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Genotyping analysis of protein S-Tokushima (K196E) and the involvement of protein S antigen and activity in patients with recurrent pregnancy loss

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ABSTRACT

Objective: Preston et al. indicated that Protein S (PS) deficiency was associated with stillbirths but not miscarriages. The PS-Tokushima missense variant was reported to serve as a genetic risk factor for deep vein thrombosis in the Japanese population. A previous cross-sectional study showed no increase in the prevalence of PS-Tokushima in patients with recurrent early pregnancy loss or in patients with intra uterine fetal death and/or fetal growth restriction. There has been limited number of prospective studies examining the pregnancy outcome in patients with both a PS deficiency and recurrent pregnancy loss (RPL). We examined the association between PS deficiency, PS-Tokushima and RPL.

Study design: The study group consisted of 355 Japanese women with two or more consecutive pregnancy losses and 101 parous women. The frequency of PS-Tokushima and the subsequent live birth rate in relation to a PS deficiency defined as low PS-specific activity (total PS activity/total PS antigen) and the carriage of PS-Tokushima were examined.

Results and conclusions: There was no significant difference in the frequency of PS-Tokushima between patients and controls. The 8 patients carriers of PS-Tokushima variant were capable of a subsequent live birth without the use of heparin. There was no significant difference in subsequent live birth rates between patients with low or normal PS-specific activity/PS activity without heparin prophylaxis after excluding miscarriages caused by an abnormal embryonic karyotype using multivariate logistic regression analysis. There was no association between PS-Tokushima and RPL and a PS deficiency or low PS activity was shown not to serve as a reliable clinical predictor of subsequent miscarriage.

Introduction

Established causes of recurrent pregnancy loss (RPL) include antiphospholipid syndrome (APS), uterine anomalies, and parental and embryonic chromosomal abnormalities [1–4]. APS, acquired thrombophilia, are the only treatable cause of RPL, and combined low-dose aspirin and heparin treatment has been shown to improve the live birth rate in patients with APS [5,6]. Heritable thrombophilia has been reported to be associated with RPL [7,8]. A cross-sectional study by Preston et al. indicated that protein S (PS) deficiency was associated with stillbirths (odds ratio(OR) 3.3, 95% confidence interval (CI) 1.0–11.3) but not miscarriages (OR 1.2, 95% CI 0.7–1.9) [7]. A meta-analysis by Rey et al. revealed that PS deficiency is associated with recurrent fetal loss (OR 14.72, 95% CI 0.99–218) and late non-recurrent fetal loss (OR7.39, 95%CI 1.28–42.63) but not with recurrent early miscarriage [8]. An association between PS deficiency and recurrent fetal loss is unclear since sample size might be insufficient because the frequency of PS deficiency is relatively small.

Protein S (PS) is a cofactor for activated protein C (APC) and degrades activated factor (F) V and FVIII [9]. The free form of PS functions as a cofactor because its cofactor activity for APC is lost when PS binds to C4b-binding protein (C4bBP). A congenital PS deficiency is a well-known risk factor for the development of deep vein thrombosis (DVT) [10].

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The PS-Tokushima (p.Lys196Glu, K196E) missense variant (PS-Tokushima) was identified in the second epidermal growth factor-like (EGF) domain of PS and reported to serve as a genetic risk factor for DVT in the Japanese population [11]. The prevalence of this variant was found to be about 1.65-1.8% in the Japanese general population [12,13] and it has not as yet been identified in Chinese, Koreans, or Caucasians [14–16].

The meta-analysis did not show that a PS deficiency increased the risk of early miscarriage at less than 10 weeks gestation [8]. In addition, the terms early miscarriage and fetal loss should be distinguished. There has been limited number of prospective studies examining the pregnancy outcome in patients with both a PS deficiency and RPL. However, a nation-wide survey found that 43.9% of facilities in Japan examined PS activity in patients with RPL [17]. A similar problem is speculated to exist worldwide.

In the cross-sectional presented here, we examined associations between PS-Tokushima variant carriage, total PS antigen, total PS activity, PS deficiency defined as low PS-specific activity (ratio = activity/antigen) and RPL. In the cohort study, we examined whether PS-Tokushima or a PS deficiency influenced the subsequent live birth rate.

Materials and methods

Patients and controls

All patients were seen at Nagoya City University Hospital between September 2008 and July 2014. The study group consisted of 355 Japanese women with two or more consecutive pregnancy losses [18,19].

All patients underwent a systematic examination, including hysterosalpingography, chromosome analysis of both partners, determination of antiphospholipid antibodies (aPLs), including lupus anticoagulant (LA), by 5×-diluted activated partial thromboplastin time (aPTT), diluted Russell's viper venom time (RVVT) and β2 glycoprotein I-dependent anticardiolipin antibody determination (β2GPI-aCL), as well as blood tests for hypothyroidism and diabetes mellitus, before a subsequent pregnancy [20]. Criteria for exclusion from the analyses included the presence of uterine anomalies and chromosomal abnormalities in either partner.

Subsequent pregnancies of all patients were followed up until December 14, 2014. Gestational age was calculated from basal body temperature (BBT) charts. Ultrasonography was performed once a week from 4 to 8 weeks of gestation. Dilation and curettage was performed on patients diagnosed as having a miscarriage. A part of the villi was cultured, and the cells were harvested after 6–22 days

Figure 1. Flowchart of patients

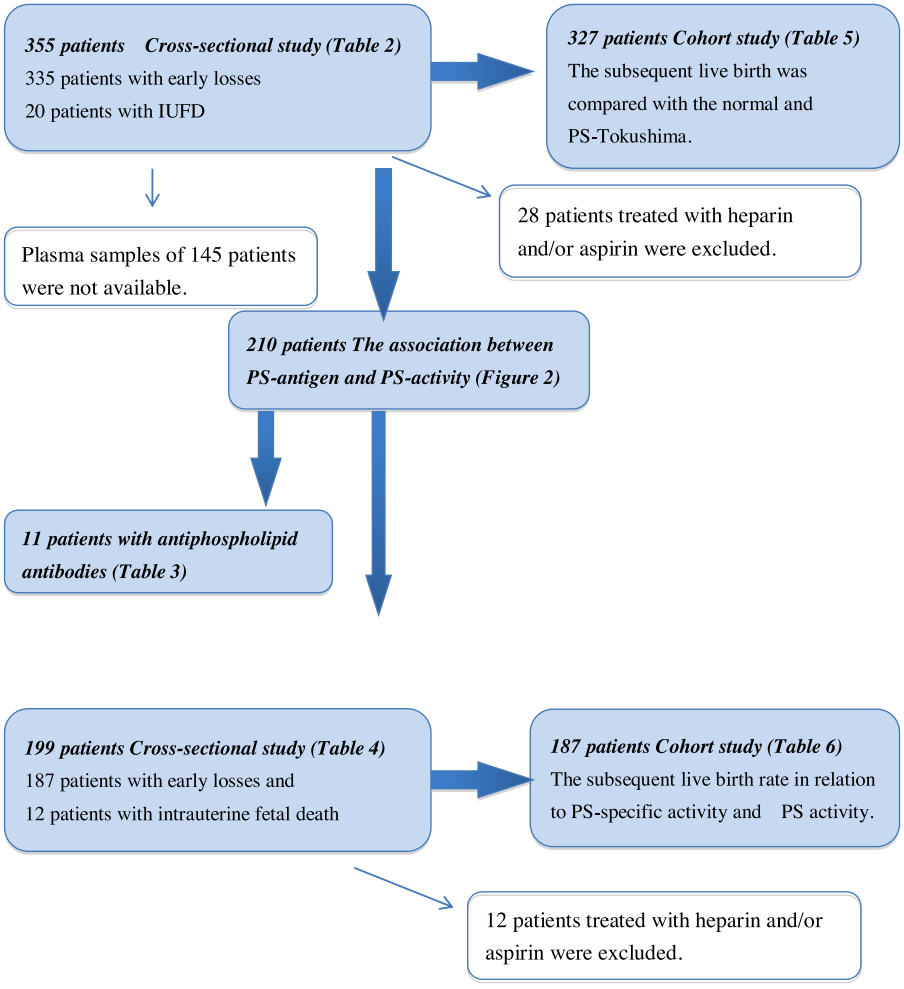

Fig. 1. A flow chart is shown to clarify the exclusion criteria. The frequencies of PS-Tokushima were compared between 355 patients and 101 controls. The total PS antigen, activity, and specific activity were compared between the 210 patients and 101 controls since only 210 patients were available to provide plasma samples. The PS activity was compared between 11 patients with aPLs and 199 patients without aPLs. The 11 patients with aPLs were excluded from further analysis.