7　無症候性の抗リン脂質抗体について

他の全身性自己免疫疾患と同様に，APSは血栓症や妊娠合併症があってはじめて「症候群」となる。無症候の場合には基本的には抗リン脂質抗体検査をする必要はないが，①SLEの診断のため，②妊娠前検査として，③血清梅毒反応や凝固時間延長の精査のために，抗リン脂質抗体がスクリーニングされることがある。無症候性の血栓症をどこまで検索すべきかについては，統一した見解はない。一方，APSの病態の基本は「血栓傾向（thrombophilia）」であり，再発が極めて多いことを考えると，特に血栓症と相関の高い抗リン脂質抗体（たとえば高力価のIgG型のβ_2グリコプロテインI依存性抗カルジオリピン抗体）が陽性である場合には，当科では全身CTを利用して下肢深部静脈および下大静脈の血栓や肺動脈血栓の有無をスクリーニングしている。また，無症候性ラクナが脳梗塞発症のリスクであることを考え，脳MRIは機会をみて施行する。これらの検査で潜在性の血栓が発見された場合は，二次予防の適応となる。

筆者ら[13]は，ロンドンの聖トーマス病院でHarrisがaCLのアッセイを確立してAPSの最初の診断基準を示した1986年に，aCLが陽性であり，その後同院で10年の観察が可能であった患者52例の10年の経過を調べた。52例中31例はAPSと診断され，ワルファリンその他の抗血栓療法が行われ，10年間での再発は29%にみられた。1986年当時にAPSの症状がなかった21例は，アスピリンの予防投与が継続あるいは断続的に行われていたが，結果的に10年間で38%に血栓症を発症した。同院はロンドンのSLEと血管炎の診療センターであり，したがって対象患者は基本的に膠原病の背景があった。SLEを対象とした別の研究[14]でも，抗β_2GPI抗体が血栓症の続発を予想するとされた。一方，最近ノルウェーで行われた一般住民を対象とした大規模観察研究では，508例の深部静脈血栓症と1,464例のコントロールを7年間フォローアップした。その結果，β_2GPI依存性aCLの静脈血栓発症の予測的価値はみられなかった[15]。

抗リン脂質抗体症候群の血栓症発症のリスクについては，抗体の種類や患者の背景によって異なっており，無症候性の抗リン脂質抗体陽性患者に対する血

栓症の一次予防に関するデータは限られている。まず日常生活で喫煙や避妊ピルなどの他の血栓リスクを減らすよう努め，薬物では習慣的にアスピリンが投与されている。Erkan ら[16]は，APS の発症を未然に防ぐべく少量アスピリン投与の意義を確認するために二重盲検試験を行った。98 人の血管および妊娠関連イベントの既往のない抗リン脂質抗体陽性者を，アスピリン 81 mg/ 日とプラセボ投与群にランダム化した。平均追跡期間 2.30 ± 0.95 年の間に，アスピリン群で 3 例の血管イベント(深部静脈血栓症 2 例，脳梗塞 1 例)，プラセボ群ではイベントはなかった(ハザード比 1.04，有意差なし)。したがって，アスピリンの抗リン脂質抗体陽性者における血栓一次予防効果は証明されなかった。

基本的に，抗血小板薬であるアスピリンは静脈血栓症や肺塞栓の予防効果がない。低強度のワルファリン（INR 1.5）の効果が予想され，現在プロスペクティブ研究が進行中であるが，結果はまだ出ていない。

以上より，血栓の既往のない患者の場合，すなわち偶然に抗リン脂質抗体の存在が証明されたような場合は，特に膠原病の背景をもたない例では，薬物による一次予防をすべきかどうかのコンセンサスはない。

（渥美 達也）

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VI-6. 抗リン脂質抗体症候群の診断

Key words: antiphospholipid antibody, anticardiolipin antibody, beta$_2$-glycoprotein I, lupus anticoagulant, antiprothrombin antibody

Point

①抗リン脂質抗体症候群(antiphospholipid syndrome：APS)は自己免疫性血栓症または妊娠合併症を臨床症状とする.

②診断にはβ_2糖蛋白(GP)I依存性抗カルジオリピン抗体あるいはループスアンチコアグラント(LA)の存在を証明することが必要.

③抗β_2GPI抗体，ホスファチジルセリン依存性抗プロトロンビン抗体も診断に有用な検査である.

④APS以外にも抗リン脂質抗体と関連する臨床症状があり，最近それらは抗リン脂質抗体関連疾患群として提唱された.

抗リン脂質抗体症候群(APS)は，抗リン脂質抗体と関連する自己免疫性血栓症および妊娠合併症と定義される．抗リン脂質抗体はリン脂質あるいはリン脂質と蛋白質の複合体に結合する自己抗体の総称であるが，APSと関連する抗リン脂質抗体の主な対応抗原はβ_2GPIとプロトロンビンであることが明らかとなった.

一方，血栓症と妊娠合併症以外にも抗リン脂質抗体と関連する臨床症状があることは以前から知られていたが，これらを一群の「抗リン脂質抗体関連疾患」として臨床研究の対象とすることが提唱された.

APSを定義する臨床症状は明確である．すなわち，APSの診断とは，APSを定義する抗リン脂質抗体の検出・同定にほかならない.

1) 概念と臨床症状

APSは，獲得性血栓傾向の原因としては頻度の高い病態のひとつとして認識され，臨床上重要な位置を占めている．現在のAPSの分類基準は，札幌クライテリア・シドニー改変[1]と呼ばれている(表1).

APSは単独で発症すれば原発性と分類されるが，約半数は全身性エリテマトーデス(SLE)に合併する．APSの病態の基本は血栓傾向である．APS患者に発症する血栓症は多様であるが，好発部位が存在する．静脈血栓としては他の血栓傾向と同様に下肢深部および表在静脈の血栓症が多く，しばしば肺塞栓を合併する．深部静脈血栓症が証明されなくとも肺血栓塞栓症が存在する場合もある．ヨーロッパ人のAPS 1,000例の集計では，90例(9%)に肺梗塞の所見がみられた[2]．その他の静脈血栓症では，網膜中心静脈閉塞がしばしばみられ，またBudd-Chiari症候群の原因としてAPSは最も頻度が高い．理由は不明だが，副腎静脈がAPSの血栓の好発部位であり，二次

表1　抗リン脂質抗体症候群診断基準(札幌クライテリア・シドニー改変分類基準, 2006)

臨床所見
1. 血栓症
　画像診断, ドプラ検査または病理学的に確認されたもので, 血管炎による閉塞を除く
2. 妊娠合併症
　a. 妊娠10週以降で, 他に原因のない正常形態胎児の死亡, または
　b. 妊娠中毒症, 子癇または胎盤機能不全による妊娠34週以前の形態学的異常のない胎児の1回以上の早産
　c. 妊娠10週以前の3回以上続けての形態学的, 内分泌学的および染色体異常のない流産

検査基準
1. 標準化されたELISA法によるIgGまたはIgM型抗カルジオリピン抗体(中等度以上の力価または健常人の99パーセンタイル以上)
2. IgGまたはIgM型抗β_2GPI抗体陽性(健常人の99パーセンタイル以上)
3. 国際血栓止血学会のLAガイドラインに沿った測定法で, LAが陽性

臨床所見の1項目以上が存在し, かつ検査項目のうち1項目以上が12週の間隔をあけて2回以上証明されるとき抗リン脂質抗体症候群と分類する.

[Miyakis S et al : International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome(APS). J Thromb Haemost 4 : 295-306, 2006]

性 Addisson 病をきたすことがある.

APS の血栓症に特徴的な点は, 静脈のみならず動脈に血栓を起こすことである. しかも APS では脳梗塞, 一過性脳虚血発作などの脳血管障害が圧倒的に多く, 虚血性心疾患が比較的少ない特徴がある. 実際に脳血管障害が動脈血栓症の90% 以上を占めている. 脳 MRI では単発性から多発性までさまざまな病巣が観察される.

妊娠合併症には妊娠高血圧症と不育症・流産がある. 流産の原因として子宮自体の異常, 染色体異常と並んで APS は最も重要である. 通常の流産が胎盤形成以前の妊娠初期に圧倒的に多いことに対して, APS 患者の流産はむしろ妊娠中・後期によく起こることが特徴である.

一方, 妊娠高血圧症やその重症型である子癇が抗リン脂質抗体陽性者に多いことが知られるようになった.

非常にまれであるが, APS の特殊型に分類される病態として, 急激に多臓器不全(とりわけ中枢神経と腎)に陥り, 重症呼吸不全, 重篤な血小板減少症を合併し致死率の高い激症型抗リン脂質抗体症候群(catastrophic antiphospholipid syndrome)がある. 播種性血管内凝固症候群(DIC)か血栓性血小板減少性紫斑病(TTP)に類似した病態を伴っていることが多い. 抗凝固療法を急に中止した場合にも起こることがある.

血栓症と妊娠合併症以外では, ①心弁膜症, ②神経疾患(特に舞踏病と横断性脊椎症が特徴), ③皮膚疾患(特にリベドー疹), ④微小血栓による腎障害, ⑤血小板減少症, の5つが抗リン脂質抗体と関連する疾患とされる. これらの抗リン脂質抗体関連疾患群は, 今後の臨床研究の対象とされ, それぞれの分類基準が示された[1].

2) 抗リン脂質抗体の検出方法と臨床的意義

抗リン脂質抗体測定の臨床的意義は, APS あるいは抗リン脂質抗体関連疾患を診断することである. APS と診断するためには抗リン脂質抗体の証明が必須である. しかし, 抗リン脂質抗体のもつ多様性のため, しばしばその判断は容易ではない.

a) 抗カルジオリピン抗体(aCL)と抗β_2GPI抗体

一連の抗リン脂質抗体の測定法のなかで, aCL は最も早くに確立された免疫学的な抗リン脂質抗体の検出法である. 当初はリン脂質であるカルジオリピンが aCL の直接の対応抗原と考えられていたが, 現在では APS と関連した aCL と, ポリクローナル B 細胞活性化を伴う膠原病(APS を合併しない SLE や Sjögren 症候群)や感染症患者にみられる非特異的な aCL は, 真の対応抗原の違いにより区別されうることがわかっている. すなわち, APS 患者に検出される aCL は

カルジオリピンとβ_2GPⅠとの複合体に結合しており，しかもその結合エピトープはβ_2GPⅠの分子上に存在する．したがってAPSに特異性の高いaCLは「β_2GPⅠ依存性aCL」と呼ばれるアッセイで検出される抗体である．このELISA法ではβ_2GPⅠの存在下および非存在下で同時にaCLの測定を行い，前者の力価が基準値を超え，かつβ_2GPⅠの存在下でのaCLの力価が非存在下での力価よりも高いものを陽性とする．β_2GPⅠ依存性と非依存性のaCLが混在している場合があり，また非依存性aCLがELISAプレート底にβ_2GPⅠが結合していない遊離カルジオリピンに結合している場合があるので，β_2GPⅠの存在でのaCL力価と非存在でのそれとを比較して判定する．

抗β_2GPⅠ抗体は直接β_2GPⅠをELISAプレートに固相化して行うアッセイであり，基本的にはβ_2GPⅠ依存性aCLと同じ抗体を検出する．クライテリアに記載され，正常範囲(カットオフ値)は定義されているものの，アッセイのわが国での標準化は不十分で，診断的意義は今後の課題である．

b) ループスアンチコアグラント(LA)

LAは，*in vitro*のリン脂質依存性凝固反応[活性化部分トロンボプラスチン時間(APTT)，カオリン凝固時間(KCT)，希釈Russell蛇毒時間(dRVVT)など]を阻害する免疫グロブリンと定義される．これらの凝固反応自体は簡易な検査であるが，臨床検査上のLAの同定はその多様性から必ずしも容易でない．また使用する試薬によって感度がかなり異なっているが，それは主に凝固試薬に含まれるリン脂質濃度に依存する．国際血栓止血学会の抗リン脂質抗体標準化委員会がLA検査のガイドラインを示している．すなわち，①APTT，KCT，dRVVTなどでリン脂質依存性凝固時間が延長していることをスクリーニングする，②ミキシングテストでこの凝固時間延長が患者血漿中にインヒビターが存在するためであることを示す，③障害血小板やリン脂質による吸収中和試験でこのインヒビターが抗リン脂質抗体であることを確証，④特定の凝固因子に対するインヒビターを除外する，のステップである．

しかし，わが国の日常臨床ではそのような煩雑な手順でLAの診断ができる施設は限られる．日常的には，APSの臨床症状があったとき，①LAに高感度のAPTT検査で凝固時間をスクリーニング，②LA確認試薬キットを用いて凝固時間延長が抗リン脂質抗体によるものであることを示す，のステップが推奨される．日本血栓止血学会標準化委員会では，多施設による検討の結果，わが国でのLAスクリーニングの標準試薬をロシュ・ダイアグノスティックスのPTT-LA(APTT検査)と定めた．本試薬による凝固時間延長はLAの検出感度が最も高かった．PTT-LAを用いたAPTT検査は保険適用となり，しかも現時点ではLA検査としてでなくAPTT検査としての収載なので，コストが低く有利である．なお，凝固時間の延長がなければLAは陰性なので，確認試験を行う必要はない．

c) ホスファチジルセリン依存性抗プロトロンビン抗体(aPS/PT)

LAに少なくとも2つのサブタイプがあり，β_2GPⅠ依存性LAおよびプロトロンビン依存性LAと呼ばれる．前者はaCLに該当するが，後者は抗プロトロンビン抗体である．ホスファチジルセリンを固相化，プロトロンビンを吸着して抗原としたものを用いてELISA法を行うとaPS/PT，APSの臨床症状やLAの存在と非常に強い相関があることが示された．LA陽性者の半数はaPS/PTが陽性であり，逆にaPS/PT陽性者は9割以上がLA陽性であった．すなわちaPS/PTはLAあるいはAPSの新しいマーカーである[3]．とりわけLAの補助診断としての意義が高いことがわかる．LAの判断は，典型的なパターンをとるものは別にして，通常は容易とはいえない．また良質な血漿サンプルがないと信頼できる結果が得られない．aPS/PTが陽性なら高い確率でLA陽性であるといえるので，ワーファリン®やヘパリンなどの抗凝固療法を施行中でLAアッセイに向かない患者の場合，あるいは何らかの理由により血漿サンプルが得られないとき，などにはaPS/PTアッセイの価値は高い．

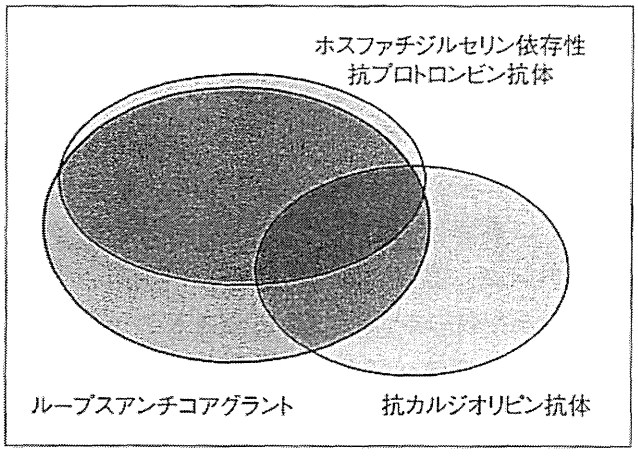

図1　抗リン脂質抗体の相互関係
抗β_2GPⅠ抗体はほぼ抗カルジオリピン抗体と同一の分布である.

d) 抗リン脂質抗体間の関係

以上のように，複数の対応抗原に対する自己抗体を複数の免疫検査法で検出することに加えて，その機能を複数の試薬を用いて複数の手順で凝固検査を行わなければならず，抗リン脂質抗体の検出は臨床検査としては最も煩雑なもののひとつである．一つひとつの検査を整備することも重要であるが，既存の検査を効率よく日常臨床に利用できるように整理することも必要である．

抗リン脂質抗体のおおよその相互関係を図1に示した．

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Genotyping analyses for polymorphisms of *ANXA5* gene in patients with recurrent pregnancy loss

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Objective: To investigate whether polymorphisms at the promoter or 5′-untranslated region of annexin A5 gene (*ANXA5*) influence miscarriage.
Design: Case-control study and nested case-control study.
Setting: Hospitals.
Patient(s): A total of 264 patients with two to nine recurrent pregnancy losses (RPLs) and 195 fertile control subjects.
Intervention(s): None.
Main Outcome Measure(s): The frequency of six single-nucleotide polymorphisms (SNPs) of the *ANXA5* gene in RPL patients versus control subjects, and subsequent live birth rate with and without risk alleles in RPL patients.
Result(s): The minor allele was significantly more frequent in RPL patients than in control subjects for SNP5 (rs1050606). The live birth rates of patients with and without risk alleles of SNP5 were 84.0% and 84.3%, respectively, after excluding cases with abnormal embryonic karyotype, with no significant difference.
Conclusion(s): The variations with the *ANXA5* gene upstream region, especially SNP5, were confirmed to be risk factors of RPL. However, presence/absence of the *ANXA5* risk allele did not have any predictive effect for subsequent pregnancy outcome. This was the first study indicating the influence of *ANXA5* SNP5 for pregnancy outcome. (Fertil Steril® 2013;100:1018–24. ©2013 by American Society for Reproductive Medicine.)
Key Words: Annexin A5, single-nucleotide polymorphisms, recurrent pregnancy loss, cohort

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Established causes of recurrent pregnancy loss (RPL) include antiphospholipid antibody syndrome (APS), uterine anomalies, and abnormal chromosomes, particularly translocations, in either partner (1–3). However, according to earlier reports, in about one-half of the cases seen at research centers, the cause remains unexplained despite conventional examinations conducted to identify the cause (4, 5). Recently, we found that an abnormal embryonic karyotype was the most frequent cause of RPL, accounting for as many as 41% of the cases (6).

APS, or acquired thrombophilia, is the only treatable cause of RPL, with combined low-dose aspirin and heparin treatment having been shown to improve the live birth rate in patients with APS (7, 8). Heritable thrombophilia has been reported to be the cause in a majority of other cases of pregnancy loss of uncertain cause

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(9, 10). However, no association has been established between hereditary thrombophilia and RPL (11).

Annexin A5 (placental anticoagulant protein, encoded by the *ANXA5* gene) normally occurs on the placental villi, and its expression appears to be decreased in the presence of antiphospholipid antibodies (aPL) (12). Annexin A5 functions as an inhibitor of coagulation by its ability to bind to the anionic phospholipids exposed on the surface of platelets (13). It is abundantly expressed in the normal placenta (14), and the encoding gene possesses a complex promoter region that is subject to intricate regulation (15). It has been shown that single-nucleotide polymorphisms (SNPs) in the promoter region of the *ANXA5* gene are significantly associated with RPL, and that women with the M2 haplotype have a 2.42-fold higher risk of pregnancy loss than noncarriers (16). More recently, it has been shown that an SNP in the 5′-untranslated region of the *ANXA5* gene (SNP5: −302T>G) serves as a major risk determinant of RPL in Japanese women (17).

In the present study, we further investigated six *ANXA5* gene SNPs in the upstream region in a cohort study of Japanese women with RPL and merged the findings with previously published reports (17) for univariate analyses. Because data on the combined effect of maternal age and genetic risk factors are still lacking, we also conducted a cohort study to examine, in relation to several factors, whether a subsequent pregnancy would result in further loss or normal delivery. This was the first cohort study to investigate *ANXA5* gene polymorphism as a molecular marker of RPL.

MATERIALS AND METHODS

Patients

We analyzed the data of 264 Japanese women with a history of unexplained RPL (defined as a history of two or more pregnancy losses) who were recruited from Nagoya City University Hospital from June 2007 to November 2012. All patients underwent systematic examination, including hysterosalpingography, chromosome analysis of both partners, determination of aPL, including lupus anticoagulant (LA), by diluted activated partial prothrombin time (aPTT), diluted Russell viper venom time (RVVT), and β2-glycoprotein I-dependent anticardiolipin antibody (18), and blood tests for hypothyroidism and diabetes mellitus, before a subsequent pregnancy. Patients with APS, uterine anomalies, and abnormal chromosomes in either partner were excluded from the analysis (19). Patients with a history of preeclampsia or abruptio placentae also were excluded.

Subsequent pregnancies of all patients were followed until February 2013. Among the patients, 225 received no medication and 39 received anticoagulant treatment although there was no evidence. Gestational age was calculated from basal body temperature charts. Ultrasonography was performed once a week from 4 to 8 weeks of gestation. Dilation and curettage was performed on the patients diagnosed as having miscarriage. Part of the villi was cultured, and the cells were harvested after 6–22 days of cultivation to analyze the chromosome. A total of 47 aborted conceptuses could be karyotyped with the use of a standard G-banding technique.

Furthermore, 195 women with at least one child and no history of infertility or miscarriage were examined as control subjects. The control subjects were recruited from Nagoya City University Hospital and Inabe General Hospital from January to April 2012. The earlier study implicated SNP5 (minor allele frequency [MAF] 0.228) as the major susceptibility locus in RPL (17). The minor allele frequency (MAF) of SNP5 was 0.228. Based on a sample size of the 264 patients already obtained, MAF of ~20% in the SNP, and a multiplicative model, 195 control subjects were needed for 80% power and 5% significance.

The allele frequencies of the six SNPs of the *ANXA5* gene were compared between the patients and control subjects. The subsequent pregnancy outcomes were compared between the 264 patients with and without the risk alleles.

This study was conducted with the approval of the Research Ethics Committee of Nagoya City University Graduate School of Medical Sciences and the Ethics Committee of Inabe General Hospital. Each patient provided written consent after being provided with a full explanation about the purpose of the study and the methods to be used.

DNA Analysis

Genomic DNA was extracted from peripheral blood samples with the Midi Blood DNA Extraction kit (Qiagen). A total of six SNPs were analyzed: rs112782763 (−467G>A at promoter; SNP1), rs28717001 (−448A>C at promoter; SNP2), rs28651243 (−422T>C at 5′-untranslated exon 1; SNP3), rs113588187 (−373G>A at 5′-untranslated exon 1; SNP4), rs1050606 (−302T>G; SNP5), and rs11538099 (−1C>T at one base upstream of the initiation codon in exon2; SNP6). All genotyping was carried out using Taqman polymerase chain reaction (PCR) assays (Applied Biosystems) in 96-well arrays that included two blank wells as negative control samples according to the manufacturer's instructions. Taqman Predesigned SNP Genotyping assay and Taqman MGB probes were used. Taqman PCR and genotyping analyses were carried out on the Applied Biosystems 7500 Fast Real-Time PCR System. The reaction mixtures were amplified in 1 μL template DNA (10 ng/μL), 12.5 μL 2× Taqman Universal Master Mix, 0.625 μL 20× primer/probe mix and 10.875 μL double-distilled H_2O in a volume of the mixture of 25 μL. The cycling conditions were as follows: initial denaturation at 95°C for 10 minutes, followed by 40 cycles at 95°C for 15 seconds and 58°C for 1 minute. The results were automatically analyzed on the Applied Biosystems 7500 Real-Time PCR System with the use of an allelic discrimination assay program (20).

Statistical Analysis

Departure from the Hardy-Weinberg equilibrium for the six SNPs was tested with the use of an exact test (21). Because previous studies have shown that the mainland Japanese population is genetically similar, we did not examine or perform corrections for the population substructure in our sample (22, 23).

For individual SNP association analyses, univariate logistic regression analyses were performed with dominant and

TABLE 1

Allele frequencies of six *ANXA5* single-nucleotide polymorphisms (SNPs) in patients with recurrent pregnancy loss and control subjects and the odds ratios (ORs) in the codominant and dominant models.

		Case	Control	OR (95% CI)	*P* value	*P* value of max-statistics	HWE	MAF
SNP1	Codominant				.187		0.233	0.110
−467G>A	G/G	206	160	Reference				
rs112782763	G/A	55	30	1.43 (0.87–2.33)				
	A/A	3	5	0.47 (0.11–1.96)				
	Dominant				.287	.443		
	G/G	206	160	Reference				
	G/A, A/A	58	35	1.28 (0.81–2.04)				
SNP2	Codominant				.071		0.086	0.108
−448A>C	A/A	207	162	Reference				
rs28717001	A/C	54	27	1.56 (0.94–2.56)				
	C/C	3	6	0.39 (0.10–1.59)				
	Dominant				.211	.266		
	A/A	207	162	Reference				
	A/C, C/C	57	33	1.35 (0.84–2.17)				
SNP3	Codominant				.086		0.154	0.122
−442T>C	T/T	205	160	Reference				
rs28651243	T/C	56	29	1.51 (0.92–2.50)				
	C/C	3	6	0.39 (0.10–1.59)				
	Dominant				.246	.267		
	T/T	205	160	Reference				
	T/C, C/C	59	35	1.32 (0.01–2.08)				
SNP4	Codominant				.137		0.214	0.107
−373G>A	G/G	207	162	Reference				
rs113588187	G/A	54	28	0.89 (0.92–2.50)				
	A/A	3	5	0.47 (0.11–2.0)				
	Dominant				.211	.381		
	G/G	207	162	Reference				
	G/A, A/A	57	33	1.35 (0.84–2.17)				
SNP5	Codominant				.109		0.099	0.218
−302T>G	T/T	155	132	Reference				
rs1050606	T/G	93	51	1.56 (1.03–2.33)				
	G/G	13	12	1.14 (0.01–2.5)				
	Dominant				.049	.100		
	T/T	155	132	Reference				
	T/G, G/G	109	63	1.47 (1.00–2.17)				
SNP6	Codominant				.044		0.086	0.108
1C>T	T/T	206	163	Reference				
rs11538099	C/C							
	T/C	55	26	1.67 (1.01–2.78)				
	C/T							
	C/C	3	6	0.40 (0.10–1.61)				
	T/T							
	Dominant				.135	.258		
	T/T	206	163	Reference				
	C/C							
	T/C, C/C	58	32	1.43 (0.89–2.33)				
	C/T, T/T							

Note: CI = confidence interval; HWE = Hardy-Weinberg equilibrium; MAF = minor allele frequency (in both patients and control subjects).

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codominant models using the presence or absence of PRL as the dependent variable. The two inheritance models were tentatively chosen because earlier studies supported a dominant model for these SNPs (16, 17). We estimated the crude odds ratios (ORs) and 95% confidence intervals (CIs) with the use of the two models. However, there remains some uncertainty about the underlying genetic model. Therefore, to examine the right model of inheritance statistically as well as to avoid multiple comparisons by fitting multiple inheritance models, we used the max-statistic, which selects the largest test statistic from the dominant, recessive, and additive models.

To characterize the linkage disequilibrium (LD) pattern, we estimated the r^2 values for all pairs of SNPs. Then, the haplotype frequencies were estimated with the use of the expectation-maximization algorithm. To evaluate the association between the haplotypes and the risk of pregnancy loss, logistic regression models were used. Haplotype analyses were conducted based on the most supported model by the max-statistic in univariate analysis.

In the cohort study, univariate logistic regression analyses were performed to examine the association of subsequent miscarriage with variable SNPs. Then, multivariate logistic analyses were performed to examine the association

Results of maximizing association statistics in the present study and combination with previous data reported by Miyamura et al.

	SNP1	SNP2	SNP3	SNP4	SNP5	SNP6
Present study						
Dominant	1.132	1.568	1.346	1.568	3.887	2.230
Recessive	1.317	2.177	2.177	1.317	0.002	2.177
Log-additive	0.365	0.400	0.318	0.581	2.408	0.705
max-statistic	1.317	2.177	2.177	1.568	3.887	2.230
P value	.443	.266	.267	.381	.100	.258
Combination with data from Miyamura et al.						
Dominant	4.955	5.826	5.300	6.393	9.943	6.393
Recessive	1.954	2.932	2.932	1.954	1.541	2.932
Log-additive	2.696	2.860	2.565	3.615	9.058	3.177
max-statistic	4.955	5.826	5.300	6.393	9.943	6.393
P value	.056	.035	.046	.025	.003	.025
OR obtained with selection of the dominant model in the combination data						
OR (95% CI), *P* value	1.52 (1.05–2.22), .0029	1.57 (1.09–2.31), .018	1.53 (1.06–2.23), .024	1.62 (1.11–2.39), .013	1.61 (1.20–2.18), .002	1.62 (1.11–2.39), .013

Note: CI = confidence interval; OR = odds ratio; SNP = single-nucleotide polymorphism.

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of subsequent miscarriage with variable SNPs, age, the number of previous miscarriages, and previous live births. The age was quadrisected (21–31, 31–34, 34–37, and 37–45 years), and the number of previous miscarriages (two, three, four, or five to nine) and previous live births (none vs. one or two) were divided so that each group had an almost equal number, and the cells with few numbers were annexed.

Statistical analyses in cross-sectional study were conducted with R software (v. 2.13.0) (24), including the SNPassoc (25) and Haplo.stat (26) packages. Analysis in the cohort study was carried out with SAS (v. 19.0). $P<.05$ was considered to denote statistical significance.

RESULTS

The mean ages (SD) of the patients and control subjects were 33.8 (4.36) and 32.1 (6.91) years, respectively. The mean numbers of previous miscarriage were 2.74 (0.96) and 0, respectively. The mean numbers of previous live births were 0.20 (0.40) and 1.53 (0.72), respectively.

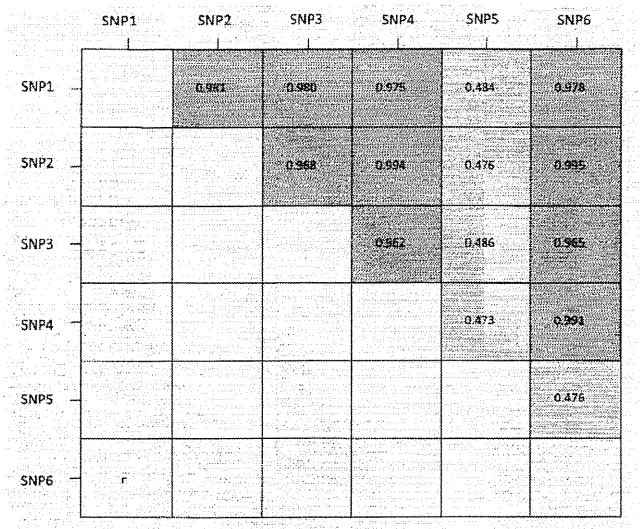
The genotype frequencies for six SNPs were found to be in Hardy-Weinberg equilibrium, suggesting neither sampling bias nor mistyping of genotyping (Table 1). The carrier frequency for the minor alleles tended to be higher in the RPL group for all six SNPs (not significant) when the dominant model was selected (SNP1: G/G vs. G/A-A/A; SNP2: A/A vs. A/C-C/C; SNP3: T/T vs. T/C-C/C; SNP4: G/G vs. G/A-A/A; SNP5: T/T vs. T/G-G/G; and SNP6: C/C vs. C/T-T/T), among which the highest significance level was obtained for SNP5 ($P=.049$; Table 1). When we selected the codominant model (SNP1: G/G vs. G/A vs. A/A; SNP2: A/A vs. A/C vs. C/C; SNP3: T/T vs. T/C vs. C/C; SNP4: G/G vs. G/A vs. A/A; SNP5: T/T vs. T/G vs. G/G; and SNP6: C/C vs. C/T vs. T/T), the highest significance level was obtained for SNP6 ($P=.044$).

Then we merged our data with the previously published Japanese data (17). Based on the results of the max-statistics, the dominant model was adopted for all six polymorphisms (Table 2). The combined data suggested that the dominant model should be selected, and statistically significant correlations were obtained for all six SNPs, with the highest level of significance for SNP5 ($P=.003$). However, the ORs were <2.0 for all six SNPs.

We next performed LD analysis among the six *ANXA5* SNPs (Fig. 1). LD evaluation revealed the existence of significant correlations among SNPs 1, 2, 3, 4, and 6. All except SNP5 exhibited a strong LD (>0.95), as reported previously (16).

Haplotype analysis indicated the presence of three major haplotypes. G-A-T-G-T-C (patient vs. control: 75.9% vs. 80.3%) and G-A-T-G-G-C (12.1% vs. 8.7%) were the major haplotypes and accounted for >85% of the subjects. The first four (G-A-T-G) SNPs constituted the N haplotype described previously (15). The haplotypes comprising all six minor alleles (A-C-C-A-G-T) was found to be the third most common (11.4% vs. 9.2%). The first four SNPs (A-C-C-A) constituted the M2 haplotype. All other haplotypes accounted for <1%. We subsequently performed a case-control study for SNPs 1–6 and for these three haplotypes. When we selected the dominant model, there was no significant difference of the

FIGURE 1



Linkage disequilibrium of the six *ANXA5* single-nucleotide polymorphisms (SNPs).

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frequency between patients and controls among the three haplotypes.

Of the 264 patients with RPL, 185 (70.1%) subsequently gave live births. Of the 79 aborted products, normal and abnormal karyotypes were 36.2% (n = 17) and 63.8% (n = 30), respectively.

Of the patients without treatment, 71.1% (160/225) gave live birth (Table 2). The subsequent live birth rates were 70.0% and 71.9% in the patients with and without the risk allele of SNP5, respectively, and the respective percentages were 84.0% and 84.3% when cases with an abnormal embryonic karyotype were excluded. Among the 39 patients who received anticoagulant treatment, the subsequent live birth rats were 63.2% and 65.0% in the patients with and without the risk allele of SNP5, respectively. Treatment had no effect on the live birth rate in patients with the risk allele of SNP5.

According to the results of the univariate analysis using SNP5, there was no increase in the pregnancy loss rate associated with the presence of the SNP5 risk allele in the 190 patients without treatment or in chemical pregnancy or miscarriage caused by an abnormal embryonic karyotype (OR 1.027, 95% CI 0.463–2.276; P=.9487).

The results of multivariate analyses using SNP5, age, number of previous miscarriages, and presence of previous live births as variables revealed the absence of any influence of the SNP5 risk allele on the subsequent pregnancy loss rate (OR 1.187, 95% CI 0.506–2.784; P=.6941). The miscarriage rate in patients with a history of five to nine miscarriages was higher than that in the patients with a history of two miscarriages (OR 6.012, 95% CI 0.976–37.037; P=.0532).

TABLE 3

Subsequent live birth rate in subjects with and without the SNP5 risk allele.

	With risk allele T/G or G/G	Without risk allele T/T
Subsequent live birth rate in 225 patients without treatment	70.0% (63/90)	71.9% (97/135)
Subsequent live birth rate in 39 patients with treatment	63.2% (12/19)	65.0% (13/20)
Live birth rate after excluding cases with treatment or chemical pregnancy (n = 215)	73.3% (63/86)	75.2% (97/129)
Live birth rate after excluding cases with treatment or an abnormal embryonic karyotype (n = 190)	84.0% (63/75)	84.3% (97/115)

Note: SNP = single-nucleotide polymorphism.

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DISCUSSION

This case-control study demonstrated a significantly higher frequency of the minor alleles in the RPL group compared with the control group for SNP5 (P=.049), but not for SNPs 1–4 and 6. Existence of an association between SNP5 and RPL was originally reported by another Japanese group (17). Miyamura et al. examined patients with a history of three or more unexplained miscarriages (3.06 ± 0.3). The other inclusion and exclusion criteria were similar to those in the present study. It was a new finding that the dominant model should be selected, because the previous study did not perform max-statistics.

Earlier studies suggested that the M2 haplotype of the *ANXA5* gene promoter was significantly associated with the risk of RPL. Bogdanova et al. demonstrated for the first time that a sequence variation in the promoter region of the *ANXA5* gene represented a risk factor for RPL in a German population (16, 27). Another analysis of the significance of M2 strengthened the initial findings in Italian patients with RPL (28). Reporter gene assays showed that M2 reduced the in vitro activity of the *ANXA5* promoter to 37%–42% of normal (16). These data suggest that these SNPs are functional and can reduce the expression of *ANXA5*, thereby influencing the risk of RPL. A recent report showed that the M2 allele in heterozygous placentas resulted in a reduced expression level of the *ANXA5* mRNA by an average of 42% compared with the normal allele (29, 30). The M2 haplotype within the ANXA5 gene was also reported as a new thrombophilic risk factor during pregnancy (31). However, we could not discern any significant difference by max-statistics or haplotype analysis even though our present study included the largest number of subjects in both patient and control groups.

LD evaluation revealed that all except SNP5 manifested a strong LD (>0.95), consistent with the earlier report (17), suggesting that SNP5 may be an independent risk factor for RPL. The significance of SNP5 has not been analyzed in Western patients with RPL.

It has been suggested that annexin A5 molecules form an anticoagulation shield on the apical surface of the placental syncytiotrophoblasts, that may, in pregnancy, be disrupted by antiphospholipid antibodies (32, 33). In a mouse study,

infusion of anti-annexin A5 antibodies induced coagulation at the surface of the syncytiotrophoblast layer, leading to fetal loss, which indicates that annexin A5 protects the fetomaternal interface from the coagulation of maternal blood (34). The syncytiotrophoblasts that possess annexin A5 protein at their surface are of fetal origin and have a half-maternal and half-paternal genome. Tranquilli et al. proposed that carrier status of the fetus of thrombophilia such as factor V polymorphism should be considered to be risk factor for intrauterine fetal death (35). A recent report demonstrated that paternal and maternal carriage of the annexin A5 M2 haplotype were equal risk factors for RPL (36). Thus, it is still not clear whether the annexin A5 protein at the syncytiotrophoblast surface originates from the maternal circulation or is produced by the fetal syncytiotrophoblasts. Functional analysis of SNP5 or SNP5 genotyping of the fetus or placenta would be warranted to further clarify our findings.

A number of articles regarding association between SNPs and RPL have been published (27). An association between heritable thrombophilia, such as the factor V Leiden mutation, prothrombin mutation, and protein S deficiency, and RPL has been reported (9, 10), although the association is not well established (11).

The present study confirmed *ANXA5* SNP5 as a risk factor for RPL. However, the subsequent live birth rate was 84.0% and 84.3% in patients with and without the risk allele of SNP5, after the exclusion of cases with an abnormal embryonic karyotype, with no significant difference. This was the first study indicating the influence of *ANXA5* SNP5 on further pregnancy outcomes. The genome-wide association study proved that the effect of the one of many kinds of SNPs associated with a common disease is very small when the OR is relatively small (37). The *ANXA5* risk allele was not found to be a reliable clinical predictor of subsequent pregnancy outcome. Therefore, we propose that testing for this allele is not needed, because it is without clinical benefit and is an unnecessary expense.

The randomized controlled trial by Kaandrop et al. concluded that there was no effect of combined heparin and aspirin treatment for unexplained recurrent miscarriage (38). The study did not deny that anticoagulant therapy might be effective for a limited number of patients with miscarriage of unexplained cause, because abnormal embryonic karyotype was found to be the most common cause in cases with an unexplained etiology (6). Adequate selection of patients based on analysis of several kinds of SNPs might improve the therapeutic effectiveness. Further study is important to find SNPs with clinical predictive benefit associated with RPL.

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ORIGINAL RESEARCH

Cognitive behavior therapy for psychological distress in patients with recurrent miscarriage

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Objective: To examine the reduction of psychiatric symptoms using individual cognitive behavior therapy (CBT) for women who suffer from recurrent miscarriage (RM) and depression and/or anxiety.

Methods: Patients with RM and a score of five or higher for K6, a self-report screening scale for depression/anxiety, were interviewed to find information about stressful situations, thoughts, and consequent behaviors that are common and potential causes of psychological distress among RM patients. We then performed individual CBT on 14 patients with RM and depression/anxiety, referring to a list from the interviews, and examined the effects of CBT by a paired *t*-test.

Results: Fourteen women received CBT. The mean number of intervention times was 8.9 sessions (standard deviation [SD], 4.6 sessions). The average Beck Depression Inventory-Second Edition and State–Trait Anxiety Inventory–state anxiety scores, self-report screening scales for depression/anxiety, decreased from 13.6 (SD, 8.2) and 49.0 (SD, 7.1) at baseline to 5.2 (SD, 4.4) and 38.0 (SD, 10.2) posttherapy, respectively. These changes were statistically significant.

Conclusion: The current preliminary open study confirmed that individual CBT was potentially useful for women with RM and depression and/or anxiety. This finding is the first step towards creating a comprehensive psychological support system for women with RM.

Keywords: spontaneous abortion, psychological support, depression, anxiety

Introduction

Miscarriage is a very common complication in pregnancy, found in 15% of the women clinically identified as being pregnant. Recurrent miscarriage (RM), defined as two or more consecutive miscarriages, develops in about 5% of couples.[1] In recent years, RM has gained social recognition as a problem related to the decreasing birthrate.[2]

Causes of RM include abnormal chromosomes in either partner, existence of antiphospholipid antibodies, and uterine anomalies. Abnormal embryonic karyotype is also known as a cause, accounting for 25%–50% of miscarriages. However, more than half of RM cases remain unexplained.[1]

Miscarriage causes mental distress such as depression, anxiety, anger, and grief. If miscarriage is repeated, mental distress is sustained.[3] Craig et al[3] and Klock et al[4] reported that mental disorder was found in two-thirds of RM patients, and Craig et al[3] also reported that their anxiety level is similar to that of psychiatric outpatients. All of these suggest that RM patients are a group of people who need appropriate mental care.

In the infertility field, many studies concerning various psychological supports have been conducted for mental distress of patients. Their outcome is divided into two major kinds, improvement of psychological status and improvement of

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pregnancy rate. Three meta-analyses lead to a general conclusion that at least one of the two is to be expected.[5–7]

A few previous studies reported that some forms of psychological support including cognitive behavior therapy (CBT) for patients with single miscarriage were useful.[8–10] In the field of unexplained RM, it has been also indicated that psychological support such as counseling and increased regular checkups in the beginning pregnancy can help raise fertility rate,[11–13] but no further progress has been made. However, unlike for infertility patients or single-miscarriage patients, little research has been done for RM patients on the effects of psychological support with an outcome of reducing anxiety and depression in daily life.

CBT is one of the psychotherapies that is generally confirmed as being effective in reducing depression and/or anxiety.[14] In this study, we preliminarily examined the possibility of reducing depression and/or anxiety of the subjects through individual CBT as the first step for constructing a total psychological support system for RM. Generally, in each CBT session, the patient and the therapist utilize specific hardships in the patient's real life to collaboratively identify and examine her perspective (thoughts, recognition) and behavior. They then devise ways to control her distress or find coping strategies for her hardship. Accordingly, having information in advance about the kind of stressful situations, thoughts, and consequent behaviors which are common and potential causes of depression and/or anxiety among RM patients helps the therapist identify a problem to be discussed and in turn smoothly conduct a session. In addition, such information could be used in the course of developing a broadly available psychological support system in the future. Therefore, we first interviewed patients about what situations often cause them distress, and then conducted preliminary individual CBT sessions referring to the previous interview results and observed the change of depression and/or anxiety.

Materials and methods

Patients

Subjects were recruited from women who visited the specialized RM outpatient care at Nagoya City University Hospital during the period of April 2008 to September 2010; recruited subjects had a history of two or more consecutive miscarriages and had no children. Two weeks after systematic RM tests and explanation about the results, patients filled in K6,[15,16] a self-report questionnaire for depression and/or anxiety, and sent it to us by mail. Patients with a score of five or higher on the K6 were selected as subjects in this study.

With those subjects, a psychiatrist (YN) conducted Diagnostic and Statistical Manual of Mental Disorders (fourth edition, text revision) (DSM-IV-TR) diagnosis with Structured Clinical Interview for DSM-IV (SCID),[17] and the psychiatrist selected those who were diagnosed with mood disorder, anxiety disorder, or adjustment disorder caused by RM-related stress, pregnancy, or childbirth. Exclusion criteria included: (1) serious physical illnesses such as autoimmune disorder, cardiac disease, or chronic respiratory disease; (2) current or past psychotic disorder, bipolar disorder, cyclothymic disorder, eating disorder, developmental disorder, learning disorder, or substance dependence according to DSM-IV-TR; (3) current depression episode, dysthymic disorder, anxiety disorder, or adjustment disorder (by DSM-IV-TR) starting before the first miscarriage; (4) being pregnant at the time of the interview or beginning of CBT; and (5) previous CBT experience. As for new pregnancy during CBT, we planned to make a go/no-go decision when it happened because pregnancy may make some subjects physically (eg, morning sickness) and psychologically (eg, anxiety about miscarriage) unstable.

In general, CBT subjects were instructed to avoid pharmacotherapy. If they were already under pharmacotherapy, they were not allowed to switch medicines during CBT, except in cases of emergency. If a cause of RM was specified, its treatment had priority over CBT.

This study was approved by the Ethics Review Committee at Nagoya City University Graduate School of Medical Sciences. The subjects received explanation in writing and submitted informed consent before entering the study.

Procedure

Before starting psychological support by CBT, a researcher in this study (YN) with over 15 years of experience in clinical psychiatry and 10 years of experience as a CBT therapist interviewed a series of ten subjects in person, for 75–90 minutes each, and collected information about mental distress concerning pregnancy and children seen in their daily situations. Another psychiatrist (TF) with over 10 years of CBT experience who has been conducting research in psychosocial factors of RM, as well as an obstetrician (MS) who specializes in RM, then examined the interviews. By checking what was said (viewpoints, recognition) and how they behaved in stressful situations, as mentioned by subjects, several features, which seemed helpful in CBT, were extracted (Table 1).

The average age of the ten subjects interviewed was 32.3 (±3.5) years and the average number of miscarriages was

Table 1 Common thoughts and behaviors among women with recurrent miscarriage who are experiencing depression and/or anxiety

1.	They believe that a woman who marries but does not have a baby is not a mature adult
2.	They believe that people who have children are happy and that people who do not have children are unhappy
3.	They have feelings of guilt that they have killed their own fetuses in uteri
4.	They are afraid of both getting pregnant and of not getting pregnant
5.	They are beleaguered with anxiety about the future and about how long their present situation will last
6.	They are uncertain if each action in their daily life is good or bad for their next pregnancy
7.	They miss many opportunities to enjoy themselves because they avoid places where there are many children and their parents, and participating in events where many mothers will be present
8.	They avoid gatherings of relatives during the summer holidays or New Year holidays, or gatherings of peers such as class reunions
9.	They and their spouses are likely to overlook women's fatigue and exhaustion
10.	Not many women understand that their idea and their husband's idea are not the same regarding having a baby

2.7 (±0.9). No significant difference was found between these and the average age of 33.0 (standard deviation [SD] ± 4.8) and the average number of miscarriages of 2.7 (SD ± 1.0) of a series of 305 patients who visited our hospital for thorough examination. The causes of miscarriage for eight subjects were unexplained. Two suffered from uterine deformity, but they did not need surgery in order to become pregnant or to maintain pregnancy.[18] The average K6 score was 8.6 (±2.5). Those who were interviewed to extract common thoughts and behaviors continued regular treatment at the obstetrics department. They also received routine psychiatric outpatient therapy if they needed it.

After common thoughts and behaviors were obtained as indicated in Table 1, 14 subjects who met the eligibility criteria provided informed consent and entered this study between October 2008 and September 2010 (Figure 1).

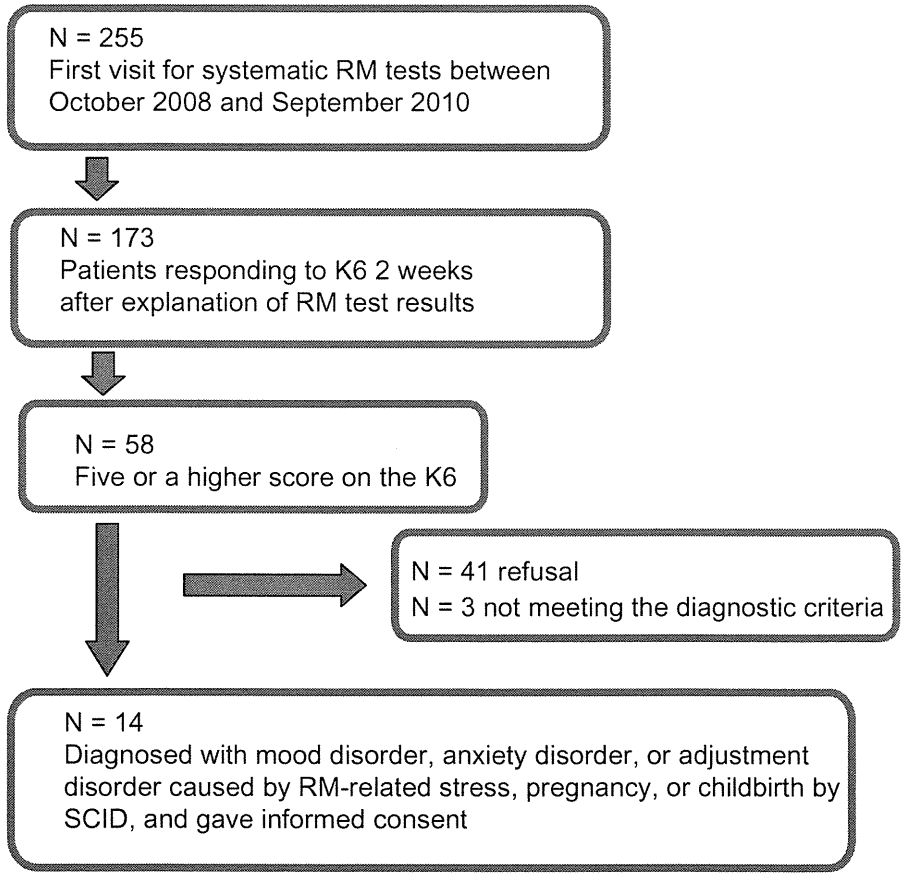

Figure 1 Patient flow chart for CBT.
Abbreviations: CBT, cognitive behavior therapy; N, number; RM, recurrent miscarriage; SCID, Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders (fourth edition); K6, 6-item self-report instrument for screening for clinical depression or anxiety in adults.

The psychiatrist (YN) conducted CBT according to the individual treatment method developed by Beck[19] as well as by Beck et al (Table 1).[20] One session lasted approximately 50 minutes. The list in Table 1 was never shown to the patient since it was made as reference to help the therapist conduct smooth and effective sessions.

The basic rule for a course of sessions was that the sessions were to be held once a week, up to 16 times in total. However, in reality, flexible decisions had to be made depending on how profoundly distressed the patient was feeling in her daily life and how much the patient's depression and/or anxiety improved. In some cases, we allowed a patient to have a session once every 2 or more weeks after the 12th session.

Measure

K6

The K6 is a 6-item self-report instrument for screening for clinical depression or anxiety in adults.[15] The items cover how frequently the respondents experienced symptoms of psychological distress during the previous 30 days. The possible total scores range from 0 to 24. According to a Japanese validation study of the K6 in a general population, the positive predictive value for major depressive disorder or any anxiety disorder according to the DSM-IV-TR criteria was 0.49 for K6 scores of 10 or more.[16] The reliability and validity of the Japanese version has been previously confirmed.[16] The internal consistency of this instrument in the current sample was sufficient (Cronbach's $\alpha = 0.89$). In this study, women with a score of five or higher on the K6 result were included as subjects.

Beck Depression Inventory-Second Edition

The Beck Depression Inventory-Second Edition (BDI-II) is a 21-item self-report instrument for measuring the severity of depression in adolescents and adults.[21] The total scores range from 0 through 63. The severity of depression is categorized based on the following BDI-II scores in Japanese samples: a score of 13 or less is regarded as minimal (or remission); 14 to 19 is regarded as mild; 20 to 28 is regarded as moderate; and 29 or greater is regarded as severe. The reliability and validity of the Japanese version has been previously confirmed.[22] The internal consistency of this instrument in the current sample was sufficient (Cronbach's $\alpha = 0.92$).

State–Trait Anxiety Inventory–state anxiety

The State–Trait Anxiety Inventory–state (STAI-s) anxiety consists of 20 items and has a maximum score of 80.[23] State anxiety refers to the degree of anxiety at a particular point. Scores of over 50 indicate an extremely high level of anxiety (neurosis level). The reliability and validity of the Japanese version has been previously confirmed.[24] The internal consistency of this instrument in the current sample was sufficient (Cronbach's $\alpha = 0.91$).

BDI-II and STAI-s anxiety were adopted because they are both simple and useful scales to check the change in depression and/or anxiety severity. Following the usual CBT procedure, they were conducted right before each CBT session to assess the patient's condition.

Statistical analysis

Wilcoxon signed rank tests were conducted before and after CBT to see any statistically-significant change of BDI-II and STAI state anxiety. All analyses were performed using IBM SPSS Statistics, version 18 (IBM Corporation, Armonk, NY, USA). All statistical tests were two-tailed, and an alpha value of less than 0.05 was considered statistically significant.

Results

Fourteen patients, who met the criteria and consented, received CBT. The features of the samples are shown in Table 2. No significant difference was found between these and a series of 305 patients who visited our hospital for thorough examination in terms of the average age and number

Table 2 Sample characteristics: demographic and symptomatic data

	Mean	SD
Age (years)	34	4
Number of previous miscarriages	2.7	1.0
BDI-II before CBT	13.6	8.2
State anxiety in STAI before CBT	49	7.1
Number of CBT sessions	8.9	4.6
	%	Number
Education		
High school graduate	28.6	4
Junior college graduate	50.0	7
College graduate or higher	21.4	3
DSM-IV diagnosis		
Major depressive disorder	35.7	5
Adjustment disorder (with depressed mood)	35.7	5
Adjustment disorder (with mixed anxiety and depressed mood)	28.6	4
Adjustment disorder (with anxiety)	7.1	1
Specific phobia	7.1	1
Panic disorder	7.1	1
Posttraumatic stress disorders	7.1	1

Abbreviations: SD, standard deviation; BDI-II, Beck Depression Inventory-Second Edition; CBT, cognitive behavior therapy; STAI, The State–Trait Anxiety Inventory; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders (fourth edition).

of miscarriages. The causes of RM for nine of the patients (64.2%) were unexplained. Four patients had uterine deformity but their possibility of maintaining pregnancy was not expected to improve much by a surgery.[13] One patient was diagnosed with hyperthyroidism but her hormone value had been stable within the normal range for over 6 months under pharmacotherapy (desiccated thyroid 75 μg).

Two patients were diagnosed with moderate- to severe-level major depression disorder according to DSM-VI-TR; their BDI-II scores exceeded 30 points at baseline. The depression and/or anxiety severity of most of the patients was around the level of adjustment disorder according to DSM-IV-TR. One patient had been in pharmacotherapy and there was no change in her medication (75 mg/day sertraline, 1 mg/day lormetazepam) during CBT sessions. There was one patient who became pregnant in the 14th week of the 16 originally planned sessions. Since she complained of high anxiety about miscarriage and asked for extended treatment, a decision was made to have a session once a month until she reached a stable period, totaling 18 sessions.

Figure 2 shows the changes in BDI-II and STAI-s anxiety before and after CBT. STAI data for four patients were lost. The average BDI-II and STAI-s scores decreased from 13.6 (SD, 8.2) and 49.0 (SD, 7.1) at baseline to 5.2 (SD, 4.4) and 38.0 (SD, 10.2) at posttherapy, respectively. These changes were statistically significant (Wilcoxon signed rank test: BDI-II n = 14, z = −3.2, $P = 0.001$; STAI-s n = 10, z = −2.4, $P = 0.016$).

Discussion

This is the first attempt of psychological support with CBT for patients with RM. This study preliminarily confirmed that CBT, in concert with references to the list of common hardships prepared in advance, can decrease depression and/or anxiety of patients with RM. It is appropriate to say that, based on the SCID diagnosis and the average BDI-II scores at baseline and posttreatment, the mild depressive state of the 14 subjects in this study recovered to normal levels. It can be also said from the average scores of STAI-s anxiety at baseline and at posttreatment that the subjects came out of their previous condition at a mildly overanxious level.

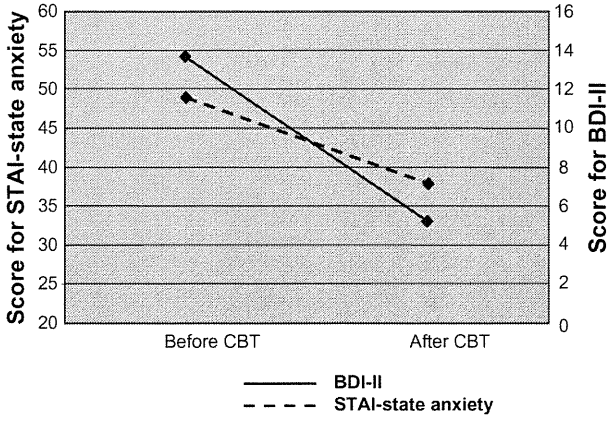

Figure 2 Comparison of BDI-II and STAI before and after CBT.
Abbreviations: BDI-II, Beck Depression Inventory-Second Edition; STAI, State–Trait Anxiety Inventory; CBT, cognitive behavior therapy.

It has been already mentioned that the childbirth rate of RM patients with an unexplained cause may be raised by increasing the number of checkups and providing counseling in early pregnancy.[8–10] Additionally, we have already shown that depression raises the possibility of miscarriage.[25] All these factors indicate that recovery from depression may lead to an increased childbirth rate. Therefore, although we were unable to examine successful birthrate this time, we should, and certainly hope to, have childbirth rate as an outcome in our future research.

However, there are several limitations in this study. First, it was an open label study with no control group. There exists a possibility that depression and/or anxiety decreased as a natural course. Second, it was left unexamined whether the positive effect could be maintained after the end of treatment, despite the fact continuing improvement of depression and/or anxiety in the life of patients is the most important outcome. Moreover, since there were only two estimating points, one at the start and another at the end of CBT sessions, the process of improvement could not be discussed. The third limitation is that the strict procedure of qualitative research, such as a qualitative and descriptive research method, was not taken in constructing Table 1. However, it was designed as reference data to conduct CBT smoothly in the first place, and in fact, Table 1 fulfilled the purpose. For example, more than half of the subjects covered items 1, 3, 5, 8, and 10 in their sessions.

We routinely hand out a form to patients to write comments freely when they visit our hospital for thorough examination. Although they often mention experiencing various kinds of psychological distress, only a few patients actually come for individual CBT. As Boivin et al[26] pointed out, it is likely that patients with mild psychological distress do not feel a need to depend on a specialist, while those experiencing severe distress do not reach a point where they want to meet with CBT therapists, or any other specialist engaging in psychological support, because these individuals worry about how much these services may cost and what the specialist may be like. Moreover, many patients might naturally be resistant to self-disclosure.

Recently, there have been many attempts concerning CBT on the Internet.[27,28] The approach using the Internet may have advantages because it can be accessed from anywhere at low cost, requires less self-disclosure, calls for less concern about compatibility with a therapist, and therefore, seems easier to start. Upon creating online CBT content in the future, the information from Table 1 will contribute considerably.

On the other hand, Wischmann[29] recommended that face-to-face consultations be used for complicated matters for infertility patients' mental distress. Therefore, for RM patients who are suffering from depression and/or anxiety, we plan to recommend a psychological support program based on CBT via the Web before inviting patients to engage in individual CBT. Once such a system takes off, we hope to conduct a randomized controlled trial with birthrate, as well as depression and/or anxiety, as study endpoints.

This pilot study, which preliminarily indicated a decrease in depression and/or anxiety for RM patients by individual CBT, was the first step towards creating a comprehensive psychological support system for RM.

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Disclosure

The authors report no conflicts of interest in this work. The authors disclosed no proprietary or commercial interest in any product mentioned or concept discussed in this article.

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Peripheral natural killer cell activity as a predictor of recurrent pregnancy loss: a large cohort study

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Objective: To determine the predictive value of preconceptional peripheral blood natural killer (pNK) cell activity in patients with recurrent pregnancy loss (RPL).
Design: Cohort study.
Setting: University department.
Patient(s): A total of 552 patients with a history of two to six consecutive miscarriages.
Intervention(s): None.
Main Outcome Measure(s): The predictive value of preconceptional pNK cell activity for subsequent miscarriage was analyzed using multivariable logistic regression analysis, with age, number of previous miscarriages, and presence/absence of previous live births and bed rest as covariates.
Result(s): Age and number of previous miscarriages, but not high pNK cell activity, were found to be independent risk factors for a subsequent miscarriage. No effect of bed rest and previous live birth on the likelihood of live birth was observed (odds ratios 1.28 [95% confidence interval 0.81–2.02] and 0.91 [0.52–1.59], respectively).
Conclusion(s): Elevated pNK cell activity was found to not be an independent risk factor for subsequent miscarriage. Clinicians should not measure the plasma NK activity as a systematic recurrent pregnancy loss examination, because its clinical significance is yet to be established. (Fertil Steril® 2013;100:1629–34. ©2013 by American Society for Reproductive Medicine.)
Key Words: Recurrent pregnancy loss, natural killer cell activity, predictor, cohort study

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Established causes of recurrent pregnancy loss (RPL) include presence of antiphospholipid antibodies in the serum, presence of uterine anomalies, and presence of abnormal chromosomes, particularly translocations, in either partner (1–3). According to previous reports, in approximately half of the cases seen at research centers, the cause of RPL remains unexplained despite conventional examinations conducted to identify the cause (4–6).

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Cytotrophoblasts that express human leukocyte antigen G (HLA-G) come in direct contact with maternal lymphocytes. Many natural killer (NK)-like large granular lymphocytes have been detected in the human decidua of early pregnancy (7). Large numbers of NK cells appear in the mid-secretory phase. Natural killer cells have been thought to play a key role in the establishment of successful pregnancy by facilitating immunologic adaptation of the semiallogenic developing embryo. Recently, Fu et al. (8) reported that recruitment of TH17 cells and local inflammation can occur at the maternal–fetal interface during natural allogenic pregnancies, and that decidual NK cells promote immune tolerance and successful pregnancy