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SELECTED **ORAL COMMUNICATIONS** SESSION 28: INVESTIGATING THE GENETIC BASIS OF REPRODUCTIVE PHENOTYPES

O-118

New insight into the genetic contribution of common variants to the development of extreme phenotypes of unexplained male infertility: a multicenter genome-wide association study

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Study question: What is the contribution of the common genetic variation to the development of unexplained male infertility due to severe spermatogenic failure (SPGF)?

Summary answer: Genetic polymorphisms of key immune and spermatogenesis loci are involved in the etiology of the most severe SPGF cases, defined by Sertoli cell-only (SCO) phenotype.

What is known already: Male infertility is a rising worldwide concern that affects millions of couples. Non-obstructive azoospermia (NOA) and severe oligospermia (SO) are two extreme manifestations characterized by SPGF. A genetic cause can be established in only around 20% of affected men, with the remaining cases being classified as otherwise unexplained. To date, the genomewide association study (GWAS) strategy, although already successfully applied in several other complex traits and diseases, was less fruitful in studies that attempted to decipher the genetic component of unexplained SPGF, mainly due to both a lack of well-powered samples in different ancestries and limitations in study design.

Study design, size, duration: We designed a GWAS for unexplained male infertility due to SPGF including a total of 1,274 affected cases and 1,951 fertile controls from the Iberian Peninsula (Spain and Portugal) and Germany. Different biostatistics and bioinformatics approaches were used to evaluate the possible effect of single-nucleotide polymorphisms (SNPs) across the whole genome in the susceptibility to specific subtypes of unexplained SPGF. Participants/materials, setting, methods: The case cohort comprised 502 SO and 772 NOA patients, who were subdivided according to histological phenotypes (SCO, maturation arrest, and hypospermatogenesis) and the outcome of testicular sperm extraction techniques (TESE) from testis biopsies. Genotyping was performed with the GSA platform (Illumina).

After quality-control and genotype imputation, 6,539,982 SNPs remained for the analysis, which was performed by logistic regression models. The datasets went through a meta-analysis by the inverse variance weighted method under fixed effects. Main results and the role of chance: Genetic associations with SCO at the genome-wide-level of significance were identified in the major histocompatibility (MHC) class II region (rs1136759, OR=1.80, P=1.32E-08) and in a regulatory region of chromosome 14 nearby the vaccinia-related kinase 1 (VRK1) gene (rs115054029, OR=3.14, P=4.37-08). VRK1 is a relevant proliferative factor for spermatogenesis that causes progressive loss of spermatogonia when disrupted in mouse models. The role of the MHC system in SCO susceptibility was comprehensively evaluated through a validated imputation method that infers classical MHC alleles and polymorphic amino acid positions. A serine at position 13 of the HLA-DRβ1 protein (defined by the risk allele of the lead variant rs1136759) explained most of the SCO association signals within the MHC class II region. This residue is located in the binding pocket of the HLA-DR molecule and interacts directly with the presented antigen. Interestingly, position 13 of HLADRβ1 is the most relevant risk amino acid position for a wide spectrum of immune-mediated disorders. The HLA-DRB1*13 haplotype (which includes the serine at position 13 and represents the strongest NOA-associated marker in Asians to date) was the strongest signal amongst the classical MHC alleles in our study cohort (OR=1.93, P=9.90E-07).

Limitations, reasons for caution: Although the statistical power for the overall analysis was appropriate, the subphenotype analyses performed had considerably lower counts, which may influence the identification of genetic variants conferring low to moderate risk effects. Independent studies in larger SCO study cohorts should be performed to confirm our findings.

Wider implications of the findings: The molecular mechanisms underlying unexplained SPGF are largely unknown. Our data suggest a relevant role of common genetic variation in the development of SCO, the most extreme histological phenotype of NOA. SCO is characterized by the loss of germ cells and, therefore, implies a considerably higher probability of unsuccessful TESE.

ABSTRACT ESHRE 2021

1. Abstract title (25)

"New insight into the genetic contribution of common variants to the development of extreme phenotypes of unexplained male infertility: a multicenter genome-wide association study "

2. Study question (25)

What is the contribution of the common variation of the genome to unexplained male infertility due to severe spermatogenic failure (SPGF)

3. Summary answer (25)

Genetic polymorphisms of key immune and spermatogenesis loci are involved in the aetiology of the most severe SPG phenotype, defined by Sertoli cell-only (SCO) phenotype.

4. What is known already (100)

Male infertility is a growing worldwide concern that affects millions of couples. Non-obstructive azoospermia (NOA) and severe oligospermia (SO) are two extreme manifestations characterised by SPGF. A genetic cause can be established only in about 20% of affected men, with the aetiology of the remaining cases classified as unexplained. To date, the genome-wide association study (GWAS) strategy, which has been successfully applied to several complex traits and diseases, has been less fruitful in defining the genetic component of unexplained SPGF, mainly due to strong limitations of the study designs and to the lack of well-powered studies in different ancestries.

5. Study design, size, duration (75)

We designed a GWAS for unexplained male infertility due to SPGF including a total of 1,274 affected cases and 1,951 fertile controls from the Iberian Peninsula (Spain and Portugal) and Germany. Different biostatistics and bioinformatics approaches were used to evaluate the possible effect of single-nucleotide polymorphisms (SNPs) across the whole genome in the susceptibility to specific subtypes of unexplained SPGF.

6. Participants/materials, setting, methods (75)

The case cohort comprised 502 SO and 772 NOA patients, who were subdivided according to histological phenotypes (including SCO, maturation arrest, and hypospermatogenesis) and the outcome of testicular sperm extraction techniques (TESE). Genotyping of DNA samples was performed with the GSA platform (Illumina). After quality-control and genotype imputation, 6,539,982 SNPs remained for the analysis, which was performed by logistic regression models. The datasets went through a meta-analysis by the inverse variance weighted method under fixed effects.

7. Main results and the role of chance (200)

Genetic associations with SCO at the genome-wide-level of significance were identified in the major histocompatibility (MHC) class II region (rs1136759, P=1.32E-08, OR=1.80) and in a regulatory region of chromosome 14 nearby the vaccinia-related kinase 1 (*VRK1*) gene (rs115054029, P=4.37-08, OR=3.14). VRK1 is a relevant proliferative factor for spermatogenesis that causes progressive loss of spermatogonia when disrupted in mouse models. The role of the MHC system in SCO susceptibility was comprehensively evaluated through a validated imputation method that infers classical MHC alleles and polymorphic amino acid positions. A serine at position 13 of the HLA-DRβ1 protein (defined by the risk allele of the lead variant rs1136759) explained most of the SCO association signals within the MHC class II region. This residue is located in the binding pocket of the HLA-DR molecule and interacts directly with the

presented antigen. Interestingly, position 13 of HLA-DRβ1 is the most relevant risk amino acid position for a wide spectrum of immune-mediated disorders. The HLA-DRB1*13 haplotype (which includes the serine at position 13 and represents the strongest NOA-associated marker in Asians to date) had the lowest P-value amongst the classical MHC alleles in our study cohort (P=9.90E-07, OR=1.93).

8. Limitations, reasons for caution (50)

Although the statistical power for the overall analysis was appropriate, the subphenotype analyses were performed with considerably lower case numbers, which may influence the identification of genetic variants conferring low to moderate risk effects. Independent studies in larger SCO study cohorts should be performed to confirm our findings.

9. Wider implications of the findings (50)

The molecular mechanisms underlying unexplained SPGF are largely unknown. Our data suggest a relevant role of common genetic variation in the development of SCO, the most extreme histological phenotype of NOA. SCO is characterised by the loss of germ cells and, therefore, implies a considerably higher probability of unsuccessful TESE.

10. Study funding/competing interest(s) (select option)

This study was mainly funded by the Spanish Ministry of Economy and Competitiveness (reference: SAF2016-78722-R). Part of this study was carried out within the frame of the German Research Foundation Clinical Research Unit 'Male Germ Cells' (DFG CRU326). We declare no conflict of interest related to this study.

11. Trial registration number (25)

Not applicable