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Original article

CHROMOSOMAL POLYMORPHISM IN BULGARIAN PATIENTS WITH REPRODUCTIVE PROBLEMS – ONE GENETIC CENTRE EXPERIENCE

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ABSTRACT

Chromosomal polymorphism is described as normal variants at chromosomal regions with no impact on the phenotype but a possible correlation to infertility and recurrent spontaneous abortions.

The **aim** of this study was to evaluate the effect of the chromosomal polymorphisms involved in families with reproductive failures in the Bulgarian population.

Material and methods: A total of 1733 patients with unexplained reproductive failures who visited the Laboratory of Medical Genetics – Varna, Bulgaria, (2004 - 2019) were investigated by conventional cytogenetic analysis GTG and CBG differential banding techniques and analyzed at the resolution 400-550 GTG bands.

Results: Chromosomal polymorphisms were found in 173 infertile patients (9,98%). The sex distribution was 6,52% males and 3,46% females. The most frequent finding was inv(9)(qh) (23,7%). The other chromosomal variants, which were found, consisted: 9qh+/- variants (15,1%); polymorphisms on the short arms of the acrocentric chromosomes (21,4%); 16qh+ (12,7%) and 1qh+ (6,9%). Y chromosome polymorphism was found in 27,4% of the males with polymorphisms. Two rare cases of polymorphism involving the centromere regions - 19qcenh+ and 20pcenh+ were also found.

Conclusion: There is growing evidence that polymorphisms may have a clinical impact on fertility and could take part in the etiology of RF. In this study, we found a significantly high percentage of polymorphisms (9,98%) among the tested patients, and they were more common among males. The statistical significance of increased incidence of chromosome variations found in our study emphasizes the need for routine evaluation of their role in families with RF in our country.

Keywords: chromosome polymorphism, variant, infertility, recurrent spontaneous abortions,

INTRODUCTION

Reproductive failure includes various common problems such as infertility (lack of pregnancy), recurrent miscarriages, stillbirths, births of children with multiple congenital anomalies and/or mental retardation.

Based on various studies, it has been found that infertility affects between 8-18% of couples of reproductive age. Each form of reproductive failure results from different molecular and cellular mechanisms, suggesting different pathways for research, diagnosis and treatment. Determining the cause of the problem in reproduction is not an easy task, so in many cases (30-40%), it is classified as having an idiopathic. Establishing parental karyotypes is part of the assessment made in cases of reproductive problems.

Chromosomal polymorphisms are described as normal variants at chromosomal regions with no impact on the phenotype, but they could correlate to infertility and recurrent spontaneous abortions (RSA). These regions consist of highly repetitive sequences of satellite DNA, which do not encode proteins. The repeated sequences, when are located on the same chromosome, could predispose to homologous unequal recombination leading to chromosomal micro-rearrangements, i.e. deletions, duplications and inversions, which can affect a clinical condition such as infertility and recurrent abortions.

This work **aims** to present one genetic laboratory experience in the evaluation of frequency, type and significance of chromosomal polymorphisms of Bulgarian patients with reproductive problems.

MATERIAL & METHODS

The study was conducted in the Laboratory of Medical genetics, University hospital "St. Marina", Varna, Bulgaria, within 15 year period (2004-2019). The design was the retrospective type of observation. The selection of patients was carried out based on documentary method (medical records) according to inclusion criteria (age over 16 years; reproductive problems - infertility, miscarriages, stillbirths, children born with disabilities and/or mental retardation; lack of anatomical, endocrinological and other conditions that would explain reproductive problems, family history on stillbirths, miscarriages, children born with malformations and/or men-

tal retardation, familial chromosomal rearrangement)

The study included 1733 patients (870 women and 863 men) aged 16-60 years with a documented clinical diagnosis of reproductive failures (repeated spontaneous miscarriages, missed abortion, infertility or other complications of pregnancy). The patients were referred by an obstetrician, andrologist or had visited the Office for genetic counselling (a division of the Laboratory of Medical Genetics) because of infertile problems.

According to the main objectives, patients were divided into three groups:

• Group I – patients with infertility with/without unsuccessful ART procedures, male factor (589);

• Group II – patients with two or more RSA (regardless of the presence or absence of a living and healthy child in the family) (743);

• Group III - patients with a combined reproductive history and patients with familial chromosomal rearrangement (401).

The participants in the study were Cell suspension was used to conduct the study. Conventional cytogenetic method (GTG and CBG differential banding techniques) and analysis of approximately 10 metaphases for each patient on the resolution 400-550 GTG bands were applied [1].

The processing of the collected primary information in connection with the performed research was supported by the possibilities of medical statistics, in particular (Variation analysis, Correlation analysis, Regression analysis, Graphical analysis). Software programs -Microsoft Excel 2016, Graph Pad Prism 9.

RESULTS

Chromosomal polymorphism was found in 9.98% (173 out of 1733 patients). The sex distribution was 113 (6,52%) males and 60 (3,46%) females. Men showed statistically significant (p=0.0001), twice as many polymorphisms (113/173; 6.52%) as women (60/173; 3.46%).

Chromosomal polymorphism of 9 chromosome

Polymorphism on chromosome 9 is present in the highest percentage – 3,9% (67/1733), 38.7% of all polymorphisms found. Inversion of the 9 heterochromatin block - inv (9)(qh) was the most common in a total of 41 patients – 2,37% (41/1733). Most patients with inv(9)(qh) were found in group II – 41,5% (17/41).

Inversion of the heterochromatin region was found simultaneously on both chromosomes 9 in two men of group II, with 2 and 4 abortions. An interesting variant of euchromatin involvement was found in a woman with long-term infertility - 46, XX, inv(9)(p13.1).

Rare variants, in which the p12 band is involved, were seen in two men: 46, XY, inv(9)(p12;q12) (with two abortions) and 46, XY, inv(9)(p12q11) (a father of a newborn with an immunodeficiency chromosome breakage syndrome). A difference in the size of the heterochromatin block (9qh+/-) was found in 26 patients (15%; 26/173): 9qh + variant with a frequency of 1,44% was highly predominant over 9qh- variant 0,05%, observed in a single patient with three abortions.

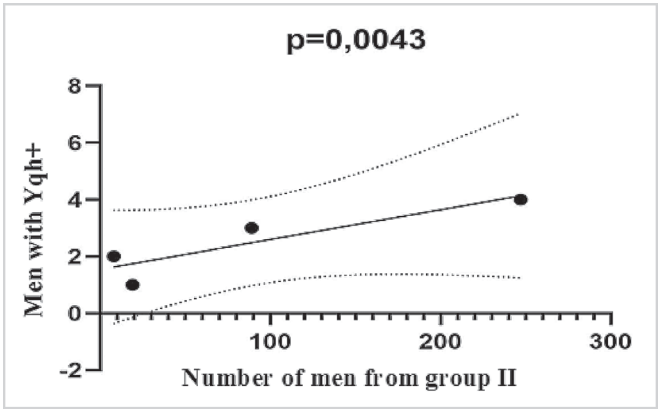
Chromosomal polymorphism of Y chromosome

We report a Y chromosome polymorphism in 31 patients – 3,6% (31/863 men with reproductive failure) presented only with variations in the length of the Y chromosome (no inversion was detected). Of these, the Yqh+ variant occupies 90.3% (28/31) and the Yqh- variant - only 9,7% (3/31).

The largest number of men with a Y chromosome polymorphism was from group I (infertility) (51,6%; 16/31 men), which correlates with data from the literature, as only three of the patients had a male factor.

Y chromosome polymorphism was presented in 32.3% (10/31) of patients from group II (with RSA) in our sample, all with Yqh+ variant. This variant correlated with statistical significance with the number of abortions (p = 0.0043) in group II (≥ 2 SPA) (fig.1).

Fig. 1. Distribution of Yqh+ by group II



Chromosomal polymorphism of acrocentric chromosomes

The acrocentric chromosome polymorphism was the second most common chromosomal polymorphism found in 21.4% (37/173), e.g. 2.1% (37/1733) of all patients analyzed. The most common chromosomal polymorphism is that on chromosome 21 (37.8% of all variants of acrocentric chromosomes). Polymorphisms on chromosome 15 and chromosome 22 show equal percentages (21.6% or 8/37).

Chromosomal polymorphism on chromosomes 1 and 16

Chromosomal polymorphism on chromosome 16, only a 16qh+ variant, was found in 22 patients (12.7%; 22/173), which represents 1.3% of all studied patients (22/1733). Patients with a polymorphism on chromosome 1 (only 1qh+ variant) were presented in the smallest proportion - 6.9% (12/173) of all detected polymorphisms or 0.7% (12/1733) of all patients examined.

Chromosomal polymorphism on two different chromosomes and rare variants

Polymorphism on two chromosomes was reported simultaneously in patients 46,XY,1qh+,Yqh+ (father of a child with trisomy 13) and 46,XX,1qh+,16qh+ (4 miscarriages). Rare polymorphisms on chromosome 19 centromere region (46, XX,19qcenh+) in a patient with 2 SPA and on chromosome 20 (46, XX,20pcenh+) in a mother of monosomy X child were observed.

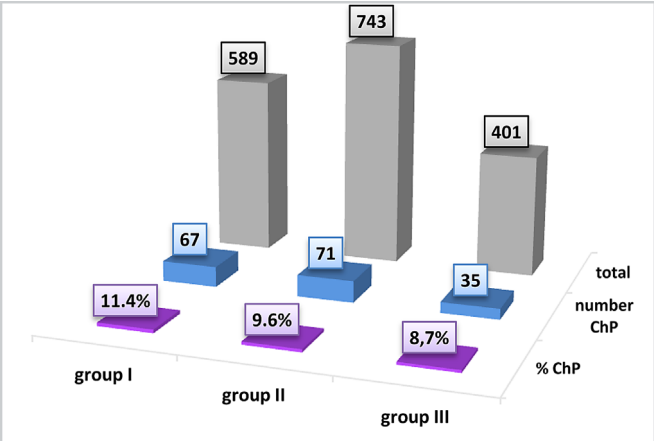
All polymorphisms found are presented in Table 1.

Table 1. Chromosomal polymorphism by sex and type

	FEMALE		MALE		p	TOTAL			p
	number	% by ChP	number	% by ChP		number	% by ChP (173)	%by total number (1733)	
Chromosome 1	5	2,9	7	4	0,7706	12	6,9	0,7	0,4376
Chromosome 9	23	13,3	44	25,4	0,0088	67	38,7	3,9	<0,001
Chromosome 16	10	5,8	12	6,9	0,8266	22	12,7	1,3	0,2544
AX	19	11	18	10,4	>0,9999	37	21,4	2,1	0,5455
ChP on 2 chromosomes	1	0,6	1	0,6	-	2	1,2	0,1	-
ChP **19qcenh+** **20pcenh+**	 1 1	 0,6 0,6	-	-	-	 1 1	 0,6 0,6	 0,05 0,05	-
Chromosome Y	-	-	31	17,9	-	31	17,9	1,8	0,05
TOTAL	60	34,7	113	65,3		173	100	9,98	p<0,001

The share of persons with chromosomal polymorphism, related to the total number of studied patients from each group, is the highest in group I – 11,4% (67/589 patients); followed by group II – 9,6% (71/743); and group III – 8,7% (35/401) (fig.2).

Fig. 2. Distribution of the chromosomal polymorphisms (ChP) by groups



The highest percentage in group I comes from patients with male factor - 21% (12/57), significantly higher frequency compared to the other patients from this group (p = 0,0044). The highest proportion of chromosomal polymorphism from group II is presented in equal percentage in patients with 4 spontaneous abortions (22,5%; 9/40 persons) and ≥ 5 spontaneous abortions (22,3%; 4/18). The analysis of hypotheses showed that with a higher number of abortions increases the possibility of detecting chromosomal polymorphism in our sample of patients (p = 0,0044).

DISCUSSION

The frequency of chromosomal polymorphism is close to that described in the literature (10-15%) [2,3] and was statistically significant (p <0.001) twice as much as in this found in the normal population (2-5%) [2, 3]. It varies widely between individuals and different population groups. Our data is a one-way trend with data from Turan, GA, et.al.[4] explained by Y chromosome variants, which are thought to have an adverse effect mainly on spermatogenesis and a corresponding negative impact on the outcome of ART procedures.

The percentage of polymorphism we found on chromosome 9 (3,9%) is statistically significantly higher (p <0.001) than in the normal population (1.5%) [5], as the inversion of the 9 heterochromatin block is slightly higher than reported by other studies –1,5% [6]. The higher percentage of chromosomal polymorphism of 9 chromosomes found in patients with RSA correlates with reported data from other studies, where this inversion is mainly associated with a higher number of abortions, i.e. can lead to the formation of genetically unbalanced gametes in the process of fertilization (with lethal deletions or large duplications), resulting in miscarriages [7].

The involvement of the rare variant, which includes the p12 band, assumes the presence of several recombination hotspots, and also, the constitutional inversions involving the pericentromeric region of chromosome 9 have breakpoints located in this region. It is the finding of these breakpoints in regions with a repetitive sequence, such as 9p12, that is important and may explain why such inversions have no clinical consequences other than infertility and RSA [6]. Data on 9qh + (1,44%) and 9qh- (0,05%) variants found in this study was comparably lower than that cited in related studies - 3,67% [8] and 8% [6] for the 9qh+ and B 1% for 9qh- [6] respectively. Some authors report an even higher frequency

of the 9qh+ variant in patients, assuming that large heterochromatin blocks can cause chromosomal abnormalities and meiotic retention, leading to abortion [9, 10].

The Y chromosome shows wide limits of variation not only between individuals but also between different population groups, and data on the clinical significance of Y chromosome polymorphisms in infertility are still conflicting. Similar to our study (90,3% for Yqh+) the most common form concerns the length of the heterochromatin region of the Y chromosome, mainly the Yqh+ variant: 91.9% [9];86% [11]; 90% [3]. The large heterochromatin block in the Yqh+ variant is thought to play a significant role in the reproduction process and is associated with suppression of gene expression, especially genes related to spermatogenesis and fertility, and hence its influence on the adverse outcome of ART procedures. On the other hand, alternating heterochromatin and euchromatin sequences in the male-specific region by inducing epigenetic changes are likely to be the reason for the association of heterochromatin variants of the Y chromosome with infertility [12]. Y chromosome polymorphism is prevalent mainly among infertile men – 29.2% [9]; 30.7% [13]; 65.1% [11], and this correlates with our study results, where we also find a higher percentage in patients with infertility (51,6%). Xu X, et al., 2016 [3], Nagvenkar P, et al., 2005 [13], Guo T, et al., 2012 [11], report Y polymorphism mostly in patients with established male factor - severe oligospermia, azoospermia, which has been suggested to have detrimental effects on male infertility by affecting a variety of physiological processes, including spermatogenesis and sperm quality. We found only three patients with confirmed male factor, assuming insufficient clinical data in our records and/or referral.

The increased presence of Y chromosome polymorphism has also been reported in couples with RSA [2, 13, 14]. In our study, the second most common Y chromosome polymorphism was the RSA group (32,3%) because this variant correlated with statistical significance with the number of abortions it is supposed to be associated with an increased risk of miscarriage given the role of heterochromatin during meiosis [13].

The frequency of the acrocentric chromosome polymorphism (2,1%) found in this study is close to that reported by Hussen DF et al. [15]. In our study, chromosome 15 polymorphism is not the most common one in contrast to that reported by Boronova et al.[16] (2.2%) and Turan GA, et al. [4] (1.7%) but is twice as high (4.62%, 8/173). According to the literature, large satellites in the short arms of acrocentric chromosomes can lead to improper chromosomal segregation during meiosis and hence to fetal loss, which is why they are more important in the study of pairs with RSA [17]. Also, the heterochromatin region around the centromeres of acrocentrics is thought to play an important role in their attachment to the dividing spindle, chromosome pairing, and cell division. Thus, disruption in these heterochromatic regions may, in fact, have implications for gene expression affecting gamete formation, fertilization, and embryogenesis [18]. Moreover, this disruption can lead to a defect in chromatid cohesion, which usually induces missegregation, increasing the risk of chromosomal aneuploidy [15].

The frequencies of chromosomal polymorphism on chromosomes 1 (0,7%) and 16 (1,3%) were similar to those reported by other studies, 1.04%[4] and 0.4%[19], respectively. The 1qh+ variant occurs with increased frequency in infertile women and men with azoospermia and is associated with multiple abortions [9]. Regarding the 16qh+ variant, our and literature data confirm no difference in the frequency in patients with RF and normal population, which suggests that this polymorphism has virtually no effect on human reproduction [10].

Chromosomal polymorphism on two different chromosomes are rare variants, probably has an aggravating circumstance on reproduction.

The highest share of people with a chromosomal polymorphism in group I (11.4%) was similar to the results of a study by Turan GA et al. [4], which reported a frequency for a group with infertility of 11,7%. However, with regard to the results reported for the other two groups in the same study, we note a difference - our values are three times higher for the group with RSA (9,6% compared to 2,9%), and vice versa, twice lower for persons from a group with a combined reproductive history (8,7% compared to 17,7%). The differences between the two studies could be explained by the population-genetic differences of the studied samples. These authors explain the importance of polymorphisms by the presence of a mechanism involving transcriptional activation of genes in the heterochromatic regions of chromosomes, which may be responsible for the association of polymorphic variants with a given clinical condition by including epigenetic mechanisms of gene regulation and control. The highest percentage found in male factor patients (21%) in our sample of patients also correlated with data from other similar studies [20], indicating its role in spermatogenesis [4].

CONCLUSION

Regarding the frequency, characteristics and significance of chromosomal abnormalities in patients with reproductive failure, the conclusions are statistically significant that:

• Chromosomal polymorphism is found more often (9.98%) in patients with reproductive failure compared to the general Bulgarian population, therefore it should be considered as a factor of reproductive importance; men show twice as high polymorphism as women

• The polymorphism on chromosome 9 has the highest frequency mainly as inv (9)(qh); mainly in group II (RSA), which confirms the association mostly with recurrent pregnancy losses;

• Y chromosome polymorphism is second in frequency, mainly as Yqh+ variant (90.3%), mainly in group I (infertility); the role of the Yqh+ variant is also confirmed in group II (recurrent abortions), as the number of abortions increases statistically significantly and the

probability of the presence of the Yqh+ variant

• The number of polymorphisms distributed by type of reproductive failure established statistical significance in participants from group II and group I over group III.

There is growing evidence that heterochromatin regions contain a significant amount of repetitive DNA, which is heterogeneous, and chromosomal variants are an expression of morphological variability associated with changes in the amount of heterochromatin. It is thought that heterochromatin in chromosomal polymorphisms can regulate gene expression by a reversible transformation between heterochromatin (non-coding DNA sequences) and euchromatin (expressed DNA sequences). It has been suggested that chromosomal heteromorphisms may play a crucial role in genomic regulation and modulation during reproduction. It is a complex biological process that requires genomic regulation and expression at different levels. This could, to some extent, clarify the lack of a clinical phenotype in carriers of heteromorphic variants outside of infertility or RSA. It is also assumed that hidden functional genes are located in the heterochromatin regions of the long arms (q) of chromosomes 1, 9, 16, and Y. They are expected to regulate cellular function in the reproductive process, and thus heterochromatin variants of these regions may be associated with reproductive failure.

The statistical significance of increased incidence of chromosome variations found in our sample of 1733 patients with RFemphasizes the need for their routine evaluation and interpretation in cytogenetic reports and consultations of these patients.

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