

Short Commentary

Cytogenetic Analysis and Thrombophilia-Associated Gene Mutations of Couples with Recurrent Miscarriage

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Abstract

Introduction: Three or more pregnancy losses before 20 weeks of gestation are usually defined as recurrent miscarriage. Parental chromosomal translocations, thrombophilic gene polymorphisms, autoimmune factors, uterine, endocrine factors associated with recurrent miscarriage (RM). Factor V Leiden, prothrombin gene mutation G20210A, protein S/Protein C/antithrombin deficiency and MTHFR mutations responsible for hereditary thrombophilia and has been included to keep a very common practice of RM pathogenesis. This study aimed to determine the incidence of these factors believed to be the effects of RM.

Materials and methods: All patients were took a full genetic analysis; full genetic examination and pedigree drawing was done to exclude known nonchromosomal causes of the anomaly. Cytogenetic analysis was done for 635 patients. The study included peripheral lymphocyte culture by a standard method using Leishmann-banding technique, centromerebanding (C-banding), and nucleolar organizing region staining was done when needed. Pyrosequencing was used to genotype the 392 individual.

Results: Male/female in patients with an inherited thrombophilia tested four types of changes, including; MTHFR C677T/1298, FV Leiden G1691A and prothrombin G20210A. Among them, 152 men and woman did not carry any mutation.

Keywords: Cytogenetics; Pyrosequencing; Recurrent miscarriage

Abbreviations: RM: Recurrent Miscarriage; MTHFR: Methylenetetrahydrofolate Reductase; C-banding: Centromeric Banding; FISH: Fluorescent *In Situ* Hybridization; CMA: Chromosomal Microarray Analysis; CNVs: Copy Number Variants; G-banding: Giemsa Banding; SNP: Single Nucleotide Polymorphism; PCR: Polymerase Chain Reaction; KCL: Potassium Chloride

Introduction

As the loss of three or more pregnancies before 20 weeks of gestation is generally defined recurrent miscarriage [1,2] And that problem affects 1–5% of the reproductive age population and is costly for patients/health care systems since the etiology generally remains unknown [3]. In human recurrent spontaneous abortion is a general phenomenon and cytogenetical rearrangements are important reason for this phenomenon [4]. Parental chromosomal translocations, thrombophilic gene polymorphisms, autoimmune factors, uterine, endocrine factors associated with RM. 40% of couples with a history of RM have no detectable factors so; they are classified as having idiopathic RM [5].

Half of the sporadic miscarriages are caused by fetal chromosome abnormalities. Cytogenetic analysis has been shown that a large portion of numerical chromosomal abnormalities anomalies (86%) and structural chromosomal abnormalities in a small proportion of cases (6%) and in addition to mosaic chromosomal abnormalities (8%) [6]. Protein S/Protein C, Factor V Leiden, prothrombin G20210A and antithrombin deficiency and methylenetetrahydrofolate reductase (MTHFR) gene changes in the pathogenesis of RM has been a very common practice. RM etiologies of inherited thrombophilia in the literature there are conflicting studies about the possible role. There was a statistically significant correlation between the cytogenetic abnormalities with recurrent miscarriages reported in a study by Choi et al. [7].

Materials and Method

Chromosomal abnormalities in patients with two consecutive

spontaneous abortions reported a high rate for this reason we have included the study of this group patients. Couples were included in the study with a loss of 2 or 3 pregnancies before 20 weeks of gestation. This study evaluated the relationship between parental chromosomes and four types of hereditary thrombophilia frequency; in male and female patients. All patients were took a full genetic analysis; full genetic examination and pedigree drawing was done to exclude known non-chromosomal causes of the anomaly. Cytogenetic analysis was done for 635 patients and 392 persons included for thrombophilia panel (MTHFR C677T-1298/FV Leiden G1691A/Prothrombin G20210A). The study included peripheral lymphocyte culture by a standard method using Leishmann-banding technique, centromere-banding (C-banding) and nucleolar organizing region staining was done when needed. It was incubated with peripheral blood lymphocytes cultured for 72 hours at about 0.5-1 ml. Lymphocyte cultures were treated with hypotonic KCl solution to obtain metaphase then were harvested by addition of colcemid for 45 min (0.075 M) treatment for 4 min and fixation using standard fixative prepared with methanol and acetic acid (3:1) [8,9]. At least 25 metaphases were scored for each patient. The best metaphases were karyotyped and the total chromosome count was usually determined in 25 cells. If mosaicism was suspected, then 100 or more cell counts were carried out for documentation for abnormal cases. Fluorescent in situ hybridization (FISH) was used if needed and the international system for human cytogenetic

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nomenclature were used for the nomenclature of human chromosomes. We used pyrosequencing to genotype 392 individuals. Pure genomic DNA obtained from peripheral blood which received EDTA tubes were performed by MagNA Pure LC DNA Isolation Kit (Roche) or manually. We chose pyrosequencing for thrombophilia panel analysis. This method, compared with conventional sequencing, including all reagents and PCR is a fast and cost-effective. SNP genotyping is also due to the high efficiency more effectively to today's challenges [10].

Results

Our patients group were calculated relative frequency; the percentage of patients with abnormal distribution were determined and the numerical and structural abnormalities (Tables 1-4). Frequencies of those values were compared with the parallel studies 392 males and females were tested for four types of hereditary thrombophilia's; MTHFR C677T/1298 polymorphisms, FV Leiden G1691A mutation and Prothrombin G20210A mutation. Among these, 152 of the men or women did not carry any of these mutations (Table 5). The most common abnormality was heterozygous for the mutation MTHFR 1298 polymorphisms in this group (n=184).

The identified polymorphic chromosomal variants		
S. No.	Variants	No. of cases
1	9qh+	32
2	Inv(9)(p11q13)	7
3	Inv(9)(p12q13)	3
4	1qh+	9
5	21ps+	7
6	Yqh+	5
7	Yqh-	4
8	22ps+	4
9	15ps+	4
10	16qh+	3
11	9qh-	2
12	14ps+	1
13	22pst+,ps+	1
14	15pst+,15cen+	1
15	1qh+,9qh+	1
16	6cen+	1
17	9qh+,15ps+	1
18	Inv(9)(p12q13),9qh+	1
19	15cen+	1

Table 1: The identified polymorphic chromosomal variants.

S. No.	Structural chromosomal abnormalities identified in this study
1	46,XY,t(1;8)(q25;q22)
2	46,XX,t(1;6)(p11;q11)
3	46,XY,t(6;14)(p23;q24)
4	46,XY,t(4;10)(p14;p13)
5	46,XX,t(11;18)(p13;q11)
6	46,XY,inv(5)(p15q31)
7	46,XX,t(11;18)(p13;q11)
8	45,XX,rob(13;14)(q10;q10)
9	46,XY,t(2;3)(q35;q25)
10	46,XX,t(1;9)(p36;q31)
11	46,XX,t(8;21)(q23;q22)
12	46,XY,t(19;20)(p13;12)
13	46,XY,t(1;2)(q25;p13)

Table 2: Structural chromosomal abnormalities identified in this study.

Authors	Total cases	Total abna.	Structural	Numerical	Variants
Dubey et al.	1484	31	22	9	21
Akgul et al.	179	21	3	15	3
Tavokina et al.	420	46	41	5	-
Rajangam et al.	3332	83	49	34	79
Espino et al.	1832	52	43	9	17
Our cases	635	20	13	7	88

Table 3: Review of literature in reproductive disorders.

S. No.	Numerical chromosomal abnormalities identified in this study
1	47,XXY[3], 46,XY[47]
2	45,X[3]/47,XXX[2]/49,XXXXX[1]/46,XX[94]
3	45,X[6]/46,XX[94]
4	45,X[5]/46,XX[95]
5	45,X[4]/46,XX[96]
6	45,X(3)/46,XX (97)
7	45,X[3]/46,XX[49]

Table 4: Numerical chromosomal abnormalities identified in this study.

	Protrombin	FV Leiden	MTHFR 677	MTHFR 1298
Heterozygous mutant	18	1	168	184
Homozygus mutant	0	41	40	56
Homozygus normal	374	350	184	152

Table 5: The genotypes of polymorphisms in this study.

Discussion

Most of chromosomal abnormalities detected in embryos and fetuses will result in spontaneous abortion Fetal chromosomal abnormalities associated with 50% of early gestation spontaneous abortion. The genetic etiology for multiple spontaneous pregnancy loss includes an unbalanced chromosome rearrangement, which may be the result of one parent being a carrier for a balanced chromosome rearrangement [4]. Sporadic abortion affects 25% women and can occur as a single isolated event. The relationship between with chromosomal abnormalities and early pregnancy loss have been established [11,12]. Other research groups all over the world apply these techniques in the late 1980s-1970s.

Chromosomal polymorphisms or variants of different sizes can be observed microscopically, are the areas showing the morphology and staining properties. It has no effect on the phenotype. A Mendelian inherited style and often found in highly variable regions chromosomes 1, 9, 16, away from the long arm of the Y chromosome and two-thirds acrocentric chromosome short arms and satellites [13,14]. In this study, the polymorphism of heterochromatic region 9qh+ was determined about 5%. This ratio is higher according to other polymorphisms. And total polymorphisms ratio is 13% in our study and total polymorphisms ratio is 635/88=13% in our study. According to reported literatures our balanced translocation results are less frequently (635/13=2%) (Table 3 and Figure 1). RM is a complex syndrome which seems to increase in frequency by thrombophilic gene aberrations. We carried out multiple studies to identified combinations of polymorphisms that could lead to an increased risk for RM [15]. Thrombophilia disorders develop because of irregular placental perfusion. For this reason pre-eclampsia is occurred. Consequently late fetal loss during pregnancy and pre-eclampsia can be seen. But the association of hereditary thrombophilia's between early pregnancy losses is not identified yet. The roles of inherited thrombophilia have been studied mostly in RM and accumulated a large amount of contradictory literature about the subject. For example, Karata et al. recently, compared the prevalence of factor V G1691A Leiden, Prothrombin G20210A, and MTHFR C677T mutations in patients with

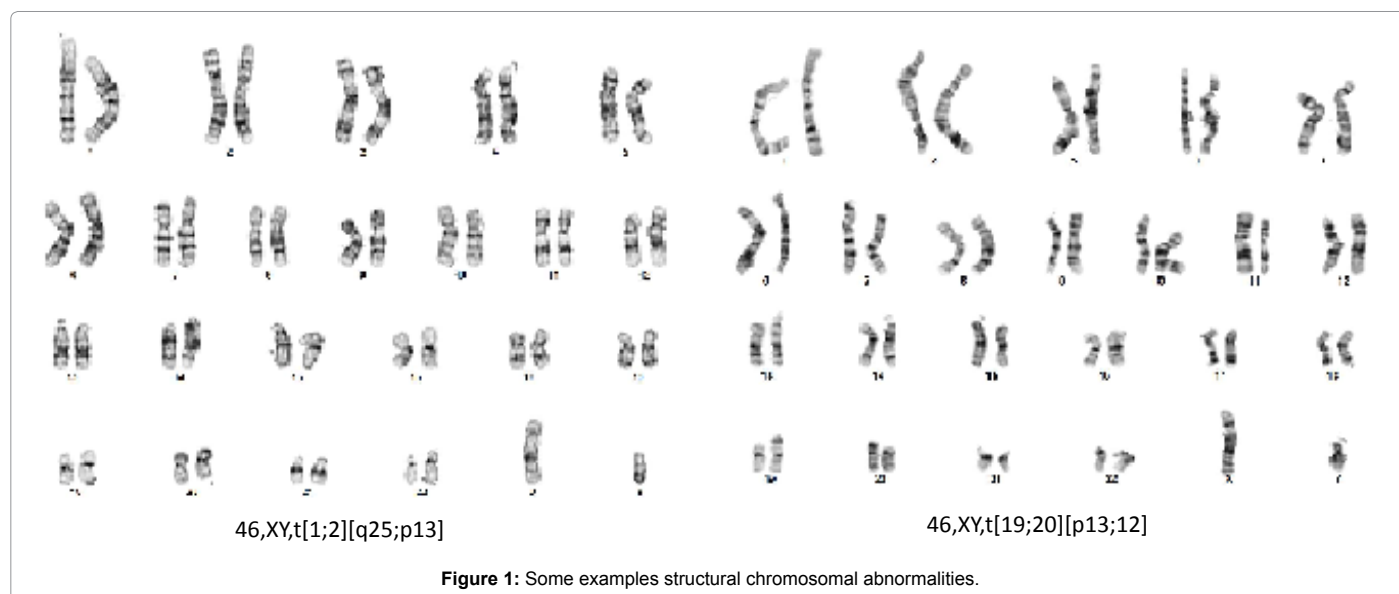


Figure 1: Some examples structural chromosomal abnormalities.

recurrent miscarriages (RM) [16]. They found an association between MTHFR C677T homozygous and heterozygous mutations with RM. G20210A and factor V G1691A was different between groups neither homozygous nor heterozygous mutations [17].

As hemimethylated promoters and monoallelic expression of CGB5 has been found in patients with recurrent miscarriage (RM), aberrant DNA methylation levels at a certain gene locus might directly lead to this disease [18]. In addition, since the levels of histone methyltransferases G9a and its associated heterochromatin marker H3K9me1/2 are significantly reduced in some cases of RM, not only the genetic mutants but also the epigenetic mutants may contribute the occurrence of RM. Interestingly, since recent study has found that G9a also plays a role in the maintenance of DNA methylation at imprinted loci in mammals, RM might be an imprinted gene associated human disorders [19,20]. Therefore, for the diagnosis of RM, it is worth to investigate the expression patterns and levels of imprinted genes in the prospective patients.

Recently, chromosomal microarray analysis (CMA) has become more commonly used. Without the need for cell culture CMA provides genome analysis with a greater resolution than traditional karyotyping standards. CMA can detect small genetic gains and losses as well. This microduplications and microdeletions in general 'copy number variants' (CNVs) are named. So as a continuation of our work, we planned the chromosomal microarray analysis for detect genomic copy number variants in couples and products of conceptions [21].

References

- Royal College of Obstetricians and Gynaecologists, 2003.
- The European Society of Human Reproduction and Embryology, 2006.
- Grande SW, Andrade AJ, Talsness CE, Grote K, Golombiewski A, et al. (2007) A dose-response study following in utero and lactational exposure to di-(2-ethylhexyl) phthalate (DEHP): Reproductive effects on adult female offspring rats. *Toxicology* 229: 114-122.
- Asgari A, Ghahremani S, Saeedi S, Kamrani E (2013) The study of chromosomal abnormalities and heteromorphism in couples with 2 or 3 recurrent abortions in Shahid Beheshti Hospital of Hamedan. *Iran J Reprod Med* 11: 201-208.
- Karaman A, Ulug P (2013) Cytogenetic analysis of couples with recurrent miscarriages: A series of 316 cases. *The New Journal of Medicine* 30: 30-32.
- Van den Berg MM, Van Maarle MC, Van Wely M, Goddijn M (2012) Genetics of early miscarriage. *Biochim Biophys Acta* 1822: 1951-1959.
- Choi TY, Lee HM, Park WK, Jeong SY, Moon HS (2014) Spontaneous abortion and recurrent miscarriage: A comparison of cytogenetic diagnosis in 250 cases. *Korean Society of Obstetrics and Gynecology* 57: 518-525.
- Hardy K, Hardy PJ (2015) 1st trimester miscarriage: four decades of study. *Transl Pediatr* 4: 189-200.
- Dutrillaux B, Couturier J (1981) The ancestral karyotype of platyrrhine monkeys. *Cytogenet Cell Genet* 30: 232-242.
- Verri A, Focher F, Tettamanti G, Grazioli V (2005) Two-step genetic screening of thrombophilia by pyrosequencing. *Clin Chem* 51: 1282-1284.
- Jonasson J, Therkelsen AJ, Lauritsen JG, Lindsten J (1972) Origin of triploidy in human abortuses. *Hereditas* 71: 168-171.
- Kajii T, Ohama K, Niikawa N, Ferrier A, Avirachan S (1973) Banding analysis of abnormal karyotypes in spontaneous abortion. *American Journal of Human Genetics* 25: 539-547.
- Petrova-Tacheva V, Alekova S, Popov B, Ivanov V (2014) Cytogenetic analysis of couples with repeated spontaneous abortions. *Trakia Journal of Sciences* 12: 238-240.
- Ivanov P, Gecheva S, Tsvyatkovska T, Georgieva G, Komsa-Penkova R et al. (2012) Inherited thrombophilic factors in women with secondary infertility. *Akush Ginekol (Sofia)* 51: 3-7.
- Torabi R, Zarei S, Zeraati H, Zarnani AH, Akhondi MM et al. (2012) Combination of thrombophilic gene polymorphisms as a cause of increased the risk of recurrent pregnancy loss. *J Reprod Infertil* 13: 89-94.
- Karata S, Aydin Y, Ocer F, Buyru A, Balci H (2012) Hereditary thrombophilia, anti-beta2 glycoprotein 1 IgM, and anti-annexin V antibodies in recurrent pregnancy loss. *Am J Reprod Immunol* 6: 251-255.
- Ocak Z, Özlü T, Ozyurt O (2013) Association of recurrent pregnancy loss with chromosomal abnormalities and hereditary thrombophilias. *African Health Sciences* 13: 447-452.
- Uuskula L, Rull K, Nagirnaja L, Laan M (2011) Methylation allelic polymorphism (MAP) in chorionic gonadotropin beta5 (CGB5) and its association with pregnancy success. *J Clin Endocrinol Metab* 96: 199-207.
- Fatima N, Ahmed SH, Salhan S, Rehman SM, Kaur J et al. (2011) Study of methyl transferase (G9aMT) and methylated histone (H3-K9) expressions in unexplained recurrent spontaneous abortion (URSA) and normal early pregnancy. *Mol Hum Reprod* 17: 693-701.
- Zhang T, Termanis A, Özkan B, Bao XX, Culley J (2016) G9a/GLP Complex maintains imprinted DNA methylation in embryonic stem cells. *Cell Reports* 15: 77-85.
- Separovic R, Diego-Alvarez D, Robinson WP, Tyson C, Qiao Y (2010) Identification of copy number variants in miscarriages from couples with idiopathic recurrent pregnancy loss. *Hum Reprod* 25: 2913-2922.