Fine mapping the MHC region identified rs4997052 as a new variant associated with nonobstructive azoospermia in Han Chinese males

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Objective: To investigate the association between genetic variants in the major histocompatibility complex (MHC) region and nonobstructive azoospermia (NOA) susceptibility.
Design: MHC region fine-mapping analysis based on previous NOA genome-wide association study (GWAS) data.
Setting: Medical university.
Patient(s): Nine hundred and eighty-one men with NOA and 1,657 normal fertile male controls.
Intervention(s): None.
Main Outcome Measure(s): The MHC region imputation assessed with SNP2HLA software, taking the specific Han-MHC database as a reference panel; statistical significance of the MHC variants calculated using logistic regression models; functional annotation based on online public databases; and phenotypic variances explained by specific groups of genetic variants estimated using the fixed effects model from individual associations.
Result(s): Two independent risk loci, rs7194 (odds ratio [OR] 1.37) at MHC class II molecules and rs4997052 (OR 1.30) at MHC class I molecules, were identified. Functional annotation showed rs7194 may tag the effect of multiple amino acid residues and the expression of *HLA-DQB1* and *HLA-DRB1*; while rs4997052 showed the effect of amino acid changes of *HLA-B* at position 116 as well as the expression of *HLA-B* and *CCHCR1*, which coexpressed with genes enriched in pathways of spermatogenesis and male gamete generation. The novel variant rs4997052 identified in our study can explain another approximately 0.66% of the phenotypic variances of NOA.
Conclusion(s): We fine-mapped the MHC region and identified two loci that independently drove NOA susceptibility. These results provide a deeper understanding of the association mechanisms of MHC and NOA risk. (Fertil Steril® 2019;111:61–8. ©2018 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: Fine mapping, GWAS, MHC, nonobstructive azoospermia

Received April 26, 2018; revised August 14, 2018; accepted August 27, 2018; published online November 28, 2018.
M.H. has nothing to disclose. M.Z. has nothing to disclose. T.J. has nothing to disclose. Y.W. has nothing to disclose. C.W. has nothing to disclose. G.J. has nothing to disclose. X.G. has nothing to disclose. J.S. has nothing to disclose. J.D. has nothing to disclose. X.W. has nothing to disclose. Z.H. has nothing to disclose.
M.H. and M.Z. should be considered similar in author order. J.D., X.W., and Z.H. jointly directed the study.
Supported by National Key Project of Research and Development Plan (2016YFC1000200, 2016YFC1000204), the State Key Program of National Natural Science of China (31530047), Cheung Kong Scholars Programme of China, Jiangsu Specially-Appointed Professor project, the science and technology innovation team of Jiangsu Province Qinglan Project, the Priority Academic Program for the Development of Jiangsu Higher Education Institutions (Public Health and Preventive Medicine) and Top-notch Academic Programs Project of Jiangsu Higher Education Institutions (PPZY2015A067), China Postdoctoral Science Foundation funded project (JX1031123201805).
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Fertility and Sterility® Vol. 111, No. 1, January 2019 0015-0282/$36.00

https://doi.org/10.1016/j.fertnstert.2018.08.052

Male factor infertility affects around half of the couples who have difficulty achieving pregnancy (1). About 10% to 15% of infertile men present with azoospermia, characterized by the absence of sperm in the ejaculate (1–3). Based on whether obstructions occur in the seminal ducts, azoospermia is classified into obstructive azoospermia (OA) and nonobstructive azoospermia (NOA) (1, 4). The latter occurs in approximately 60% of azoospermic men and 1% of adult men. Multiple studies have identified genetic abnormalities—including Y chromosome micro/macrodeletions, chromosomal inversions/translocations, aneuploidy, autosomal chromosome mutations, and epigenetic alterations—as risk factors for NOA cases (5–7).

Previous genome-wide association studies (GWASs) in Han Chinese men have identified 10 loci as associated with the risk of NOA (8–10). Three of these variants (rs3129878, rs498422, and rs7194) were found in the major histocompatibility complex (MHC) locus, a dense region containing approximately 150 genes that encode the human leucocyte antigen (HLA) immunoregulatory proteins (11). These three variants were further verified as NOA-risk loci by subsequent association and meta-analysis of HLA and NOA in the Han Chinese or Japanese population (12–14). However, because of the extensive linkage disequilibrium (LD) existing in the MHC region, it has been difficult to pinpoint the specific risk-associated variants.

In addition, multiple classic HLA alleles in MHC locus (MHC class I alleles: *HLA-A*33*, *HLA-B*13* and **44*; MHC class II alleles: *HLA-DPB1*04:01*, *DQB1*06:04* and *DRB1*13:02*) have also been suggested to confer a risk for NOA (13,15–18), but there were inconsistent observations among these studies. This inconsistency could possibly have resulted from the complex structure of the MHC, the limited number of HLA loci analyzed, or the relatively small sample sizes of the previous studies (19, 20). Furthermore, because each amino acid residue is typically assigned to multiple classic alleles, the NOA risk effect at a certain amino acid position could go undetected if only classic alleles and single-nucleotide polymorphisms (SNPs) are assessed.

Recently, Zhou et al. (21) performed a deep sequencing of the entire 5-Mb MHC region in the Han Chinese population and constructed a Han-MHC reference database, including SNPs, indels, amino acid polymorphisms, and classic HLA alleles of high accuracy. This is now the largest database of the MHC region in the Han Chinese population. In this study, using the Han-MHC database as a reference panel, we imputed all missing SNPs and HLA variants in the MHC region for our existing NOA GWAS genotype data and then explored the MHC associations to NOA susceptibility comprehensively.

MATERIALS AND METHODS

Because our study was built on a joint analysis of existing data and no other patients were enrolled in the present study, ethics permission was not necessary.

Study Participants

We used data from our previous genome-wide association study of 981 individuals with NOA and 1,657 male controls, and detailed sample information and quality control procedures were described previously elsewhere (8). In brief, all cases had been identified as idiopathic NOA and were selected on the basis of comprehensive andrologic testing, including examination of medical history, physical examination, semen analysis, scrotal ultrasound, hormone analysis, karyotyping, and Y chromosome microdeletion screening (8). The controls were normal fertile men who had fathered one or more healthy children. For the following MHC region fine-mapping, we extracted the genotypes of SNPs located in the MHC region (defined as from 29 to 34 Mb on chromosome 6, NCBI Build 37), and a total of 1,709 SNPs were included in this study.

Imputation of HLA Variants

We applied HLA imputation to the NOA GWAS data set with the software SNP2HLA (22) using the HLA imputation reference panel of the Han Chinese population (n = 10,689) (21). In all, 1,709 SNPs in MHC region were used to impute genotypes of the two- and four-digit classic HLA alleles, amino acid polymorphisms, SNPs, and indels for HLA genes (*HLA-A*, *HLA-B*, *HLA-C*, *HLA-DRB1*, *HLA-DQA1*, *HLA-DQB1*, *HLA-DPA1*, and *HLA-DPB1*). Postimputation quality control was performed to filter the variants with imputation quality (INFO) less than 0.8 and minor allele frequency less than 5% in controls. SNP2HLA used the "best-guess" genotypes for imputed variants encoded as [1] minor and major alleles for biallelic variants (for example, two-allele SNPs, two-residue amino acid positions) or [2] presence and absence of each allele for multiallelic variants (for example, multi-allele SNPs, multiresidue amino acid positions, indels, and two-digit and four-digit classic HLA alleles). Finally, a total of 16,180 variants, including 15,018 SNPs, 580 indels, 513 amino acid polymorphisms, 34 two-digit classic alleles, and 35 four-digit alleles, were kept for further evaluation (Supplemental Fig. 1, available online).

Association Analysis of HLA Variants with NOA Risk

For association analysis, we defined all the HLA variants as biallelic variants (including biallelic SNPs, two- and four-digit biallelic classic HLA alleles, and biallelic HLA amino acid polymorphisms for respective residues) and multiallelic HLA amino acid polymorphisms for each amino acid site. For biallelic variants, we performed association analyses using an additive logistic regression model with the first principle component as adjustment (8).

For multiallelic HLA amino acid polymorphisms, an omnibus test was used to evaluate the association of each amino acid site. The details of omnibus test have been described in previous studies (23–25). In brief, the omnibus test uses a log-likelihood ratio test to compare the likelihood of the null logistic model (only including the first principle

component) against the likelihood of the fitted logistic model including m − 1 alleles at the site (one allele was selected as the reference). A total of 51 amino acid sites were multiallelic amino acid polymorphisms. As a result, we included 16,231 variants in the following stepwise conditional analysis to identify the statistically independent effects. In a forward stepwise manner, we included the most significant HLA variant as a covariate in the next model and repeated the same step until no variants reached the study-wide significance threshold, which we set to be $P_{fdr}<.20$ (26).

Functional Analysis of the Two Independent Associations

To investigate the potentially functional associations, we used data from the Genotype-Tissue Expression (GTEx v6p) to perform the expression quantitative trait loci (eQTL) analysis in testis tissues (http://www.gtexportal.org/home/eqtls/). To further map the variants onto potentially regulatory elements, we annotated SNPs using the histone Chip-seq (H3K27AC, H3K4ME1, H3K4ME3) peaks, DNase peaks, and transcription binding sites from ENCODE Project Consortium (http://genome.ucsc.edu/ENCODE). We also linked the SNP to its three-dimensional (3D) interacting gene using 3D chromatin looping data (http://cbportal.org/3dsnp/).

To identify coexpressed genes, we performed genome-wide expression correlation analysis using a linear regression model based on the GTEx database. After Bonferroni correction, the statistically significantly coexpressed genes were then used for GO enrichment analysis, which was implemented in the R package clusterProfiler (27).

Variance Explained

We estimated the phenotypic variances explained by specific groups of genetic variants using the fixed effects from individual associations, as described previously elsewhere (28). Variants identified in our current study and those in the MHC region or non-MHC regions reported in previous GWASs were used to calculate the respective variances by assuming the prevalence of NOA to be 5/1,000, 10/1,000, and 15/1,000 separately (10).

RESULTS

Summary of the Previously Reported Loci

Among the three NOA-related variants in MHC region, two variants, rs3129878 at *HLA-DRA* and rs7194 at *HLA-DRA*, were statistically significantly associated with NOA risk in our results. However, we did not confirm the association of rs498422 at *C6orf10* with NOA even though the association direction was consistent (Supplemental Table 1, available online).

Association Analysis of Classic HLA Alleles with NOA Risk

After quality control, 69 classic HLA alleles were available for association analysis (Supplemental Table 2, available online). A total of 22 two-digit or four-digit classic alleles were statistically significantly associated with NOA risk ($P_{fdr}<.20$). Previous analyses of the relationship between HLA antigens and idiopathic azoospermia have shown a strong linkage of *HLA-A*33*, *−B*13*, *−B*44*, *−DPB1*04:01*, *−DQB1*06:04*, and *−DRB1*13:02* to the susceptibility of idiopathic NOA in Japanese men (13,15–18). Here we replicated the proposed *HLA-DRB1*13:02* association in Han Chinese men (OR 1.32, $P=.013$, $P_{fdr}=.129$), implying a critical role for this classic allele in conferring risk for NOA.

Association Analysis of HLA Amino Acid Polymorphisms with NOA Risk

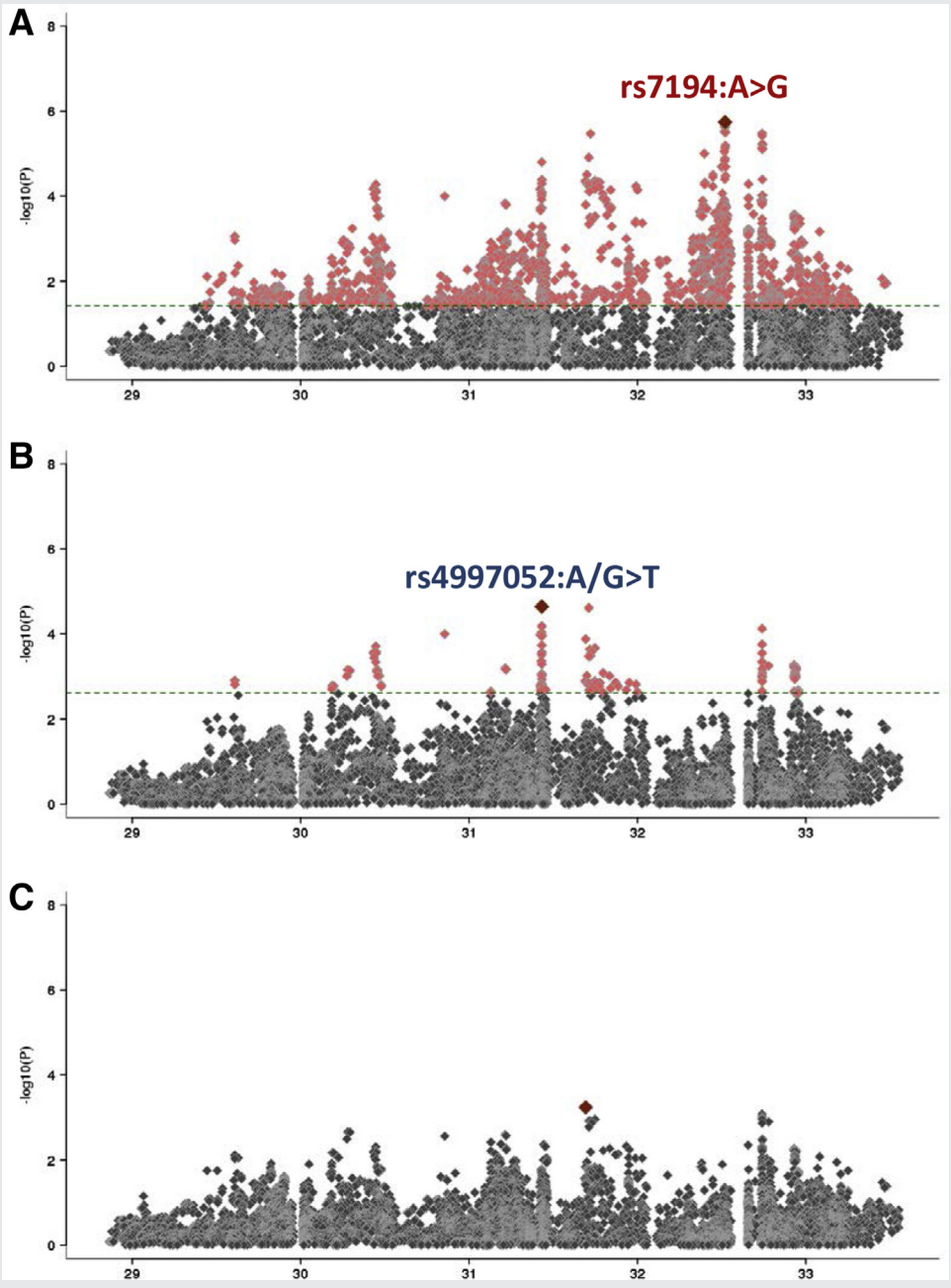
Of the 513 amino acid polymorphisms that survived quality control, 158 amino acid polymorphisms were statistically significantly associated with NOA risk ($P_{fdr}<.20$) (Supplemental Table 3, available online). The top statistically significant association signals ($P<1\times10^{-3}$) were located in three HLA genes (*HLA-DQB1*, *HLA-B*, and *HLA-DRB1*). These amino acid polymorphisms were in high linkage disequilibrium (LD) within the respective genes (Supplemental Fig. 2, available online) and were led by HLA-DQB1 residues Arg55 (OR 1.30, $P=7.37\times10^{-6}$, $P_{fdr}=4.78\times10^{-3}$), HLA-B residues Ser116 (OR 0.79, $P=8.08\times10^{-5}$, $P_{fdr}=.014$), and HLA-DRB1 residues Gly73 (OR 0.74, $P=1.10\times10^{-4}$, $P_{fdr}=0.014$), respectively.

In addition, 22 multiallelic amino acid sites were also found to be statistically significantly associated with NOA risk using an omnibus association test ($P_{omnibus}$ from 3.33×10^{-6} to 3.23×10^{-2}) (see Supplemental Table 3). The most statistically significant multiallelic amino acid site was HLA-DQB1 residues position 55 ($P=3.33\times10^{-6}$, $P_{fdr}=3.23\times10^{-3}$). There are three possible residues (Arg, Leu, Pro) at the amino acid position 55 of HLA-DQB1. Among them, Arg55 showed a risk effect for NOA (OR 1.30, $P=7.37\times10^{-6}$, $P_{fdr}=4.78\times10^{-3}$) whereas Leu55 was protective (OR 0.77, $P=1.00\times10^{-3}$, $P_{fdr}=.039$). The other residue Pro55 exhibited no statistically significant effect on NOA risk.

Independent HLA Associations Drive NOA Risk

To identify the independent association signals, we implemented a stepwise assessment of HLA gene contributions to NOA risk (Supplemental Table 4, available online). We noted that the most statistically significant association was SNP rs7194:A>G (OR 1.37; 95% CI, 1.21–1.56, $P=1.43\times10^{-6}$), which was reported by our previous NOA GWAS (Fig. 1A). When we conditioned on rs7194, we observed a statistically significant independent association at a nonsynonymous coding SNP of *HLA-B* (rs4997052:A/G>T, OR 1.30; 95% CI, 1.15–1.46, $P=2.26\times10^{-5}$) (Fig. 1B). No independent association was observed when we conditioned on these two risk variants (Fig. 1C). Multivariate regression analysis incorporating these two associated HLA variants showed the two associations were independent from each other (Table 1).

FIGURE 1



Regional association plots of HLA loci independently associated with nonobstructive azoospermia (NOA) risk. HLA variants, including single-nucleotide polymorphisms (SNPs), indels, classic alleles, and amino acid polymorphisms, were tested for NOA association, using the imputed best-guess genotypes. In each panel, the diamonds represent −log10 (*P* values) for the variants, and the green line marks P_{fdr}=0.20. (A) The strongest association was SNP rs7194:A>G (OR 1.37; $P=1.43 \times 10^{-6}$) in *HLA-DRA* locus. (B) After adjusting for rs7194, the strongest independent signal was SNP rs4997052:A/G>T (OR 1.30; $P=2.26 \times 10^{-5}$) in *HLA-B* locus. (C) After conditioning for rs7194 and rs4997052, we found no additional independent associations in the MHC region.

Huang. NOA-associated variants in MHC region. Fertil Steril 2018.

Functional Exploration of rs7194 Risk

We first investigated whether the effect of rs7194 could be explained by amino acids or classic HLA alleles. Using a reference panel of the Han Chinese population, we noted that rs7194 was in low LD ($r^2 < 0.4$) with any of the statistically significantly NOA-associated amino acids or classic alleles (Supplemental Table 5, available online), which suggested that the effect of rs7194 may not be derived from amino acid changes or the role of classic alleles directly.

To further determine the potential functional variants tagged by rs7194, we evaluated genetic variants in the flanking region (1 Mb upstream or downstream) of rs7194 at

TABLE 1

Associations of the HLA variants with nonobstructive azoospermia in Han Chinese men.

HLA variants	Reference allele	Effect allele	EAF		OR (95% CI)[a]	*P* value[a]
			Cases (981)	Controls (1,657)		
rs7194	A	G	0.31	0.24	1.36 (1.20–1.55)	2.59×10^{-6}
rs4997052	A/G	T	0.39	0.33	1.30 (1.15–1.46)	2.26×10^{-5}

Note: CI = confidence interval; EAF = effect allele frequency; OR = odds ratio.
[a] Calculated from multiple regression model including principal component analysis and single-nucleotide polymorphisms.

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6p21.32 (Supplemental Table 6, available online). We found that, even though in low LD, the associations of multiple amino acid residues of *HLA-DQB1* and *HLA-DRB1* were eliminated after adjusting for rs7194. Moreover, we also found the expressions of *HLA-DQB1* and *HLA-DRB1* were statistically significantly associated with rs7194 in the testis (Supplemental Fig. 3A and B, available online).

To explore the potential regulatory mechanism, we mapped the statistically significantly associated variants in LD ($r^2 > 0.4$) with rs7194 to the regulatory elements based on data from ENCODE (Supplemental Table 7, available online). We identified multiple variants tagged by H3K27AC histone modification, which indicated these candidate variants may regulate HLA gene expression by modulating an enhancer function.

Functional Exploration of rs4997052 Risk

SNP rs4997052 was a triallelic polymorphism (T, G, and A) within exon 3 of *HLA-B*. Presence of the T allele conferred increased risk for NOA (OR 1.30; 95% CI, 1.16–1.47, $P=1.57 \times 10^{-5}$). By contrast, the presence of the G allele would result in reduced risk for NOA (OR 0.79; 95% CI, 0.70–0.89 $P=8.08 \times 10^{-5}$). There was no statistically significant effect of the A allele on NOA risk ($P=.79$) (see Supplemental Table 4).

Our LD and conditional analysis suggested rs4997052 to be a new susceptibility locus as it was independent from the previous GWAS reported variants (Supplemental Tables 8 and 9, available online). Notably, the corresponding amino acid position for rs4997052 was HLA-B amino acid position 116. There are five possible residues (Ser, Leu, Phe, Asp, and Tyr) at the position 116. Among them, Tyr116 and Asp116 showed risk effects for NOA, while Ser was protective. The other residues Leu and Phe exhibited no statistically significant effects on NOA risk (Supplemental Table 3). Because position 116 is located in the peptide-binding groove of the HLA-B protein (Supplemental Fig. 4, available online), the changes at position 116 of HLA-B could influence antigen-presentation ability or protein stability.

Given that the SNP rs4997052 was not available in GTEx database, we tested the NOA-associated SNPs in LD with it ($r^2 > 0.4$) for eQTL effects in testis tissues instead (Supplemental Table 10, available online). Two SNPs, rs2074496 and rs1050654, located at the exon of the *HLA-B* gene, had a strong eQTL effect on *HLA-B* (Supplemental Fig. 3C and D). Leveraging ENCODE it was evident that these two SNPs had a strong signal in the promoter region, tagged by H3K4Me3 histone modification (Supplemental Table 11, available online), suggesting their role as an active transcription start site.

We also noted another strong effect involving rs709054 ($r^2=0.48$), which was related to *CCHCR1* expression (see Supplemental Fig. 3E). Rs709054 is located 200 kb upstream of *CCHCR1*, and the 3D chromatin looping data showed that *CCHCR1* was a 3D interacting gene of rs709054 in prostate tissue (Supplemental Fig. 5, available online). Therefore, it is reasonable to assume that rs709054 could have an effect on *CCHCR1* through chromatin looping.

Of particular interest was that *CCHCR1* was predominantly expressed in the testis (Supplemental Fig. 6, available online). The GO enrichment analysis further showed that genes coexpressed with *CCHCR1* in testis were enriched in pathways of spermatogenesis ($P_{nominal}=2.37 \times 10^{-16}$, first rank) and male gamete generation ($P_{nominal}=3.14 \times 10^{-16}$, second rank) (Supplemental Table 12, available online), constituting highly statistically significant evidence of the involvement of *CCHCR1* in azoospermia.

Variance Explained by the Independent HLA Variants

On the basis of the variants identified in our study and those reported by previous GWAS, we estimated the proportion of phenotypic variance attributable to these variants assuming the prevalence of NOA to be 5/1,000, 10/1,000, and 15/1,000, respectively (Table 2). The novel variant identified in our study could explain 0.57%, 0.66%, and 0.73%, and the GWAS-reported three SNPs in MHC region account for 0.77%, 0.89%, and 0.99%, respectively. In total, all these 11 variants together can explain 5.78%, 6.71%, and 7.37% of the phenotypic variance at the respective prevalence.

DISCUSSION

Major histocompatibility complex genes play essential roles in controlling susceptibility to autoimmune diseases, and fine-mapping of the MHC region has achieved great successes for immune-related diseases in identifying independent loci and pinpointing functional variants (29–31). In this study, we performed a fine-mapping analysis on 2,638 unrelated case-control subjects to systematically dissect the genetic basis of NOA within the MHC region. Our results implicated two variants in conferring the risk for NOA. The SNP rs7194 at MHC class II molecules was reported by our previous

TABLE 2

Heritability estimates for our identified and GWAS reported variants in nonobstructive azoospermia.

Model[a]	h2(SE) observed scale	h2(SE) Liability scale Prevalence 5/1,000	Prevalence 10/1,000	Prevalence 15/1,000
The one new independent HLA variant	1.12%	0.57%	0.66%	0.73%
SNPs in MHC region identified by GWAS (3 SNPs)	1.50%	0.77%	0.89%	0.99%
SNPs in non-MHC regions identified by GWAS (7 SNPs)	7.34%	3.95%	4.60%	5.06%
Combined	10.43%	5.78%	6.71%	7.37%

Note: GWAS = genome-wide association study; MHC = major histocompatibility complex; SNP = single-nucleotide polymorphism.
[a] Variants identified in our study, and previous GWAS studies identified HLA variants in the MHC region or non-MHC regions, were used to estimate the heritability variances of nonobstructive azoospermia risk separately.

Huang. NOA-associated variants in MHC region. Fertil Steril 2018.

GWAS, but rs4997052 at MHC class I molecules was a new NOA-associated locus that acted independently.

The SNP rs7194 may tag the effect of multiple amino acid residues and the expression of *HLA-DQB1* and *HLA-DRB1* in testis tissues. These two genes belong to the class II HLA genes encoding MHC class II proteins, which mediate antigen presentation to CD4+ T cells to induce immune responses (32). The relationship between autoimmunity in the testis and NOA has been discussed before. Previous studies have suggested that antisperm antibodies are associated with HLA class II genes (33). In addition, Spiess et al. (34) used gene expression analysis on testicular biopsies and found the expression of inflammation-related genes was increased in the testicular tissue of NOA patients. Moreover, immunohistochemical analyses by Hussein et al. (35) showed an exaggerated immune response in NOA cases. In this study, we also found the expression of two HLA genes, HLA-DQB1 and HLA-DRB1, were statistically significantly associated with rs7194 in testis using GTEx testis data. Therefore, the variations in HLA class II region might evoke an immune response against testicular microenvironmental antigens, leading to testicular azoospermia through autoimmune inflammatory responses.

In addition to previously reported loci, we also identified a new nonsynonymous mutation (rs4997052) in the class I HLA gene of *HLA-B*. The *HLA-B* gene contains eight exons. Exon 1 encodes the leader peptide, exon 2 and 3 encode the alpha 1 and alpha 2 domains which both bind the peptide, exon 4 encodes the alpha 3 domain, exon 5 encodes the transmembrane region, and exons 6 and 7 encode the cytoplasmic tail (36). The amino acid position 116 corresponding to rs4997052 was located at the exon 3 of HLA-B and thus a variant at this position might have an effect on the antigen presentation capability of a class I molecule.

An interesting finding in our study was the association of gene *CCHCR1*. The variant rs4997052 might also be a proxy of SNP rs709054 that interacted with gene *CCHCR1* and influenced its expression in testis tissues. The gene expression profiling from the GTEx database revealed that *CCHCR1* was expressed predominantly in the testis compared with other normal tissues, suggesting its possible role in spermatogenesis. The role of *CCHCR1* in germ cell development was also supported by GO enrichment analysis based on coexpressed genes in the testis, which showed multiple significant spermatogenesis-related pathways, including spermatogenesis, male gamete generation, spermatid differentiation, spermatid development, and germ cell development. This evidence suggests that *CCHCR1* acts as a NOA susceptibility gene, possibly by regulating the expression of genes associated with spermatogenesis. However, the exact mechanism of *CCHCR1* needs further validation through in vitro and in vivo experiments.

In summary, we first dissected the MHC region for NOA using existing GWAS data and identified two loci that were independently associated with NOA risk. The results provided a deeper understanding of the GWAS-reported associations and identified additional susceptibility loci that had been missed in previous studies due to the limitations of genotyping chips. The variants identified in this study provide additional insight into the pathogenesis of NOA, highlighting autoimmune inflammatory responses in the development of NOA. These variants may serve as biomarkers for high-risk population identification as well as indicate a potential therapeutic target of NOA in the future.

However, our study was limited by its moderate sample size, so further validation studies with larger sample sizes are warranted. In addition, the histology (such as semen analysis data or testicular tissue data) and testicular sperm extraction data of the patients were not available to us, and the associations between HLA variants and clinical outcomes after NOA diagnosis remain unknown.

These results have expanded our understanding of the mechanisms of NOA and may be helpful for the development of therapeutic targets in the future. Overall, the identification of new susceptibility genes will improve our understanding of the pathogenesis of NOA and offer novel targets for future biological research.

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Mapeo fino de la región de MHC rs4997052 identificada como una nueva variante asociada a azoospermia no obstructiva en hombres chinos han

Objetivo: Investigar la asociación entre variantes genéticas en la región correspondiente al complejo mayor de histocompatibilidad (MHC) y la susceptibilidad a azoospermia no obstructiva (NOA).

Diseño: análisis de mapeo fino de la región de MHC teniendo en cuenta los datos previos del estudio de asociación de amplificación-genómica en NOA.

Lugar: Universidad Médica.

Paciente (s): novecientos ochenta y un paciente con diagnóstico de NOQ y 1.657 hombre con fertilidad normal como control.

Intervención (es): Ninguna.

Variable Resultado principal (es): La región de MHC evaluada con el software SNP2HLA, tomando como panel de referencia específico la base de datos Han-MHC; se obtuvo significancia estadística en las variantes MHC calculadas usando modelos de regresión logística; anotaciones funcionales según las bases de datos online públicas; la variación fenotípica explicada por variantes genéticas de grupos específicos usando el modelo de efectos fijos tras asociaciones individuales.

Resultado (s): Se identificaron dos loci de riesgo independiente; rs7194 (odds ratio (OR):1,37) en las moléculas de clase II de MHC y rs4997052 (OR 1,30) en las moléculas de clase I de MHC. Se describió como anotación funcional que el rs7194 puede identificar el efecto de múltiples residuos de amino ácidos y la expresión del HLA-DQB1 y HLA-DRB1; mientras que el rs4997052 mostró efectos en los cambios de aminoácidos en la molécula HLA-B en posición 116, así como la expresión de HLA-B y CCHCR1, la cual se co-expresa con genes enriquecidos en vías de producción del gameto masculino y espermatogénesis. La variante novedosa rs4997052 identificada en nuestro estudio puede llegar a explicar el 0,66% de las variaciones fenotípicas de la NOA.

Conclusión (es:): Se realizó mapeo fino de la región de MHC y se identificaron dos zonas que de forma independiente son susceptibles de desarrollar NOA. Estos resultados aportan un entendimiento mayor sobre los mecanismos de asociación entre MHC y riesgo de NOA.