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ARTICLE

M2/ANXA5 haplotype as a predisposition factor in Malay women and couples experiencing recurrent spontaneous abortion: a pilot study

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Abstract Recurrent spontaneous abortion (RSA) is a prevalent condition among the Malay population of Malaysia, where carriage risk of conventional hereditary thrombophilia factors has been generally ruled out. The contribution of *M2/ANXA5*, a common haplotype in the annexin A5 gene promoter, was evalauted for RSA in Malay. Seventy-seven women who had experienced two or more unexplained RSA and 41 available male partners were selected for study, with 360 population controls recruited from healthy Malay individuals. Incidence of M2 carriage and odds ratios were calculated between control and patient groups, and clinically defined subgroups and RSA risk was evaluated. *M2/ANXA5*, found in 42.2% of the general Malay population, was associated with greater risks for women with primary and secondary RSA with early (gestational week 5–15) losses. The risk was somewhat higher in Malay couples when both partners were carriers and a trend of higher prevalence was seen for the male partners patients who had experienced RSA. M2 carriage seems to be a risk factor with unusually high incidence in Malay women and couples with primary and secondary RSA with 'early' spontaneous abortions. The associated male partner risk confirms the proposed role of *M2/ANXA5* as a genetic trait impeding embryonic anticoagulation.

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Introduction

Recurrent spontaneous abortion (RSA) is a central topic in reproductive medicine and an aggravating condition of women's health. About one in five women worldwide have suffered at least one spontaneous abortion and one in 20 have had two or more spontaneous abortions (Dudley and Branch, 1989). Generally 30–40% of RSA cases remain unexplained, thus classified as 'idiopathic', and are thought to be caused by a variety of genetic, physiological and environmental conditions (Kutteh et al., 1999). Hereditary thrombophilia is increasingly recognized as a factor for adverse pregnancy outcome (Bogdanova and Markoff, 2010) and mounting evidence shows that impaired placental perfusion increases the risk for RSA (Grandone and Margaglione, 2003; Tiscia et al., 2009; Tüttelmann et al., 2013; Younis and Samueloff, 2003). The predisposing role of genetic thrombophilia has been revealed in several clinical studies (Rodger et al., 2010), and historically has focused on Factor V Leiden (FVL) or prothrombin G20210A (PTm) variants for European populations and their descendants (Rey et al., 2003). In contrast, these lesions have been documented as virtually absent in Africans and Asians from previous studies (Rees et al., 1995, 1999).

Malays are an ethnic group of Austronesian people that comprise 60% of the indigenous Malaysian population. Depending on a variety of environmental and socioeconomic conditions spontaneous abortion and RSA rates have been reported as quite high among Malays, about 33% (Arshat et al., 1985), but the genetic aspect has been scarcely studied. Published reports confirm the rather low incidence of FVL and PTm in Malay women experiencing RSA (Ayadurai et al., 2009; Yusoff et al., 2002), with estimated prevalence of 1% and 0.3%, respectively. Institutional data concerning the number of pregnancy losses for the year 2013 in Hospital Sultan Abdul Halim, Sungai Petani show that a high proportion (779/892 [87.3%]) occurred in Malay women. The spontaneous abortion rate in Malay women at this hospital in 2013 was 20.8% from a total of 3740 admissions, although no records were available on RSA.

In 2007, a new hereditary factor for thrombophilia-mediated RSA was identified, termed 'M2', a haplotype in the proximal core promoter region of the annexin A5 (*ANXA5*) gene (Bogdanova et al., 2007). The haplotype is present in about 15% of European populations studied so far (Tiscia et al., 2009; Tüttelmann et al., 2013), in 11% of the Japanese (Miyamura et al., 2011), and confers about a two-fold elevated RSA risk in relevant patient groups. Available evidence links carriage of *M2/ANXA5* with reduced expression of *ANXA5* in chorionic placenta (Chinni et al., 2009; Markoff et al., 2010), and associated similar risks of male carriers in couples who have experienced RSA point to a possibly impeded embryonic anticoagulant function (Rogenhofer et al., 2012; Tüttelmann et al., 2013).

The aim of the present study was to determine the population incidence of *M2/ANXA5* in Malays and the possible risk role of the haplotype in patients and couples who have RSA according to RSA and embryonic categories.

Materials and methods

Study populations

The present genetic association study complied with the ethical guidelines of the institutions involved, and was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. Approvals were received from the Human Ethics Research Committee of the Universiti Sains Malaysia (approved 12 January 2012, reference USMKK/PPP/JEPeM [245.3.(2)]) and from the National Institutes of Health, Ministry of Health, Malaysia (approved 30 June 2012). The study was entered in the Malaysian National Medical Research Register (NMRR), ID: NMRR-11-1044-9519. Informed consent was obtained from all participants examined.

Malay origin for all patient and control participants was verified across three generations that did not have intermarriage. Patiens who had experienced RSA (n = 77) were selected from the routine work-up of pregnant women attending the Department of Obstetrics and Gynaecology at the Sultan Abdul Halim Hospital, Sungai Petani, between January and September 2013. Male partners of 41 of these women agreed to participate in the study upon signing informed consent. Patients who have experienced RSA were pre-screened for potential causes of their repetitive spontaneous abortions as described previously (Li et al., 2002; Rogenhofer et al., 2012). Uterine anomalies, endocrinological dysfunctions (polycystic ovary syndrome according to the Rotterdam criteria (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004) and thyroidal dysfunctions, if anamnestic) were excluded, as well as fetal and parental chromosomal disorders (numerical aberrations). Inherited thrombophilia (FVL, PTm) and deficiencies in anti-thrombotic factors (protein C, protein S, factor XII, antithrombin III) were ruled out. All patients were non-smokers.

Basic clinical data of patients who had experienced RSA and male partners are presented in **Table 1**. Recurrent spontaneous abortion was defined as primary if two or more repeated consecutive spontaneous abortions occurred before the 20th gestational week and no viable pregnancy outcomes were recorded; as secondary, with two or more repeated spontaneous abortions before the 20th gestational week, after a live birth; and as tertiary if two or more repeated spontaneous abortions occurred before the 20th gestational week were recorded, interspersed with live births. These working definitions were compiled according to the Practice Committee of the American Society for Reproductive Medicine (2008) reviewed by Tulandi et al. (2014) and combined with definitions by Carp (2007).

According to fetal development at the time of pregnancy loss (Heuser et al., 2010), patients were stratified into three subgroups: subgroup 1, embryonal losses (gestational weeks 5–9); subgroup 2, 'early' fetal losses (gestational weeks 10–15); and subgroup 3, 'late' fetal losses, (gestational weeks >15).