An Overview of Genetic and Molecular Factors Responsible for Recurrent Pregnancy Loss

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ABSTRACT Recurrent pregnancy loss usually results from disorders that cause intrauterine fetal damage, such as maternal or paternal chromosomal abnormalities. About 15 to 20 percent of all recognized pregnancies end in a first- trimester spontaneous abortion. Parents who are carriers of structural abnormalities have a higher risk of miscarriage because of the aberrations in genetic information may not segregate properly into the reproductive cells. This may be due to translocations, inversions, deletions, and duplications causing pregnancy loss. It is believed that between 3 and 5 percent of recurrent miscarriages are due to genetic factors, about 7 percent are caused by chromosome defects, 15 percent to hormonal defects, and 10 to 15 percent to anatomical defects. The most common cause of early pregnancy losses are chromosomal abnormalities that occur by chance, except in the case of parental chromosomal rearrangements and are not under any controllable influences. This review focuses on the genetic and molecular abnormalities that may contribute to this clinical problem and delineates strategies for genetic evaluation and clinical management in subsequent pregnancies

INTRODUCTION

Pregnancy loss is a common problem which results in the spontaneous loss of a pregnancy before 24 weeks' gestation, when the foetus has a chance of survival outside the womb. Recurrent Pregnancy Loss (RPL) is defined as two or more consecutive spontaneous abortions. RPL affects about 1 percent of the child-bearing population and presents couples with this disorder of formidable challenge in successfully having a family (Ford and Schust 2009). Although a few cases of RPL are sporadic, most couples experience genetic basis as a challenging clinical dilemma. A variety of possible etiologies have been described for both sporadic and RPL (Stray et al. 1984). Using the very sensitive pregnancy tests that are available now, it is known that one out of two pregnancies end in very early miscarriage (Macklon et al. 2002). In the past the majority of these would have been passed off as late or heavy menses. Even after a clinically diagnosed pregnancy, one out of 5- 6 pregnancies end in a miscarriage between 4th and 20th week of gestation (*Gracia et al. 2005*). This review focuses on the genetic abnormalities and molecular factors that may contribute to this clinical problem and delineates strategies for genetic

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evaluation and clinical management in subsequent pregnancies.

The proposed causes of RPL are parental chromosomal abnormalities, uterine anatomic anomalies, endometrial infections, endocrine etiologies (leuteal phase defect, thyroid dysfunction, uncontrolled diabetes mellitus), antiphospholipid syndrome, inherited thrombophilias, alloimmune causes and environmental factors (Kutteh 1999) as mentioned in Table 1. Among the various proposed etiologies, genetic factors appear to be highly associated with reproductive loss (Byrne et al. 1994; Sierra and Stephenson 2006).

Table 2 shows numerical and structural variations in the chromosomes resulting RPL. Chromosome abnormalities are the cause for pregnancy loss in 50 to 80 percent of cases, depending on maternal age and gestational age at time of the loss (Hogge et al. 2003). Cytogenetically ab-normal embryos are usually aneuploid because of sporadic events, such as meiotic nondisjunction, or polyploid from fertilization abnormalities. Incidence of numerical and structural chromosome abnormalities in spontaneous abortuses is reported to be almost half (47.9%) ,9.8 percent, polyploid (mostly triploid) 8.6 percent 45, X, and 26.8 percent trisomic for one or another chromosome (Miller and Therman 2001).

Autosomal trisomies are involved in 50 percent of the cytogenetically abnormal abortuses

Table 1: Etiologies of pregnancy loss

Genetic Causes	o Aneuploidy
	o Somatic
	o Sex chromosome
	o Mendelian disorders
	o Multifactorial disorders
	o Parental chromosomal abnormalities (translocations)
	o Chromosomal inversions. (Sullivan et al. 2004)
Immunologic Causes	o Autoimmune causes (Festin et al. 2007)
	o Alloimmune causes (Porter and Scott 2000)
Anatomic Causes	
	o Uterine müllerian anomaly
	o Uterine septum (the anomaly most commonly associated with pregnancy loss)
	o Hemiuterus (unicornuate uterus)
	o Bicornuate uterus
	o Diethylstilbestrol-linked condition
	o Acquired defects (eg, Asherman syndrome)
	o Incompetent cervix
	o Leiomyomas
	o Uterine polyps. (Saravelos and Cocksedge 2010.)
Infectious Causes	*Listeria monocytogenes, Toxoplasma gondii*, rubella, herpes simplex virus (HSV), measles, cytomegalovirus, and coxsackieviruses. (Ford and Schust 2009).
Environmental	o Smoking
	o Excessive alcohol consumption
	o Caffeine (Vibeke 2003)
Endocrine Factors	
	o Diabetes mellitus
	o Antithyroid antibodies
	o Luteal phase deficiency (Arredondo and Noble 2006)
Hematologic Disorders	
	o Thromboplilia (Robertson et al. 2006)

Table 2: Numerical and structural chromosomal variations in the abortus resulting in recurrent pregnancy loss

Numerical variations in chromo- somes	Trisomy 21 Trisomy 13 Trisomy 18 45, XO 47, XXY 45,X/46,X 45,X/47,XXX 45,X/45,i(X)/46,XY	Stephenson et al. 2002 Diego-Alvarez et al. 2006. Dubey et al. 2005
Structural variations in chromo- somes	46,XX/46X,r(X) 46,XX/47,XX,+17 46,XY/47,XX,+marker 46,XX,dup(4) (12q-q21) 46,XY,del(12) (q13-q14) 46,XX,t(7;14) (q36;q11.2) 46,XY,inv(9) (p11;q13) 46,XX,22pstk+* 46,XX,9qh+* 46,XX,13ps+,14ps+* 46,XX,14ps+* 46,XY,14ps+* 46,XX,15p+* 46,XX,21ps+* 46,XX,22ps+* 46,XY,14ps+,21ps+*	Vajira et al. 2009 Dubey et al. 2005

Dup- duplication, del-deletion, inv- inversion, *Polymorphic chromosomal variants
This table referes only to the Numerical and structural chromosomal variations in the abortus resulting in recurrent pregnancy loss. The other factors ar mentioned in the manuscript.

in the first trimester as summarized in Table 3. They may arise *de novo* because of meiotic non-disjunction during gametogenesis in parents with normal karyotypes (Lebedev and Nazarenko 2001). Viable trisomies have been observed for chromosomes 13, 16, and 21. Turner syndrome is frequently observed and most common sex chromosomal abnormality which accounts for 20-25 percent of cytogenetically abnormal abortuses (Uematsu et al. 2002). Triploidy and tetraploidy are related to abnormal fertilization and are not compatible with life. Triploidy is found in 16 percent of abortions, with fertilization of a normal haploid ovum by dispermy as

the primary pathogenic mechanism (Zaragoza et al. 2000). Tetraploidy occurs in approximately 8 percent of chromosomally abnormal abortions, resulting from failure of an early cleavage division in an otherwise normal diploid zygote. Structural chromosomal abnormalities occur in approximately 3 percent of cytogenetically abnormal abortuses. These problems in men often leads to low sperm concentrations, abnormal sperms or teratozoospermia male infertility, and therefore, a reduced likelihood of pregnancy and increased miscarriage (Puscheck et al. 2007).

Translocations are the most common types of structural abnormalities and can be balanced or unbalanced (Midro et al. 1991). Slightly more than one half of unbalanced rearrangements result from abnormal segregation of Robertsonian translocations and the rest arise *de novo* during gametogenesis. In reciprocal translocations, there is an exchange of material between non-homologous chromosomes (Ogasawara et al. 2004). The unbalanced distribution of the chromosomes involved in the translocation, leads to partial trisomy for one chromosome and partial monosomy for the other chromosome. Balanced

Table 3: Structural chromosome abnormalities of the carrier couples with repeated miscarriage

	Female	Male	References
Reciprocal Translocations	46,XX,t(14;X)(q11;q12)	46,XY, t(5;12)(p15.1;p12.2)	MED FA 2011
	46,XX, t(7;10)(p21;p13)	46,XY, t(6;17)(q21;q24.2)	Stephenson 2006
	46,XX, t(4;6)(q35.2;q12)	46,XY, t(8;10)(p21.3;q24.3)	Mokate et al. 2006
	46,XX, t(3;6)(q12;q12)	46,XY, t(1;17)(p36.2;q23.1)	Goddijn 2004
	46,XX, t(4;6)(q31.3;q21)	46,XY, t(5;9)(q23.2;q22.3)	Sugiura-Ogasawara 2004
	46,XX, t(8;11)(q11.23;q24.2)	46,XY, t(11;13)(p13;q22.3)	
	46,XX, t(2;6)(q33;q23)	46,XY, t(8;18)(q11.2;q21.3)	
	46,XX, t(7;18)(q11.21;q21.1)	46,XY, t(7;13)(p13;q21.2)	
	46,XX, t(2;4)(q36.3;q13.3)	46,XY, t(6;13)(q10;q10)	
	46,XX, t(4;9)(q35;q31)	46,XY,t(5;14)(q11.2;q32.1)	
	46,XX, t(1;6)(q42.1;q24.2)	46,XY,t(5;7)(p13;p15)	
	46,XX, t(8;12)(q22;q22)	46,XY,t(1;6)(p36.1;p22.1)	
	46,XX, t(4;15)(q33; q26)	46,XY, t(8;17)(q24.1;p13.1)	
	46,XX, t(7;10)(q31.2;q23.2)	46,XY,t(1;3)(q21;q25)	
	46,XX, t(11;12)	46,XY,t(2;13)(q35;q32)	
	46,XX,t(2;12)(q13;q24.31)	46,XY,t(5;12)(q35.1;q24.1)	
	46,XX, t(3;19)(q25.1;q13.3)	46,XY,t(5;17)(q33.1;q25.3)	
	46,XX,t(1;7)(q32.1;q32)	46,XY,t(6;16)(q25.3;p13.3)	
	46,XX,t(1;10)(q23;22.3)	46,XY,t(8;12)(p23.1;p13.3)	
	46,XX,t(1;11)(p34.3;q13)	46,XY,t(10;13)(q23.3;q13)	
	46,XX,t(1;16)(p35.3~p36.1;p13.3)	46,XY,t(11;21)(p15.4;q22.1)	
	46,XX,t(2;8)(p15;q13.1)		
	46,XX,t(2;12)(p25;q13)		
	46,XX,t(3;6)(q25~q26;q23~q24)		
	46,XX,t(3;6)(q25;q23.1)		
	46,XX,t(3;15)(p13;q26.1)		
	46,XX,t(4;6)(q31.1;q22.32)		
	46,XX,t(4;10)(q13.3;q24.3)		
	46,XX,t(5;12)(p15.1;q22)		
	46,XX,t(11;12)(p15.4;p13.2)		
	46,XX,t(11;20)(q13;p13)		
	46,XX,t(14;18)(q22;q11.2)		
	46,XX,t(16;20)(q24;p13)		
	46,XX,t(17;18)(q21.1;q12.2)		
	mos46,XX[20]/46,X,t(X;14)(p21.1;q21)[10]		
Robertsonian Translocations	45,XX,der(13;22)(q10;10)	45,XY,der(13;14)(q10;q10)	Mokate et al. 2006
	45,XX,der(13;14)(q10;q10)[a]	45,XY, der(13;14)	Stephenson 2006
	Y;22 translocations	45,XY,der(13;14)	Goddijn 2004
	46,XX,der(22)t(Y;22)(q12;p12/13)	45,XY, der(13;q14q)	
	46,XX,der(22)t(Y;22)(q12;p12/13)		
	45,XX, der(13;14)(q10;q10)		
	45,XX, der(13;14)(q10;q10)		
	45,XX, der(13q;22q)(q10;q10)		
	45,XX, der(13;14)(q10;q10) 45,XX, der(13;14)(q10;q10)		
	45,XX, der(13;14)(q10;q10)		
	45,XX,der(13;14)(q10;q10)		
	45,XX, der(13;14)(q10;q10)		
	45,XX, der(13;14)		
Pericentric Inversions	46, XX,inv (1) (p32q44)	46,XY,inv(2)(p11.2q13)	Gaber et al. 2010
	46, XX,inv (9) (p12q12)	46,XY,inv(5)(p15.3q35)	Figen Celep and Ahmet
	46,XX,inv(8)(p11.22q13.1)	46,XY,inv(9)(p13q13)	Karagüzel 2008.
	46,XX,inv(9)(p13q13)	46,XY, inv(17)(p13.1q25.3)	Stephenson 2006
	46,XX, inv(3)(p21.33q22.2)	46,XY, inv10(p11.2q21.2)	Mozdarani et al. 2007.
	46,XX, inv(6)(p12.2p25.1)	46,XY inv (9)(p11q12)	
	46,XX, inv(2)(p24.2q21.1)		
	46,XX, inv(7)(q22.2q21.1) pat		
	46,XX, inv(2)(p11.2q13)		
	46,XX, inv (9)(p11q12)		
Paracentric Inversions	46,XX,inv(6)(p21.3p25)	46,XY,inv(12)(q15q24.1)	Gaber et al. 2010
	46,XX.ish inv(8)(p21p23)[b]		Stephenson 2006
	46,XX,inv(11)(q21q23)		Goddijn 2004
	46,XX ,inv(2)(p11q13)		Sugiura-Ogasawara 2004

[a]The carrier of this translocation is known to have autosomal benign myopathy.
[b]The inv(8)(p21p23) was confirmed by fluorescence *in situ* hybridization.
[c]The inversion was only detected in second instance after the birth of a child with congenital abnormalities (see Discussion).

translocations account for the largest percentage of these karyotypic abnormalities. They can cause pregnancy loss because segregation during meiosis results in gametes with duplication or deficiency of chromosome segments (Bhasin et al. 1973).The severity of the phenotype depends on the chromosomes involved and on the positions of their breakpoints. Other structural rearrangements, such as inversions or ring chromosomes, are relatively rare. These chromosomal abnormalities can be associated with congenital malformations and mental retardation, as well as spontaneous abortion. Other chromosome abnormalities include chromosome inversions, sex chromosome mosaicism and ring chromosomes. Single gene defects might also be responsible for multiple miscarriages, but will not be detected by a karyotype (Macklon 2002).

Genetic Abnormalities/ Mendelian Disorders

Certain genetic mutations, such as the autosomal dominant disorder leading to myotonic dystrophy, may predispose a patient to infertility or even miscarriage (Fiorentino et al. 2005). The cause of the abortion in this disease is unknown, but it may be related to abnormal gene interactions combined with disordered uterine function and implantation defects. Other presumed autosomal dominant disorders associated with RPL include lethal skeletal dysplasias, such as thanatophoric dysplasia and type II osteogenesis imperfecta (Souka et al. 2001). Maternal disease associated with increased fetal wastage includes connective tissue disorders (Hernández et al. 2002), such as Marfan syndrome (Yudaeva 2009), Ehlers-Danlos syndrome homocystinuria (Bick et al. 2003) and pseudoxanthoma elasticum (Byrne et al. 1994; Bick 2008). Hematologic abnormalities associated with RPL include dysfibrinogenemia (Blumenfeld and Brenner 1999), factor XIII deficiency (Pauer et al. 2003), congenital hypofibrinogenemia (Zdziarska et al. 2009) and afibrinogenemia (Bick 2008), and sickle cell anemia (Ilham et al. 2002) .Women with sickle cell anemia are at increased risk for fetal loss, possibly because of placental-bed microinfarcts (Hsu et al. 2007).

Genes Involved

Biological processes for maintaining normal pregnancy requires a series of differential gene expression. There are 30 genes showing different levels of expression between normal and RPL patients (Baek 2004). In addition, other research groups have also identified a number of genes that are expressed aberrantly in pregnancy failure. Table 4 shows mutation in several genes which are involved in immunity, angiogenesis, apoptosis pathways in association with RPL (Kilpatrick 2000; Takakuwa et al. 2003). A detailed functional analysis for these genes during normal pregnancy will help to identify pregnancies with a high risk of RPL and how to manage those pregnancies.

Table 4: Immunity- and angiogenesis-related genes involved in recurrent pregnancy loss.

Immunity-related Genes	
PP14 (placental protein 14)	Dalton et al. 1998; Baek et al. 2002
hCG	France et al. 1996; Zayed et al. 2001; Baek et al. 2002
MUC1	Serle et al. 1994; Hey et al. 1995; Baek et al. 2002
CA-125	Dalton et al. 1998
Annexin II	Aarli et al. 1997
Mannan binding protein	Kilpatrick 2000
LIF (leukaemia inhibitory factor)	Choudhury and Knapp 2001
Indoleamine 2,3-dioxygenase	Heikkinen et al. 2003
CD95'	Hoshimoto et al. 2002
PIBF (progesterone-induced blocking factor)	Laskarin et al. 2002
HLA-DRB1	Takakuwa et al. 2003
CD69	Ramhorst et al. 2003
Angiogenesis-related Genes	
MMP-2 (matrix metalloproteinase-2)	Jokimaa et al. 2002; Choi et al. 2003
MMP-9 (matrix metalloproteinase-9)	Choi et al. 2003
Fibronectin	Pijnenborg et al. 2000; Choi et al. 2003
Integrin	Choi et al. 2003
PAI (plasminogen activator inhibitor)	Choi et al., 2003
TGF-β (transforming fibroblast growth factor-β)	Choi et al. 2003
VEGF (vascular endothelial growth factor)	Choi et al. 2003
BFGF (basic fibroblast growth factor)	Choi et al. 2003
TIMP-1 (tissue inhibitors of metalloproteinases-1)	Jokimaa et al. 2002

MTHFR Gene

Methylenetetrahydrofolate reductase (MTH-FR) is a rare genetic defect that leads to complications in pregnancy (Ivy et al. 2007). *MTHFR* gene produces an enzyme called methylene-

tetrahydrofolate reductase and mutation in the gene inhibits the production of this enzyme, result in hyperhomocytenemia, which is an elevated level of an enzyme homocysteine found in blood plasma. When the body is deficient in ethylenetetrahydrofolate reductase, its ability to absorb folate, such as folic acid, is inhibited. Folic acid and B9 are both essential to the development and health of the fetus. Because of a mother with MTHFR's inability to efficiently metabolize folic acid and vitamin B9, the disorder has been linked to a variety of pregnancy complications such as congenital malformations. Elevated levels of homocysteine have been associated with placental disease, preeclampsia and RPL (Foka et al. 2003).

CYP Family Genes

Among the members of the CYP family, *CYP1A1*, *Cyp1A2*, *Cyp2C*, *CYP2D6*, *Cyp2E1*, *Cyp2F1*, *Cyp3A4*, *Cyp3A5*, *Cyp3A7* and *Cyp4B1* were found to be expressed in the placenta during the first trimester (Jauniaux et al. 2000), although the functional activities of *Cyp3A* and *Cyp4B* were not detected. The importance of *CYP1A1* and *Cyp2E1* in relation to pregnancy is substantiated further by the increased risk of miscarriage with maternal smoking and alcohol consumption. Experimental evidence indicates that during pregnancy, there is increased expression of *CYP2D6* (Wadelius et al. 1997), which metabolizes about one-quarter of clinically important medications. However, the exact role played by the enhanced *CYP2D6* levels in pregnancy remains obscure. Recent studies have demonstrated that the *CYP* family gene polymorphisms significantly influence reproductive conditions. Wang *et al.* (2002) reported that *CYP1A1* gene polymorphism was associated with a reduction in birth weight among women who smoked cigarettes in the United States. We previously demonstrated that *CYP17* gene polymorphism was associated with risks of RPL (Sata *et al.* 2003b) and intrauterine fetal growth restriction in the Japanese population (Yamada *et al.* 2004). Recent reports indicate the presence of dioxins, which are strong inducers of *CYP1A1* expression, in human follicular fluid (Tsutsumi *et al.* 1998). This suggests the possibility of constant induction of *CYP1A1* at low levels in this tissue. The presence of a hyper-inducible allele in such an instance may increase the levels of *CYP1A1* to the extent that it can cause severe damage to the fetus.

The glutathione-*S*-transferase (GST) families of enzymes are being important members of phase II detoxification pathways, catalyse the conjugation of a variety of electrophilic substances to glutathione, facilitating their elimination from the body. Usually, the foreign substances activated by the phase I reactions are acted upon by the GST enzymes. Moreover, GST enzymes also play an important role in regulation of reduced glutathione levels, and thereby redox reserves of the individual. Hence any decrease in the GST activity can lead to accumulation of the products of phase I activity which can cause severe damage. GST enzymes are believed to play a crucial role in female reproduction as suggested by their presence in placenta and ovarian follicles in excessive amounts (Zusterzeel et al. 1999). Among the various GST enzymes, GSTP1 is reported to be the predominant isoform in placenta, suggesting a possible role for this enzyme in pregnancy (Knapen et al. 1999 a, b).

Factor V Leiden Mutation

Factor V Leiden mutation (FVL) is an autosomal dominant hemostatic disorder that predisposes affected persons to venous thromboembolic events (Grandone et al. 1997). Pregnancy is a hypercoagulable state since antiphospholipid antibodies (APL), an acquired thrombophilic defect, have been established as an important and treatable cause for pregnancy loss at all gestational ages (Rai et al. 1997). The potential role of thrombophilic defects may play in adverse pregnancy outcome is under investigation. One such thrombophilic defect is Factor V Leiden, a common mutation (G→A at nucleotide position 1691 in the Factor V gene) which is associated with a significant increased risk for systemic venous thrombosis (Dahlback 1995). Factor V Leiden has also been reported in association with placental thrombosis (Rai et al. 1996). *De novo* resistance to activated protein C can develop during pregnancy among persons without the factor V Leiden mutation (Bertina et al. 1994; Cumming et al. 1996) and that these persons may have an increased risk for pregnancy-related venous thromboembolism finally leads to pregnancy loss.

Natural Killer Cells

In the past decade, considerable effort has been made to identify cellular constituents and processes putatively underlying immune-based RPL. Natural killer (NK) cells have been the cells most extensively studied, primarily because they constitute the predominant leukocyte population present in the endometrium at the time of implantation and in early pregnancy (Yamada 1994). Cytokines produced by peripheral blood NK cells in RPL can inhibit blastocyst development (Polgar 2002). Given the evidence for modulation of NK cells by the hormonal system as well as the involvement of uNK cells in normal pregnancy, NK and uNK cells have been the focus of investigation of many studies trying to understand the pathophysiology of unexplained RPL (Chrysoula 2005).

It has been suggested that cell-mediated (Th1) immunity toward trophoblast cells is suppressed in normal pregnancy, and that perhaps failure of such suppression contributes to loss of the pregnancy in cases of unexplained RPL (Souza et al 2002). In response to trophoblast antigens, peripheral blood mononuclear cell (PBMC) from women with RPL have been shown to produce *interferon,* tumor necrosis factor (TNF), and TNF-β, whereas PBMC of reproductively normal women produce interleukin (IL)-10 (Hill et al 1995). In addition, after *in vitro* stimulation of PBMC with phorbol myristate acetate and ionomycin, CD3$^+$ CD8– T helper cells from women with RPL have significantly higher Th1/Th2 cytokine ratios of *Interferon*-4, TNF-/IL-4, and TNF-/IL-10 compared with control multiparous women (Kwak-Kim et al. 2003). The above findings suggest that Th1 immunity to trophoblast is associated with RPL, whereas Th2 immunity is associated with a successful pregnancy (Choi et al. 2000).

Preliminary observation revealed that telomerase activity was suppressed in chorionic villi tissues obtained from RPL patients, supporting the hypothesis that aberrant expression of apoptosis related genes during development is also involved in RPL. Signalling pathways leading to apoptosis converge on a common machinery of cell destruction activated by a family of cysteine proteases that cleave proteins at aspartate residues (caspases), members of the tumour necrosis factor (TNF) receptor (TNF R) superfamily, and members of the Bcl 2 family proteins.

Aoki and co-workers (1995) observed increased preconceptional NK cell activity, as measured by a chromium-51 release cytotoxicity assay, in 68 women with unexplained RPL compared with controls. The discrepancies observed in preconceptional NK activity between RPL patients and controls in these studies could derive from the use of fresh *vs.* cryopreserved PBMC, as well as differences in the methods used to assess cytotoxicity. (Shimada et al. 2004).

Recent studies reported that apoptosis-related genes during development and its aberrant expression are also a major eitiology for RPL (Baek 2004). Signalling pathways leading to apoptosis which initiate the cell destruction by a family of cysteine proteases that cleave proteins at aspartate residues (caspases), members of the tumour necrosis factor (TNF) receptor (TNF-R) superfamily, and members of the BCL-2 family proteins. Higher expression levels of apoptosis-related genes such as caspase 3,6, 7, 8, 9, 10, 12, BAD, BAX, BID, Fas and FasL were shown in chorionic villi from RPL patients than those from normal patients (Choi et al, 2003), as listed in Table 5.

Table 5: Apoptosis- and other groups of genes involved in recurrent pregnancy loss

Apoptosis-related Genes	
Caspase 3	Choi et al. 2003
Caspase 6	Choi et al. 2003
Caspase 7	Choi et al. 2003
Caspase 8	Choi et al. 2003
Caspase 9	Choi et al. 2003
Caspase 10	Choi et al. 2003
Caspase 12	Choi et al. 2003
BAD	Choi et al. 2003
BAX	Choi et al. 2003
BID	Choi et al. 2003
Fas	Choi et al. 2003
FasL	Choi et al. 2003
Other Groups of Genes	
Cathepsin H	Jokimaa et al. 2002
Globin	Baek et al. 2002
Unknown 1	Baek et al. 2002
Unknown 2	Baek et al. 2002

CONCLUSION

Couples with pregnancy loss produce chromosomally abnormal embryos in a significantly higher percentage than those not having this reproductive problem which are mainly due to non-dysjunction. Once an unbalanced translocation in the fetus / child has been identified, parental karyotype is essential. Molecular analy-

sis such as PCR-based subtractive hybridization showed a limited number of genes in chorionic villi from RPL patients. Since the PCR-based cDNA subtractive hybridization analysis may not represent all genes differentially expressed, it is expected that there should be more genes that are involved in the process of establishing and maintaining pregnancy. Thus, further research is necessary to confirm the clinical relevance of these genes identified for abnormal expression in RPL patients. Therefore, finding any aberrant expression of these genes may delineate general health during pregnancy and a better understanding of the physiological significance of these genes may help in controlling the management of subsequent pregnancies. Therapeutic intervention is guided by the underlying cause of RPL. In all cases, emotional support is important in caring for these often anxious couples, and may enhance therapeutic success

GENETIC EVALUATION AND TESTING RECOMMENDATIONS

Couples may have had a prior evaluation with a reproductive endocrinologist, gynecologist, maternal fetal medicine specialist, or other specialists, and testing (e.g., antiphospholipid antibodies, ultrasounds) may have been pursued previously to rule out other causes of RPL. A referral to a genetics specialist is warranted when the prior evaluations yielded normal results, and during pregnancy, medical, and family history evaluations suggest the possibility of a genetic cause for the couple's RPL, as well as to address any implications for other family members when a genetic etiology is identified. When possible, chromosomal analysis on fetal tissue from products of conception (POC) should be considered, in addition to a thorough pathological evaluation of the fetus and placenta. Routine karyotyping of each partner is standard and testing the woman for the factor V Leiden and prothrombin G20210A mutations should be considered. Testing for the less common thrombophilias (anticoagulants protein C, protein S, and antithrombin III) should be reserved for women with a personal and/or family history of venous thromboembolism. Testing for methylene tetrahydrofolate reductase (*MTHFR*) mutations in a woman with RPL is not justified, according to currently available studies. The use of specialized chromosomal studies such as comparative genome hybridization, subtelomeric studies, interphase studies on sperm and assays for skewed X-inactivation patterns are not warranted at this time, as their clinical utility is yet to be determined.

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