

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



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2. Letter of Offer (Research Grant)

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Puan

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

Tajuk Projek	: <i>The Roles Of Gene Mutation And Phenotypes In Contributing Towards Donors Rh Negative(Del) Related To Adverse Effect Of Transfusion Reaction</i>
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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.

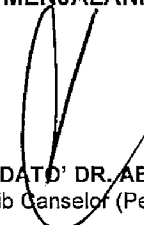
3. Bagi pihak Universiti kami mengucapkan tahniah kepada puan kerana kejayaan ini dan seterusnya diharapkan berjaya menyiapkan projek ini dengan cemerlang.

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 antisera. 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 that are differing of 36 out of 417 amino acids (Van Kim, Colin, and Cartron 2006). Large number of differences between these two proteins trigger 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 genes are 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 techniques over the years have now been explained by more recent studies using molecular techniques. The D negative phenotype is 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 expressed by DEL phenotype compared 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 in most DEL donors being typed as RhD-negative. Nowadays, molecular techniques have widely been used to reveal the weak expression of DEL phenotype. Frequencies of the RH gene complex not only showed differences in ethnicity but also in the genetic background of the Rh antigen. Majority of Caucasians 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 or pseudogene (RHD ψ) (C.-P., Shao, J-H Maas, M. Kohler 2002; Flegel 2011; Gu et al. 2014). In contrast in the Asian population, RHD ψ 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 approximately 30% in Asian RhD-negative donors and 0.1% in Caucasian RhD (Nuchnoi et al. 2015).

DEL phenotype derives from several mechanisms, including splice-site mutation, missense mutation, frameshift mutation and a long deletion of the RHD gene. DEL is 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 of DEL phenotype