A rare variant in the *IL22RA2* gene is associated with unexplained recurrent pregnancy loss in Han Chinese women

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Abstract

Introduction: Recurrent pregnancy loss (RPL) occurs in ~1–2% of reproductive women. Previous studies have suggested that altered expression and polymorphisms of interleukin-related genes may be involved in RPL. The aim of this study was to explore the potential presence of interleukin-22 receptor subunit α2 (IL22RA2) mutations in Han Chinese women with unexplained recurrent pregnancy loss (URPL).
Material and methods: A total of 328 Han Chinese women with URPL were analyzed for potential mutations in the *IL22RA2* gene by sequencing all of the exons. Additionally, 615 Han Chinese control women without miscarriage history were analyzed. Evolutionary conservation and in silico analyses were performed to predict the potential pathogenicity of IL22RA2 mutations.
Results: A rare variant, p.Arg204* (c.610C>T), was identified in 4 out of 328 women with URPL. In contrast, this rare variant was absent in 615 Han Chinese control women without miscarriage history and had a significantly higher mutation frequency when compared with 10,588 Chinese control women from The China Metabolic Analytics Project (ChinaMAP), 4245 East Asians, or 60051 individuals across the world from the Exome Aggregation Consortium (ExAC) database. Evolutionary conservation analysis indicated that this rare variant was highly conserved from human to zebrafish, and in silico analysis suggested that it may be pathogenic.
Conclusions: An enrichment of a potentially pathogenic rare variant in the *IL22RA2* gene was observed in URPL patients for the first time, suggesting that this rare variant may be associated with the development of URPL. However, further functional assays should be performed to confirm the potential role of this rare variant in URPL.

Key words: recurrent pregnancy loss, IL22RA2, rare variant, Han Chinese.

Introduction

Recurrent pregnancy loss (RPL), a highly heterogeneous condition, id defined as two or more sequential abortions before the 20th week of gestation [1, 2]. This condition affects approximately 1–2% of fertile couples, and is

a common public reproductive concern causing both physical and emotional burdens [1, 3]. A number of risk factors have been proposed to be associated with RPL, including anatomic abnormalities of the female reproductive tract, immunological and endocrine aberrations, genetic factors, coagulation protein defects, and environmental factors [4–7]. However, ~50% of RPL cases are not attributable to these known risk factors, and these cases are classified as unexplained recurrent pregnancy loss (URPL) [8–10].

Successful pregnancies require the induction of maternal tolerance to fetal tissues, where natural killer (NK) cells migrate and adjust the secretion of cytokines to regulate the invasion of trophoblast cells into the uterus [11–13]. Among the complex URPL-related factors, those involved in abnormal activation of inflammatory processes in immune rejection and suppression of immune regulators have attracted considerable attention [14–16]. Multiple studies have suggested that genetic variants in immune-associated genes confer increased risk for URPL. A genetic variant in the interleukin 1β (IL1B) gene (-511T>C) was shown to be associated with RPL women in South Korea, and carriers of the -511C allele cases had a higher proportion of NK cells than -511T carriers [17]. Another study found that the tumor necrosis factor-α (TNF-α) rs1800630 (-863C>A) variant could confer an increased risk of RPL in women in South Korea [18]. Likewise, certain genetic variations in pro-inflammatory or anti-inflammatory genes were also found to affect the risk of RPL in different ethnic populations of women, including the rs2275913 polymorphism in interleukin 17 (IL-17) [19], rs187238 polymorphism in interleukin 18 (IL-18) [20], rs1518111 and rs1800871 polymorphisms in interleukin 10 (IL-10) [21], and rs1800796 polymorphism in interleukin 6 (IL-6) [22].

A previous study found that the IL-22 level was elevated after an inflammatory stimulus at the maternal-fetal interface in pregnant mice, indicating potential involvement of IL-22 in protection against inflammation-induced preterm birth [23]. Furthermore, a recent study showed that the expression of IL-22 and its soluble IL-22 receptor, IL22RA2, was downregulated in human villi tissues and trophoblasts in RPL women, and the decreased IL-22/IL22RA2 expression could suppress the growth and proliferation of trophoblasts [24], implicating that the IL-22/IL22RA2 signaling pathway may play crucial roles in the development of RPL.

Due to the potential role of IL-22 signaling in URPL [25–27], we thus hypothesized that mutations or aberrant expression of IL22RA2, an inhibitor of IL22, may be present in women with URPL. Here, we analyzed potential mutations in the *IL22RA2* gene in a cohort of 328 Han Chinese women with URPL, and a potential pathogenic mutation was identified in 4 out of these samples.

Material and methods

Samples

A total of 328 sporadic Han Chinese URPL women, as well as 615 Han Chinese control women without miscarriage history, were enrolled in Jiangxi Provincial Maternal and Child Health Hospital (Nanchang, China), between January 2018 and March 2019. This study was approved by the Institutional Ethics Committee of Jiangxi Maternal and Child Health Hospital. All patients provided written informed consent, and all experiment protocols were in accordance with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards and approved by the Institutional Review Board of Jiangxi Maternal and Child Health Hospital prior to the study.

DNA extraction and mutation analysis

Blood samples from both control and URPL subjects were collected and stored at –80°C. The genome DNA (gDNA) was extracted using Axygen AxyPrep Blood Genomic DNA Miniprep Kit AxyPrep Blood Genomic DNA Miniprep Kit (Axygen Biosciences, Union City, CA, USA). Spectrophotometric analysis was performed to evaluate the quality and quantity of the isolated gDNA.

Genotype screening was performed to identify the potential mutations in the *IL22RA2* gene among the 328 Han Chinese URPL cases. In brief, all of the coding exons and corresponding intron/exon boundaries of the *IL22RA2* gene were amplified with a set of primer pairs (Table I). The PCR re-

Table I. Primers for mutational analyses of the IL22RA2 gene

Gene	Target region	Annealing	PCR amplicon [bp]	Forward primers (5′-3′)	Reverse primers (5′-3′)
IL22RA2	Exon 2	52°C	252	aattgtagttgcttcaagg	cttgattggactcagatga
IL22RA2	Exon 3	58°C	352	atgcaggtattccactga	tatcctgaccttggcttc
IL22RA2	Exon 4	54°C	286	gaaatgtttctatgagag	tattctggggtctgaaat
IL22RA2	Exon 5	50°C	366	ctatcatggtggtattgc	tcactagccgtgctccgg
IL22RA2	Exon 6	56°C	320	ataatgcgtctgtatataa	cagaagaggacatctcata
IL22RA2	Exon 7	61°C	260	atgcagctgtgacttaat	tctcagggagctttagaa

actions were carried out in a Thermal Cycler 2720 (Applied Biosystems, Foster City, CA, USA), with a final volume of 30 µl, containing 2.5 mM dNTPs (Takara Biotechnology), 2.5 mM $MgCl_2$ (Takara Biotechnology), 1 U of LA Taq (Takara Biotechnology), and 0.5 µM forward and reverse primers. The PCR cycling conditions consisted of an initial activation at 94°C for 5 min followed by 35 amplification cycles of denaturation at 94°C for 1 min, annealing at 50–61°C (Table I) for 30 s and extension at 72°C for 30 s, followed by the final extension reaction at 72°C for another 7 min. The PCR products were purified on spin columns (Tiangen, Beijing, China) and were directly sequenced by using the forward and reverse primers and BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) on an ABI 3730 Automatic Capillary DNA Sequencer (Applied Biosystems, USA).

Evolutionary conservation analysis of IL22RA2 p.Arg204* mutation

Evolutionary conservation analysis was performed to predict the potential pathogenicity of the IL22RA2 p.Arg204* mutation with seventeen species from GenBank, including human (NP_443194), rhesus monkey (XP_014992847), mouse (NP_839989), rat (XP_017445018), Chinese tree shrew (XP_006144418), dolphin (XP_026935228), cattle (XP_010806925), dog (XP_013967785), horse (XP_001503623), sea lion (XP_027977220), cat (XP_003986643), bat (XP_005859191), red fox (XP_025853613), koala (XP_020842817), camel (XP_010982040), turtle (XP_024063403), and zebrafish (NP_001038744).

In silico analysis of IL22RA2 p.Arg204* mutation

Two bioinformatic programs, MutationTaster (http://mutationtaster.org/) [28] and SIFT (http://sift.jcvi.org/) [29], were used to analyze the potential pathogenicity of the IL22RA2 p.Arg204* nonsense mutation. All these bioinformatic analyses were carried out on July 6th, 2019. These programs automatically assessed the mutation as either pathogenic or benign.

Statistical analysis

The statistical analysis was performed with the statistical software package SPSS version 18.0 (SPSS, Inc., Chicago, IL, USA). Fisher's exact test was used to compare the age and number of pregnancy losses between URPL participants with and without IL22RA2 mutation, and the difference in frequency of the IL22RA2 rare variant between our URPL cohort and local control women, as well as control samples from The China Metabolic Analytics Project (ChinaMAP) and Exome Aggregation Consortium (ExAC) databases. All tests were two-sided, and a p-value < 0.05 was considered statistically significant.

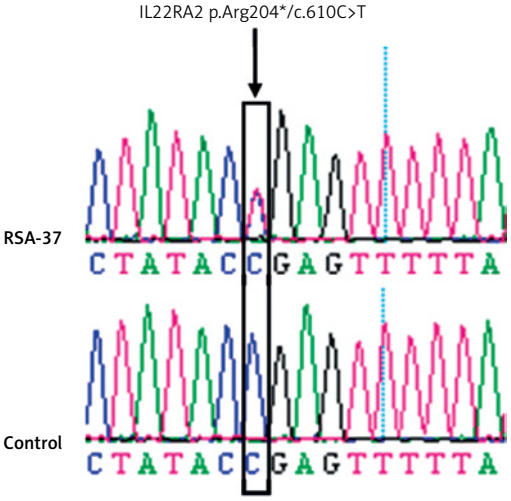

Figure 1. Representative sequencing electropherograms of IL22RA2 p.Arg204* (c.610C>T) mutation; the arrow indicates locations of the mutations

Results

Sample characteristics

The mean age of these participants was 30.6 ±5.6 years (range: 18–45), and the average pregnancy loss was 2.4 (range: 2–7). All of these participants had no childbearing history. In addition, the mean age of the 615 control women was 29.2 ±6.3 years (range: 22–39); all of the controls had a history childbirth and no history of pregnancy loss.

IL22RA2 mutation

A heterozygous nonsense mutation in IL22RA2, p.Arg204*/c.610C>T, was identified in 4 out of 328 URPL participants (1.22%, 4/328) (Figure 1). The age of the 4 participants with IL22RA2 mutation

Table II. Frequency comparison of IL22RA2 mutation among our 328 women with URPL, 615 local controls, 10,588 Chinese samples from ChinaMAP database, and 60051 samples from ExAC database

SNP ID	Cases (N = 328)	Normal controls (N = 615)	P-value[a]	East Asians in ExAC	P-value[a]	Global frequency in EXAC	P-value[a]	Global frequency in ChinaMAP	P-value[a]
rs184626014	4/656	0/1230	0.027	6/8490	< 0.0001	20/120102	< 0.0001	43/21176	< 0.0001

[a]*P-value, Fisher's exact test.*

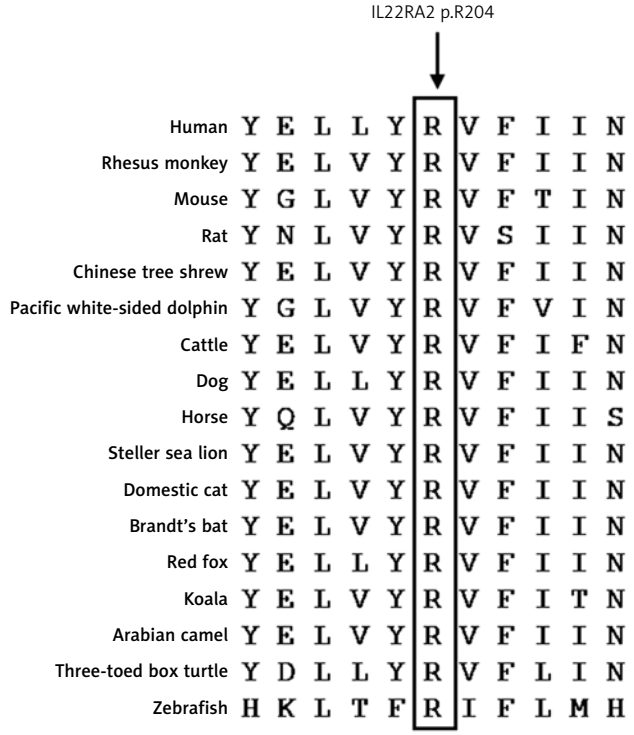

Figure 2. Evolutionary conservation analysis of IL22RA2 p.Arg204 residue from human to zebrafish

was 20, 25, 28, and 38 years, and they had experienced 2, 3, 3, and 4 pregnancy losses, respectively. Notably, this mutation was not detected in 615 Han Chinese control women without miscarriage history (Table II). When compared with 120,102 Han Chinese controls in the ChinaMAP database, 4245 East Asians or 60051 individuals across the world from the ExAC database, a positive association between IL22RA2 p.Arg204* and URPL was observed (Table II). The age (p = 0.56) and number of pregnancy losses (p = 0.87) in IL22RA2-mutated URPL participants did no differ significantly from those without the mutation.

In silico functional annotation of the IL22RA2 mutation

Two different software programs were used to predict the potential pathogenicity of the IL22RA2 p.Arg204* mutation. This mutation was predicted to be "Disease causing" and "Deleterious" by MutationTaster and SIFT, respectively. Evolutionary conservation analysis indicated that the "Arg" residue at 204 in the IL22RA2 protein was highly conserved among 17 vertebrate species from human to zebrafish (Figure 2).

Discussion

Previous studies have found that IL-22 level was elevated after an inflammatory stimulus at the maternal-fetal interface in pregnant mice, indicating potential involvement of IL-22 in protecting against inflammation-induced preterm birth [23]. Furthermore, decreased expression of IL-22/IL22RA2 in trophoblasts in RPL patients could suppress the growth and proliferation of trophoblasts, suggesting that the IL-22/IL22RA2 signaling pathway may play important roles in URPL [24].

In the present study, we analyzed all exons of the *IL22RA2* gene in 328 Han Chinese URPL women and 615 Han Chinese control women without miscarriage history. A rare variant in IL22RA2, p.Arg204* (c.610C>T), predicted to be pathogenic with in silico programs, was found to be associated with Han Chinese URPL women in our cohort. Also, the frequency of p.Arg204* (c.610C>T) in URPL women was significantly higher than that in 10,588 Chinese control individuals from ChinaMAP, 4245 East Asian individuals, and 60,051 individuals from the world population in the ExAC dataset. This comparison should be treated with caution, as the control population contains both male and female individuals, and we lacked detailed information regarding the disease status of the control population. Combined with the observation that IL-22/IL22RA2 signaling pathway plays a crucial role in the pathogenesis of URPL [24], we speculated that the IL22RA2 p.Arg204* (c.610C>T) rare variant may confer genetic risk to Han Chinese URPL women.

The present study had several limitations. First, the analyzed sample size was relatively small, so further validation of the association with a larger sample size is needed. Second, although in silico prediction results have suggested that this variant

is potentially pathogenic, *in vitro* and *in vivo* functional assays are necessary to test its pathogenicity.

In conclusion, for the first time, we observed enrichment of a potentially pathogenic rare variant in the *IL22RA2* gene in Han Chinese URPL patients, suggesting that this rare variant may be associated with the development of URPL. However, further comprehensive functional assessments are essential to determine the potential role of this rare variant in the development of URPL.

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Ethical approval

Ethics approval for this study was obtained from the Institutional Review Board of Jiangxi Maternal and Child Health Hospital (JXFYBJY-2017009) and an informed consent was obtained from each patient prior to this study. The study was conducted according to the Declaration of Helsinki.

Conflict of interest

The authors declare no conflict of interest.

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