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# No gender differences in the frequencies of HLA-DRB3/B4/B5 heterozygotes in newborns and adults in Koreans

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HLA class II haplotypes often contain a second expressed HLA-DRB locus tightly linked to the classical HLA-DRB1 locus on the haplotype, which can be either HLA-DRB3, -DRB4 or -DRB5. These encode the HLA-DR51, -DR52 or -DR53 supertypic specificities and mark the ancestral lineages. HLA-DRB3/B4/B5 heterozygote excess in Welsh male newborns has been reported, suggesting a possibility of male-specific major histocompatibility complex (MHC)-mediated prenatal selection. However, it has not been confirmed in newborns of other ethnic groups or in adult populations. We analyzed the HLA-DRB1 and HLA-DRB3/B4/B5 genes in Korean newborns and healthy adults to examine whether MHC-mediated prenatal or postnatal selection exists. A total of 1,038 newborns (cord blood registry, 516 males and 522 females) and 2,082 healthy adults (hematopoietic stem cell donor registry, 1,111 males and 971 females) were HLA typed. HLA-DRB1/B3/B4/B5 DNA typing was performed using Dynal RELI™ HLA-DRB SSO Kit (Dyanl Biotech, Wirral, U.K.). Genotype frequencies and homozygosity and heterozygosity rates for DRB3/B4/B5 supertypic loci were compared between males and females in newborns and adults. There were no significant differences in the HLA-DRB3/B4/B5 homozygosity and heterozygosity rates between males and females in both newborns and adults. In the comparison between newborns and adults, homozygosity rate was significantly higher in newborn females than in adult females (31.0% vs 25.0%, $p = 0.01$). Whether there is an age-related change from newborns toward adults has not been well studied in other populations, and further studies are warranted. In conclusion, male-specific heterozygosity excess reported in Welsh newborns has not been observed in Korean population, and there might be some ethnic differences in the gender-specific prenatal selection events.

**Key words:** age, gender, heterozygosity, HLA-DRB, homozygosity

The HLA system is known to be the most polymorphic genetic system in human. The existence of an advantage for HLA heterozygosity is suggested by the major histocompatibility complex (MHC) restriction of T cell responses to pathogens (Doherty and Zinkernagel, 1975). Theoretically, it is more evident in the pathogens which mutate frequently and escape immune surveillance effectively (Nowak et al., 1991). HLA heterozygosity for class I loci (A, B, and C) delayed the onset of acquired immunodeficiency syndrome (AIDS) among patients infected with human immunodeficiency virus-type 1 (HIV-1), whereas individuals who were homozygous for one or more loci progressed rapidly to AIDS and death (Carrington et al., 1999). Similarly, the hypothesis can be applied to HLA class II loci and resistance to disease during hepatitis B virus infection has been reported to be greater in heterozygotes than in homozygotes for HLA-DQ-DR region (Thursz et al., 1997).

HLA class II gene has peculiar structural polymorphism in that the number of HLA-DRB locus is different in different individuals. The DRB3 gene which encodes HLA-DR52 molecule is present on HLA-DRB1*03, *11, *12, *13 and *14 haplotypes; DRB4 which encodes HLA-DR53 is on HLA-DRB1*04, *07 and *09 haplotypes, and DRB5 which encodes DR51 is on HLA-DRB1*15 and *16 haplotypes. HLA-DRB1*01, *08 and *10 haplotypes do not have a second expressed DRB gene. Different supertypic loci (HLA-DRB3/B4/B5) cannot occur on the same

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haplotype, they behave like and can be treated as allelic specificities despite not being truly allelic, ie, not encoded by the same locus (Andersson et al., 1994).

Dorak et al. (2002) suggested that heterozygosity for the members of different class II supertypic families may be better markers for 'functional' heterozygosity at the HLA-DRB1 locus. They insisted that selection does not favor genotypes consisting of HLA-DRB1 alleles from the same supertypic group even if it is heterozygous for classical HLA-DR alleles such as HLA-DRB1*04 and *07 (both in the HLA-DR53 family), or HLA-DRB1*03 and *13 (both in the HLA-DR52 family). While there was no significant change in heterozygosity rates between males and females at DRB1, the proportion of males carrying two DRB1 specificities from different ancestral lineages was significantly increased than that of females in Welsh newborns (Dorak et al., 2002). This result suggests a more favorable outcome for male concepti heterozygous for supertypical haplotypes. On the contrary, increased HLA-DR53 homozygosity rate in males than in females was reported in Turkish newborns (Sunguroglu et al., 1998).

Thus, it is not certain whether there is heterozygosity or homozygosity advantage in prenatal selection in males and this issue has not been confirmed in newborns of other ethnic groups or in adult populations. We analyzed the HLA-DRB1/B3/B4/B5 genes in Korean newborns and healthy adults to examine whether any male-specificity of MHC-mediated prenatal or postnatal selection exists. This study was approved by the Institutional Review Board of the Seoul National University Hospital (H-0704-020-205).

For newborn samples, 1,038 cord blood units obtained from Korean women consecutively registered to the AllCORD (Public Cord Blood Bank in Seoul Boramae Hospital) from Aug 2006 to Aug 2007 were analyzed. Korean ethnicity of the newborns was ascertained when the mother consented to virological and HLA testing. For adult samples, peripheral blood samples of 2,082 healthy Korean adults of hematopoietic stem cell donation volunteers registered to the KONOS (Korean Network for Organ Sharing) were analyzed: consecutively registered 1,111 males from Nov 2001 to May 2002 and consecutively registered 971 females from Jan 1998 to May 2002 (median age 22, age range 18–47).

Genomic DNA was prepared from blood samples using a QIAamp DNA Blood Mini Kit (QIAGEN, Valencia, CA, U.S.A.) or a LaboPass$^{TM}$ Genomic DNA Extraction Kit (COSMO Genetech, Seoul, Korea). DRB1 alleles were determined using Dynal RELI$^{TM}$ HLA-DRB SSO Kit (Dynal Biotech Ltd., Wirral, U.K.). The typing kit also detects the presence of the supertypic HLA-DRB (HLA-DRB3/B4/B5) genes.

Phenotype frequencies of HLA-DRB1 alleles and HLA-DRB3/B4/B5 specificities and frequencies of homozygous or heterozygous genotypes for HLA-DRB3/B4/B5 loci were estimated by direct counting. Phenotype or genotype frequencies were calculated from the number of subjects possessing the phenotype or genotype divided by the total number of subjects in the sample. It was performed in each of newborn and adult group according to the sex. Expected homozygosity or heterozygosity rates were calculated from the allele frequencies (which were calculated by direct counting, assuming homozygosity in case of only 1 detectable allele) in the same sample of population assuming Hardy-Weinberg equilibrium. Pearson's $\chi^2$ was used for the analysis of all $2 \times 2$ tables concerning observed frequencies. The level of significance was set at $p < 0.05$.

Phenotype frequencies of HLA-DRB1 and HLA-DRB3/B4/B5 specificities are shown in Table 1. The frequencies

Table 1. Phenotype frequencies (%) of HLA-DRB1 alleles and HLA-DRB3/B4/B5 supertypic specificities

| | Newborns | | | Adults | | |
|---|---|---|---|---|---|---|
| | Total (n = 1,038) | Males (n = 516) | Females (n = 522) | Total (n = 2,082) | Males (n = 1,111) | Females (n = 971) |
| DRB1*01 | 11.8 | 13.4 | 10.3 | 12.4 | 13.5 | 11.2 |
| DRB1*02 | 22.4 | 22.9 | 21.8 | 23.9 | 24.2 | 23.6 |
| DRB1*03 | 3.7 | 3.5 | 3.8 | 3.7 | 3.2 | 4.3 |
| DRB1*04 | 34.4 | 34.3 | 34.5 | 34.6 | 33.8 | 35.5 |
| DRB1*07 | 14.5 | 13.6 | 15.3 | 13.3 | 12.6 | 14.0 |
| DRB1*08 | 18.3 | 20.3 | 16.3 | 18.6 | 18.9 | 18.3 |
| DRB1*09 | 20.6 | 21.1 | 20.1 | 17.7 | 17.9 | 17.5 |
| DRB1*10 | 3.9 | 3.1 | 4.6 | 3.8 | 3.6 | 4.0 |
| DRB1*11 | 9.3 | 10.1 | 8.6 | 9.5 | 9.4 | 9.6 |
| DRB1*12 | 14.1 | 13.8 | 14.4 | 15.0 | 15.0 | 15.0 |
| DRB1*13 | 19.8 | 18.6 | 21.1 | 21.4 | 21.5 | 21.2 |
| DRB1*14 | 15.5 | 14.0 | 17.0 | 15.2 | 15.8 | 14.6 |
| DRB3 | 54.1 | 52.7 | 55.6 | 56.0 | 55.4 | 56.6 |
| DRB4 | 59.4 | 59.7 | 59.2 | 58.5 | 57.4 | 59.7 |
| DRB5 | 22.4 | 22.9 | 21.8 | 24.0 | 24.2 | 23.7 |

were not different between males and females in both newborns and adults. They were also not different between newborns and adults. Genotype frequencies and homozygosity and heterozygosity rates for HLA-DRB3/B4/B5 supertypic loci are shown in Table 2. Genotype frequencies were not different between males and females in both newborns and adults. Homozygosity and heterozygosity rates for HLA-DRB3/B4/B5 loci were not significantly different between males and females in both newborns and adults. However, in the comparison between newborns and adults, homozygosity rate was significantly higher in newborn females than in adult females (31.0% vs 25.0%, $p = 0.01$). The difference was most marked in DRB4/DRB4 homozygosity rate, but the significance was marginal (15.7% vs 12.2%, $p = 0.054$). On the other hand, heterozygosity rate tended to be lower in newborn females than in adult females (39.5% vs 43.2%, $p = 0.17$).

Genotype frequencies and homozygosity and heterozygosity rates for HLA-DRB3/B4/B5 loci were compared between Welsh (Dorak et al., 2002) and Korean newborns in the present study (Table 3). Although increased frequency of heterozygotes in males was evident in Welsh (53.73% vs 39.25%, $p = 0.003$), its frequency was not different between males and females in Koreans (38.6% vs 39.5%). Significantly decreased frequency of homozygotes in males was also noted in Welsh (22.89% vs 32.71%, $p < 0.03$), and its frequency tended to be decreased in males in Koreans (26.3% vs 31.0%, $p = 0.10$).

The effect of HLA on prenatal selection has been reported in Welsh and Turkish newborns with inconsistent results. Dorak et al. (2002) reported that the frequency of HLA-DRB3/B4/B5 heterozygotes was significantly higher in male than in female newborns in Welsh (n = 415) and they suggested that heterozygosity for HLA-DRB3/4/5 could be a better marker for male-specific prenatal selection. On the contrary, the frequency of HLA-DR53 homozygotes was reported to be higher in

Table 2. Genotype frequencies and homozygosity and heterozygosity rates (%) for HLA-DRB3/4/5 supertypic loci

| | Newborns | | | Adults | | |
|---|---|---|---|---|---|---|
| | Total (n = 1,038) | Males (n = 516) | Females (n = 522) | Total (n = 2,082) | Males (n = 1,111) | Females (n = 971) |
| DRB3/DRB3 | 11.2 | 9.7 | 12.6 | 11.9 | 12.3 | 11.3 |
| DRB3/DRB4 | 23.0 | 23.3 | 22.8 | 24.0 | 22.1 | 26.1 |
| DRB3/DRB5 | 7.9 | 7.4 | 8.4 | 7.7 | 7.8 | 7.6 |
| DRB3/Blank | 12.1 | 12.4 | 11.7 | 12.4 | 13.1 | 11.6 |
| DRB4/DRB4 | 14.8 | 13.9 | 15.7 | 12.1 | 12.1 | 12.2 |
| DRB4/DRB5 | 8.1 | 7.9 | 8.2 | 9.8 | 10.0 | 9.5 |
| DRB4/Blank | 13.5 | 14.6 | 12.4 | 12.7 | 13.2 | 12.0 |
| DRB5/DRB5 | 2.7 | 2.7 | 2.7 | 1.4 | 1.4 | 1.5 |
| DRB5/Blank | 3.7 | 4.9 | 2.5 | 5.0 | 5.0 | 5.0 |
| Blank/Blank | 3.1 | 3.3 | 2.9 | 3.0 | 2.9 | 3.1 |
| Homozygotes | 28.7 | 26.3 | 31.0* | 25.4 | 25.7 | 25.0* |
| Heterozygotes | 39.0 | 38.6 | 39.5 | 41.5 | 40.0 | 43.2 |

Blank means the haplotypes lacking an HLA class II supertype (HLA-DRB3/B4/B5).
*: $p = 0.01$, newborn females vs adult females.

Table 3. Comparison of supertypic genotype frequencies and homozygosity and hetrozygosity rates (%) between Welsh and Korean newborns

| | Welsh* | | | | Korean | | | |
|---|---|---|---|---|---|---|---|---|
| | Total (n = 415) | Males (n = 201) | Females (n = 214) | $p$ | Total (n = 1,038) | Males (n = 516) | Females (n = 522) | $p$ |
| DRB3/DRB3 | 14.70 (14.06) | 10.95 (13.92) | 18.22 (14.14) | < 0.04 | 11.2 (10.7) | 9.7 (9.7) | 12.6 (11.6) | 0.13 |
| DRB3/DRB4 | 23.13 (23.55) | 27.86 (22.83) | 18.69 (24.21) | < 0.03 | 23.0 (24.3) | 23.3 (23.0) | 22.8 (25.5) | 0.85 |
| DRB3/DRB5 | 11.08 (13.28) | 13.43 (14.99) | 8.88 (11.58) | 0.19 | 7.9 (8.2) | 7.4 (8.0) | 8.4 (8.4) | 0.52 |
| DRB4/DRB4 | 9.40 (9.86) | 6.96 (9.37) | 11.68 (10.37) | 0.14 | 14.8 (13.8) | 13.9 (13.6) | 15.7 (14.0) | 0.42 |
| DRB4/DRB5 | 12.05 (11.12) | 12.44 (12.30) | 11.68 (9.92) | 0.81 | 8.1 (9.3) | 7.9 (9.4) | 8.2 (9.2) | 0.86 |
| DRB5/DRB5 | 3.86 (3.13) | 4.98 (4.04) | 2.80 (2.37) | 0.37 | 2.7 (1.6) | 2.7 (1.6) | 2.7 (1.5) | 0.98 |
| Other genotypes** | 25.78 (25.00) | 23.38 (22.52) | 28.04 (27.41) | 0.28 | 32.3 (32.2) | 35.1 (34.7) | 29.5 (29.8) | 0.05 |
| Homozygotes | 27.95 (27.07) | 22.89 (27.34) | 32.71 (26.93) | < 0.03 | 28.7 (26.0) | 26.3 (24.9) | 31.0 (27.2) | 0.10 |
| Heterozygotes | 46.27 (47.95) | 53.73 (50.12) | 39.25 (45.71) | 0.003 | 39.0 (41.7) | 38.6 (40.4) | 39.5 (43.1) | 0.77 |

Numbers in brackets are the expected frequencies for each genotype.
*: Dorak et al., 2002.
**: DRB3/Blank, DRB4/Blank, DRB5/Blank, and Blank/Blank.

male than in female newborns in Turkish (Sunguroglu et al., 1998). However, the sample size (n = 134) was rather small and the significance of the difference was marginal (12.3% vs 3.3%, $p = 0.05$) in this study. On the other hand, homozygosity for HLA-DRB4*01 (DR53) was also reported to be a male-specific genetic risk factor for childhood acute lymphoblastic leukemia (Dorak et al., 1999).

In our study, we have analyzed the effect of HLA on prenatal selection in Korean newborns with a much larger sample size (n = 1038) than the previous two studies in Welsh and Turkish newborns. There was no difference in HLA-DRB3/B4/B5 heterozygosity rates between male and female newborns (38.6% vs 39.5%) in our population, which was quite different from the finding of a significantly increased heterozygosity rate observed in male newborns in Welsh population (53.73% vs 39.25%, $p = 0.003$) (Table 3). Although of marginal significance, decreased HLA-DRB3/B4/B5 homozygosity rate in male than in female newborns (26.3% vs 31.0%, $p = 0.10$) in our population was in line with the finding observed in Welsh newborns (22.80% vs 32.72%, $p < 0.03$). We have studied both newborns and adults in this study, and it was of interest that the increased HLA-DRB3/B4/B5 homozygosity rate in female newborns declined toward female adults (31.0% vs 25.0%, $p = 0.01$) to a similar level to that of male adults (Table 2). Whether there is an age-related change from newborns toward adults has not been well studied in other populations, and further studies are warranted.

In conclusion, male-specific increase of HLA-DRB3/B4/B5 heterozygosity previously reported in Welsh newborns has not been observed in Korean population. There might be some ethnic differences in the gender-specific prenatal selection events. In our study of newborns and adults in parallel, an increased HLA-DRB3/B4/B5 homozygosity rate in female newborns declined toward adults. To clarify the issues related with the effects of HLA on prenatal selection and on age-related changes, further studies in other populations are needed.

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