

## CHAPTER FOUR

### DISCUSSION

The Mi subsystem consists of a different low incidence antigens of the MNS blood group system phenotypes, a group of them are known to be clinically significant in Asian populations and the prevalence varies depending on the ethnic groups. As a result, the PCR-SSP technique was used in this study to screen for and detect the *GYP* hybrid gene (GP.Mur, GP.Bun, GP.Hop, GP.HF or GP.Hut) of common MNS variants – due to the primers specificity - in Malaysian populations from genomic DNA samples by using specific primers.

From the previous studies of serological tests and Real-time PCR which have been done on this research project by Nadarajan & Shanmugam (2005); of the 1723 healthy blood donors that were tested for the presence of Mi<sup>a</sup> antigens using the GAMA-210 antibody, 79 (4.6%) samples were reactive. The prevalence of Mi<sup>a</sup> positivity was nearly the same between Malays and Chinese; 5.4% and 5.3% respectively, whereas it was less in Indians (1.6%). The *GYP B-A-B* gene recombination events was detected in 41 (51.9%) out of the 79 samples that were Mi<sup>a</sup> positive serologically using Real-time PCR method. Hence, these results clearly indicate that the prevalence of the Mi was detected with a higher percentage in Chinese and Malays and lesser in Indians blood donors. In addition, the detection of *GYP B-A-B* hybrid gene indicates the presence of GP.Mur, GP.Bun, GP.Hop or GP.HF of the common MNS variants.

#### 4.1 PCR-SSP Amplification Results

Obviously, from positive results of serological tests and Real-time PCR that were done by Nadarajan & Shanmugam (2005) to detect the presence of Mi<sup>a</sup> antigen of the Mi subsystem, although the positive results were included the different ethnic groups (Malays, Chinese and Indians). However, in the PCR-SSP technique results, the *GYP* hybrid gene (GP.Mur, GP.Bun, GP.Hop, GP.HF or GP.Hut) of the Mi was identified in the archived genomic DNA samples of Malays and Chinese only. On the other hand, it was not detected in the 6 samples of Indians ethnic group. In addition, for the genomic archived DNA samples of the foreigners that were included in this study, although the 3 samples of them were Mi<sup>a</sup> positive serologically, but there was no *GYP* hybrid gene detected by PCR-SSP technique.

## 4.2 Comparison between Mi in Malaysian populations

In this study, the prevalence of *GYP* hybrid gene of Mi Antigens was detected in Malaysia populations by PCR-SSP technique and confirmed the previous studies that were done by Real-time PCR. However, the prevalence of *GYP* hybrid gene was identified in a higher percentage in Malaysian Chinese (70.6%) than in Malays (44.1%), whereas it was not detected in Indian ethnic groups. In addition, results of PCR-SSP for different samples included in the study that showed positivity are same with samples screened with the Real-time PCR. This indicates that both methods; Real-time PCR or PCR-SSP is capable of detecting the polymorphisms. In addition, due to the primers specificity; PCR-SSP can detect (GP.Mur, GP.Bun, GP.Hop, GP.HF or GP.Hut) of *GYP* hybrid gene according to the primer specificity. However, Real-time PCR detects *GYP* (*B-A-B*) hybrid gene only (GP.Mur, GP.Bun, GP.Hop or GP.HF). Thus, the *GYP* hybrid gene that was detected by PCR-SSP can be GP.Mur, GP.Bun, GP.Hop or GP.HF.

For the GP.Mur, GP.Bun, GP.Hop and GP.HF of the *GYP* hybrid gene, the GP.Mur (Mi.III) was commonly detected among Chinese populations in different geographic locations in Asian countries with a percentage that are nearly similar; for instance, 7.3 % among Chinese in Taiwan. 6.28 % in Hong Kong Chinese blood donors (Broadberry & Lin, 1994). In 1997, Blumenfeld & Huang mentioned that the incidence of MiIII in Taiwan varies among different ethnic groups. Therefore, among the Malaysian Chinese that have *GYP* hybrid gene, they are most likely Mi.III (GP.Mur) phenotype that can be confirmed by doing DNA sequencing. While in the Malays ethnic group, there are no previous studies done for detection of *GYP* hybrid genes. Similarly, for Indians samples that were Mi<sup>a</sup> positive serologically and because they have no *GYP* hybrid gene detected by PCR-SSP,

they cannot be GP.Mur (Mi.III) or GP.Bun (Mi.VI) or GP.Hop (Mi.IV) or GP.HF (Mi.X) and may be any one of the other glycoprotein gene mutation.

### **4.3 Recommendation for Further Studies**

For future studies on the subject, complete DNA sequencing is recommended for the conformation of *GYP* hybrid gene for all samples that were identified by PCR-SSP technique; especially for the samples of Indians that were Mi<sup>a</sup> positive serologically and there was no *GYP* hybrid gene identified.

## CHAPTER FIVE

### CONCLUSION

The study demonstrated and confirmed the presence of common variants of Mi Antigens of the MNS blood group system; by using PCR-SSP technique from archived genomic DNA samples. The *GYP* hybrid gene was identified in the Chinese and Malays of the Malaysian populations whereas in Indians ethnic group it was not detected.

In addition, there are no previous studies done for the presence of *GYP* hybrid gene of Mi in Malays ethnic group by using PCR-SSP technique. According to the studies that have been done regarding GP.Mur (Mi.III) of Mi Antigens in different Asian countries, hence, for the Malaysian Chinese samples that shown *GYP* hybrid gene are probably GP.Mur (Mi.III) that should be confirmed by DNA sequencing. While in Indians ethnic group, they are possible to be other Mi phenotype than GP.Mur (Mi.III) or GP.Bun (Mi.VI) or GP.Hop (Mi.IV) or GP.HF (Mi.X) and can be due to other mutation as they do not show a *GYP* hybrid gene.

In conclusion, this study confirmed the presence of *GYP* hybrid genes in particular the common MNS variants in Malaysian Chinese and Malays blood donors using PCR-SSP and shows that the Mi subsystem can be detected in Asian populations other than Chinese.