SHORT REPORT

Phenotype of triploid embryos

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The phenotypes of triploid fetuses and placentae are now well established and known to correlate with parental origin of the extra haploid set of chromosomes. In fetuses, it is not clear whether there is a direct parent of origin effect on the fetus itself or if the phenotypes are the result of growth differences influenced by abnormalities in growth and function of the placenta. Examining the phenotype of triploid embryos at an earlier stage in gestation, when the placenta effects may be less pronounced, could help clarify this question. A phenotype characteristic of triploidy in the embryonic period has been described; however, parental origin was not determined in these embryonic cases. In the present study, a population of triploid embryos is assessed to determine if there is a correlation between parental origin and phenotype. Parental origin was determined in 27 first trimester miscarriages. Digyny accounted for 19 cases and diandry for eight cases. Assessment of embryonic phenotype with parental origin showed no correlation between the phenotype of the embryo and parental origin of the extra haploid set. While there may be subtle effects of imprinting on embryonic development, they are not as obvious as they are in the mouse, consistent with the general trend of fewer imprinted genes in human beings compared with the mouse.

Triploidy is a common occurrence in human gestation, present in 2–3% of pregnancies. It often culminates in early spontaneous abortion but occasionally results in the fetal or newborn period with the birth of an abnormal fetus or infant. Triploidy may be the result of either digyny (extra haploid set from mother) or diandry (extra haploid set from father). Digynic triploidy predominates in fetuses, and diandry accounts for about 50–60% of early triploid spontaneous abortions.[1–3] Within the group of early spontaneous abortions, digyny has been reported to predominate in cases of <8.5 weeks gestational age or those in which an embryo is present (embryos are, by definition, <10 weeks gestational age).[2 3] Diandry is more common in cases >8.5 weeks gestational age and in cases that were >10 weeks gestational age in which an embryo or fetus was not identified.[2 3]

Two distinct phenotypes observed in triploid fetuses have been shown to be associated with parental origin of the triploidy.[1 2] The diandric phenotype is characterised by a normally sized or mildly symmetrically growth retarded fetus with normal adrenal glands, and is associated with an abnormally large, cystic placenta with histological features known as partial hydatidiform mole (PHM). The digynic phenotype is characterised by marked asymmetric intrauterine growth restriction, marked adrenal hypoplasia, and a very small, non-molar placenta. While triploid fetuses may have a wide variety of congenital anomalies such as complete syndactyly of the third and fourth fingers, syndactyly of the toes, abnormal genitals, and cardiac, urinary tract, and brain anomalies, these abnormalities do not appear to differ between the digynic and diandric triploids. This fact raises the possibility that the parent of origin effect observed is a manifestation of altered intrauterine growth, perhaps mediated through placental phenotype or function, determined by parental origin.

In mice, different phenotypes have been observed between digynic and diandric triploid mice, in both embryos and placentas.[4 5] In human beings, triploid embryo phenotypes have been described without correlation with parental origin of triploidy.[6]

Because of the clear fetal and placental phenotypes that correlate with parental origin in triploidy, evidence from mice indicating that parental origin phenotypic differences are manifest in embryos, and evidence that suggests there may be a triploid phenotype in human embryos, we assessed embryonic and placental phenotype and parental origin in cases of triploidy in the embryonic period.

MATERIALS AND METHODS

The study proposal was approved by the clinical research ethics board at the University of British Columbia and the research review board at Children's and Women's Health Centre of British Columbia. In total, 33 triploid embryos were examined from miscarriage specimens examined in the fetal pathology laboratory at the Children's and Women's Health Centre. The cases were examined by one of two fetal pathologists. In all cases, the embryo was examined to assess developmental stage and presence of developmental inconsistencies and focal abnormalities. Embryos classified as growth disorganised (GD) were subdivided into four categories: GD1, defined as an intact empty sac; GD2, a nodular embryo; GD3, a cylindrical embryo with distinguishable cephalic and caudal poles; and GD4, an embryo in which external development is not consistent with any stage.[6] Embryos that were not GD were assessed for developmental stage and presence of focal abnormalities. Placental tissue was submitted for histological examination of placenta morphology, including presence or absence of PHM. Amnion and chorion or chorionic villi were submitted for cytogenetic analysis, and villus tissue was frozen for DNA extraction. Scrolls from decidua submitted for histology were used to obtain maternal DNA for assignment of parental origin. Of the 33 cases identified, maternal DNA was obtained for 27, thus determination of parental origin was limited to those cases. In the remaining cases, the decidual DNA was either degraded or had high levels of embryo contamination and could not be used.

Parental and meiotic origin was determined by comparing microsatellite marker inheritance patterns in the triploid placenta with that of the maternal decidua. Markers used mapped near to (<1.5 cM) the centromere (http://cedar.genetics.soton.ac.uk/pub) and were from multiple chromosomes (2, 3, 7, 12, 13, 15, 16, 21, and X). Digyny as the result of an error in the first meiotic division (MI) should show non-reduction to homozygosity at all centromeric markers for

Abbreviations: GD, growth disorganised; hCG, human chorionic gonadotrophin; PHM, partial hydatidiform mole

which the mother is heterozygous and those arising from errors in the second (MII) should show reduction at all centromeres.

RESULTS

Of the 27 embryos, 2 were intact empty sacs (GD1), 3 were nodular embryos (GD2), 3 were embryos with inconsistent development (GD4), 10 were morphologically normal, and 9 were abnormal. Of the nine abnormal embryos, two showed features that met the criteria for probable triploid as described by Harris *et al* (table 1), while the other embryos had only one of the criteria (such as tan deposits on head or lower spine or delayed limb development) or had other abnormalities such as neural tube defect or abnormal cervical flexion.

Parental origin was determined in all 27 cases. Digyny accounted for 19 cases and diandry for eight. The majority of digynic cases were the result of errors in the second meiotic division (10/18 assessed). In one case of maternal origin, there was reduction to homozygosity for markers listed as 1–7 cM from the centromere of three different chromosomes (D2S139, D15S11, AR) with non-reduction of markers near the centromeres of five other chromosomes (D8S166, D9S886, D12S87, D16S3093, D16S409, and D21S1911) consistent with ''dieggy''—that is, appearing to have come from two eggs, as has been reported elsewhere.[7]

Assessment of embryonic phenotype with parental origin showed no correlation between the phenotype of the embryo and parental origin of the extra haploid set. GD, normal, and abnormal embryos, including those with the features previously outlined, were seen in both digynic and diandric triploidy. The two GD1 embryos were digynic, as were two of three GD2 and two of three GD4 embryos. One of the two embryos with the ''triploid phenotype'', eight of 10 normal embryos, and four of seven of the other abnormal embryos were digynic.

Of the eight cases of paternal origin, one showed definite PHM while four others showed some features of PHM (villus oedema with cistern formation in three, villus oedema with cistern and lacey trophoblast in one). One case showed scant villi without PHM, one showed hydropic degeneration, and one was normal. All maternal cases were normal or showed only degenerative changes.

DISCUSSION

Triploidy is known to have effects on embryonic/fetal and placental development. Attempts have been made to delineate a specific embryonic phenotype while recognising that triploid embryos show a range of phenotypes from severe disorganisation to relatively normal embryos.[6 8 9] Fetal and placental phenotypes have been established and have been correlated with parental origin of the triploidy.[2 3 6 8–16] It has not yet been established whether the parental origin effect in triploid fetuses is the result of an imprinting effect on the fetus itself or on the placenta, the phenotype of which then determines the growth and phenotype of the fetus. There are findings that support the latter interpretation, including the fact that the phenotype of digynic triploids is essentially one of marked asymmetric intrauterine growth restriction. The growth restriction is always associated with marked adrenal hypoplasia, which, because fetal adrenal growth occurs under the influence of human chorionic gonadotrophin (hCG), most likely reflects lack of sufficient hCG produced by the very small digynic placentas, as documented on triple screening testing.[17 18] Conversely, diandric triploid fetuses usually have better growth (and may have normal growth parameters), and show no features of adrenal hypoplasia, reflecting the increased placental volume and/or the syncytiotrophoblastic hyperplasia with increased levels of hCG encountered in those cases. These findings are in keeping with studies that indicate that imprinted gene expression

Table 1 Triploid embryo: phenotype and parental origin

No.	Gestational age (weeks)	Karyotype	Embryo	Placenta: diandric only	Parental origin	Meiotic origin: digynic only
1	8	69,XXY	GD4		Maternal	MII
2	10	69,XXY	Stage 19		Maternal	MI
3	18	69,XXX	tan deposits (''triploid phenotype'')	PHM	Paternal	
4	8	69,XXX	DA 37 days Frontal protuberance		Maternal	MI
5	9	69,XXY	Stage 17: DA 42–44 days	Normal	Paternal	
6	9	69,XXY	DA 26–30 days Absence cervical flexion		Maternal	MII
7	12	69,XXX	DA 35–38 days		Maternal	MII
8	10	69,XXY	Inconsistent development, tan deposits		Maternal	MII
9	14	69,XXY	Microcephaly, tan deposits (''triploid phenotype'')		Maternal	MI
10	8	69,XXY	41 days DA, tan deposits	Scant villi, no PHM	Paternal	
11	?9–10	69,XXY	GD2	Hydropic villi	Paternal	
12	5	69,XXY	GD2		Maternal	MI
13	9–10	69,XXX	35–38 days DA		Maternal	MI
14	6	70,XXX,+2	GD2		Maternal	MII
15	Unknown	69,XXY	Stage 16, DA 37–42 days		Maternal	MII
16	17	69,XXX	GD4	Cystic villi, ?early PHM	Paternal	
17	15	69,XXX	DA 53 days	Hydropic villi with cisterns ?early PHM	Paternal	
18	17	69,XXY	GD4		Maternal	MI
19	Unknown	68,XX	Stage 16, DA 38 days		Maternal	MI
20	10	68,XXY,−22	GD1		Maternal	MII
21	9–10	69,XXY	Stage 17, absent cervical flexion,		Maternal	MII
22	Unknown	71,XXX,+6,+21	GD1		Maternal	MII
23	7–8	69,XXX	Stage 15,		Maternal	MI
24	9–10	69,XXX	Stage 16,		Maternal	MII
25	15	69,XXY	37–42 days DA, discolourations	Villus oedema with cisterns, ?PHM	Paternal	
26	10–11	69,XXX	Stage 17		Maternal	?"Dieggy"
27	14–15	69,XXY	Green discolourations	Oedema,cisterns, lacey trophoblast ?PHM	Paternal	

appears to be more important in placental than in embryonic development.[19]

The phenotype of triploid human embryos has been assessed without correlation with parental origin of the triploidy. Harris *et al* examined triploid embryos and found 10 severely GD, 4 normal, and 22 abnormal embryos, of which 6 were characterised by less severe growth disorganisation (GD4).[6] These latter 26 triploid embryos were compared with 40 non-triploid embryos using assessment of a combination of four embryonic and placental features: retarded limb development, facial dysplasia, subectodermal haemorrhage, and cystic chorionic villi. This combination of features was not unique to triploidy, being observed in both normal and trisomic embryos, but was most often seen in triploid embryos. The authors concluded that 80% of abnormal embryos with at least three of the features were triploid (excluding those in which cystic villi was the third defining feature, there were 7/22 abnormal triploid embryos with three of the defining characteristics). In our experience, embryos with the features assessed by Harris *et al* occur in a minority of triploid embryos, with GD embryos, embryos with isolated abnormalities, and apparently morphologically normal embryos, usually with development correlating to 37–42 days, being regularly encountered.

In mice, the effects of digyny and diandry in triploid embryogenesis have been assessed in both spontaneous and induced triploidy. The development of mouse triploids appears to be dependent on genetic background, with the expression of triploidy varying between different strains. In an induced digynic triploidy in A strain mice, early triploid embryos (6.5–7.5 days) were classified as normal, while 10 day embryos showed poor development of embryonic structures, particularly mesodermal derivatives, with less affected extraembryonic tissues.[4] Triploid embryos did not survive past the 12th day of gestation. The phenotypes of diandric and digynic embryos have been assessed in C57BL × CBA mice and it was found that in diandric triploids, nine of 44 cases (20.5 %) were empty gestational sacs and the others showed normal development at various stages.[5] The embryos observed were morphologically normal but smaller than their chromosomally normal counterparts. In contrast, two of the 18 digynic triploids (11.1%) were empty gestational sacs and the other embryos were all morphologically abnormal with abnormal cephalic regions. There was no difference in the extraembryonic membranes between the two groups or in comparison with chromosomally normal controls. The lack of effect of parental origin on extraembryonic membranes in triploid mice contrasts with the changes observed in diploid digynic and diandric mice derived from nuclear transfer experiments, in which differences in extent of development were observed in both embryonic and extraembryonic tissues. The diploid digynic embryos showed small but advanced embryos (25 somite stage) with poor development of the extraembryonic tissues, whereas diploid diandric embryos showed poor embryo development (6–8 somite stage) with extensive (more than normal) trophoblast.[20] As both experiments (triploid and diploid) were performed in the same strain of mice, the differences suggest that triploidy alters the imprinting effect on extraembryonic tissues—that is, parent of origin effects are less profound than complete absence of one parental genome. As the descriptions of the digynic triploid mouse embryos are similar to the phenotype described in some human triploid embryos, our hypothesis was that those with the phenotype characteristic of triploidy would be digynic while the more normal embryos would be diandric, as in the mouse model.

Our results, however, suggest that the effects of triploidy in human embryos are non-specific, causing generalised growth disorganisation and other non-specific abnormalities, and also allowing for at least superficially normal early embryonic development. The placenta does show effects related to parental origin, as has been extensively documented, with the diandric cases showing PHM features more often than not.

Our results further illustrate that, in the early embryonic period, digyny predominates as the parental origin of triploidy. In a previous study of 22 triploid embryos, 13 were digynic, 7 were diandric, and 2 were uninformative.[2] With the present cases, there are 47 embryo cases in which parental origin was determined: 32 digynic and 15 diandric. These findings are in keeping with those of others and illustrate, as outlined by Zaragoza *et al*, that the relationship between developmental age and origin of triploidy is not straightforward.[7] It appears that digyny predominates in those cases with development of an embryo and in the fetal phase of development, while diandric triploids generally present at the interface of the embryonic and fetal periods, and are less likely to have discernible embryos or fetuses. The reasons for this are not understood. The placentas of digynic triploids are small, which would seem to make them less likely to survive in utero, possibly accounting for the predominance of digyny in the early embryonic period. Those placentas with PHM may be more likely to survive past the embryonic period, but the larger placental volume and high hCG levels of PHM may lead to earlier clinical presentation or miscarriage, as is the case of true hydatidiform moles. The reasons for survival of digynic triploids compared with diandric triploids into the fetal period are not clear. Possibly, as in mouse strain differences, this depends on genetic background/modifiers.

Triploid embryonic phenotypes do not correlate with parental origin of triploidy, and one of the phenotypes previously thought to be suggestive of triploidy is observed rather infrequently in this population. There do not appear to be growth differences between the diandric and digynic triploid embryos, suggesting that such differences develop later in gestation. These findings also suggest that the growth differences that make up the phenotypes observed in triploid fetuses may be placentally mediated rather than the direct effect of imprinting in the fetus. Factors that determine development of PHM in diandric triploids and intrauterine survival of digynic triploids remain to be elucidated.

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