[12, 13]. Interestingly, the mouse MHC is embedded within the t-complex and intermingled with several recessive lethals[14, 15].

The present study investigated the HLA-DR53 homozygosity rate in a group of newborn babies to gain further insight into the male-specific increase and to see whether a prenatal (pre- or post-zygotic) mechanism may be responsible for it. It was expected that a newborn population which has not been subject to any postnatal selection process would be ideal for this purpose.

Material and Methods

Anonymous cord blood samples and sex of the babies were obtained from the Zübeyde Hanım Maternity Hospital in Ankara, Turkey. Altogether 134 samples were collected in a period of three months with no selection. All the samples were from Turkish babies born to families living in the Ankara province. DNA was extracted using standard methods from cord blood samples anti-coagulated with sodium EDTA.

HLA-DQA1 and HLA-B RFLP analyses were done as described elsewhere using the restriction endonuclease TaqI and the cDNA probes 10-8 and pB250, respectively[16]. These RFLP typing systems yield five alleles at each locus. One allele of each locus is strictly associated with a common serological HLA specificity. These are the 5.8 kb DQA1 allele and HLA-DR53, and 2.6b HLA-B allele and HLA-B7 (in addition, 3.2kb HLA-B corresponds to HLA-B57, but this is extremely rare in the Turkish population). Therefore, it was possible to estimate gene frequencies and homozygosity rates for these alleles by direct counting.

Statistical evaluation of the differences was made using the Fisher's exact test when a number was less than 5 in a 2 x 2 table as well as the Z-test for two proportions from independent groups. In confirmation of the previously shown male-specific higher homozygosity rate, a one-tailed p value was used. Because the direction of the difference was specified in the hypothesis, a one-tailed test was deemed to be appropriate. The observed rates were compared to expected rates using the Z-test for sample proportion versus hypothesized value.

Results

The total and sex-specific allele frequencies and homozygosity rates are presented in Tables I and II. They only significant difference was noted between male- and female-specific homozygosity rates for the 5.8kb RFLP band of the

DQA1 locus which corresponds to HLA-DR53 ($p = 0.05$ by Fisher's exact test; $p = 0.03$ by Z-test). The HLA-DR53 allele frequency was not significantly different between the two groups (Table III). No other genotype of either locus showed a similar trend.

Table I: HLA-B Allele Frequencies (AF) and Homozygosity Rates (HR) (%)

		Total n = 134	Male n = 73	Female n = 61
Allele A	AF	36.9	35.6	38.5
9.0kb	HR	14.9	12.3	18.0
Allele B	AF	22.4	21.0	23.0
4.2kb	HR	4.5	2.7	6.6
Allele C	AF	35.1	8.4	31.1
3.4kb	HR	15.7	17.8	6.6
Allele D	AF	0.0	0.0	0.0
3.2kb/HLA-B57	HR	0.0	0.0	0.0
Allele E	AF	5.2	4.1	6.6
2.6kb/HLA-B7	HR	0.0	0.0	0.0

The nomenclature for alleles is arbitrary (see ref. 16).
Allele D and homozygosity for allele E (HLA-B7) were not observed at all.

Table II: HLA-DQA1 Allele Frequencies (AF) and Homozygosity Rates (HR) (%)

		Total n = 134	Male n = 73	Female n = 61
Allele A	AF	11.6	14.4	8.2
2.7kb	HR	1.5	2.7	0.0
Allele 1B	AF	16.8	13.0	21.3
6.7kb	HR	0.7	1.4	0.0
Allele 1C	AF	15.3	15.8	14.8
7.4kb	HR	3.7	5.8	1.6
Allele 2	AF	27.2	26.0	31.5
4.8kb	HR	9.0	9.6	8.2
Allele 3	AF	29.1	30.8	21.3
5.8kb/HLA-DR63	HR	8.2	12.3	3.3

The nomenclature for alleles is from Ref. 17.

Table III: Sex-Specific Gene Frequencies (GF) and Homozygosity Rates (HR) of HLA-DR53 and Homozygosity Rates for a Supertypic DR53 Haplotype (%)

			Newborns		Adults
		Total n = 134	Boys n = 73	Girls n = 61	(Female) n = 65
HLA-DR53	GF	29.1	30.3[a]	27.0	25.4[b]
HLA-DR53	HR	8.2	12.3[c]	3.3	4.6

a: The difference between boys and girls is not significant and originates from the difference in the homozygosity rates.
b: Yields an expected homozygosity rate of 6.45% calculated by the formula hr = gz.
c: For comparison to the homozygosity rate in girls p = 0.05.

Discussion

The present study aimed to distinguish a prenatal mechanism from a postnatal one causing the male-specific increase in the HLA-DR53 homozygosity rate. Although the numbers are relatively small for a population study, the mechanism of increased homozygosity for HLA-DR53 in healthy adult males reported previously seemed to be a prenatal one as the same increase was noted in newborns. This sex-specific difference in the homozygosity rates may be due to an increase in males or a decrease in females, or both.

In the other two British studies, the homozygosity rate for DR53 in adult males was higher than the expected rate calculated from the gene frequency of the same population. As a control group for an ongoing young-onset breast cancer study, 65 females from Ankara were typed at the DQA1 locus also by RFLP analysis. In that group, the HLA-DR53 gene frequency was 25.4 percent (Table III). This frequency yielded an expected homozygosity rate of 6.45 percent. The observed rate of 12.33 percent was significantly higher (p = 0.02, Z-test), in line with the findings of the previous larger studies. The general excess of males in the newborn population is well/recognized; this excess gradually disappears later in life[18]. The sex-differential in the homozygosity rate for HLA-DR53 seems to correlate to the overall male excess among live births.

On the other hand, the female-specific homozygosity rate in newborns is non-significantly lower than the excepted rate, probably due to small numbers (3.28% vs 6.45%). If this trend persists and reaches statistical significance in a larger group, it will have important implications. This might turn out to be the immunogenetic basis for a higher abortion rate of female fetuses[19, 20].

A plausible model would be that homozygosity for HLA-DR53 is increased due to preferential transmission of HLA-DR53 at the pre-zygotic level, with the excess being eliminated during the prenatal period in female fetuses and postnatally in males. In line with the increased abortion rate in female fetuses, there is a male predominance in childhood malignancies[21]. The male-specificity of HLA-

DR53 association in the most common childhood malignancy, leukemia, supports this model[5]. The long-suspected link in the genetics of spontaneous abortions and cancer[9, 22], and even more strikingly, an increased leukemia risk among the survivors of threatened abortions[23, 24], imply that abortions and childhood malignancies may serve the same evolutionary purpose. In light of the currently available data, we suggest that HLA-DR53 homozygosity may be a factor in the common background of abortions and leukemia susceptibility.

The results of the present study lead the way to further studies to follow up the findings which have been shown in three different populations to date. Considering the possible implications of these findings in genetics, predictive epidemiology and preventive medicine, we find it would be worthwhile to take further steps, such as increasing the size of the sample analyzed in this study and extending it to healthy adults to examine the role of HLA-DR53 in longevity. The DNA samples collected can also be used for a comprehensive (class I, II, III) HLA polymorphism study at the DNA level for the first time in the Turkish population.

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