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Studies of non-disjunction in trisomies 2, 7, 15, and 22: does the parental origin of trisomy influence placental morphology?

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Abstract

Recently, there have been several molecular studies of trisomic fetuses and liveborns which have examined the parent and meiotic stage of origin of non-disjunction. However, little is known about the possible phenotypic effects of the origin of trisomy. For trisomic spontaneous abortions, no distinct phenotype has been described, although some have been reported to have features, such as trophoblastic hyperplasia, similar to hydatidiform moles. In the present report, we describe molecular and histological studies of spontaneous abortions with trisomies 2, 7, 15, or 22, conditions occasionally linked to trophoblastic hyperplasia.

Our results provide strong evidence for chromosome specific mechanisms of non-disjunction, with trisomy 2 having a high frequency of paternally derived cases and trisomy 7 typically originating postzygotically. In studies correlating parental origin of trisomy with phenotype, we found no difference in the proportion of cases with trophoblastic hyperplasia, fetal tissue, nucleated red blood cells, or hydropic villi among paternally or maternally derived trisomies 2, 7, 15, or 22. However, paternally derived trisomies tended to abort earlier than maternally derived trisomies. This suggests that parental origin might affect the developmental stage at which abortion occurs but not other features of placental phenotype.

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Keywords: genomic imprinting; hydatidiform mole; trisomy; trophoblast

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Trisomy is the most common chromosome abnormality in humans, occurring in about 4% of all clinically recognised pregnancies.[1] Nearly all trisomic conceptuses miscarry and as many as 25% of all spontaneous abortions are the result of trisomy.[1]

Most molecular studies of trisomy have focused on the parent and meiotic stage of origin of non-disjunction, using fetuses or liveborns with trisomy 16,[2] trisomy 18,[3] or trisomy 21,[4] among others. These studies have shown that most trisomy originates from maternal meiotic non-disjunction and that factors such as abnormal recombination play an important role in its genesis.[5]

However, little is known about the phenotypic effects of the origin of trisomy, that is, whether or not the parent or meiotic stage of origin influences the phenotype of the trisomic conceptus. Some data are available from studies of people with Down syndrome and specific phenotypic abnormalities such as leukaemia[6] or congenital heart disease.[7] These studies have failed to find a relationship between the parental origin of the extra chromosome 21 and either leukaemia or heart disease, but have provided evidence for other, subtler differences in the origin of trisomy. For example, Zittergruen *et al*[7] suggested that recombination was decreased in meioses leading to Down syndrome with congenital heart defects by comparison with cases without heart disease and Shen *et al*[6] suggested that Down syndrome patients with leukaemia were more likely to be homozygous for regions of chromosome 21 than were Down syndrome patients without leukaemia.

As an alternative approach, we have been interested in examining trisomies in spontaneous abortions to determine whether there might be a relationship between the origin of trisomy and the placental phenotype of the abortus. We have been especially interested in studying the parental origin of trisomy since it is clear that there is a "genome wide" parent of origin effect on placental phenotype. That is, studies have shown that the paternal genomic imbalance in androgenetic diploid and diandric triploid conceptuses may lead to the development of complete and partial hydatidiform moles, respectively, spontaneous abortions characterised by hydropic changes in chorionic villi, and proliferation of trophoblastic tissues.[8–10] Thus, genomic imprinting clearly plays a significant role in determining the phenotype of complete and partial molar pregnancies.

Similar to the situation for hydatidiform moles, we hypothesised that genomic imprinting might play a role in the placental phenotype of trisomy; that is, for chromosomes having imprinted loci, the presence of two paternal and one maternal chromosomes might lead to molar changes.[11] In this event, increased dosage of a single paternal chromosome, rather than of the entire haploid set as seen in androgenetic diploidy and diandric triploidy, would be responsible for the molar phenotype.

Changes in placental morphology alone, or with respect to the origin of the additional chromosome, have not been clearly defined for trisomies. Nevertheless, there have been several reports suggesting the presence of features similar to hydatidiform moles in association with specific trisomies. Most notably, hydropic changes and trophoblastic proliferation have

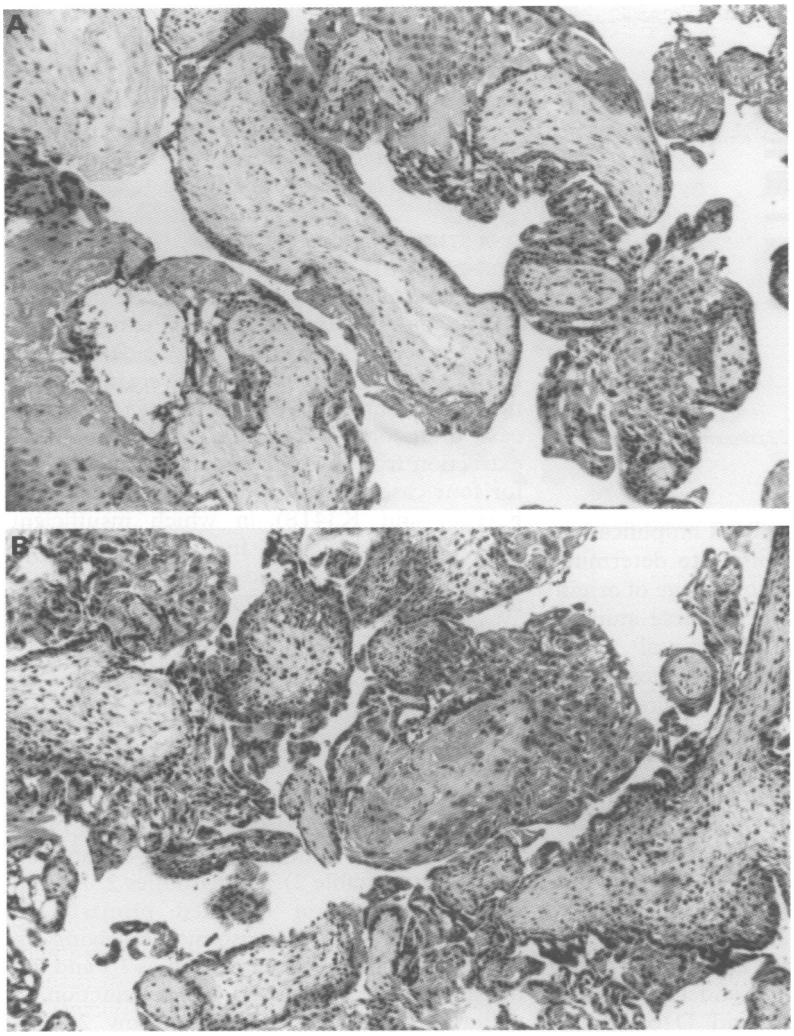

Figure 1 Histological features of trophoblast hyperplasia. (A) Grade 1 trophoblast hyperplasia: focal circumferential hyperplasia involving predominately syncytiotrophoblast surrounds the group of small villi on the right side of the figure. (B) Grade 2 trophoblast hyperplasia: all villi are surrounded by a more florid proliferation of both cytotrophoblast and syncytiotrophoblast (H&E).

been described in cases of trisomies 2, 7, 15, 16, and 22.[11-15] For trisomy 16, virtually all cases are maternal in origin,[2] so the molar changes cannot be explained simply by paternal chromosomal imbalance. However, for the other trisomies paternal overexpression remains a possibility. Chromosomes 7 and 15 have been shown to be imprinted in studies of Silver-Russell syndrome and Angelman/Prader-Willi syndromes,[16 17] and while there is no evidence for imprinting for chromosomes 2 and 22, phenotypic effects in the placenta have not been examined.[18 19] Furthermore, at least for chromosome 2, paternal trisomies are thought to be common.[20]

In this report, we describe molecular and histological studies of spontaneous abortions, investigating the association between placental phenotype and parental origin of the extra chromosome in trisomies 2, 7, 15, and 22, trisomies previously reported to have molar changes or trisomies for chromosomes known to be imprinted, or both. Our results suggest that neither the parent nor the stage of origin of trisomies 2, 7, 15, and 22 plays a significant role in the development of trophoblastic hyperplasia or other obvious phenotypic features such as presence or absence of hydropic villi, fetal tissue, or nucleated red blood cells. However, our results suggest that parent of origin might play a role in the stage of development at which abortion occurs of the trisomic conceptus. This suggests that imprinting may be important in certain aspects of placental development in trisomic conceptuses, but for others, such as trophoblastic hyperplasia, chromosomal imbalance for specific chromosomes alone may be sufficient.

Methods

STUDY POPULATION

The study population consisted of 111 fetuses with non-mosaic, single trisomy 2, 7, 15, or 22, ascertained from cytogenetic studies of spontaneous abortions at University Hospitals of Cleveland, Northside Hospital in Atlanta, and Kapiolani-Children's Medical Center in Honolulu. A total of 66 cases were collected in Cleveland (13 cases of trisomy 2, 10 of trisomy 7, 20 of trisomy 15, and 23 of trisomy 22), and all of these were examined histologically as well as studied for the mechanism of origin of trisomy. The remaining 45 cases were ascertained in Atlanta or Honolulu, and for these only the DNA marker studies of origin of trisomy were performed. Preliminary DNA results in 34 of these cases (14 cases of trisomy 15 and 20 cases of trisomy 22) have been described previously.[21]

Of the 111 cases, 55 involved "complete" families in which blood samples were obtained from both parents. The remaining 56 cases involved "incomplete" families in which tissue was obtained from paraffin blocks, with maternal material being derived from decidua; thus, in these cases fetal and maternal, but not paternal, DNA samples were analysed.

CYTOGENETIC STUDIES

Culturing and cytogenetic analysis of blood and tissue samples were performed using standard procedures.[22] For each case, we attempted to score 10-15 cells.

HISTOLOGICAL STUDIES

Haematoxylin-eosin stained tissue sections for 66 cases of trisomies 2, 7, 15, or 22, ascertained at University Hospitals of Cleveland, were examined microscopically. For each case, the degree of trophoblastic hyperplasia was scored from 0-3 (0=absent, 1=low grade/focal or diffuse hyperplasia, 2=high grade/focal or diffuse partial mole-like hyperplasia, 3=high grade/focal or diffuse complete mole-like hyperplasia; see fig 1 for examples) and the developmental stage (<6.0 weeks, 6.0-8.5 weeks, 8.5-11.5 weeks, >11.5 weeks) of each abortus was estimated by histological criteria. In addition, histological evaluation included the presence or absence of uniformly hydropic villi, fetal tissue, and villous nucleated red blood cells. The presence of nucleated red blood cells was taken as indirect evidence of yolk sac development or villous vasculogenesis. Further details of the histological analysis for trophoblastic hyperplasia and developmental stage are described elsewhere.[23]

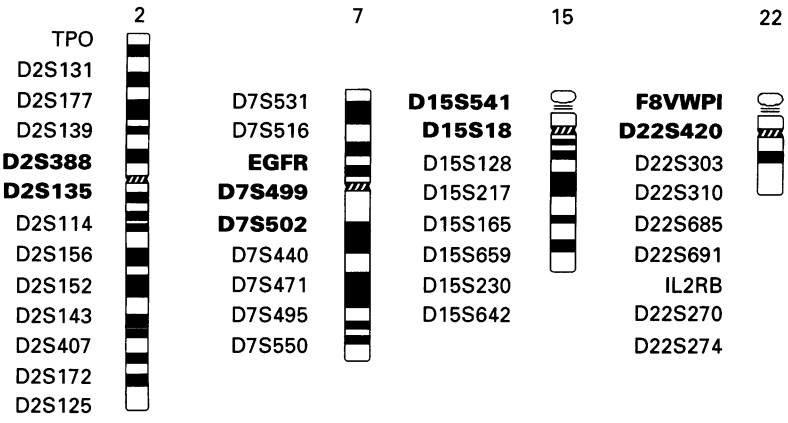

Figure 2 Microsatellite markers for chromosomes 2, 7, 15, and 22, showing the physical order of the markers. Pericentromeric loci are shown in bold.

DNA STUDIES

Each case was analysed by PCR amplification of microsatellite polymorphisms to determine the parental and meiotic/mitotic stage of origin of the extra chromosome. For these studies, DNA was extracted from parental blood samples and fetal tissue as previously described[24] or, alternatively, was obtained from maternal decidua and fetal tissue dissected from paraffin blocks.[25] The DNA polymorphisms consisted of 13 markers for chromosome 2, nine for chromosome 7, eight for chromosome 15, and nine for chromosome 22 with the approximate physical locations of the loci provided in fig 2. The sets of loci were chosen to span the entire chromosome and pericentromeric markers (D2S388 and D2S135 for chromosome 2; EGFR, D7S499, and D7S502 for chromosome 7; D15S541 and D15S18 for chromosome 15; F8VWPI and D22S420 for chromosome 22) were included for the meiotic stage determinations. For chromosomes 2 and 7, the pericentromeric markers flank the centromere and are estimated to be 9 cM and 5 cM apart, respectively (Genome Data Base). For chromosomes 15 and 22, the pericentromeric markers are estimated to be located within 10 megabases of the centromere (Genome Data Base). Additional mapping and PCR primer information for each locus are available from the Genome Data Base. For the studies, DNA was amplified in a total volume of 25 μl, using standard conditions and "low or high touchdown" protocols[26] or using an annealing temperature of 55-65°C. PCR products were then separated by polyacrylamide gel electrophoresis and visualised by autoradiography.

Examples of determinations of the parental and meiotic/mitotic stage of origin of non-disjunction are shown in fig 3 for cases with "complete" families (n=55) and those involving "incomplete" families (n=56). In brief, the parental origin of the additional chromosome was determined by comparing DNA polymorphisms of the fetus and parents; most such determinations were based on at least three informative markers. The meiotic/mitotic stage of origin of non-disjunction was then determined by examining the results at pericentromeric loci, as previously described.[27]

Results

For all 111 cases, detailed information on chromosome constitution, maternal age, and origin of non-disjunction is shown in table 1. Histological studies of placental morphology were conducted on a subset of 66 cases and this information is also given in table 1.

DNA STUDIES OF THE ORIGIN OF NON-DISJUNCTION

The results of the DNA studies for parental and meiotic/mitotic stage of origin are summarised in table 2. Parental origin was determined for 104 of the 111 cases. We were unable to make a parental origin assignment for a single case of trisomy 7 (A2), in which the DNA extraction from paraffin tissue had failed, and for four cases of trisomy 22 (K3272, K3296, K3323, and K3418) in which insufficient material was available. In addition, parental origin was not assigned for two cases with incomplete families (A73, 47,XX,+2; A620, 47,XX,+22) in which the results were consistent with maternal origin but based on fewer than five markers.

Of the 104 cases in which parental origin was determined, 91 (87.5%) were maternal in origin and 13 (12.5%) were paternal in origin. The proportion of paternal cases was highest for trisomy 2 (29%) and lowest for trisomy 22 (3%).

We determined the stage of non-disjunction for 102 cases (table 2). For trisomies 2, 15, and 22, non-disjunction occurred primarily in meiosis. For the two trisomies involving an acrocentric chromosome, trisomies 15 and 22, origin was typically from non-disjunction in maternal meiosis I, but for trisomy 2 non-disjunction occurred in either maternal or paternal meiosis I. Unlike the other trisomies, mitotic non-disjunction was the predominant cause of trisomy 7, accounting for eight (57%) of the 14 cases.

HISTOLOGICAL STUDIES OF PLACENTAL MORPHOLOGY

For 66 cases of trisomies 2, 7, 15, and 22, we examined phenotypic features of the abortus including the presence and degree of hyperplasia (grades 1-3) and the presence or absence of fetal tissue, nucleated red blood cells, and uniformly hydropic villi (table 1). Trophoblastic hyperplasia was observed in 19 (29%) cases and nucleated red blood cells were present in 48 (73%) cases. Fetal tissue and hydropic villi were relatively uncommon features of trisomies 2, 7, 15, and 22, being observed in nine (14%) cases and four (6%) cases, respectively.

From table 1, it is also possible to calculate the frequency of trophoblastic hyperplasia among trisomies 2, 7, 15, and 22. Trophoblastic hyperplasia occurred in six of 10 (60%) cases of trisomy 7, 10 of 20 (50%) cases of trisomy 15, and three of 23 (13%) cases of trisomy 22. None of the 13 cases of trisomy 2 had trophoblastic hyperplasia.

THE ASSOCIATION OF PLACENTAL MORPHOLOGY WITH THE ORIGIN OF NON-DISJUNCTION

Subsequently, we correlated the results of the histological and DNA studies to determine

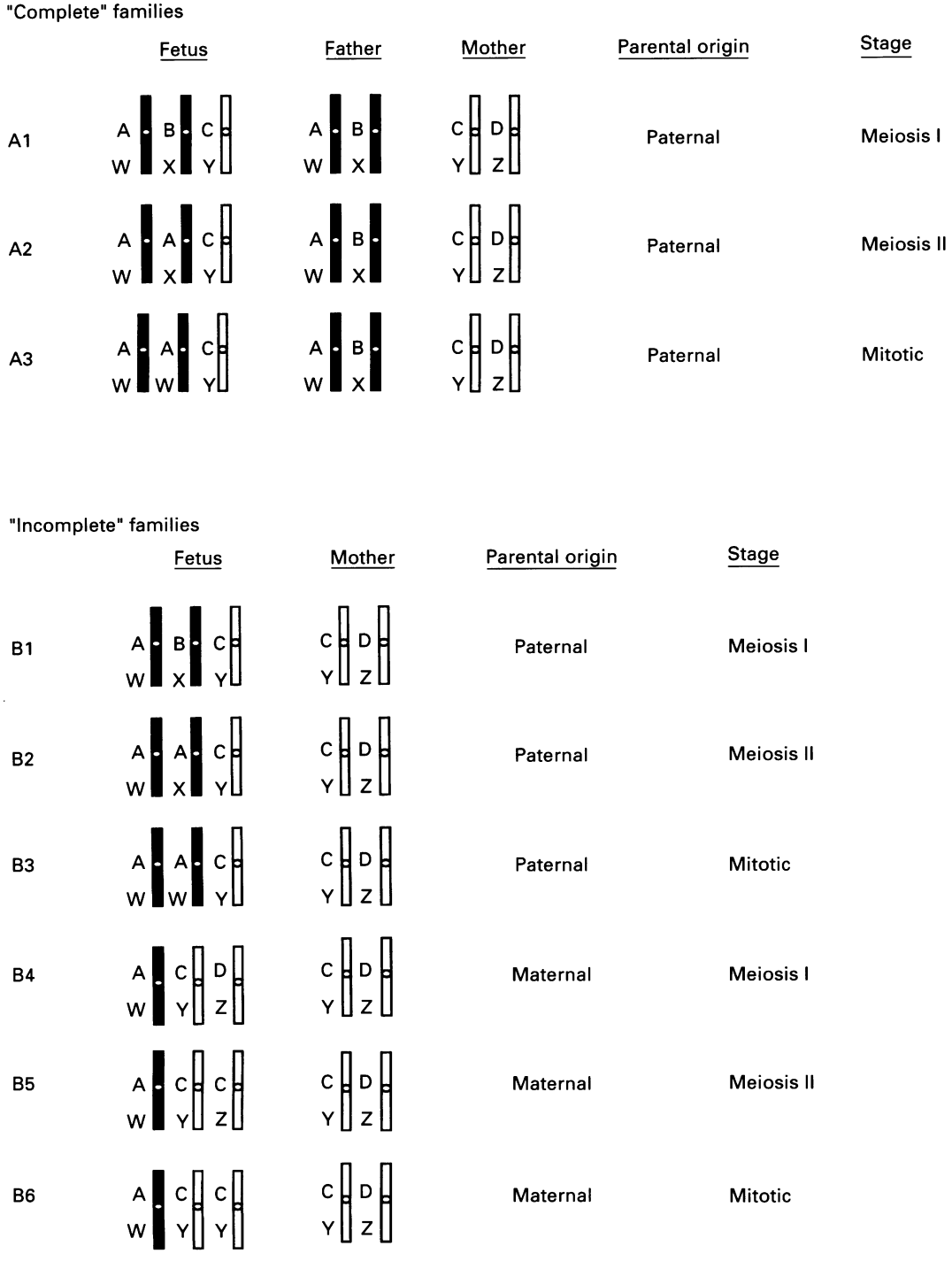

Figure 3 Examples of parent and stage of origin determinations for "complete" and "incomplete" families. (A) Complete families. For families in which DNA samples were available from both parents and the trisomic fetus, the parental origin of trisomy was determined by comparing polymorphisms of the three samples; for the sake of simplicity, examples A1-A3 are restricted to trisomies of paternal origin. For stage of origin determinations, we first identified markers that were heterozygous in the parent of origin, and then asked whether heterozygosity was maintained (non-reduction) or reduced to homozygosity (reduction) in the trisomic fetus. Trisomies were scored as arising at meiosis I if pericentromeric markers were not reduced (A1), at meiosis II if pericentromeric markers were reduced but at least one other marker was not reduced (A2), and in mitosis if all markers, pericentromeric and otherwise, were reduced (A3). (B) Incomplete families. Determinations of the parent and stage of origin had to be modified for those families in which DNA samples were available only from the mother and trisomic fetus. We concluded a paternal origin if the fetus shared only one of three alleles with the mother (B1-B3). We concluded a maternal origin if results at each of five or more microsatellite markers were consistent with that interpretation (B4-B6); previous studies conducted in our laboratory indicate that, in the presence of such "sharing" of maternal and fetal alleles at each of five or more microsatellite markers, the trisomy is virtually always maternally derived. Determinations of the stage of origin were similar to those for the complete families; that is, non-reduction at a pericentromeric marker indicated a meiosis I error (B1 and B4), reduction at a pericentromeric marker but non-reduction at another marker(s) a meiosis II error (B2 and B5), and reduction at all markers a mitotic error (B3 and B6). Note that in making determinations for the paternally derived cases, we assumed that the father was heterozygous at all loci.

whether or not the parental origin of trisomy influenced the placental phenotype. In this analysis, the proportion of cases with trophoblastic hyperplasia, fetal tissue, nucleated red blood cells, or hydropic villi was compared among trisomies of paternal and maternal origin. We observed no difference in the frequencies of any of the features among paternally and maternally derived trisomies. For example, we observed no difference in the frequency of

Table 1 Summary of results for the cytogenetic, molecular, and histological studies of trisomies 2, 7, 15, and 22

ID No*	Chromosome constitution	Mat age	Dev stage (wk)	TH†	Fetal tissue‡	Nucleated red blood cells	Hydropic villi	Origin
Trisomy 2								
A55§	47,XX,+2	34	6.0–8.5	0	–	–	–	PAT MI
A73§	47,XX,+2	41	6.0–8.5	0	–	+	–	Unknown¶
A195	47,XY,+2	28	6.0–8.5	0	–	+	–	MAT MI
A262	47,XX,+2	38	<6.0	0	–	–	–	MAT MI
A554	47,XX,+2	28	<6.0	0	–	–	–	MAT MI or MII
A555§	47,XY,+2	23	6.0–8.5	0	–	–	+	MAT MI
A628§	47,XY,+2	37	6.0–8.5	0	–	+	–	MAT MI or MII
A662§	47,XX,+2	23	<6.0	0	–	–	–	PAT MI
A752§	47,XY,+2	25	6.0–8.5	0	–	–	+	PAT MI
A838§	47,XY,+2	37	6.0–8.5	0	–	–	+	MAT MI or MII
A853§	47,XY,+2	40	8.5–11.5	0	–	–	–	MAT MII
A992§	47,XX,+2	27	6.0–8.5	0	–	–	–	MAT MI or MII
A1037§	47,XX,+2	39	6.0–8.5	0	–	+	–	MAT MI or MII
S300	47,XY,+2	34	nt	nt	nt	nt	nt	PAT MI
S338	47,XX,+2	26	nt	nt	nt	nt	nt	MAT
S361	47,XY,+2	23	nt	nt	nt	nt	nt	MAT MI or MII
S379	47,XX,+2	31	nt	nt	nt	nt	nt	MAT MI
S1447	47,XX,+2	34	nt	nt	nt	nt	nt	MAT mitotic
S1498	47,XX,+2	30	nt	nt	nt	nt	nt	PAT MI
Trisomy 7								
A2§	47,XX,+7	36	6.0–8.5	2	–	+	+	Unknown
A6§	47,XX,+7	36	>11.5	0	+	+	–	MAT mitotic
A123§	47,XY,+7	41	<6.0	0	–	+	–	MAT mitotic
A179§	47,XX,+7	17	6.0–8.5	1	–	+	–	PAT mitotic
A420§	47,XX,+7	30	6.0–8.5	2	–	+	–	PAT mitotic
A475§	47,XX,+7	26	8.5–11.5	0	–	–	–	MAT MI
A758§	47,XY,+7	24	6.0–8.5	0	+	+	–	MAT MII
A793§	47,XY,+7	34	<6.0	2	–	–	–	MAT MI
A862§	47,XY,+7	34	6.0–8.5	1	–	+	–	MAT MI or MII
A1000§	47,XY,+7	32	8.5–11.5	1	–	+	–	MAT mitotic
S33	47,XY,+7	30	nt	nt	nt	nt	nt	MAT mitotic
S213	47,XX,+7	41	nt	nt	nt	nt	nt	MAT MII
S785	47,XY,+7	29	nt	nt	nt	nt	nt	MAT mitotic
S851	47,XY,+7	30	nt	nt	nt	nt	nt	MAT mitotic
S1422	47,XY,+7	37	nt	nt	nt	nt	nt	MAT MII
Trisomy 15								
A46	47,XY,+15	37	8.5–11.5	1	–	+	–	MAT MI
A96§	47,XY,+15	18	6.0–8.5	2	–	+	–	MAT MI
A99	47,XX,+15	31	6.0–8.5	0	–	+	–	PAT MII
A265§	47,XX,+15	41	6.0–8.5	1	–	+	–	MAT MI or MII
A317§	47,XX,+15	44	8.5–11.5	1	–	+	–	MAT MI
A342§	47,XX,+15	38	8.5–11.5	0	–	+	–	MAT MI or MII
A359§	47,XY,+15	33	8.5–11.5	0	–	+	–	MAT MI
A379§	47,XY,+15	39	6.0–8.5	0	+	+	–	PAT MII
A405	47,XY,+15	38	6.0–8.5	0	–	+	–	MAT MI
A496§	47,XX,+15	27	8.5–11.5	1	–	+	–	PAT MII
A502§	47,XX,+15	34	8.5–11.5	0	+	+	–	MAT MI
A581§	47,XY,+15	32	8.5–11.5	2	–	+	–	MAT MI
A593	47,XY,+15	34	6.0–8.5	0	–	+	–	MAT MI or MII
A781§	47,XX,+15	39	8.5–11.5	1	–	+	–	MAT MI or MII
A849	47,XY,+15	32	<6.0	0	–	+	–	MAT MI
A885	47,XX,+15	32	8.5–11.5	1	–	+	–	MAT MI
A952§	47,XX,+15	33	6.0–8.5	0	–	–	–	MAT MI
A953§	47,XX,+15	32	8.5–11.5	1	–	+	–	MAT MI
A984§	47,XX,+15	27	8.5–11.5	2	–	+	–	MAT MI
A1003§	47,XX,+15	31	8.5–11.5	0	–	+	–	MAT MI
S104	47,XX,+15	34	nt	nt	nt	nt	nt	MAT MI or MII
S112	47,XY,+15	38	nt	nt	nt	nt	nt	MAT MI or MII
S287	47,XY,+15	38	nt	nt	nt	nt	nt	MAT MI
S359	47,XY,+15	42	nt	nt	nt	nt	nt	MAT MII
S387	47,XX,+15	38	nt	nt	nt	nt	nt	MAT MI
S467	47,XX,+15	34	nt	nt	nt	nt	nt	PAT MI or MII
S494	47,XX,+15	33	nt	nt	nt	nt	nt	MAT MI or MII
S550	47,XY,+15	28	nt	nt	nt	nt	nt	MAT MI or MII
S557	47,XX,+15	32	nt	nt	nt	nt	nt	PAT MII
S687	47,XY,+15	37	nt	nt	nt	nt	nt	MAT MI
S811	47,XX,+15	31	nt	nt	nt	nt	nt	MAT MI or MII
S814	47,XX,+15	38	nt	nt	nt	nt	nt	MAT MI or MII
S826	47,XY,+15	36	nt	nt	nt	nt	nt	MAT MI
S827	47,XX,+15	35	nt	nt	nt	nt	nt	MAT MII
Trisomy 22								
A38§	47,XX,+22	39	6.0–8.5	2	–	+	–	MAT MI or MII
A54§	47,XX,+22	27	6.0–8.5	0	–	+	–	PAT MI or MII
A84§	47,XY,+22	35	6.0–8.5	0	–	+	–	MAT MI or MII
A93§	47,XX,+22	36	6.0–8.5	0	+	+	–	MAT MI
A131§	47,XY,+22	33	8.5–11.5	0	–	+	–	MAT MI or MII
A133§	47,XX,+22	37	6.0–8.5	0	+	+	–	MAT MI
A212§	47,XY,+22	39	6.0–8.5	0	–	+	–	MAT
A306§	47,XX,+22	38	8.5–11.5	0	–	–	–	MAT MI or MII
A320§	47,XX,+22	36	8.5–11.5	0	–	+	–	MAT MI or MII
A435§	47,XY,+22	25	6.0–8.5	0	–	+	–	MAT MI or MII
A453§	47,XY,+22	37	<6.0	0	–	+	–	MAT MI or MII
A454§	47,XY,+22	35	<6.0	0	+	+	–	MAT MI
A464§	47,XY,+22	43	8.5–11.5	1	–	+	–	MAT MI
A620§	47,XX,+22	26	6.0–8.5	0	–	+	–	Unknown¶
A624§	47,XY,+22	36	8.5–11.5	0	–	–	–	MAT MI or MII
A768§	47,XX,+22	34	8.5–11.5	0	–	+	–	MAT MI or MII
A817§	47,XY,+22	32	8.5–11.5	0	+	–	–	MAT MI

Table 1 Continued

*ID No**	*Chromosome constitution*	*Mat age*	*Dev stage (wk)*	*TH†*	*Fetal tissue‡*	*Nucleated red blood cells*	*Hydropic villi*	*Origin*
A827§	47,XX,+22	30	8.5–11.5	1	–	+	–	MAT MI or MII
A886§	47,XY,+22	38	8.5–11.5	0	–	+	–	MAT MII
A898§	47,XX,+22	36	8.5–11.5	0	–	+	–	MAT MI
A977§	47,XY,+22	35	8.5–11.5	0	+	–	–	MAT MI or MII
A1004§	47,XY,+22	40	8.5–11.5	0	–	–	–	MAT MI
A1013§	47,XX,+22	36	8.5–11.5	0	–	–	–	MAT MI
S51	47,XX,+22	35	nt	nt	nt	nt	nt	MAT MI
S133	47,XX,+22	40	nt	nt	nt	nt	nt	MAT MI
S286	47,XY,+22	40	nt	nt	nt	nt	nt	MAT MI
S298	47,XX,+22	40	nt	nt	nt	nt	nt	MAT MI
S351	47,XY,+22	40	nt	nt	nt	nt	nt	MAT MI
S419	47,XY,+22	35	nt	nt	nt	nt	nt	MAT MI
S461	47,XX,+22	37	nt	nt	nt	nt	nt	MAT MI
S661	47,XX,+22	28	nt	nt	nt	nt	nt	MAT MI or MII
S672	47,XY,+22	32	nt	nt	nt	nt	nt	MAT MI
S686	47,XX,+22	35	nt	nt	nt	nt	nt	MAT MI
S764	47,XX,+22	36	nt	nt	nt	nt	nt	MAT MI
S820	47,XY,+22	32	nt	nt	nt	nt	nt	MAT MI
K3217	47,XY,+22	35	nt	nt	nt	nt	nt	MAT MI
K3272	47,XY,+22	31	nt	nt	nt	nt	nt	Unknown
K3296	47,XY,+22	29	nt	nt	nt	nt	nt	Unknown
K3323	47,XX,+22	32	nt	nt	nt	nt	nt	Unknown
K3359	47,XX,+22	23	nt	nt	nt	nt	nt	MAT MI or MII
K3400	47,XX,+22	33	nt	nt	nt	nt	nt	MAT MI or MII
K3418	47,XX,+22	27	nt	nt	nt	nt	nt	Unknown
K3450	47,XX,+22	42	nt	nt	nt	nt	nt	MAT MI or MII

*A = ascertained in Cleveland, S = ascertained in Atlanta, K = ascertained in Honolulu.
†TH = trophoblastic hyperplasia; scoring: 0 = absent, 1 = diffuse, 2 = high grade/partial mole-like hyperplasia, 3 = high grade/complete mole-like hyperplasia.
‡+ = present, – = absent, nt = not tested.
§Fetal and maternal genotypes only.
¶Results consistent with maternal origin but based on fewer than five markers.

Table 2 Parental origin and stage of origin of trisomies 2, 7, 15, and 22

Trisomy	*Total No*	*Maternal meiotic*			*Paternal meiotic*			*Mitotic*		*Unknown*
		I	*II*	*I or II*	*I*	*II*	*I or II*	*Mat*	*Pat*	
2	19	4	1	6	5	0	0	1	0	2*
7	15	2	3	1	0	0	0	6	2	1
15	34	17	2	10	0	4	1	0	0	0
22	43	20	1	15	0	0	1	0	0	6*

*For both trisomy 2 and trisomy 22, one of the unknown cases was maternally derived but we were unable to determine whether the additional chromosome originated in meiosis or mitosis.

*Table 3 Parental origin and trophoblastic hyperplasia of trisomies 2, 7, 15, and 22**

Trisomy	*Paternal*			*Maternal*		
	+	–	*nt*	+	–	*nt*
2	0	3	2	0	9	4
7	2	0	0	3	4	5
15	1	2	2	9	8	12
22	0	1	0	3	18	16
Total	3	6	4	15	39	37

*Includes cases in which parental origin could be determined.
+ = hyperplastic, – = non-hyperplastic, nt = not tested.

hyperplasia in the paternally derived trisomies, three of nine (33%) cases, versus that for maternally derived trisomies, 15 of 54 (33%) cases, nor was there any evidence that individual trisomies (trisomies 2, 7, 15, or 22) had differing levels of trophoblastic hyperplasia among paternally or maternally derived cases (table 3).

The results of the DNA studies on the mechanism of origin of trisomy were also compared with the histological observations to determine whether there was an association between the stage of origin of non-disjunction and placental phenotype. We observed no obvious difference in the frequencies of trophoblastic hyperplasia, fetal tissue, nucleated red blood cells, or hydropic villi in trisomies of meiosis I, meiosis II, or mitotic origin. In addition, the sex chromosome constitution for each trisomic abortus was compared with the histological observations for placental phenotype. There was no difference in the frequency of any phenotypic feature between XX and XY trisomies, suggesting that the sex chromosomes do not play a major role in placental phenotype.

Finally, we compared the results of the DNA studies with the histological evaluation for age of development of the abortus (table 4). There was no obvious association between developmental age and the stage of origin of trisomy. However, we observed a significant increase in the proportion of paternally derived cases which aborted early (<6 weeks or 6.0-8.5 weeks); eight of nine (89%) paternally derived cases were evaluated as less than 8.5 weeks compared to 26 of 54 (48%) maternally derived cases (χ^2=5.75, p<0.05). While the small sample size precluded formal analysis of individual trisomies, this trend was observed for each of the four trisomies.

*Table 4 Parental origin and stage of development of trisomies 2, 7, 15, and 22**

Trisomy	*Origin*	*<6 wk*	*6.0–8.5 wk*	*8.5–11.5 wk*	*>11.5 wk*
2	Maternal	2	6	1	0
	Paternal	1	2	0	0
7	Maternal	2	2	2	1
	Paternal	0	2	0	0
15	Maternal	1	5	11	0
	Paternal	0	2	1	0
22	Maternal	2	6	13	0
	Paternal	0	1	0	0
Total	Maternal	7	19	27	1
	Paternal	1	7	1	0

*Includes cases in which parental origin could be determined.

Discussion

NON-DISJUNCTION OF AUTOSOMAL TRISOMIES

While the primary purpose of this study was to analyse parental origin effects on phenotype, it also generated information on the stage of origin of non-disjunction for trisomies 2, 7, 15,

and 22, and provides further evidence for chromosome specific non-disjunction patterns. In previous studies of autosomal trisomies 16, 18, and 21, it has been shown that non-disjunction predominately originates from maternal meiosis I.[2-4] This study, surveying a larger collection of cases, confirms our earlier report that this is also true for trisomies 15 and 22.[21] However, the results of the present study suggest different aetiologies for trisomies 2 and 7. Trisomy 2 was found to have a substantial paternal contribution and more than half of the cases of trisomy 7 originated from mitotic non-disjunction. This latter observation is consistent with molecular studies of eight pregnancies with confined placental mosaicism of trisomy 7, in which seven cases resulted from a somatic error and one case was the result of a maternal meiosis I error.[28]

ORIGIN AND PLACENTAL PHENOTYPE

We hypothesised that paternally derived trisomies involving chromosomes with imprinted loci might have abnormal placental phenotypes. To test this, we first conducted a detailed histological study of trisomies 2, 7, 15, and 22, evaluating specific phenotypic features, primarily trophoblastic hyperplasia as well as fetal tissue, nucleated red blood cells, hydropic villi, and the developmental stage of the abortus. Next, we determined the parent and meiotic/mitotic stage of origin of the additional chromosome involved and correlated these observations with placental phenotype. The results provide no evidence that either parent or meiotic/mitotic stage of origin is associated with the development of trophoblastic hyperplasia, fetal tissue, nucleated red blood cells, or hydropic villi in any of the trisomies examined; however, the results provide suggestive evidence that the parental origin of trisomy influences the stage of development of the abortus.

Our finding of an association between the parental origin of the additional chromosome and the developmental age of the trisomic abortus suggests that genomic imprinting plays a role in the relative viability of the conceptus. In the mouse, studies have shown that genomic imprinting influences both extent of development and viability of embryos. For example, mouse conceptuses with paternal uniparental disomy for distal chromosome 7 die much earlier than do those with maternal uniparental disomy 7.[29] In humans, a similar effect has been observed in studies of triploidy. Among triploid spontaneous abortions, gestational ages are significantly advanced among androgenetic triploids by comparison with gynogenetic triploids,[9 23] while most triploids which reach term are of maternal origin.[30] Our results on trisomy, although based on a limited number of cases, suggest the existence of a parental origin effect on the viability of the trisomic conceptus.

In contrast, our results provide no evidence that the parental origin of trisomies 2, 7, 15, and 22 is associated with molar features, such as trophoblastic hyperplasia and hydropic villi, or the development of fetal tissues. There are several possible explanations for the lack of any such parental origin effect. For example, since different loci have been implicated in the growth and development of the placenta,[31] there may be a number of mechanisms leading to trophoblastic hyperplasia, some involving imprinted loci and others simply involving the overexpression of non-imprinted loci. It is possible that those involving imprinted loci do not depend on the overexpression of paternally expressed loci but are related to the underexpression of maternally expressed loci. Both types of dosage imbalance are present in androgenetic diploid and diandric triploid moles but which of the two is responsible for the abnormal phenotype is uncertain. In chromosomally normal conceptions, paternal and maternal contributions are equivalent, but in complete moles and triploids these relationships are altered. In androgenetic diploid and diandric triploid moles, paternal contributions represent all and two-thirds of the genome, respectively, while maternal contributions are decreased to none and to one-third, respectively. In the present study on trisomy we have focused on the effect of increased dosage of paternal genetic material. In paternally derived trisomies, the paternal contribution is increased by a single chromosome compared to that of normal diploid conceptions and the maternal contribution to the entire genome remains essentially the same. Thus, our approach tests only for the effect of increased dosage of paternally expressed loci and not for a decrease of maternally expressed loci. Our results indicate that this type of imbalance is insufficient to cause trophoblastic hyperplasia.

An alternative approach to test whether or not the underexpression of a single imprinted chromosome might play a role in abnormal placental phenotype would be to screen for uniparental disomy. This has been postulated as the explanation for the small proportion of complete moles which are biparental conceptuses.[32] A genome wide analysis to screen for uniparental disomy might be performed to test this hypothesis.

Finally, our failure to identify a parental origin effect may simply mean that genomic imprinting in trisomies is unimportant in the genesis of trophoblastic hyperplasia, as well as in the other features we examined. Rather, the cause of the hyperplasia may be imbalance of specific chromosomal material, leading to the overexpression of non-imprinted genes which are involved in trophoblastic growth. For example, for trisomies 7 and 15, several loci encoding growth factors, cytokines, and receptors expressed in placental trophoblasts, such as IGF1R, EGFR (erb B), IL6, HGF, and HGFR (met), have been mapped to these chromosomes.[31 33] Overexpression of these genes in trisomy may lead to trophoblast proliferation. Future studies may involve the production of trophoblasts overexpressing these loci by gene targeting in the mouse; analysis of its effect on the growth and development of the placenta may provide insight into the factors which play a role in the abnormal phenotype seen in molar pregnancies.

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