Genetic Variants in MIR3142HG Contribute to the Predisposition of IgA Nephropathy in a Chinese Han Population

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Keywords
IgA nephropathy · MIR3142HG polymorphisms · Lee's grade · Multifactor dimensionality reduction analysis

Abstract

\textbf{Background:} The study aimed to evaluate the association of genetic variants in MIR3142HG with the predisposition of IgA nephropathy (IgAN) in a Chinese Han population. \textbf{Methods:} Six single-nucleotide polymorphisms (SNPs) in MIR3142HG were chosen for genotyping among 417 IgAN cases and 424 healthy controls using Agena MassARRAY technique. Logistic regression models adjusted for age and gender were used to calculate odds ratios (ORs) and 95\% confidence intervals (CI). Haploview and multifactor dimensionality reduction analysis were used to analyze the role of combined SNPs in IgAN risk. \textbf{Results:} Rs17057846-AA genotype (OR = 2.11, 95\% CI: 1.04–4.27, \(p = 0.039\)) and rs58747524-CC genotype (OR = 1.89, 95\% CI: 1.06–3.38, \(p = 0.032\)) had the higher risk for IgAN developing in the overall. Interestingly, rs7727115 had a reduced risk for IgAN in females, while rs17057846, rs2961920, and rs58747524 were related to the increased susceptibility to IgAN in females and the subjects with age \(\leq\) 35 years; moreover, rs17057846 and rs58747524 conferred to the higher risk for Lee's grade \(\geq\) III IgAN (\(p < 0.05\) for all). Besides, the combination of rs1582417, rs7727115, and rs2961920 was the best model (testing accuracy = 0.5468, CVC = 10/10, \(p < 0.001\)) to predict IgAN predisposition compared to the single SNP alone. \textbf{Conclusions:} Our study firstly indicated that rs17057846 and rs58747524 in MIR3142HG contributed to the elevated risk for IgAN in a Chinese Han population. These results might provide a new insight for the molecular mechanism in the progression of IgAN.

Introduction

IgA nephropathy (IgAN), a common primary glomerulonephritis globally, is a main reason of renal failure and chronic kidney disease [1]. IgAN is the result of the predominant IgA deposition in the glomerular mesangium along with a small amount of IgG, IgM, or complement C3; most patients present with hematuria or proteinuria [2, 3]. There is a marked regional difference in the prevalence of IgAN; in which prevalence is modest in Ameri-
cans, higher in Europeans, and highest in Asians [4]. In China, IgAN accounted for 45.26% of primary glomerular disease [5]. IgAN has been shown to be a multifacto-
rial polygenic disease. Although the exact etiology of IgAN remains unknown, susceptibility to IgAN is influ-
enced by both genetic and environmental factors [6]. Many evidence indicated that genetic factors are one of the primary factors affecting the pathogenesis of IgAN. Recently, some studies have identified genetic suscepti-
bility genes for IgAN occurrence, including IFN-γ, IT-
GAM-ITGAX, ST6GAL1, and FCRL3 [7–9]. However, there are many risk polymorphisms to be uncovered.

MicroRNAs (miRNAs) are small noncoding RNAs with about 18–25 nucleotides that act as posttranscription-
al regulators by binding mRNAs to inhibit gene expression [10]. Emerging evidence has suggested that altered expres-
sion of miRNAs plays important roles in the genesis and development of several immune diseases, such as IgAN [11]. MicroRNA-146a (miR-146a) has been reported to be an important part in the immune and inflammatory re-
sponse, by negatively regulating the release of the proin-
flammatory chemokines [12]. Recent studies reported that the elevated expression of miR-146a occurred in intra-re-
nal and urinary of IgAN patients and was related to with clinical and histological severity of IgAN [13]. These stud-
ies suggested that miR-146a might have a crucial part in the occurrence and development of IgAN. Single-nucleotide polymorphisms (SNPs) in miRNAs gene may affect the maturation, expression, binding and/or specificity of the target mRNA, which in turn affects the occurrence of dis-
eses [14]. MIR3142HG (chromosome 5q33.3) is the host gene for miR3142 and miR-146a [15]. A study showed that rs2910164 in MIR3142HG was not related to IgAN risk from a Chinese Han population [16]. However, the genetic relationship of other variants in MIR3142HG with IgAN predisposition has not been reported.

Here, six SNPs in MIR3142HG were selected for geno-
typing to examine the association between genetic vari-
ants in MIR3142HG and IgAN predisposition at allele, genotype, and haplotype and SNP-SNP interaction inter-
face. We also determined the relationship of MIR3142HG SNPs with age, gender, and disease progression of IgAN among a Chinese Han population.

**Subjects and Methods**

**Study Subjects**

In order to ensure the accuracy and credibility of the research results, we used G*power 3.1.9.7 software (https://stats.idre.ucla.
edu/other/gpower/) to estimate the sample size before this study. The specific parameters were set as follows: effect size $d = 0.2$, a error probability $= 0.05$, and power $(1-\beta \text{ err prob}) = 85\%$. This calculation yielded a sample consisting of at least 361 cases and 361 controls. Here, a total of 841 subjects including 417 IgAN cases and 424 healthy controls were recruited from the First Af-
filiated Hospital of Xi’an Jiaotong University [17, 18], which was larger than the total sample size recommended by G*power. All participants were of Han Chinese descent from northwestern Chi-
na. All IgAN patients diagnosed were histologically confirmed by renal biopsy. Patients with cancer, systemic and autoimmune dis-
eases, infection, diabetes mellitus, hypertension, severe liver failure, secondary IgAN, or other renal diseases were excluded. Healthy controls were enrolled from the same hospital and veri-
ified with normal urinalysis. The exclusive criteria for controls in-
cluded history of renal diseases, diabetes, hypertension, and im-
paired vital organs. Demographic characteristics and clinical in-
formation were collected by questionnaires and medical records. This study protocol was reviewed and approved by the First Af-
filiated Hospital of Hainan Medical College, approval number (Ethical approval No.: Med-Eth-Re [2018] 10), and conformed to Helsinki Declaration. Written informed consent was got from all participants.

**DNA Extraction, SNP Selection and Genotyping**

Five milliliters of venous blood (5 mL) were collected into EDTA anticoagulant tubes. DNA was isolated from peripheral blood using a commercially available DNA extraction kit (Gold-
Mag Co. Ltd., Xi’an, China). DNA purity and concentration were assessed by Nanodrop spectrophotometer (Thermo, Waltham, MA, USA).

Six SNPs (rs1582417, rs2431689, rs7727115, rs17057846, rs2961920, and rs58747524) in the MIR3142HG gene were selected based on (1) the variations of MIR3142HG through the eGRCh37 (http://asia.ensembl.org/Homo_sapiens/Info/Index) database in the CHB and CHS population; Hardy-Weinberg equilibrium (HWE) $>0.01$, minor allele frequency (MAF) $>0.05$, and min ge-
notype $>75\%$, using Haplovew software, (2) the related literature of MIR3142HG polymorphisms [19, 20], (3) a MAF $>0.05$ based on the database of 1000 Genomes Project and the call rate $>95\%$. The frequency distribution of candidate polymorphisms in differ-
ent ethnic groups was evaluated through HaploReg v4.1 database (https://pubs.broadinstitute.org/mammals/haploreg/haploreg.
php) and dbSNP (http://www.ncbi.nlm.nih.gov/SNP/). The poten-
tial functions of these polymorphisms were evaluated through the HaploReg v4.1 and RegulomeDB (https://regulome.stanford.
edu/regulome-search/).

The genotypes of MIR3142HG SNPs were detected by Agena MassARRAY platform (Agena, San Diego, CA, USA). The Mass-
ARRAY platform is based on MALDI-TOF (matrix-assisted laser desorption/ionization – time of flight) mass spectrometry. The analytical accuracy of MALDI-TOF MS is quite high, 0.1–0.01% of the determined mass [21, 22]. The genotyping steps for MassAR-
RAY iPLEX were based on manufacturer’s protocol, as following: (1) DNA templates containing SNPs were amplified by PCR, and PCR products were treated with alkaline phosphatase to neutralize unincorporated nucleotides. (2) A single-base extension reaction was then performed to extend the PCR fragments by one base into the SNPs. (3) The resin purification reaction was performed, and the purified resin extension products were transferred to Spectro-
CHIP Assay using the purpose-built dispenser Agena Bioscience
MIR3142HG Variants Were Associated with IgA Nephropathy Risk

(4) Due to the different bases of polymorphism sites, the different terminal bases of extension products will lead to the difference of molecular weight after extension. The size of the product molecular weight was detected using MALDI-TOF mass spectrometry analysis. Primer's design (online suppl. Table S1; see www.karger.com/doi/10.1159/000525484 for all online suppl. material) and data interpretation of genotyping results were performed by the corresponding supporting software. About 5% of the samples were repeated genotyping for the quality control, and the results were consistent.

Statistical Analysis

Variables were shown as the mean ± standard deviation (SD), or frequency and percentage. Comparisons of characteristics between patients with IgAN and healthy controls were undertaken using χ² test or sample t test, as appropriate. Genotype frequencies in cases and controls were analyzed for departure from HWE using χ² test. Logistic regression models adjusted for age and gender were used to calculate odds ratios (ORs) and 95% confidence intervals (CI). Pairwise linkage disequilibrium (LD) was produced Haploview 4.2 software, and the correlation of MIR3142HG haplotypes with IgAN risk was evaluated by logistic regression model. To analyze MIR3142HG SNP-SNP interaction in IgAN predisposition, Multifactor dimensionality reduction (MDR) analysis was used. Data analyses were performed using SPSS 20.0 (SPSS Inc., Chicago, IL, USA), PLINK 1.0.7, Haploview, and MDR software. A p < 0.05 was on behalf of statistical significance.

Results

Participants’ Characteristics

Here, 417 IgA patients (33.15 ± 12.09 years, 272 males and 145 females) and 424 healthy controls (33.32 ± 11.08 years, 276 males and 148 females) were recruited. The features of IgAN cases and control subjects are displayed in Table 1. There were not significantly different in age (p = 0.832) and gender (p = 0.968) between cases and controls. Significant differences were observed in urine RBC, urinary cast, serum albumin, serum creatinine, serum uric acid, hemoglobin, and fibrinogen between two groups (all p < 0.001).

Table 1. Characteristic of patients with IgA nephropathy and health controls

| Characteristic                        | Cases       | Controls    | p value |
|---------------------------------------|-------------|-------------|---------|
| N                                     | 417         | 424         |         |
| Age, year (mean±SD)                   | 33.15±12.09 | 33.32±11.08 | 0.832   |
| Gender, n (%)                         |             |             |         |
| Male                                  | 272 (65.2)  | 276 (65.1)  |         |
| Female                                | 145 (34.8)  | 148 (34.9)  |         |
| Lee’s grade, n (%)                    |             |             |         |
| <III                                  | 135 (32.4)  |             |         |
| ≥III                                  | 270 (64.7)  |             |         |
| Missing                               | 12 (2.9)    |             |         |
| Course of disease, months, n (%)      |             |             |         |
| >27                                   | 97 (23.3)   |             |         |
| ≤27                                   | 313 (75.1)  |             |         |
| Missing                               | 7 (1.7)     |             |         |
| Urine RBC/μL                          | 48.54±47.53 | 10.91±19.16 | <0.001  |
| Urinary cast/μL                       | 4.56±18.07  | 0.39±0.53   | <0.001  |
| Serum albumin (g/L)                   | 36.72±18.22 | 46.98±3.07  | <0.001  |
| Serum creatinine (μmol/L)             | 151.89±166.04 | 66.54±13.48 | <0.001  |
| Serum uric acid (μmol/L)              | 388.89±173.62 | 336.72±98.69 | <0.001  |
| Hemoglobin (g/L)                      | 126.76±23.86 | 149.93±18.97 | <0.001  |
| Fibrinogen (g)                        | 3.78±1.27   | 2.93±0.31   | <0.001  |
| Urine protein (g/day)                 | 2.34±4.79   |             |         |
| Serum cholesterol (mmol/L)            | 4.95±1.97   |             |         |
| Serum IgA (g/L)                       | 2.76±1.13   |             |         |
| Serum C3 (g/L)                        | 1.05±0.25   |             |         |
| Serum C4 (g/L)                        | 0.27±0.09   |             |         |
| Blood urea nitrogen (mmol/L)          | 8.12±5.67   |             |         |
| Blood β2 microglobulin (μg/L)         | 2,967.03±2,142.04 |             |         |
| Urine β2 microglobulin (μg/L)         | 601.62±837.22 |             |         |

RBC, red blood cell. p values were calculated by χ² test or the Student's t test. p < 0.05 indicates statistical significance.
The Contribution of Genetic Variants in MIR3142HG to IgAN Susceptibility

The detail of candidate SNPs in MIR3142HG is shown in Table 2. The call rate of genotyping was more than 98.5%, and the frequencies of these variants in the controls and cases were not deviated from HWE. The potential function of these polymorphisms by HaploReg v4.1 database and RegulomeDB database was displayed in Table 2. By HaploReg annotation, we found that the selected SNPs were associated with regulation of promoter and/or enhancer histone, DNase, proteins bound, motifs changed, and selected eQTL hits. Based on RegulomeDB database, these SNPs were found to be related to TF binding, any motif, DNase peak. The genotype and allele frequencies distribution are revealed in Table 3 and online supplementary Table S2. We found AA genotype of rs17057846 had an increased risk for IgAN developing compared to GG (OR = 2.10, 95% CI: 1.03–4.28, \( p = 0.042 \)) and GG-GA genotypes (OR = 2.11, 95% CI: 1.04–4.27, \( p = 0.039 \)). Besides, rs58747524-CC genotype confers to the higher susceptibility to IgAN in the homozygote (OR = 1.89, 95% CI: 1.06–3.38, \( p = 0.032 \)) and recessive (OR = 1.80, 95% CI: 1.02–3.19, \( p = 0.031 \)) models.

### Table 2. The details of candidate SNPs in MIR3142HG

| SNP ID   | Chr: position | Alleles (Ref/Alt) | MAF cases | MAF controls | Call rate, % | HWE-p cases | HWE-p controls | Frequency | Haploreg* | Regulome DBc |
|----------|---------------|------------------|-----------|--------------|--------------|-------------|----------------|-----------|-----------|-------------|
| rs1582417| 5:160470494   | G/A              | 0.352     | 0.392        | 99.4         | 0.318       | 0.685          | 0.06      | 0.32      | 0.39        | 0.23        | 0.427        | Promoter histone marks, enhancer histone marks, DNAse, proteins bound, Motifs changed, selected eQTL hits |
| rs2431689| 5:160472115   | G/A              | 0.144     | 0.152        | 98.8         | 0.238       | 1.000         | 0.02      | 0.31      | 0.12        | 0.22        | 0.150        | Promoter histone marks, enhancer histone marks, DNAse, proteins bound, Motifs changed |
| rs7727115| 5:160474732   | G/T              | 0.296     | 0.313        | 100.0        | 0.540       | 0.498         | 0.30      | 0.23      | 0.31        | 0.11        | 0.354        | Promoter histone marks, enhancer histone marks, DNAse, Motifs changed, selected eQTL hits |
| rs17057846| 5:160475306   | G/A              | 0.215     | 0.192        | 99.9         | 0.191       | 0.347         | 0.15      | 0.12      | 0.21        | 0.11        | 0.073        | Promoter histone marks, enhancer histone marks, DNAse, Motifs changed |
| rs2961920| 5:160484499   | C/A              | 0.434     | 0.404        | 98.7         | 0.242       | 0.266         | 0.57      | 0.69      | 0.40        | 0.78        | 0.341        | Enhancer histone marks, proteins bound, Motifs changed |
| rs58747524| 5:160485772   | T/C              | 0.276     | 0.235        | 99.5         | 0.559       | 0.418         | 0.26      | 0.10      | 0.23        | 0.08        | 0.258        | Enhancer histone marks, proteins bound |

SNP, single-nucleotide polymorphism; MAF, minor allele frequency; HWE, Hardy-Weinberg equilibrium; O(HET), observed heterozygosity frequency; E(HET), expected heterozygosity frequency; AFR, African; AMR, American; ASN, Asian; EUR, European. a Data from Haploreg (https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php). b Data from dbSNP (http://www.ncbi.nlm.nih.gov/SNP/). c Data from RegulomeDB (https://regulome.stanford.edu/regulome-search/).
Stratification Analyses for the Association of MIR3142HG SNPs with IgAN Risk

Stratified analyses by gender, age, and Lee’s grade were performed to assess the genotypic effects of MIR3142HG SNPs on IgAN risk in different subgroups. Interestingly, rs7727115, rs17057846, rs2961920, and rs58747524 were related to IgAN susceptibility in females but not in males (Table 4). For rs7727115, GT genotype had a reduced risk of IgAN occurrence compared to GG genotype (OR = 0.59, \( p = 0.035 \)) in females. Rs17057846 (AA vs. GG, OR = 5.96, \( p = 0.023 \); and AA vs. GG-GA, OR = 6.03, \( p = 0.021 \)) and rs2961920 (AA vs. CC, OR = 2.14, \( p = 0.035 \); AA vs. CC-CA, OR = 2.00, \( p = 0.033 \); and additive, OR = 1.39, \( p = 0.055 \)) were related to the enlarged IgAN risk in females. We also found that rs58747524 had a higher risk for IgAN predisposition under allele (OR = 1.52, \( p = 0.031 \)), genotype (OR = 8.12, \( p = 0.007 \)), recessive (OR = 7.80, \( p = 0.007 \)), and additive (OR = 1.56, \( p = 0.027 \)) models.

The results of age-stratification analyses displayed that rs17057846 (AA vs. GG, OR = 3.15, \( p = 0.019 \); and AA vs. GG-GA, 3.08, \( p = 0.020 \)) and rs2961920 (AA vs. CC, OR = 1.72, \( p = 0.048 \)) contributed to the increased IgAN susceptibility at age ≤35 years, as shown in Table 4. Besides, rs58747524 also conferred to the higher risk for IgAN incidence among the subjects with age ≤35 years (allele: OR = 1.48, \( p = 0.005 \); genotype: OR = 2.71, \( p = 0.010 \); dominant: OR = 1.53, \( p = 0.017 \); recessive: OR = 2.36, \( p = 0.024 \); and additive: OR = 1.51, \( p = 0.004 \)). Stratification by Lee’s grade (Table 5), rs17057846 (AA vs. GG, OR = 2.19, \( p = 0.048 \)) and rs58747524 (CC vs. TT, OR = 2.03, \( p = 0.028 \); and CC vs. TT-TC, OR = 1.94, \( p = 0.035 \)) conferred to the higher risk for Lee’s grade ≥III IgAN.

Table 3. The correlation of genetic variants in MIR3142HG with IgA nephropathy susceptibility

| SNP ID    | Model     | Genotype | Case | Control | Adjusted by age and gender | OR (95% CI) | \( p \) value |
|-----------|-----------|----------|------|---------|-----------------------------|-------------|---------------|
| rs17057846| Allele    | G        | 653  | 685     | 1                           | 1.15 (0.91–1.46) | 0.243         |
|           | A        | 179      | 163  | 1       |                             |             |               |
|           | Genotype | GG       | 261  | 273     | 1                           | 0.99 (0.74–1.32) | 0.925         |
|           | GA       | 131      | 139  | 1       |                             |              |               |
|           | AA       | 24       | 12   | 1       |                             | 2.10 (1.03–4.28) | 0.042*        |
|           | Dominant | GG       | 261  | 273     | 1                           | 1.07 (0.81–1.42) | 0.618         |
|           | GA-AA    | 155      | 151  | 1       |                             |              |               |
|           | Recessive| GG-GA    | 392  | 412     | 1                           | 2.11 (1.04–4.27) | 0.039*        |
|           | AA       | 24       | 12   | 1       |                             |              |               |
|           | Log-additive |       | 1.15 | 0.91–1.46 | 0.244                     |             |               |
| rs58747524| Allele    | T        | 601  | 647     | 1                           | 1.24 (0.99–1.54) | 0.056         |
|           | C        | 229      | 199  | 1       |                             |              |               |
|           | Genotype | TT       | 220  | 244     | 1                           | 1.12 (0.85–1.49) | 0.422         |
|           | TC       | 161      | 159  | 1       |                             | 1.89 (1.06–3.38) | 0.032*        |
|           | CC       | 34       | 20   | 1       |                             |              |               |
|           | Dominant | TT       | 220  | 244     | 1                           | 1.21 (0.92–1.59) | 0.173         |
|           | TC-CC    | 195      | 179  | 1       |                             |              |               |
|           | Recessive| TT-TG    | 381  | 403     | 1                           | 1.80 (1.02–3.19) | 0.043*        |
|           | CC       | 34       | 20   | 1       |                             |              |               |
|           | Log-additive |       | 1.24 | 1.00–1.55 | 0.055                     |             |               |

SNP, single-nucleotide polymorphism; OR, odds ratio; 95% CI, 95% confidence interval. \( p \) values were calculated by logistic regression analysis with adjustments for age and gender. * \( p < 0.05 \) respects the data is statistically significant.
Table 4. Stratification by age and gender for the correlation of genetic variants in MIR3142HG with IgA nephropathy predisposition

| SNPs ID   | Model     | Genotype | Sex stratification | Age stratification |
|-----------|-----------|----------|--------------------|--------------------|
|           |           |          | males              | females            | >35 years          | ≤35 years          |
|           |           |          | OR (95% CI)        | p value            | OR (95% CI)        | p value            |
| rs7727115 | Allele    | G        | 1.01 (0.78–1.31)   | 0.925              | 1.17 (0.83–1.65)   | 0.380              |
|           | Genotype  | GG       | 1.15 (0.81–1.63)   | 0.447              | 1.14 (0.71–1.84)   | 0.582              |
|           |           | GT       | 0.86 (0.46–1.60)   | 0.634              | 1.41 (0.62–3.19)   | 0.409              |
|           |           | TT       | 1.09 (0.78–1.53)   | 0.602              | 1.19 (0.75–1.86)   | 0.460              |
|           | Dominant  | GG       | 0.80 (0.44–1.47)   | 0.479              | 1.33 (0.60–2.91)   | 0.483              |
|           |           | GT-TT    | 1.01 (0.78–1.32)   | 0.923              | 1.17 (0.83–1.66)   | 0.378              |
|           | Recessive | GG-GT    | 1.01 (0.79–1.42)   | 0.705              | 1.17 (0.83–1.66)   | 0.378              |
|           | Log-additive |        | 1.12 (0.85–1.46)   | 0.423              | 1.12 (0.85–1.46)   | 0.423              |
| rs17057846| Allele    | G        | 1.06 (0.79–1.42)   | 0.705              | 1.17 (0.83–1.66)   | 0.378              |
|           | Genotype  | GG       | 1.00 (0.69–1.43)   | 0.980              | 0.95 (0.64–1.41)   | 0.792              |
|           |           | GA       | 1.34 (0.57–3.14)   | 0.502              | 1.29 (0.96–1.73)   | 0.097              |
|           |           | AA       | 1.03 (0.73–1.46)   | 0.871              | 1.20 (0.84–1.71)   | 0.307              |
|           | Dominant  | GG       | 1.34 (0.58–3.11)   | 0.495              | 1.14 (0.37–3.50)   | 0.817              |
|           |           | GA-AA    | 1.06 (0.79–1.42)   | 0.706              | 1.30 (0.96–1.76)   | 0.086              |
|           | Recessive | GG-GA    | 1.06 (0.79–1.42)   | 0.706              | 1.30 (0.96–1.76)   | 0.086              |
|           | Log-additive |        | 1.12 (0.85–1.46)   | 0.423              | 1.12 (0.85–1.46)   | 0.423              |
| rs2961920 | Allele    | C        | 1.02 (0.80–1.30)   | 0.884              | 1.30 (0.99–1.91)   | 0.061              |
|           | Genotype  | CC       | 1.14 (0.78–1.67)   | 0.493              | 1.01 (0.61–1.68)   | 0.966              |
|           |           | CA       | 0.99 (0.59–1.66)   | 0.960              | 1.22 (0.83–1.79)   | 0.317              |
|           |           | AA       | 1.10 (0.77–1.59)   | 0.593              | 1.32 (0.91–1.90)   | 0.144              |
|           | Dominant  | CC       | 1.10 (0.57–1.45)   | 0.687              | 1.52 (0.94–2.46)   | 0.087              |
|           |           | CA-AA    | 1.12 (0.80–1.30)   | 0.884              | 1.29 (0.96–1.76)   | 0.052              |
|           | Recessive | CC-CA    | 1.02 (0.79–1.31)   | 0.879              | 1.29 (1.00–1.67)   | 0.052              |
|           | Log-additive |        | 1.12 (0.85–1.46)   | 0.423              | 1.12 (0.85–1.46)   | 0.423              |
| rs58747524| Allele    | T        | 1.12 (0.85–1.46)   | 0.423              | 1.22 (0.83–1.79)   | 0.317              |
|           | Genotype  | TT       | 1.14 (0.80–1.62)   | 0.479              | 1.39 (0.97–2.00)   | 0.071              |
|           |           | TC       | 1.21 (0.61–3.23)   | 0.589              | 2.71 (1.27–5.77)   | 0.010*             |
|           |           | CC       | 1.15 (0.82–1.61)   | 0.426              | 1.53 (1.08–2.16)   | 0.017*             |
|           | Dominant  | TT       | 1.12 (0.85–1.46)   | 0.425              | 1.51 (1.14–2.00)   | 0.004*             |
|           |           | TC-CC    | 1.14 (0.59–2.21)   | 0.691              | 1.23 (1.12–4.95)   | 0.024*             |
| SNP, single-nucleotide polymorphism; OR, odds ratio; 95% CI, 95% confidence interval. p values were calculated by logistic regression analysis with adjustments for age and gender. * p < 0.05 respects the data is statistically significant.
Variants Were Associated with IgA Nephropathy Risk

Furthermore, the haplotype structures of six SNPs in MIR3142HG and haplotype association analysis were further investigated. Haplotype block was composed of rs2431689, rs7727115, rs17057846, rs2961920, and rs58747524 polymorphisms, as exhibited in Figure 1. The haplotypes frequencies distribution was shown in online supplementary Table S3. The association between MIR3142HG haplotype and IgAN susceptibility was in-
vestigated, however, there were no significant relationship of these haplotypes with IgAN risk ($p > 0.05$, online suppl. Table S3).

**Influence of MIR3142HG SNP-SNP Interaction on the Predisposition of IgAN**

MDR analysis was carried out to evaluate the influence of MIR3142HG SNP-SNP interaction models on the predisposition of IgAN. The dendogram (Fig. 2) shows that SNP-SNP interaction of MIR3142HG had strong redundant effect. Moreover, we found that the combination of rs1582417, rs7727115, and rs2961920 was the best model to predict IgAN (testing accuracy = 0.5468, CVC = 10/10, $p < 0.001$, Table 6).

**Discussion**

In this study, six SNPs in MIR3142HG were examined to assess the effect of genetic variants of MIR3142HG in IgAN occurrence in a Chinese Han population. We found that rs17057846-AA genotype and rs58747524-CC genotype had the higher risk for IgAN developing. Interestingly, the risk-association of MIR3142HG variants with IgAN susceptibility might depend on gender, age, and Lee’s grade. Besides, the best model of SNP-SNP interaction for IgAN predisposition was the combination of rs1582417, rs7727115, and rs2961920. These results suggest that MIR3142HG polymorphisms might have a chief part in the susceptibility to IgAN among the Han Chinese population. To us, this study was firstly to discover the relationship between genetic polymorphism of MIR3142HG and IgAN predisposition.

IgAN has been defined as a complex immune disease marked by the deposits of immune complexes containing IgA with ensuing glomerular injury. Recently, numerous studies have addressed the potential role of immune-related miRNAs in the occurrence and development of IgAN, such as miR-146a. MiR-146a has several molecular targets implicated in innate and adaptive immune responses. The upregulation of MiR-146a levels are reported to suppress the activity of NF-kB pathway by down-regulating TRAF6 and IRAK1, which is an important part in IgA pathogenesis [23, 24]. At present, a large amount of evidence indicated that abnormal expression of miR-146a was associated with several renal diseases, such as diabetic nephropathy, lupus nephritis, and IgAN [13, 25, 26]. Compared with normal controls, renal level of miR-146a was identified a marked increase in IgAN patients and the level in kidney tissue was related to IgAN progression [27]. In addition, exosomal miRNA (miR-146a) might potentially serve as novel noninvasive biomarkers for IgAN [28], suggesting that miR-146a was significantly associated with IgAN. MIR3142HG is a larger long noncoding RNA host gene for miR3142 and miR-146a. RNA sequencing data showed that MIR3142HG might be processed to produce miR-146a but not miR3142 in inflammation [29]. Taken together, we hypothesized that genetic polymorphisms in MIR3142HG might have a crucial role in the occurrence of IgAN.

Here, we firstly found that rs17057846 and rs58747524 variants of MIR3142HG contributed to the higher risk for...
IgAN predisposition in a Chinese Han population. Previously, miR-146a SNPs have been reported to impact miR-146a function or expression further to result in genetic susceptibility to diseases [30, 31]. After functional annotation analysis, HaploReg [32] annotated that these SNPs might be associated with promoter histone marks, enhancer histone marks, DNase, proteins bound, motifs changed and selected eQTL hits of MIR3142HG, suggesting that MIR3142HG variants might affect IgAN occurrence by regulating gene expression. Introns play an important role in gene regulation and expression. Several studies provided increasing evidence to support that intronic SNPs confer susceptibilities by affecting gene expression [33–35]. Therefore, we hypothesized that MIR3142HG polymorphisms, especially rs17057846 and rs58747524 may affect the expression of MIR3142HG to contribute to the risk of IgAN. However, the potential function of these polymorphisms needs to be confirmed by further experiments.

Considering age, sex, and Lee’s grade as risk factors for IgAN prevalence, we conducted the stratified analysis to investigate the relationship between genetic variants in MIR3142HG and IgAN predisposition. Previous studies in white patients show a male-incidence in IgAN, but the male-prevalence is not obvious in Asian patients [36]. MIR3142HG rs2961920 was reported to be associated with asthma [37], but no studies have analyzed the SNPs with IgAN risk to now. Stratified by gender, we found that rs7727115 had a reduced risk for IgAN occurrence while rs17057846, rs2961920 and rs58747524 were related to the increased susceptibility to IgAN in females, but not in males. In addition, IgAN frequency is higher in children and young adults than in elderly individuals [38]. The results of age-stratification analyses displayed that rs17057846, rs2961920, and rs58747524 contributed to the increased IgAN susceptibility at age ≤35 years. Based on these results, we speculated the gender- and/or age-specific effects for the risk-association of MIR3142HG polymorphisms with IgAN susceptibility, which might be correlated with sex hormones. Moreover, our results revealed that rs17057846 and rs58747524 conferred to the higher risk for the Lee’s grade ≥III IgAN, suggesting that the risk-associated SNPs in MIR3142HG might be used to predict progression of IgAN. However, these hypotheses need to be further verified in a large sample. Given that IgAN is a complex disease, the analyses of SNP-SNP interaction may help in finding IgAN risk factors. Therefore, MDR analysis was performed to determine the potential SNP-SNP interactions, and we found that the combination of rs1582417, rs7727115, and rs2961920 was the best model to predict the predisposition of IgAN occurrence compared to the single SNP alone.

Several potential limitations should not be neglected. First, there was selection bias in the subjects from a same hospital. Hence, the results of our study need to be further verified in other ethnicities populations. Second, only six polymorphisms in MIR3142HG were evaluated and the risk correlation of other SNPs of MIR3142HG required to investigate in future. Third, due to the lack of environmental information the role of gene-environment interaction in IgAN susceptibility was not analyzed in this study. Fourth, data on the potential function these SNPs in MIR3142HG were predicted in in silico analysis only; thus, further functional assay is necessary to explore the functions and the underlying mechanisms of these polymorphisms. Nevertheless, our study supports the role of MIR3142HG in IgAN occurrence among the Chinese Han population.

**Conclusion**

In summary, we firstly found that rs17057846 and rs58747524 in MIR3142HG were related to the higher risk for IgAN occurrence in a Chinese Han population. We also found evidence that the risk-association of MIR3142HG genetic variants with IgAN susceptibility might depend on gender, age, and Lee’s grade. These results indicated that MIR3142HG genetic polymorphisms might be involved in IgAN pathogenesis among a Chinese Han population and provide a new insight for the pathogenesis and progression of IgAN.

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**Statement of Ethics**

This study protocol was reviewed and approved by the First Affiliated Hospital of Hainan Medical College, approval number (Ethical approval No.: Med-Eth-Re [2018] 10). Written informed consent was got from all participants.
Conflict of Interest Statement

The authors declare that they do not have any conflict of interests.

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Author Contributions

Yun Cao: writing – original draft and conceptualization. Ru Wang and Haizhen Zhang: methodology and data curation. Peiming Zhai and Jiali Wei: conceptualization and writing – review & editing.

Data Availability Statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.
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