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Pereza, Nina; Črnjar, Ksenija; Buretić-Tomljanović, Alena; Volk, Marija; Kapović, Miljenko; Peterlin, Borut; Ostojić, Saša

Source / Izvornik: **Fertility and Sterility, 2013, 99, 1663 - 1667**

Journal article, Published version
Rad u časopisu, Objavljena verzija rada (izdavačev PDF)

https://doi.org/10.1016/j.fertnstert.2013.01.101

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:184:262926

Y chromosome azoospermia factor region microdeletions are not associated with idiopathic recurrent spontaneous abortion in a Slovenian population: association study and literature review

Nina Pereza, M.D.,[a] Ksenija Črnjar, M.D.,[a] Alena Buretić-Tomljanović, Ph.D.,[a] Marija Volk, M.D.,[b] Miljenko Kapović, M.D., Ph.D.,[a] Borut Peterlin, M.D., Ph.D.,[b] and Saša Ostojić, M.D., Ph.D.[a]

[a] Department of Biology and Medical Genetics, Faculty of Medicine, University of Rijeka, Rijeka, Croatia; and [b] Clinical Institute of Medical Genetics, Department of Gynecology and Obstetrics, University Medical Centre, Ljubljana, Slovenia

Objective: To investigate the potential association of Y chromosome microdeletions with idiopathic recurrent spontaneous abortion (IRSA) in a Slovenian population and compare our results with those of previously published studies in different populations, with the intention of clarifying the potential impact of Y chromosome microdeletions on IRSA.
Design: Case–control and association study.
Setting: Departments of gynecology and obstetrics and university-based research laboratory.
Patient(s): Male partners of 148 couples with at least three spontaneous pregnancy losses of unknown etiology, and 148 fertile men.
Intervention(s): Multiplex polymerase chain reactions.
Main Outcome Measure(s): Azoospermia factor (AZF) regions were tested for Y chromosome microdeletions according to European Academy of Andrology/European Molecular Genetics Quality Network guidelines. The PubMed database was searched to retrieve articles linking Y chromosome microdeletions and susceptibility to IRSA.
Result(s): None of the IRSA or control men had microdeletions in the AZFa, AZFb, or AZFc regions. A total of nine previous studies examined the relationship between Y chromosome microdeletions and IRSA, yielding contradictory results, which we discuss in detail.
Conclusion(s): On the basis of our comparisons, it is unlikely that Y chromosome microdeletions contribute to IRSA and are therefore currently not recommended for the routine evaluation of IRSA couples. (Fertil Steril® 2013;99:1663–7. ©2013 by American Society for Reproductive Medicine.)
Key Words: Male infertility, microdeletions, miscarriage, pregnancy, spermatogenesis

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Received November 26, 2012; accepted January 7, 2013; published online February 11, 2013.
N.P. has nothing to disclose. K.Č. has nothing to disclose. A.B.-T. has nothing to disclose. M.V. has nothing to disclose. M.K. has nothing to disclose. B.P. has nothing to disclose. S.O. has nothing to disclose.
This research is part of the project "Genetic factors in etiology of recurrent spontaneous abortion" (no. 062-0000000-3548), supported by the Ministry of Science, Education and Sports, Zagreb, Croatia, as well as Slovenian Research Agency grant P3-0326 (Gynecology and reproduction: Genomics and stem cells). The results of this study are part of the Human Miscarriage Genetic Network (HuMGeN) international project started by research groups from Slovenia and Croatia.
Reprint requests: Saša Ostojić, M.D., Ph.D., Department of Biology and Medical Genetics, Faculty of Medicine, University of Rijeka, B. Branchetta 20, 51000 Rijeka, Croatia (E-mail: sasa.ostojic@medri.uniri.hr).

Fertility and Sterility® Vol. 99, No. 6, May 2013 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2013.01.101

Recurrent spontaneous abortion (RSA), defined as three or more spontaneous pregnancy losses with the same partner before the 24th week of gestation, represents a separate entity in human infertility, affecting 3%–5% of couples trying to conceive (1, 2). Although RSA comprises a large group of heterogeneous conditions of genetic and nongenetic etiology, the cause cannot be identified in approximately 50% of couples

(idiopathic RSA; IRSA). The known causes are usually classified as either fetal or maternal, whereas the potential contribution of male factors, such as sperm quality, paternal age, and gene mutations, remains largely unexplored (3, 4). As a result, clinical investigation of RSA couples focuses mostly on the evaluation of the mother and conceptus, whereas both parents are included only in the cytogenetic analysis (5).

Several studies have indicated that the sperm genome and epigenome provide specific molecular regulatory factors required for fertilization and proper embryonic development (6, 7). Consequently, it has been suggested that abnormalities of the sperm genome, including chromosome aberrations, chromatin fragmentation, oxidative stress, and Y chromosome microdeletions, might be the underlying cause in some cases of IRSA (8–10).

Y chromosome microdeletions are a well-established cause of spermatogenetic impairment and male infertility (11, 12). Even though the actual prevalence of clinically relevant microdeletions is still unclear, severely oligozoospermic or azoospermic men have the highest risk of carrying a Y chromosome microdeletion (4). Various studies have reported significant differences in the prevalence of microdeletions in infertile men, ranging from 5% to 35%, which can be attributed to different patient selection criteria and laboratory techniques used to detect the microdeletions (13–15). Y chromosome microdeletions commonly occur in the band q11.23, at the azoospermia factor (AZF) locus, which is further subdivided into AZFa, AZFb, and AZFc regions. These microdeletions are classified as one of six classic types: AZFa, AZFb, AZFc, AZFbc, AZFabc, and partial AZFc, all of which result in different degrees of spermatogenetic failure (12, 16); however, the function of genes located in the AZF region and the exact genotype–phenotype correlation are unknown.

Considering that the initiation and maintenance of a viable pregnancy depends on the integrity of the paternal genome, several studies attempted to evaluate the association between Y chromosome microdeletions and IRSA but yielded contradictory results (8, 10, 17–23). To investigate the potential association of Y chromosome microdeletions with IRSA in a Slovenian population, we performed a case–control study in male partners of IRSA women. Furthermore, we compared our results with those of previously published studies in different populations with the intention of clarifying the potential impact of Y chromosome microdeletions on IRSA.

MATERIALS AND METHODS

Patients and Control Subjects

All subject samples were collected at the Institute of Medical Genetics, Department of Obstetrics and Gynecology, University Medical Centre, Ljubljana, Slovenia from 2005 to 2011, according to a protocol approved by the Slovenian National Ethics Committee. Written informed consent to participate in this study was obtained from all individuals. This study was approved by the Slovenian National Ethics Committee. All subjects included in the study were Slovenian.

The patient group consisted of 148 men of 148 couples with a history of three or more spontaneous abortions of unknown etiology before the 24th week of gestation. These couples were previously recruited for case–control genetic association studies investigating the role of different genetic polymorphisms in the etiology of IRSA and were selected using strict criteria. The inclusion criteria for women were as follows: no endocrine, autoimmune, metabolic, or other systemic disorders and no previous venous or arterial thrombosis or uterine anatomic abnormalities. Additionally, both partners had normal karyotypes. Characteristics of IRSA couples have been described in detail previously (24, 25).

The control group consisted of 148 unrelated, healthy Caucasian men who had at least two children and whose partners had never experienced spontaneous abortions or any other pregnancy complications.

DNA Extraction

Peripheral venous blood samples (3–5 mL) were collected from IRSA couples and controls, and genomic DNA was extracted from peripheral blood leukocytes by standard procedure using a commercially available kit (FlexiGene Kit; Qiagen), according to the manufacturer's instructions. Extracted DNA was stored at −20°C.

Molecular Analysis

The AZFa, AZFb, and AZFc regions were tested for Y chromosome microdeletions using multiplex polymerase chain reaction systems according to recommendations made by the European Academy of Andrology/European Molecular Genetics Quality Network (EAA/EMQN) guidelines provided for diagnostic laboratories (26). Two multiplex reactions (A and B) were used for the analysis of the three AZF regions on the Y chromosome. Each multiplex contained five sequence-tagged sites (STS) spanning AZFa–c regions: sY86, sY127, and sY254 for multiplex A; sY84, sY134, and sY255 for multiplex B; and two control fragments used in both multiplexes—the sex-determining region of the Y chromosome (*SRY*/sY14) and zinc finger protein of Y chromosome/zinc finger protein of X chromosome (*ZFY*/*ZFX*). The *ZFY*/*ZFX* was used as an internal control, whereas the *SRY* gene was examined to confirm the sex of the donor. According to the EAA/EMQN guidelines, this primer set enables the detection of almost all clinically relevant deletions and >95% of all AZFa, AZFb, and AZFc deletions reported in the literature (26).

All reactions were performed in a thermal cycler (Mastercycle Personal; Eppendorf). Amplification conditions started with an initial activation step of 5 minutes (94°C), followed by 35 cycles of 60 seconds denaturation (94°C), 60 seconds annealing (58°C), and 60 seconds elongation (72°C), plus a final elongation step of 10 minutes (72°C).

The polymerase chain reaction products were separated by electrophoresis on a 2% agarose gel stained with ethidium

TABLE 1

Comparison of the present study and previously published studies investigating the association of Y chromosome microdeletions and IRSA.

Reference	Patients (n)	Controls (n)	STS	Spermiogram	No. (%) of patients with microdeletions	Population
Present study	148	148	AZFa: sY84, sY86 AZFb: sY127, sY134 AZFc: sY254, sY255 + SRY, ZFY	NA	0	Slovenian
Dewan et al. (17)	17	18	AZFb: DYS220 (sY129) AZFc: DYF85S1 (sY150), DYF86S1 (sY152) + DYS262 (sY67)	NA	14 (82)[a]	American
Karaer et al. (19)	43	43	AZFb: DYS220 (sY129) AZFc: DYS235 (sY150), DYS236 (sY152), DYS237 (sY153)	32 NS 1 OS 6 TAS	7 (16): 5 in DYS220 2 in DYS220 + DYS237	Turkish
Lu et al. (20)	51	NA	AZFa: sY84, sY86 AZFb: sY127, sY134 AZFc: sY254, sY255 + SRY, ZFY	NA	0	Chinese
Kaare et al. (10)	40	NA	AZFa: sY81, sY82, sY84, Y6HP35pr (DYS274, sY85), sY86 AZFb: Y6D14pr (DYS205, sY113), sY117, Y6PHc54pr (Y6H54), sY127, sY134, sY144, sY145, DYS220 (sY129) AZFc: Fr15-lipr, Y6HP52pr (DYS239, sY156), sY147, sY149, sY254, sY255, sY277, sY283, sY157, sY158, sY159, DYF85S1 (sY150), DFY86S1 (sY152) + SRY, DYS262 (sY67), sY160, sY88, sY182, sY151, sY94, sY95, sY97, sY102, sY105	NA	0	Finnish
Wettasinghe et al. (23)	76	120	AZFa: sY84, sY86 AZFb: sY127, sY134 AZFc: sY254, sY255 Other: sY1191, sY1291, sY1201, sY1206, sY1161 + SRY, ZFY	41 NS 5 OS 2 AS 2 AS/OS	0	Sinhalese
Bellver et al. (8)	30	30	AZFa: sY81, sY84s, sY86 AZFb: sY182, sY121, sYPR3, sY124, sY127, sY128, sY130, sY133, sY134 AZFc: sY145, sY152, sY242, sY208, sY254, sY255 + sY157	NS	0	Spanish
Venkatesh et al. (22)	48	20	AZFa: sY84, sY86 AZFb: sY127, sY134 AZFc: sY254, sY255 + SRY, ZFY	16 OS	0	Indian
Ghorbian et al. (18)	100	100	AZFa: sY84, sY86 AZFb: sY127, sY134 AZFc: sY254, sY255, sY150, sY152 + SRY	NA	0	Iranian
Piña-Aguilar et al. (21)	71	66	AZFa: sY84, sY86 AZFb: sY127, sY134, DYS220 (sY129) AZFc: sY254, sY255, sY150, sY152, DYF85S1 (sY150), DYF86S1 (sY152) + SRY, DYS262 (sY67), DYF87S1 (sY153)	NA	0	Mexican

Note: NA = not available; NS = normozoospermia; OS = oligozoospermia; TAS = teratoasthenozoospermia; AS = asthenozoospermia.
[a] Results of the analysis for each patient are not provided in the article.

Pereza. Y chromosome microdeletions in IRSA. Fertil Steril 2013.

bromide and visualized under ultraviolet light. Genomic DNA from a man with an already confirmed deletion (AZFb) was used as a positive control, whereas negative control DNA was obtained from a woman.

Literature Search Strategy

The PubMed database was searched to retrieve articles linking Y chromosome microdeletions and susceptibility to IRSA published up to September 2012 and without language

restrictions. The following key words were used: "recurrent spontaneous abortion," "recurrent miscarriage," "recurrent pregnancy loss," "Y chromosome microdeletions," and "AZF microdeletions."

RESULTS

Molecular Analysis of Y Chromosome Microdeletions

All eight STS loci were detected in all subjects, thereby confirming that none of the 148 patients and 148 controls had microdeletions in the AZFa, AZFb, or AZFc regions.

Literature Search Strategy

A total of nine publications evaluating the potential role of Y chromosome microdeletions in IRSA have been retrieved. These studies differ to a large extent, using different methodologies and yielding contradictory results (Table 1). Couples with three or more spontaneous abortions were included in all studies, with the exception of the one by Venkatesh et al. (22), in which patients with two or more spontaneous abortions were investigated. Studies in which patient selection criteria were not described were not included in this analysis (27). A positive correlation between Y chromosome microdeletions and IRSA was reported in two of these studies, whereas the other seven, as well as the present study, failed to confirm these results.

DISCUSSION

In the present study we investigated the potential association between Y chromosome microdeletions and IRSA, studying the largest number of male partners of IRSA women yet published. We used the six STS loci recommended by the EAA/EMQN guidelines in the analysis and found that none of the IRSA or control men had microdeletions in the AZFa, AZFb, or AZFc regions, indicating that these do not contribute to the etiology of IRSA in a Slovenian population.

In addition, we searched the available literature and found that nine previous studies examined the relationship between Y chromosome microdeletions and IRSA. A positive correlation between Y chromosome microdeletions and IRSA was found in two of these studies, both of which were subject to much discussion owing to the disputable patient selection criteria and the methodology used for detection of microdeletions, which did not conform to the EAA/EMQN guidelines.

In the first study, Dewan et al. (17) found Y chromosome microdeletions set in the AZFb (DYS220/sY129) and/or AZFc (DYF85S1/sY150 and DYF86S1/sY152) regions in 14 of 17 IRSA men (82%). Another STS found to be deleted in IRSA men, the DYS262/sY67, is located outside the AZF region. These results indicate that IRSA men have multiple deleted regions on Y chromosome, which is highly unlikely in a normal male (23). Although Dewan et al. (17) reported to have used four STSs—one located in the AZFb region (DYS220) and three in the AZFd region (sY150, DYS236/sY152, and DYS237/sY153)—there is no confirmed evidence that the AZFd zone exists. Therefore, all STSs referred to as AZFd in this study correspond to the AZFc zone (26, 28, 29). Additionally, the authors did not use the five Yq STS markers recommended by Repping et al. (30) for further delineation of the AZFc deletions. Although the AZFc deletions are relatively frequent, the contribution of different partial AZFc deletions to male infertility varies and requires further genotype–phenotype correlations before indications can be given for their use in clinical routine (30, 31). Another weakness of the study by Dewan et al. (17) is the small number of patients who were selected from a tertiary referral center and were likely to have had failed evaluations and treatments before referral.

In the second study, Karaer et al. (19) reported that Y chromosome microdeletions were found in 7 of 43 men (16%). All of these men had microdeletions in the AZFb region (DYS220/sY129), whereas two men had a microdeletion in both the AZFb (DYS220/sY129) and AZFc (DYS237/sY153) regions. Piña-Aguilar et al. (29) pointed out that both of these deletions are polymorphic traits that cannot be considered true microdeletions but rather polymorphisms or methodologic mistakes not fulfilling the criteria for an accurate diagnosis (26, 28). Moreover, all of the IRSA men carrying microdeletions in this study had normal spermiograms, which is not typical of AZFb microdeletions.

In the remaining seven studies (8, 10, 18, 20–23) as well as in our study, no correlation was found between Y chromosome microdeletions and IRSA. Y chromosome microdeletions were evaluated according to the EAA/EMQN guidelines in all of these studies, and Bellver et al. (8), Ghorbian et al. (18), Kaare et al. (10), Piña-Aguilar et al. (21), and Wettasinghe et al. (23) used several additional STSs for the screening of AZFb and AZFc subdeletions. Although none of these studies confirmed the presence of Y chromosome microdeletions in peripheral blood lymphocytes, this does not exclude the possibility of sperm chromosome abnormalities arising through de novo mutations.

In conclusion, on the basis of our and previously reported results, it is unlikely that Y chromosome microdeletions are associated with IRSA and are therefore not recommended for the routine evaluation of IRSA couples.

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