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Review article

Molecular investigation of uniparental disomy (UPD) in spontaneous abortions

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ABSTRACT

Objective: About 10–15% of all clinically recognized pregnancies end as spontaneous abortions while at least 50% of pregnancies are lost before reaching term gestation. Genetic abnormalities are responsible for ≥50% of all early miscarriages. The aim is to indentify associations between UPD and abortions and regarding UPD as pathogenetic mechanism possibly to understand the role of imprinted genes or recessive mutations in abortions.
Study design: To determine additional factors causing spontaneous abortions we searched for uniparental disomies (UPD) which is known to be associated with distinct birth defects as per the chromosome involved and parental origin. Studies were carried on DNA of 68 cases of first trimester spontaneous abortions and DNA of their parents. We examined tissue from aborted fetuses, especially in the first trimester, with molecular techniques to detect UPD to chromosomes that contain imprinting genes.The inheritance of each region of the chromosome was determined by comparing the genotypes obtained from abortion and parental DNA.
Results: Of the 68 cases of spontaneous abortions investigated, 324% were found to be biparental inheritance or were uninformative in locus that they were examined, 4118% were matUPD, 147% trisomy for a chromosome, 8,8% patUPD and 294% matUPD and trisomy for a certain chromosome. Most cases of UPD found on chromosomes 21 and 14. Many of those are found in combination with chromosomes 13, 20 and 22.
Conclusions: UPD might be a common finding among spontaneous abortuses. UPD can be a cause of miscarriage if localized to regions of chromosomes with imprinted genes which control embryogenesis and fetal development and or can activate a recessive mutation in genes which are essential for early embryogenesis.

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Introduction

About 10–15% of all recognized pregnancies end as spontaneous abortions while at least 50% of pregnancies are lost before reaching term gestation [1,2,4]. The majority of spontaneous abortions (SM) occur during the first trimester [1,3,4]. Repetitive spontaneous first trimester spontaneous abortions as well as second and third trimester fetal deaths are considered as recurrent pregnancy losses (RPL) and represent 1% of all pregnancies [5]. The risk of spontaneous abortions in a clinically recognized pregnancy is rising from 12% in women younger than 20 years to 26% in women aged over 40 years [6–8].

It is well known that chromosome abnormalities are responsible for more than 50% of the abnormal genetic abnormalities in all early spontaneous abortions [2–4] (Table 1).

The term uniparental disomy (UPD) was first introduced by Engel in 1980 [10]. UPD is the presence of both copies of a chromosome, or part of a chromosome, from only one parent and with the subsequent absence of the corresponding copies from the other [11,12]. UPD can either be maternally (matUPD) or paternally (patUPD) derived. There are two different types of UPD: isodisomy (iUPD): the inheritance of two copies of one parental chromosome and heterodisomy (hUPD): the inheritance of two different homologous chromosomes from one parental chromosomal pair [13–15].

UPD can be formed either in the entire chromosome or in part or a region of a chromosome (segmental UPD interstitial or telomeric). 11% of all UPD cases are segmental [15–17]. Complex UPD is UPD of a part or a whole chromosome when is associated with a chromosomal aberration [14,18,19].

The most likely mechanisms of formation of UPD are trisomy and monosomy rescue. Mechanisms like post fertilization error and gamete complementation are also contributing in UPD formation but are rare [10,16,20,21].

The frequency of UPD depends on that of aneuploidy, which is rather high in human gametes. When these abnormal gametes are involved in fertilization, there is an increased risk for the pregnancy to be lost during the first trimester [10,17,22]. Taking into account the frequency of trisomies in spontaneous abortions stemming from meiotic errors in human gametes, the frequency of UPD is estimated at 1.65 per 1000 conceptuses [13,21,23].

There are several problems associated with UPD: a) trisomy mosaicism (placental or embryonic), b) abnormal genomic imprinting and c) homozygosity of autosomal recessively inherited mutations.

Trisomy mosaicism can be formed either with hUPD and iUPD with the aneuploid cell line apparently often confined to the placenta and somatically undetected. Although miscarriage could result from mosaicism [12,24], true mosaicism only accounts for about 0.125–0.2% of early miscarriages [17,25,26]. Most mosaicism is demonstrated as confined placental mosaicism (CPM) and accounts for 0.7–2% [25,27,28]. CPM may lead to pregnancy loss and intrauterine growth restriction, in up to 20% of cases [1,26,29]. CPM with trisomy 16 has the higher pregnancy loss rate [1,13,24].

In genomic imprinting, a small group of genes (so called "imprinted genes") are expressed from only one parental allele, either maternal or paternal. It is known that imprinting is tissue specific and the genes controlling growth, cell division and development of trophoblast and embryonic tissues at the early stages, are imprinted and important for fetal and placenta development [30,31] (Table 2). Thus it is logical to propose that a UPD of a chromosome carrying imprinted genes affects their normal monoallelic expression and it could be hypothesized that spontaneous abortions could be one of the groups with an elevated observed frequency of UPD [1,32].

The phenotypic effect of UPD is also determined by a possible homozygotization of recessive mutant genes as a consequence of isodisomy. Upon mutations in genes which are essential for early embryogenesis, UPD may lead to the arrest of intrauterine development, or in spontaneous abortions [16,32].

Table 1
Genetic abnormalities of spontaneous abortions.

	% of cases	References
Chromosomal Abnormalities		
Structural anomalies	≤10%	[36]
Numerical abnormalities	≥90%	[29,24]
monosomy X	20%	[24]
Polyploidy	20%	[24]
Triploidy	6%	[35]
autosomal trisomies	60%	[24,29]
trisomy 16	20%-30%	[24]
trisomy 21	20%	[24,29,36]
trisomy 22	20%	[24,29,36]
Parental balanced translocation	3%-6%	[24]
Confined placental mosaicism (CPM)	20%	[24,30,19]
Single gene disorders	10%	[24,36]
Thrombophilia	10%	[9]
X-linked conditions	10%	[36]

Material and methods

Cases

Studies were carried on DNA of 68 cases of first trimester spontaneous abortions and DNA of their parents. All of the parents in these cases agreed to allow the use of parental and fetal materials for analysis, after being given understandable and detailed information on this study and its purposes. Peripheral blood of the parents, placenta and extraembryonic tissues from the abortion, were obtained for each case.

Most of them represented cases of early embryolethality diagnosed during ultrasound examination of pregnant women. 72 embryos were examined of which 61 (84.7%) were spontaneous abortions with no apparent clinical characteristics, while the remaining 11 (15.3%) showed clinical features which either resulted in intrauterine death or in pregnancy termination. The embryonic material represented, as a rule, by fetal sac tissues were provided by Department of Obstetrics and Gynaecology, hospital of Ioannina. The gestation age varied from 5 to 16 weeks and was 8.5 ± 2.6 weeks, on average.

Molecular analysis

Of the 72 samples received, successful molecular analysis was possible in 68 samples. Genomic DNA was extracted from placenta and extraembryonic tissues of spontaneous abortuses using QIAamp Blood Mini Kit and from peripheral blood lymphocytes of parents by the standard method.

Search for uniparental disomies for nine human chromosomes was carried out by analysis of the pattern of inheritance of alleles of polymorphic DNA markers localized on these chromosomes from

Table 2
Imprinting genes and UPD.

Gene	Locus	expression	UPD	Role and consequences	Syndrome
GRB10	7q12.2	B	m	–	SRS
PEG1/ MEST	7q32.2	P	m	regulates placental and fetal growth	SRS (5%) and IUGR
PEG10	7q21	P	m	When down expressed causes abortion	SRS
IGF2	11p15.5	p	p	play a role in fetal development and is a potent embryonic growth factor	BWS (20%) SRS
H19	11p15.5	m	p	is essential for the growth of embryonic and extraembryonic tissues	SRS
PHLDA2	11p15.5	m		Plays a role in regulating placenta and fetal growth	IUGR
KCNQ10T1	11p15.4	m	P	increased risk of embryonal tumor formation and plays a role in embryonic and placental growth	BWS, absence causes neonatal lethality in the mouse (10%)
MEG3/ GTL2	14q32.2	m	P	placenta size and organization, and prenatal lethality in mice	phenotypes similar to paternal UPD14
DLK1	14q32.2	p	M	Play a role in neuroendocrine differentiation	phenotypes similar to maternal UPD14
UBE3A	15q11.2	m	P		AS

parents. A panel of 25 dinucleotide markers which were selected based on high heterozygosity (ranging from 0.91 to 0.65 with most being 0.8 or higher), was used for analysis of chromosome inheritance. The information about sequence of oligonucleotide primers and conditions of amplification was obtained from the genomic database (www.gdb.org).

PCR products were analysed by separation according to their length on 12% polyacrylamide gel electrophoresis visualised by silver staining. The inheritance of each chromosome was determined by comparing the genotypes obtained from abortion and parental DNA. Determination of UPD was based on at least two informative markers. This analysis was not designed to identify or exclude partial or segmental UPD. However, in a few cases proved either uninformative, or only eliminated the possibility of UPD inherited from only one of the parents, further testing was performed using random polymorphic markers for the uninformative chromosomes until our objective of excluding holochromosomic UPD was met.

A DNA marker (SRY) which is located in gene region for the determination of the sex in humans was used for the determination of sex of the samples.

From the entire sample, we can exclude the maternal contamination in: a) all male samples, for the reason that carry the Y chromosome, b) samples that either by molecular or cytogenetic analysis have diagnosed with a trisomy for a specific chromosome, c) samples that after molecular analysis bearing zones that correspond to zones of paternal origin in one or more chromosomes and d) 46,XX samples, which have diagnosed with patUPD.

But we cannot exclude the existence of maternal contamination in some female fetuses which show matUPD and normal karyotype (46, XX). For this reason, we tested all the samples which were diagnosed with matUPD in more than one chromosome. These samples did not produce visible bands of paternal origin when tested with polymorphic DNA markers and it was not possible to analyze karyotype.

Bioinformatics

Using bioinformatics (http://genome.ucsc.edu) we searched for genes in the regions of polymorphic markers that were used for molecular analysis, mutations in which are possibly related to embryonic development (Table S1).

Results

Of the 72 cases studied a successful molecular evaluation was performed in 68 cases (94.5%). Polymorphic analysis has revealed the following results as shown in Table 3.

Table 3
Molecular analysis with polymorphic markers results.

Number of samples			Result	Rate (%)
♀	♂	tot		
14	8	22	Normal-Non informative	324%
26	2	28	matUPD	4118%
5	1	6	patUPD	8,8%
1	–	1	Trisomy 13	147%
1	–	1	Trisomy 18	
5	3	8	Trisomy 21	
1	–	1	Trisomy 14 και 21	294%
1	–	1	Trisomy 14, 21 και 22	

Originally a way to exclude maternal contamination in fetal tissues is to check the area of the SRY gene of the Y chromosome responsible for sex determination in humans. After the molecular analysis 55 samples were female (764%) and 17 were male (236%).

Nine samples of the 26 female samples that were diagnosed with matUPD were examined for maternal cell contamination. The seven of them were founded to be contaminated with maternal cells and were excluded from the study.

The number of cases and rates for matUPD, patUPD and trisomy in each marker used for the molecular analysis of the samples are presented in Table 4.

Using bioinformatics we found several genes, which recessive mutations are responsible for clinical features such as developmental delay, embryonic development or are associated with clinical syndromes that may lead to embryonic arrest and possibly spontaneous abortions. Those genes are presented in Table S1.

Discussion

Our study was designed to examine tissue from aborted fetuses, especially in the first trimester, with molecular techniques to detect UPD to chromosomes that contain imprinting genes.

The trisomy 21 (20%) is the second most observed trisomy in spontaneous abortions after trisomy 16 (20–30%) [27,33]. From the molecular analysis, we found that trisomy 21 was observed at a greater rate than other trisomies. In our samples trisomy 21 is the most frequent trisomy observed as well as more frequently appeared UPD21, where it can be the result of rescue trisomy 21. Also we observed that our samples exhibit large percentage of random chromosomal rearrangements.

According to the literature the rate of observed matUPD compared with patUPD is larger, which is confirmed by our own results [10]. This difference in rate of maternal and paternal UPD is due to the fact that most events of not segregation of chromosomes in meiosis occurring during the formation of eggs in approximately

Table 4
Cases of UPD and trisomy in each polymorphic marker that were used.

Chromosome	Marker	Chromosome Region	Number of examined families						IN %	NI %
			total	BPI	UPD		Tr	NI		
					mat	pat				
7	D7S645	7q11.22	13	1	1	0	0	11	15.4%	84.6%
13	D13S1295	13q34	8	1	0	0	1	6	25%	75%
	D13S1243	13q12.12	20	1	1	0	1	17	15%	85%
14	D14S608	14q12	55	15	12	3	2	23	58%	42%
	D14S1279	14q31.3	11	1	2	1	0	7	36.4%	63.6%
	D14S267	14q32.2	11	10	0	0	0	1	91%	9%
15	D15S542	15q11.2	2	0	2	0	0	0	100%	0%
	GABRB3	15q12	2	0	1	0	0	1	50%	50%
16	D16S3121	16q24.3	11	0	2	1	1	7	36.4%	63.6%
	D16S3045	16p12.3	10	10	0	0	0	0	100%	0%
	D16S3018	16q23	19	3	2	1	0	13	31.6%	68.4%
18	D18S59	18p11.32	19	6	4	1	3	5	73.7%	26.3%
	D18S1141	18q23	7	0	0	0	1	6	14.3%	85.7%
20	D20S96	20q13.1	10	6	4	0	0	0	100%	0%
	D20S171	20q13.32	9	0	1	1	0	7	39.5%	60.5%
	D20S162	20p12.2	4	0	1	0	0	3	25%	75%
21	D21S11	21q21.1	17	2	7	1	3	4	76,5%	235%
	D21S1437	21q21.1	55	6	14	3	5	27	51%	49%
	D21S1446	21q22.3	64	16	9	4	6	29	54.7%	45.3%
	D21S1270	21q22	15	2	1	1	1	10	33,4%	666%
22	D22S1138	22q11.21	25	0	0	0	0	25	0%	100%
	D22S1169	22q13.32	5	0	1	1	0	3	40%	60%
	D22S281	22q12	23	7	5	1	1	9	61%	39%

BPI: Biparental Inheritance, Tr: Trisomy, NI: Non Informative, IN: Informative.

50% (maternal meiosis I) despite the spermatozoa (5%) [22,34]. There is a higher frequency of aneuploidy in oocytes (20%) than in sperm (10%) because the oocyte has a less active machinery to eliminate chromosomal mistakes when compared to the spermatocyte [20,22].

According to our results the percentage of observed matUPD is larger compared with patUPD. A total of 35 samples showed UPD at a ratio of about 6:1 (6 matUPD: 1 patUPD). Mainly are UPD of a section of chromosome (segmental UPD) for chromosomes 7, 13, 14, 16, 18, 20, 21 and 22 or for the entire chromosome for chromosome 21. Most cases of UPD found on chromosomes 21 and 14. Many of those are found in combination with chromosomes 13, 20 and 22.

Conclusion

UPD of chromosome regions or chromosomes containing imprinted genes or recessive mutations can lead to severely harmful phenotypes or to spontaneous abortions if are involved genes which control embryogenesis and fetal development.

Trisomic rescue and UPD should be highly actual fields of research, considered as important for epigenetic gene regulation. Thus, cytogenetic tools like fluorescence in situ hybridization could also be of interest in UPD-research. The genetic background of a 'UPD-patient', needs to be thouroughly investigated by molecular and cytogenetics methods since a UPD event is associated with one third of the chromosomal rearrangements in general. To avoid abortion is necessary to examine embryos for UPD with PGD techniques.

Disclosure of interests

No, there are no conflict of interests that I should declare.

Contribution to authorship

L.I. conceived the idea with the support of S.M., designed and performed the experiments, analyzed the data and wrote the manuscript.

S.M. conceived the original idea, contributed with the necessary reagents, materials and analysis tools and supervised the findings of this research

P.M. and FG contributed with fetal and parental materials essential for the research and supervised the findings of this research with S.M

F.G verified the numerical results and helped with the corrections of the manuscript

All authors discussed the results and contributed to the final manuscript.

Details of ethics approval

All of the parents have given their consent and agreed to allow the use of parental and fetal materials for analysis, after being given understandable and detailed information on this study and its purposes. No other ethics approvals were necessary.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ejogrb.2019.03.004.

References

[1] Warren Jennifer E, Silver Robert M. Genetics of pregnancy loss. Clin Obstet Gynecol 2008;51(1):84–95.
[2] Zhang YX. Genetic analysis of first-trimester miscarriages with a combination of cytogenetic karyotyping, microsatellite genotyping and array CGH. Clin Genet 2009;75(February (2)):133–40.
[3] Jacobs P, Hasshold TJ. Chromosome abnormalities origin and etiology in abortions and livebirths Human Genetics. New York: Springer- Verlag; 1987. p. 233.
[4] Nagaishi Masaji, et al. Chromosome abnormalities identified in 347 spontaneous abortions collected in Japan. J Obstet Gynaecol Res 2004;30 (June (3)):237–41.
[5] Rai R, Regan L. Recurrent miscarriage. Lancet 2006;368:601–11.
[6] Frias Jr AE, Luikenaar RA, Sullivan AE, et al. Poor obstetric outcome in subsequent pregnancies in women with prior fetal death. Obstet Gynecol 2004;104:521–6.
[7] Nybo Andersen AM, Wohlfahrt J, Christens P, et al. Maternal age and fetal loss: population based register linkage study. BMJ 2000;320(7251):1708–12.
[8] Okuyama K. Miscarriage and maternal age. Shusanki Igaku 1992;22:1227–31.
[9] Werner EF, et al. Thrombophilias and stillbirth. Clin Obstet Gynecol 2010;53(3) 617–6.
[10] Shaffer LG, Agan N, Goldberg JD, Ledbetter DH, Longshore JW, Cassidy SB. American College of Medical Genetics statement of diagnostic testing for uniparental disomy. Genet Med 2001;3(May–June (3)):206–11.
[11] Kotzot D. Review and meta-analysis of systematic searches for uniparental disomy (UPD) other than UPD 15. Am J Med Genet 2002;111:366–75.
[12] Kotzot D. Uniparental disomy (UPD) other than 15: phenotypes and bibliography updated. Am J Med Genet 2005;136A:287–305.
[13] Kotzot D. Prenatal testing for uniparental disomy: indications and clinical relevance. Ultrasound Obstet Gynecol 2008;31:100–5.
[14] Kotzot D. Complex and segmental uniparental disomy updated. J Med Genet 2008;45:545–56 originally published online June 4, 2008.
[15] Spence JE, Perciaccante RG, et al. Uniparental disomy as a mechanism for human genetic disease. Am J Hum Genet 1988;42(2):217–26.
[16] Engel E. A fascination with chromosome rescue in uniparental disomy: Mendelian recessive outlaws and imprinting copyrights infringements. Eur J Hum Genet 2006;14(November (11))1158–69 E pub 2006 May 17.
[17] Liehr T. Cytogenetic contribution to uniparental disomy (UPD). Mol Cytogenet 2010;3(8).
[18] Berend SA, Horwitz J, McCaskill C, Shaffer LG. Identification of uniparental disomy following prenatal detection of Robertsonian translocations and isochromosomes. Am J Hum Genet 2000;66:1787–93.
[19] Kotzot D. Complex and segmental uniparental disomy (UPD) – review and lessons from rare chromosomal complements. J Med Genet 2001;38:497–507.
[20] Robinson WP. Mechanisms leading to uniparental disomy and their clinical consequences. Bioessays 2000;22(May (5)):452–9.
[21] Engel E, DeLozier-Blanchet CD. Uniparental disomy, isodisomy, and imprinting: probable effects in man and strategies for their detections. Am J Hum Genet 1991;40:432–9.
[22] Martin RH. Distribution of aneuploidy in human gametes. Comparison between human sperm and oocytes. Am J Med Genet 1991;39:321.
[23] Fritz B, Aslan M, Kalscheuer V, et al. Low incidence of UPD in spontaneous abortions beyond the 5th gestational week. Eur J Hum Genet 2001;9:910–6.
[24] Van Opstal D, Van Den Berg C, Deelen WH, Brandenburg H, Cohen-Overbeek TE, Halley DJ, et al. Prospective prenatal investigations on potential uniparental disomy in cases of confined placental trisomy. Prenat Diagn 1998;18(1):35–44.
[25] Robinson WP. Meiotic origin of trisomy in confined placental mosaicism is correlated with presence of fetal uniparental disomy, high levels of trisomy in trophoblast, and increased risk of fetal intrauterine growth restriction. Am J Hum Genet 1997;60(4):917–27.
[26] Hansen WF. Maternal uniparental disomy of chromosome 2 and confined placental mosaicism for trisomy 2 in a fetus with intrauterine growth restriction, hypospadias, and oligohydramnios. Prenat Diagn 1997;17(5):443–50.
[27] Hassold T. A cytogenetic study of 1000 spontaneous abortions. Ann Hum Genet 1980;44:151–78.
[28] Yong PJ, Marion SA, Barrett IJ, et al. Evidence for imprinting on chromosome 16: the effect of uniparental disomy on the outcome of mosaic trisomy 16 pregnancies. Am J Med Genet 2002;112: 123–13.
[29] Lebedev IN, Ostroverkhova NV, Nikitina TV, Sukhanova NN, Nazarenko SA. Molecular cytogenetic characteristics of chromosome imbalance in cells of spontaneous human abortion fetuses with low proliferative activity in vitro. Genetika 2003;39(8):1111–22.
[30] Hurst LD, McVean GT. Growth effects of uniparental disomies and the conflict theory of genomic imprinting. Trends Genet 1997;13(11):436–43.
[31] Preece MA, Moore GE. Genomic imprinting, uniparental disomy and foetal growth. Trends Endocrinol Metab 2000;11(7):270–5.
[32] Nikitina TV, et al. Evaluation of the role of uniparental disomy in early embryolethality of man Russian. J Dev Biol 2004;35(4)238–46 Translated from Ontogenez, Vol. 35, No. 4, 2004, pp. 297–306. Original Russian Text Copyright © 2004.
[33] Nagaishi M, Yamamoto T, Iinuma K, et al. Chromosome abnormalities identified in 347 spontaneous abortions collected in Japan. J Obstet Gynaecol Res 2004;3:237–41.
[34] Ginsburg C, Fokstuen S, Schinzel A. The contribution of uniparental disomy to congenital development defects in children born to mothers at advanced childbearing age. Am J Med Genet 2000;95(5):454–60.
[35] Ostrer H, Wilson DI, Hangley NA. Human embryo and early fetus research. Clin. Genet. 2006;70(August (2)):10–98.
[36] Zhang YX, Zhang YP, Gu Y, Guan FJ, Li SL, Xie et al JS. Genetic analysis of first-trimester miscarriages with a combination of cytogenetic karyotyping, microsatellite genotyping and arrayCGH. Clin Genet. 2009;75(February (2)):133–40.