Tetrasomy 16 in the mouse: a more severe condition than the corresponding trisomy

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SUMMARY

Although an understanding of the phenotypic similarities and differences between individuals trisomic and tetrasomic for the same chromosome or chromosome segment is of considerable theoretical interest with regard to the relationship of the phenotypic features of aneuploid states to changes in the dosage of specific genes, the data obtainable from human cases of tetrasomy do not permit firm conclusions to be drawn. However, a mouse model system based on the intercrossing of animals doubly heterozygous for two Robertsonian translocation (metacentric) chromosomes with monobrachial homology can be used to generate both tetrasomic and trisomic embryos simultaneously. With mice carrying the two metacentric chromosomes, \( Rb(11.16)_{2}H \) and \( Rb(16.17)_{3}Lub \), which share chromosome 16 in common, embryos with tetrasomy 16 and trisomy 16 have been generated. The rate of non-disjunction in both males and females was 34 to 36 \%, and the observed overall frequency of tetrasomics in embryos up to 10 days of age was 3.3 \%, very close to the expected 3.1 \%. Although trisomy 16 and tetrasomy 16 embryos could not be distinguished by size from one another or from diploid embryos at the sixth day of gestation, the tetrasomy 16 embryos were very small and delayed in development at day 10 and clearly distinguishable from the trisomics. Fusion of the anterior neuropore had not occurred, rotation of the embryo was incomplete, and somite formation was retarded. By day 12, all of the tetrasomy 16 embryos were either dead or dying. By contrast, virtually all trisomy 16 embryos were alive at day 10, although about 60 \% were retarded in development. The same was also the case on day 12 for trisomy 16 embryos obtained from mothers that were naturally mated. However, superovulation of the mothers, which caused a doubling of the number of implants, resulted in a significant decrease in the frequency of trisomy 16 embryos and possibly of tetrasomy 16 embryos as well. This finding is consistent with the suggestion that the viability of aneuploid embryos, which may be at a proliferative disadvantage, is highly dependent upon intrauterine conditions. These exogenous factors notwithstanding, it is clear that tetrasomy 16 has much more deleterious effects on embryonic development than does trisomy 16 and that four doses of the genes on chromosome 16 result in developmental derangements greater than are produced by three doses of the same genes.

INTRODUCTION

While trisomy is the most commonly observed autosomal aneuploidy in man, only a few reports have been made of full or, more usually, partial autosomal tetrasomy in either mosaic or pure form (Balestrazzi \textit{et al.} 1983; Batista, Vianna-Morgante \& Richiere-Costa, 1983; Garcia-Cruz \textit{et al.} 1982; Schinzel \textit{et al.} 1981; * Present address: Département d'Embryologie et de Tératologie expérimentales, Institut de Zoologie, Université de Fribourg, CH-1700 Fribourg, Switzerland.† Author for reprint requests: Department of Pediatrics, University of California.

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Understanding the phenotypic similarities and differences between individuals trisomic and tetrasomic for the same chromosome or chromosome segment is of considerable theoretical interest with regard to attempts to relate the phenotypic features of aneuploid states to changes in the dosage of specific genes (Epstein, 1986). Unfortunately, the conclusions that can be drawn from the human tetrasomy data are quite limited because of the scarcity of cases, the difficulties in matching unrelated translocations (two translocations for a similar region do not necessarily involve exactly the same loci), and the problems associated with investigations of human prenatal development. The existence of these limitations in the study of tetrasomy in man suggests a need for an animal model system in which specific tetrasomies can be generated and analysed.

A method for generating embryos and foetuses trisomic for whole autosomes already exists (Gropp & Kolbus, 1974; White, Tjio, Van de Water & Crandall, 1974) and is based on the use of animals, usually males, doubly heterozygous for two Robertsonian translocation (metacentric) chromosomes that have monobrachial homology (i.e. share a chromosome arm in common) (Gropp, Kolbus & Giers, 1975). A high degree of non-disjunction occurs in such animals (Gropp & Winking, 1981) and results in the generation of both nullisomic and disomic gametes. The latter, upon fertilization of a normal gamete, produces trisomic progeny. Depending on the specific metacentric chromosomes used, all of the mouse autosomal trisomies can be produced, and this breeding system has been used in a variety of studies of aneuploidy in the mouse (Epstein, 1985a, b). Since the same combinations of chromosomes can be carried by both males and females, it would be predicted that a breeding scheme employing pairs of animals with identical sets of metacentric chromosomes should result in the conception of embryos tetrasomic or nullisomic for the common chromosome arm, as well as the already observed trisomies and monosomics (Fig. 1). Such a breeding scheme had previously been used to study trisomy 19 (White, Tjio, Van de Water & Crandall, 1972) and non-disjunction (Searle & Beechey, 1982) and had been suggested by Ford (1975) as a means to produce nullisomic embryos. A preliminary report of its use to generate embryos with tetrasomy 15 has recently appeared (Beechey, Kirk & Searle, 1985).

In this report, we describe the generation and phenotypic properties of mouse embryos tetrasomic for chromosome 16. These embryos, while able to implant and survive to midgestation, are much more severely affected than are siblings with trisomy 16. In addition, we describe some previously unreported aspects of the development of trisomy 16 embryos and of a possible relationship between intrauterine conditions and the survival of aneuploid embryos.

MATERIALS AND METHODS

(A) Production of aneuploid embryos

Female mice doubly heterozygous for two metacentric Robertsonian translocation chromosomes, \( Rb(11.16)2H \) and \( Rb(16.17)32Lub \) were mated with doubly heterozygous males of the
same type to generate aneuploid embryos and diploid controls. Both homozygous strains were obtained from Dr Heinz Winking and the late Prof. Alfred Gropp, Lübeck, Germany, and were maintained in our laboratory as closed outbred colonies. The day the vaginal plug was observed was considered as day 0. At the appropriate day of gestation, the pregnant females were killed, the embryos removed and their appearance recorded. Some of them were photographed before the genotype was determined by karyotype analysis.

(B) Karyotype analysis

Embryos were karyotyped by modifications of the method described in Magnuson, Smith & Epstein (1982). Day-3 (preimplantation) embryos were removed from the uteri of superovulated females (4 to 5 weeks old) and incubated for 3–4 h in modified Whitten’s medium (Golbus & Epstein, 1974) containing 0·08 μg ml⁻¹ of vinblastine (Velban, GIBCO). After incubation, the mitotically arrested embryos were individually transferred to a microdrop of a hypotonic solution (0·75 % sodium citrate) in a well of a microtitre plate and incubated at room temperature for 5 min and then on ice for up to 1 h. One embryo at a time was then transferred to a new well containing a 7:3 methanol:acetic acid fixative. After a few seconds, the embryo was transferred to a microscope slide and immediately covered with a microdrop of a 1:4:5 lactic acid (85 % syrup solution): distilled water: acetic acid solution. Once the fixed embryo was seen to break apart, it was washed with an excess of fixative and dried thoroughly. The slides were stained with 3 % Giemsa (Gurr) for 10 min. The genotype was determined by counting the number of acrocentric and metacentric chromosomes per metaphase plate (Fig. 1). Tetrasomic embryos have 42 chromosome arms distributed as 34 acrocentrics and 4 metacentrics while trisomics have 41 chromosome arms and 3 metacentrics and diploid embryos have 40 arms and 2 metacentrics.

Day-6 (early postimplantation) embryos were dissected from the uteri of hormonally induced females (8 to 12 weeks old) and incubated 4–6 h in Dulbecco’s modified Eagle’s medium supplemented with 10 % foetal calf serum and 0·04 μg ml⁻¹ of vinblastine. The karyotyping procedure was the same as that for preimplantation embryos, except that the hypotonic solution was 0·6 % sodium citrate.

RESULTS

(A) Survival of aneuploid embryos

Forty-two superovulated Rb2H/Rb32Lub females were mated with Rb2H/Rb32Lub males and sacrificed at various stages of development. The mean litter size (number of preimplantation embryos or implantation sites) was 23·1, 14·7, 15·1, and 18·2 at, respectively, 3, 6, 10, and 12 days of gestation. Twenty-three additional naturally mated females were dissected at days 10 and 12 and had a mean of 9·8 and 10·2 implantation sites, respectively.

Among the 593 embryos successfully karyotyped, 20 were tetrasomic for chromosome 16 (3·4 %). The frequency in embryos 10 days or less in gestational age was 3·3 %. The frequency of tetrasomy per 100 diploid embryos ranged from 1·8 in day-12 superovulated females to 7·8 in day-10 superovulated females (Table 1).
Fig. 1. Production of specific nullisomic (Ns), monosomic (Ms), diploid (2n), trisomic (Ts) and tetrasomic (Tets) mouse embryos. In the first cross (P generation), a female homozygous for a Robertsonian translocation chromosome is mated with a male homozygous for another Robertsonian translocation chromosome which has one chromosome arm in common with the female's metacentric. The resulting F₁ generation mice, doubly heterozygous for the two metacentrics, have a high rate of non-disjunction which will generate 25–40% (Gropp, Kolbus & Giers, 1975) of gametes either nullisomic or disomic for the shared chromosome arm. When two F₁ mice are crossed together, four different types of aneuploids are generated (F₂ generation) together with diploid embryos. Only the three pairs of chromosomes involved in the metacentrics are shown here. Except for a very low frequency of non-disjunction involving mainly chromosomes 11 and 17 (White et al. 1972; Gropp & Winking, 1981), the other 34 acrocentrics segregate normally.
Table 1. Number and frequency of aneuploid embryos in the cross Rb(11.16)2H/Rb(16.17)32Lub × Rb(11.16)2H/Rb(16.17)32Lub

<table>
<thead>
<tr>
<th>Sample size</th>
<th>Number of diploid embryos</th>
<th>Monosomy 16</th>
<th>Trisomy 16</th>
<th>Tetrasomy 16</th>
<th>Other chromosome abnormalities*</th>
</tr>
</thead>
<tbody>
<tr>
<td>From superovulated females</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 3</td>
<td>47</td>
<td>24 (51-1)</td>
<td>29 (61-7)</td>
<td>1 (2-1)</td>
<td>4 (8-5)</td>
</tr>
<tr>
<td>Day 6</td>
<td>97</td>
<td>0 (0)</td>
<td>36 (37-1)</td>
<td>5 (5-2)</td>
<td>3 (3-1)</td>
</tr>
<tr>
<td>Day 10</td>
<td>77</td>
<td>0 (0)</td>
<td>29 (37-7)</td>
<td>6 (7-8)</td>
<td>5 (6-5)</td>
</tr>
<tr>
<td>Day 12</td>
<td>55</td>
<td>0 (0)</td>
<td>9 (16-4)</td>
<td>1 (1-8)</td>
<td>1 (1-8)</td>
</tr>
<tr>
<td>From naturally mated females</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 10</td>
<td>19</td>
<td>0 (0)</td>
<td>8 (42-1)</td>
<td>1 (5-3)</td>
<td>2 (10-5)</td>
</tr>
<tr>
<td>Day 12</td>
<td>96</td>
<td>0 (0)</td>
<td>31 (32-3)</td>
<td>6 (6-2)</td>
<td>1 (1-0)</td>
</tr>
</tbody>
</table>

* Included are non-specific monosomic, trisomic, double trisomic, triploid, and one triploid–tetrasomic embryos.

Approximately equal frequencies of monosomy 16 and trisomy 16 embryos were found at the early blastocyst (day 3) stage (Table 1). However, the mean cell number was significantly lower ($P<0.005$, Student's t-test) in monosomic than in diploid or in trisomic littermates (38 ±3 [s.e.m.], 52 ±2 and 49 ±3 cells per embryo, respectively). The single tetrasomy 16 embryo karyotyped at this stage had 51 cells. At day 6, no monosomic embryos were found and the ratio
Table 2. General appearance of the trisomic 16 (Ts) and tetrasomic 16 (Tets) embryos and their diploid littermates (2n) at midgestation

<table>
<thead>
<tr>
<th>Appearance of the embryos</th>
<th>Superovulated females</th>
<th>Naturally mated females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 10</td>
<td>Day 12</td>
</tr>
<tr>
<td></td>
<td>2n: Ts: Tets</td>
<td>2n: Ts: Tets</td>
</tr>
<tr>
<td>Normal to slightly small</td>
<td>73: 12: 6</td>
<td>48: 4: 6</td>
</tr>
<tr>
<td>Retarded, alive</td>
<td>4: 17: 6</td>
<td>4: 1: 6</td>
</tr>
<tr>
<td>Dying</td>
<td>3: 4: 1</td>
<td></td>
</tr>
<tr>
<td>Membranes only</td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

of trisomics to disomics was lower, although not significantly, than before implantation.

On the tenth day of development, the proportion of trisomy 16 embryos from either superovulated or naturally mated females was the same as at day 6. Two days later, no change was found in the naturally mated females, but superovulated one carried significantly fewer (P < 0.05, chi-squared test) trisomy 16 embryos (Table 1). The same difference was observed when the numbers of diploid and chromosomally abnormal embryos were related to the total number of implantation sites (Fig. 2).

(B) Severity of the trisomic and tetrasomic states

Trisomic and tetrasomic embryos could not be distinguished by size from normal littermates at the 6th day of gestation. However, the tetrasomic embryos were very delayed in development at day 10 (Fig. 3, Table 2). Although a beating heart and onset of somite and allantois formation was noted, fusion of the anterior neuropore had not occurred (Fig. 4B) and the rotation of the embryo was incomplete (Fig. 3C, Fig. 4A, B). The most advanced day-10 tetrasomic embryo had only 17 somites (Fig. 4D), compared with 29.8 ± 1.4 (s.d.) and 26.8 ± 1.9 somites in diploid and trisomic siblings, respectively. At day 12, the tetrasomic embryos were either dying (Table 2, Fig. 5D, E, F) or were so resorbed that only extraembryonic membranes were found. At this stage, only one out of seven tetrasomics still had a beating heart.

An extremely deformed and retarded day-10 embryo (Fig. 3D) had 53 acrocentric and 4 metacentric chromosomes, giving a total of 61 chromosome arms. We assume that this triploid–tetrasomic embryo resulted from the combination of diploid and haploid–disomic gametes. Alternatively, it could have been generated by a dispermic fertilization, with one of the three gametes being disomic.

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Fig. 3. Light micrographs of day-10 embryos from superovulated (A, B, C) and naturally mated (D) females and their respective karyotypes. (A) Normal diploid embryo with 36 acrocentric and 2 metacentric chromosomes; (B) trisomy 16 with 35 acrocentric and 3 metacentric chromosomes; (C) tetrasomy 16 with 34 acrocentric and 4 metacentric chromosomes; and (D) triploid–tetrasomy 16 with 53 acrocentric and 4 metacentric chromosomes. The slight growth retardation of the trisomic embryo and the severe retardation of the tetrasomic and triploid–tetrasomic embryos are apparent. Bar equals 1 mm.
At day 10 about 60% of the trisomics were smaller than the diploid controls (Table 2, Fig. 3). Two days later, the discrepancy between the normal-appearing trisomics (Fig. 5B) and the retarded ones (Fig. 5C) was even greater, the latter being in an advanced stage of resorption (Table 2). Surprisingly, no intermediate form of abnormal phenotype was observed, suggesting the existence of two distinct populations of trisomics. The existence of these populations was not correlated with the sizes of the litters or the ages of the females.

DISCUSSION

As expected, tetrasomic embryos were actually generated by an intercross between mice doubly heterozygous for two metacentric chromosomes that share one arm in common (Fig. 1). Nullisomic embryos, also predicted by the breeding

Fig. 4. Tetrasomy 16 embryos at the 10th day of gestation, from hormonally induced (A,C,D) and naturally mated (B) Rb2H/RB32Lub females. The embryo in (D) was the most advanced day-10 tetrasomic observed. Bar equals 1 mm.
scheme to be generated in equal numbers, were not detected at the early blastocyst stage. Preliminary results from the karyotyping of earlier stages suggest that nullisomics might undergo one, perhaps, at a maximum, two divisions, before they die (S. Debrot, A. Zweig, C. J. Epstein, unpublished observations). In previous experiments (Magnuson et al. 1985) we found that the \( Rb2H/Rb32Lub \) males, when mated with ICR females, generate 17% monosomics and 17% trisomics when analysed after 2 days of development, giving a male non-disjunction rate of 34%. Using these data, we calculated that a 36% rate of non-disjunction in the doubly heterozygous females best fits with our day-3 results. We therefore conclude that superovulated \( Rb2H/Rb32Lub \) females have a rate of non-disjunction similar to that of doubly heterozygous males and that when intercrossed \( Rb2H/Rb32Lub \) heterozygotes will generate about 3% tetrasomic embryos (or 6 tetrasomics per 100 diploids). The frequency of tetrasomics found at day 10,
7.8 per 100 diploids (Table 1), is consistent with this expectation and indicates that all tetrasomics probably implant and survive to midgestation.

The findings with the tetrasomic embryos make it clear that four copies of chromosome 16 have a greater adverse effect on embryonic development than do three copies. Van der Hoeven & de Boer (1984) found a similar situation when the translocation T(1;13)70H was used to generate tetrasomy for the small regions of chromosome 1 and 13 involved in the 1;13 translocation chromosome. This tetrasomy (actually a double partial tetrasomy) is a prenatal lethal, while mice that are trisomic for the same regions survive and are fertile. Similarly, Beechey, Kirk & Searle (1985) found that tetrasomy 15 results in death by day 9–9.5, whereas trisomy 15 embryos at the same stage were still alive although they were about a third smaller than normal in size and had exencephaly. Therefore, for the two whole chromosome tetrasomies and one double partial tetrasomy studied to date, the tetrasomic state results in developmental abnormalities more severe than do the corresponding trisomies. On the other hand, Beechey & Speed (1981) described a male tetrasomic for the extra small 5;12 translocation chromosome of T(5;12)31H. This animal, although viable, was infertile, smaller than normal, and had a shortened head and small testes. A slightly retarded foetus with a twisted spine and the same genotype was also found at 12.5 days of gestation (Beechey, Kirk & Searle, 1980). These abnormalities are not much more severe than were found with the equivalent trisomy which resulted in male sterility and mild growth retardation. Overall, the situation in the mouse is reminiscent of what has been reported in man, with the effects of aneuploidy ranging from little or no specific phenotypic differences between tetrasomics and trisomics to the existence of clearly distinguishable syndromes with the tetrasomics being more severely affected (Epstein, 1986).

In addition to its usefulness for producing tetrasomic (and, potentially, nullisomic) embryos, the breeding system described here, with both parents bearing doubly heterozygous chromosomes, may also be of value in the study of trisomy (White et al. 1972). Trisomic embryos will be generated in a higher proportion than in the traditional mating of a doubly heterozygous male with a normal 'all acrocentric’ female. For example, with the Rb2H/Rb32Lub heterozygotes used in this study, 47 rather than 27 trisomics per 100 diploids can be expected when two double heterozygotes are intercrossed rather than just one being mated to a wild-type animal.

Our data do not reveal any differences in the incidence of aneuploidy between superovulated and naturally mated females in embryos analysed at 10 days of gestation (Fig. 2A,B). However, a significant loss of aneuploid embryos is observed two days later in superovulated females (Fig. 2C). We interpret this result as indicating that a selection process may be operating within the uterus. In a crowded situation (20.5 implants/female after superovulation versus 10.2 after natural mating), a greater competition for either nutrients or space could occur at midgestation which could place trisomic and tetrasomic embryos at a proliferative disadvantage. This conclusion would be consistent with the suggestion of Boué,
Philippe, Giroud & Boué (1976) and Gropp (1981) that the time of death of aneuploid foetuses could be determined in part by the ability of the aneuploid placenta to serve the metabolic needs of the embryo or foetus. In a marginal situation, with an already hypoplastic placenta (Gearhart, Oster-Granite & Hatzidimitriou, 1985), trisomy 16 embryos could be quite vulnerable to the local effects of uterine crowding.

The separation of the trisomy 16 embryos into two populations, some being grossly normal or only mildly abnormal while the others are severely delayed, could suggest a critical developmental threshold about or shortly before the 10th day of development. Some trisomies may be so delayed in development at this stage that they do not recover and are resorbed, while the others are able to cross this threshold and continue through a programme of fairly normal development up to and beyond day 12. Trisomy 16 foetuses have been reported to survive until or even shortly beyond birth (Miyabara, Gropp & Winking, 1982). It is of interest to note, in the context of a barrier to embryonic development, that the 9th day of gestation is often considered in the mouse to be a critical one, since it appears to be the stage most sensitive to various exogenous insults (Rugh, 1968). Furthermore, it also represents the time at which the most lethal of the mouse trisomies result in embryonic death (Gropp, 1978).

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