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Molecular blood group typing in Banjar, Jawa, Mandailing and Kelantan Malays in Peninsular Malaysia

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ABSTRACT

In this study we genotyped ABO, Rhesus, Kell, Kidd and Duffy blood group loci in DNA samples from 120 unrelated individuals representing four Malay subethnic groups living in Peninsular Malaysia (Banjar: n = 30, Jawa: n = 30, Mandailing: n = 30 and Kelantan: n = 30). Analyses were performed using commercial polymerase chain reaction–sequence specific primer (PCR–SSP) typing kits (BAG Health Care GmbH, Lich, Germany). Overall, the present study has successfully compiled blood group datasets for the four Malay subethnic groups and used the datasets for studying ancestry and health.

1. Introduction

Blood group antigens are polymorphic and immunogenic proteins or glycoproteins located on the exofacial surface of red blood cell membranes [1]. Currently, 308 blood group antigens are recognized and 270 of these markers have been classified into 30 blood group systems by the International Society of Blood Transfusion (ISBT) Committee on Terminology for Red Cell Surface Antigens [2].

The majority of blood centres routinely provide ABO and RHD matched blood [3]. However, other red cell antigens (RBC) are also immunogenic (e.g. Kell, Kidd and Duffy) and should also be compatible between donors and recipients especially for those who may require multiple blood transfusions. Failing to provide fully compatible blood may cause haemolytic transfusion reaction and can be a major challenge for transfusion-dependent patients who have become sensitized to low frequency RBC antigens. In this respect, population data can be used to search for panels of potential donors and to plan more effective donor recruitment strategies. Many studies have shown that there are pronounced differences in blood group antigen frequencies between geographically and historically unrelated populations [1,4–10]. Thus, determination of RBC antigen variation at sub-population level can provide useful information for local transfusion agencies, especially in a multi ethnic country like Malaysia has which received multiple waves of migrations in prehistoric (e.g. Negrito, Senoi and Proto-Malays) and historic (e.g. Chinese, Indians and Arab) time [11,12]. The Malays (i.e. Deutro-Malays) that formed a major part of population in Peninsular Malaysia are thought to be the descendants of Austronesian speakers (i.e. Proto-Malays) with genetic admixture from other ethnicities including Chinese, Indians and Arabs. There was later migration of Austronesian

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speakers into Peninsular Malaysia from Island of Southeast Asia such as Sumatera (Rawa and Minangkabau Malays), Sulawesi (Banjar Malays) and Java (Jawa Malays). These groups of people are identified as Malay subethnic groups and live in various places in Peninsular Malaysia [11,13].

The present study was aimed to genotype ABO, Rhesus, Kell, Kidd and Duffy blood group in four Malay subethnic groups (Banjar, Jawa, Mandailing and Kelantan Malays). The Banjar Malays were originated from Banjarmasin, Indonesia [14]. They migrated to Peninsular Malaysia by the end of the 19th and early of 20th century and settled in the west coast (Johor, Selangor and Perak) of Peninsular Malaysia [14]. The Jawa Malays were originated from Java Island and migrated to Peninsular Malaysia since the 17th century [15,16] and most of them inhabit the west coast of Peninsular Malaysia such as Selangor, Perak and Johor [17]. The Mandailing Malays are people of North Sumatera who migrated to Peninsular Malaysia in the early of 18th century [18]. They were settled in mining areas such as Selangor, Perak and Negeri Sembilan [18]. In contrast, Kelantan Malays are the indigenous people living in Kelantan, a state in the north-east of Peninsular Malaysia. Archaeological evidences showed that the Kelantan Malays existed in Peninsular Malaysia since Mesolithic era [19] and have a strong relationship with other empires (China and Pattani) in the north of Peninsular Malaysia [20].

This study provides for the first time ABO, Rhesus, Kell, Kidd and Duffy blood group data for four selected Malaysian subethnic groups (Banjar, Jawa, Mandailing and Kelantan Malays). Blood group data for the other Malaysia subpopulations (i.e. Malays, Chinese and Indians) have been previously reported by Musa et al. [21]. These data can now be compared with the new datasets collected from the four Malay subethnic groups and are presented in detail for the first time here. Findings from the present survey are anticipated to have benefits in ancestry studies and for transfusion medicine.

2. Methods

2.1. Samples

Blood samples were collected with informed consent from 120 healthy and unrelated individuals. These volunteers belong to four Malay subethnic groups: Banjar (n = 30), Jawa (n = 30), Mandailing (n = 30) and Kelantan (n = 30). The study was approved by the Human Ethical Committee, Universiti Sains Malaysia.

2.2. DNA extraction

Genomic DNA was extracted from blood sample using QIAamp blood DNA Mini Kit (Qiagen®, Hilden, Germany). The genomic DNA was stored at –20 °C until required for further use.

2.3. ABO, Rhesus, Kell, Kidd and Duffy blood group typing

DNA samples were typed for ABO, Rhesus, Kell, Kidd and Duffy blood groups using polymerase chain reaction–sequence specific primer (PCR–SSP) typing kits (BAGene ABO-Type variant, RH-Type and KKD-Type kits; BAG Health Care GmbH, Lich, Germany). Genotyping was performed according to the manufacturer's protocols and PCR products were separated on 1.5% ethidium bromide stained agarose gels. Gel images were seen under UV light and were recorded using Geliance 600 Imaging System and GeneSnap software (PerkinElmer, MA, USA). Amplification product patterns were interpreted using the manufacturer provided BAGene evaluation software (BAG Health Care GmbH, Lich, Germany).

2.4. Statistical analysis

Phenotype frequencies were obtained by direct counting. Homogeneity between datasets was tested using Fisher's exact test with limiting significance value set at <0.05 [22]. Principle coordinate (PCO) analysis was carried out using Multivariate Statistical Package 3 (MVSP3: Kovach Computing Services, UK; http://www.kovcomp.com/mvsp). Finally, probability of finding a phenotype match between randomly selected donors and recipients was calculated according to the published formula [23].

3. Results

Phenotype frequencies of the ABO, Rhesus, Kell, Kidd and Duffy blood groups observed in the present survey are given in Table 1. RBC phenotype frequencies for Malays, Chinese and Indians reported by Musa et al. [21] are also included in Table 1 as reference populations for comparison with our newly collected data for the Malay subethnic groups. For ABO blood group system, the B blood group phenotype was observed as the most common type in Banjar (0.47) and Mandailing (0.50) Malays, O and B phenotypes in Jawa Malays (0.30 each) and A phenotype in Kelantan Malays (0.40). In contrast, there are similar distributions of Rhesus, Kell (monomorphic), Kidd and Duffy blood group phenotype frequencies across all four Malay subethnic groups where DCCee, K–k+, Jk(a+b+) and Fy(a+b–) phenotypes were recorded to be the most frequent (0.70–0.83, 1, 0.47–0.57 and 0.57–0.93, respectively).

Exact tests of population differentiation for each blood group system and each pair of datasets listed in Table 1 are shown in Table S1. In general, there are no significant differences between our four newly studied Malay subethnic groups and the reference Malay population. However, significant differences were observed between Malays/Malay subethnic groups and Indians for Rhesus and Duffy blood groups and between Indians and Chinese for ABO, Rhesus, Kidd and Duffy blood groups. A PCO plot (Fig. 1) was used to examine genetic relationships between the Malay subethnic groups and a wider group of other populations. Generally, the Malay subethnic groups are scattered to the right of axis 2 and above axis 1 and are closest to other Austronesian groups rather than the other more distantly related populations. Blood group data collected in the present survey and those reported by Musa et al. [21] were also used to estimate probabilities of match between randomly selected donors and recipients for blood transfusion. Probability values are shown in Table S2.