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

Type

Research paper

Keywords

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Abstract

Introduction
Recurrent pregnancy loss (RPL) occurred in ~1-2% of reproductive women. Previous studies have implicated that altered expression and polymorphisms of interleukin-related genes might be involved in RPL. The aim of this study was to explore the potential presence of interleukin-22 receptor subunit alpha-2 (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 the potential mutations in the IL22RA2 gene by sequencing all of the exons. Furthermore, 615 Han Chinese control women without miscarriage history were also 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 URPL samples. 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 10588 Chinese control samples 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 this rare variant was highly conserved from Human to Zebrafish, in silico analysis implicated this rare variant might be pathogenic.

Conclusions
An enrichment of a potentially pathogenic rare variant in the IL22RA2 gene was observed in URPL patients, for the first time, implicating this rare variant might be associated with the development of URPL. However, further functional assays would be performed to confirm the potential role of this rare variant in URPL.

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Introduction

Recurrent pregnancy loss (RPL) is a highly heterogeneous condition and 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 becomes 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% 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 required the induction of maternal tolerance to fetal tissues, where natural killer (NK) cells migrated and adjusted the secretion of cytokines, to adjust 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 attracted much attention [14-16]. Multiple studies have suggested that genetic variants in immune-associated genes conferred increased risk for URPL. A genetic variant in the interleukin 1 beta (IL1B) gene (-511T>C) was shown to be associated with RPL women in South Korea, and the -511C allele cases possessed higher proportion of NK cells than -511T carriers [17]. Another study found that tumor necrosis factor-alpha (TNF-α) rs1800630 (-863C>A) variant could confer an increased risk of RPL women

in South Korea [18]. Likewise, certain genetic variations in pro-inflammatory or anti-inflammatory genes were also shown to affect the risk for 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].

Prior study has found that IL-22 level was increased after inflammatory stimulus at the maternal-fetal interface in pregnant mice, indicating a potential involvement of IL-22 in protecting from 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 trophoblast [24], implicating that IL-22/IL22RA2 signaling pathway might 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, the inhibitor of IL22, might existed in URPL women. Here, we analyzed the 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.

Materials 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 signed an informed consent and, all experiment protocols were in accordance with the 1964 Helsinki declaration 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 ºC. 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 check 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 was amplified with a set of primer pairs (Table 1). The PCR reactions 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 of $MgCl_2$ (Takara Biotechnology), 1 U LA Taq (Takara Biotechnology), 0.5 μM forward and reverse primer. The cycle parameters for PCR 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 ºC (Table 1) for 30 sec and extension at 72 °C for 30 sec, and 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 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 loss between URPL samples with and without IL22RA2 mutation, and the frequency difference of IL22RA2 rare variant between our URPL samples and local control women, and control samples from the ChinaMAP and ExAC databases. All tests were two-sided and a P-value <0.05 was considered statistically significant.

Results

Sample characteristics

The age of these samples was 30.6 ± 5.6 years (range, 18-45), and the average pregnancy loss was 2.4 (range, 2-7). All of these samples had no childbearing history. In addition, the age of the 615 control women was 29.2 ± 6.3 years (range, 22-39), all of these controls had childbirth history while 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 samples (1.22%, 4/328) (Fig. 1). The age of the 4 samples with IL22RA2 mutation was 20, 25, 28 and 38 years old, respectively, and they experienced 2, 3, 3 and 4 pregnancy loss, respectively. Notably, this mutation was not detected in 615 Han Chinese control women without miscarriage history (Table 2). When compared with 120102 Han Chinese controls in ChinaMAP database, 4245 East Asians or 60051 individuals across the world from ExAC database, a positive association between IL22RA2 p.Arg204* and UPRL was observed (Table 2).

The age (P=0.56) and number of pregnancy loss (P=0.87) in IL22RA2-mutated URPL samples exhibit no significant differences with that of URPL samples without IL22RA2 mutation.

***In silico* functional annotation of the IL22RA2 mutation**

Two different software programs were used to predict the potential pathogenicity of 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 the 17 vertebrate species from Human to Zebrafish (Fig. 2).

Discussion

Prior studies have found that IL-22 level was increased after inflammatory stimulus at the maternal-fetal interface in pregnant mice, indicating a potential involvement of IL-22 in protecting from inflammation-induced preterm birth [23]. Furthermore, decreased expression of IL-22/IL22RA2 in trophoblasts in RPL patients could suppress the growth and proliferation of trophoblast, implicating that IL-22/IL22RA2 signaling pathway might 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 sample cohort. Also, the frequency of p.Arg204* (c.610C>T) in URPL women

was significantly higher than that in 10588 Chinese control samples from The China Metabolic Analytics Project (ChinaMAP), 4245 East Asian and 60051 individuals from the world population in the ExAC dataset. Noted that this comparison should be treated with caution as the control population contains both male and female individuals, and we lacked the detailed information regarding the disease status of the control population. Combined with the observations that IL-22/IL22RA2 signaling pathway played crucial role in the pathogenesis of URPL [24], we speculated that the IL22RA2 p.Arg204* (c.610C>T) rare variant, might confer genetic risk to Han Chinese URPL women.

The present study had several limitations. First, the analyzed sample size was relatively small, further validation of the association result with larger sample size would be needed. Second, although in silico prediction results have implicated this variant was potentially pathogenic, *in vitro* and *in vivo* functional assays would be need to test its pathogenicity.

Conclusion

For the first time, we observed an enrichment of a potentially pathogenic rare variant in the IL22RA2 gene in Han Chinese URPL patients, implicating this rare variant might be associated with the development of URPL. However, further comprehensive functional assessments would be essential to define the potential role of this rare variant in the development of URPL.

Acknowledgements

We thank all participants involved in this study. This study was supported by grants

from the National Natural Science Foundation of China (81560255) and the Science Foundation of Jiangxi Province (20171BBG70118, 20171BAB205068 and 20181BBG70018).

Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

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Figure legends

Figure 1. The representative sequencing electropherograms of IL22RA2 p.Arg204* (c.610C>T) mutation, the arrow refers to locations of the mutations.

Figure 2. The evolutionary conservation analysis of IL22RA2 p.Arg204 residue from Human to Zebrafish.

Table 1. The primers for the 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

Table 2. The frequency comparison of IL22RA2 mutation among our 328 URPL samples, 615 local controls, 10588 Chinese samples from ChinaMAP database, and 60051 samples in the ExAC database.

SNP ID	Case (N=328)	Normal control (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.

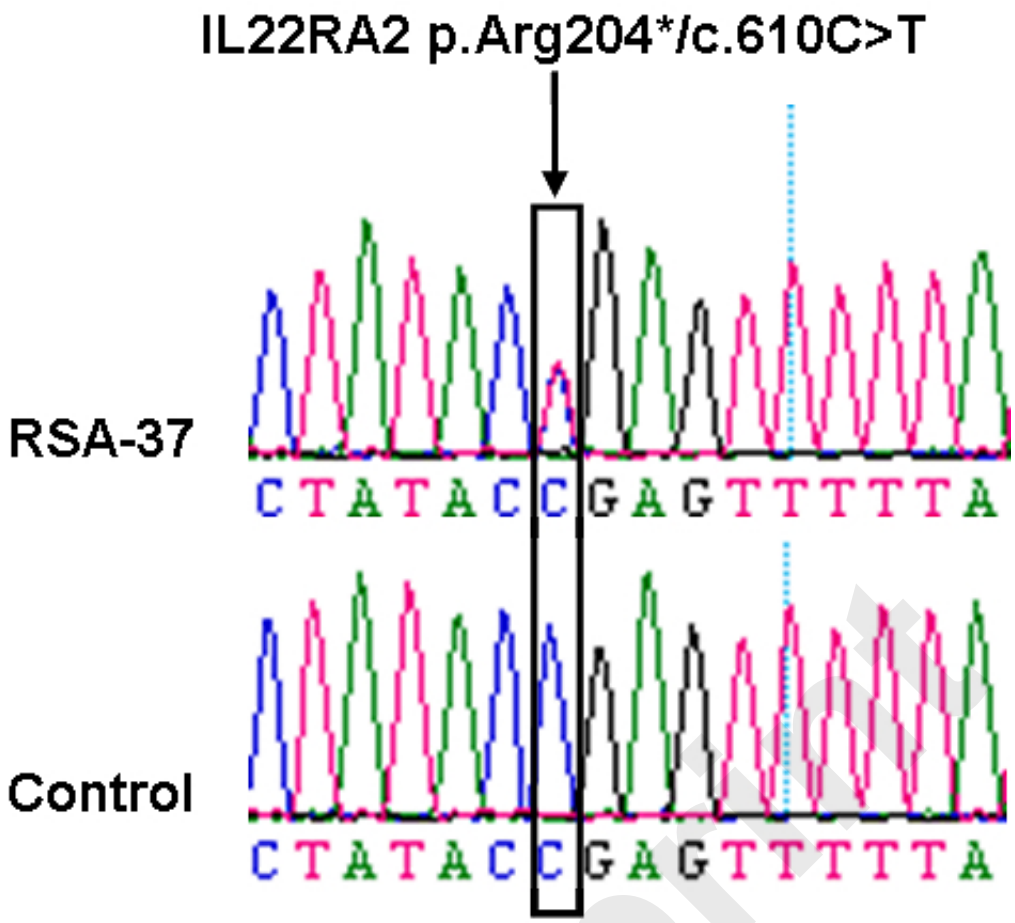

Fig.1

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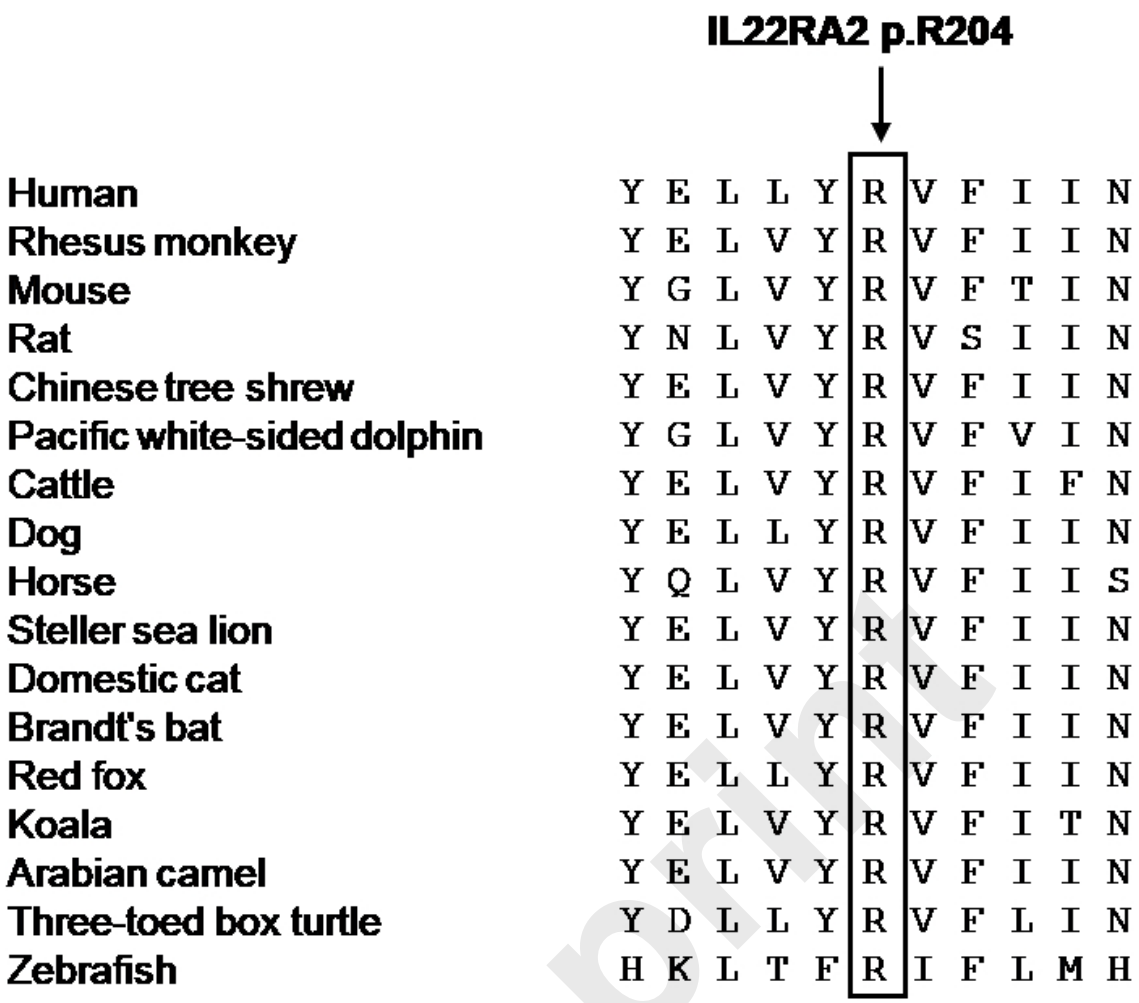

Fig.2