Molecular Insights of Rhesus Negative Donors with DEL Phenotype in National Blood Centre, Malaysia

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DISEMBER 2016

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2. Letter of Offer (Research Grant)

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Surat Kami : 600-RMI/FRGS 5/3 (26/2014)
Tarikh : 01 Julai 2014

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Puan

KELULUSAN SKIM GERAN PENYELIDIKAN FUNDAMENTAL (FRGS) FASA 1/2014

Tajuk Projek	:	*The Roles Of Gene Mutation And Phenotypes In Contributing Towards Donors Rh Negative(Del) Related To Adverse Effect Of Transfusion Reaction*
Kod Projek	:	600-RMI/FRGS 5/3 (26/2014)
Kod Rujukan Penaja	:	FRGS/1/2014/SKK10/UITM/02/2
Bidang	:	Sains Kesihatan dan Klinikal (Sains Kesihatan Bersekutu)
Tempoh	:	01 Julai 2014 - 30 Jun 2016 (24 bulan)
Peruntukan Diluluskan (KPM)	:	RM122,580.00
Perkhidmatan Penyelidikan	:	RM 6,129.00 (5%)
Peruntukan Pengoperasian	:	RM116,451.00 (95%)
Ketua Projek	:	Dr. Mazura Binti Bahari

Dengan hormatnya perkara di atas adalah dirujuk.

2. Sukacita dimaklumkan pihak Kementerian Pendidikan Malaysia (KPM) telah meluluskan kertas cadangan penyelidikan puan untuk di biayai di bawah Skim Geran Penyelidikan Fundamental (FRGS) Fasa 1/2014.

3. Bagi pihak Universiti kami mengucapkan tahniah kepada puan kerana kejayaan ini dan seterusnya diharapkan berjaya menyiapkan projek ini dengan cemerlang.

4. Untuk tujuan mengemaskini, pihak puan adalah diminta untuk menandatangani perjanjian FRGS, melengkapkan semula kertas cadangan penyelidikan dan menyusun perancangan semula bajet yang baru di dalam sistem MyGRANTS seperti yang diluluskan. Sila lihat lampiran bagi tatacara tambahan untuk pengurusan projek.

Sekian, harap maklum.

"SELAMAT MENJALANKAN PENYELIDIKAN DENGAN JAYANYA"

Yang benar

PROFESOR DATO' DR. ABU BAKAR ABDUL MAJEED
Penolong Naib Canselor (Penyelidikan)

5.2 Enhanced Executive Summary

Background: DEL is the most weakly expressed of D antigen. Rhesus genotyping regarding DEL phenotypes has been intensively studied and varied in different populations. To date, there is a paucity of data for DEL phenotype in Malaysian population, thus the purpose of this study was to analyze the genotype of DEL phenotype among RhD negative donor in Malaysia.

Materials and Methods: A total of 322 Rh negative blood samples were collected from National Blood Center. The Rhesus phenotype was determined by testing the patient's red blood cells with the five standard antiserums. Genomic DNA was extracted and analysed by SSP-PCR to screen the RHD specific polymorphism located in RHD Exon 4 and Exon 7. Samples that were positive were further test for RHD 12227A polymorphism.

Results: Among 322 blood donors documented as Rh negative, 155 (48.1%) were came from Indian ethnics, followed by Malays 95 (29.5%), Chinese were 48 (14.9%) and 17 (5.3%) were from minor ethnics. Twenty one samples (6.5%) confirmed as DEL phenotype by present of RHD 1227A allele. Among 21 DEL positive samples, the most frequent Rh phenotypes were CCee and Ccee (15/21).

Conclusion: In conclusion, frequency of DEL phenotype was lower in our country (6.5%) compared to other Asian country. All DEL phenotype was shown to have RHD 1227A allele. This study added to the understanding of molecular mechanisms underlying DEL phenotypes in our population and provided useful information for adopting suitable genotyping strategies in future.

5.3 Introduction

Rhesus (Rh) blood group D antigen is the largest group of all 33 known blood group systems (Kappler-gratias et al. 2014). Rh blood group system divided into two groups which is RhD positive and RhD negative determined by the presence or absence of RhD protein respectively.Most blood groups are encoded by single genes with alleles that differ by only one or a few amino acids. On the contrary, the Rh gene encoded by two proteins thatare differing of 36 out of 417 amino acids (Van Kim, Colin, and Cartron 2006). Large number of different between this two proteins trigger to the strong antigenicity of the RhD protein though explains why exposure to RhD can result in a potent immune response in a D-negative individual. Both gene located in a tail-to-tail orientation toward the end of the short arm of chromosome 1 (p34–36) with physical distance between 30 000 base pairs that contained SMP1 gene and *Rhesus box* (Wagner, Franz F, Flegel 2002).

The RhD antigen can differ in both the quantity of antigen expressed and the qualitative nature of the antigen. Serological technique over the years has now been explained by more recent studies using molecular technique. The D negative phenotype caused mainly by a series of changes in the RhD protein, which alter the phenotype of the D antigen. Therefore, based on their phenotype and molecular structure, these RHD alleles are classified as true negative, partial D, weak D and DEL. DEL is the most weakly expressed of D antigen. Usually, 30 or less copies of the D antigen per RBCs will be express by DEL phenotype compare with 1500 to 7000 sites for weak D and 30,000 antigen sites for normal D (Li et al. 2009; Sandler et al. 2014; Wagner et al. 2005).

The routine serological typing does not distinguish RhD negative from the DEL phenotype resulting most of DEL donors are typed as RhD-negative. Nowadays, molecular technique has widely used to reveal the weak expression of DEL phenotype. Frequencies of the RH gene complex not only showed differences in the ethnicity but also in the genetic background of the Rh antigen. Majority Caucasian that phenotype as RhD negative is associated with the deletion of RHD between the upstream and downstream Rhesus boxes but in Africans about 25% of RhD negative Africans have an inactive RHD gene of pseudogene (RHDψ) (C.-P., Shao. J-H Maas, M. Kohler 2002; Flegel 2011; Gu et al. 2014). In contrast in the Asian population, RHDψ is rare, and a certain percentage of RhD-negative individuals have DEL phenotype or RHD-CE-DS hybrid gene(Chen et al. 2004; Gu et al. 2014). The prevalence of DEL is approximately30% in Asian RhDnegativedonors and 0.1% in Caucasian RhD(Nuchnoi et al. 2015).

DEL phenotype derive from several mechanisms, including splice-site mutation, missense mutation, frameshift mutation and a long deletion of the RHD gene.DEL most commonly reported in individuals of Chinese, Korean and Japanese ethnicity(Chen et al. 2004; Fukumori et al. 2000; Gu et al. 2014; Li et al. 2009; Nuchnoi et al. 2015). Previous studies through molecular analysis showed that the RHD1227A allele is the prevalent causal mutation for DEL individuals in East Asian and could be the genetic marker for detection DEL phenotype