

Patterns of D Chromosome Involvement in Human (DqDq) and (DqGq) Robertsonian Rearrangements

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In 1966 it was noted that Robertsonian translocations in the 13-trisomy syndrome tended to be of type t(DqDq) rather than t(DqGq) [1]. In 1967 autoradiographic data were presented suggesting that t(DqDq) translocations might be nonrandom in composition [2]. This was subsequently confirmed [3, 4]. In 1968 autoradiographic information on t(Dq21q) translocations indicated that they, too, were nonrandom in composition [5]. This paper contains further information on the autoradiographic identification of chromosomes in t(DqDq) and t(DqGq) translocations in an attempt to delineate the overall patterns of composition and perhaps eventually to gain insight into the mechanisms by which Robertsonian translocations are formed.

AUTORADIOGRAPHIC METHODS

Lymphocytes were cultured for 72 hours and continuously late-labeled beginning six hours before harvest with tritiated thymidine (specific activity 2.0 c/millimole at a concentration of 1 m μ /milliliter medium). Metaphase figures were photographed before and after stripping for autoradiography with Kodak AR-10 film [6]. The resultant autoradiographs were scored as to the late-labeling patterns of the D chromosomes. The D chromosomes were distinguished [7, 8] as follows: the no. 13 chromosomes were heavily labeled with grains mainly over the distal and sometimes the middle third of the long arm; the no. 14 chromosomes were slightly less heavily labeled with grains mostly over the centromeric region and proximal third of the long arm; and the no. 15 chromosomes were lightly labeled with grains mainly over the centromeric area. We considered the translocated D chromosomes to be those with the missing labeling patterns. In cases with t(DqDq) translocations, we also tried to analyze the labeling pattern of the translocation chromosome.

Patients

Lymphocytes from 19 unrelated persons [four with t(DqDq) and 15 with t(DqGq)] were studied (table 1). The four persons with t(DqDq) were ascertained as follows:

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TABLE 1
 AUTORADIOGRAPHIC ANALYSIS OF ROBERTSONIAN REARRANGEMENTS

Type of Translocation	D Chromosomes Involved	No. of Cases	Individual Laboratory No.
t(DqDq)	13 and 14	3	3/69; 99/69; 102/69
t(DqDq)	14 and 15	1	92/69
t(Dq21q)*	14	11	85/68; 121/68; 122/68; 123/68; 177/68; 236/68; 240/68; 4/69; 5/69; 38/69; 39/69
t(Dq21q)*	15	3	75/68; 50/69; 51/69
t(DqGq)†	14	1	93/69

* Ascertained through individuals with Down's syndrome.

† Ascertained through a phenotypically normal individual (see text).

case 3/69 was phenotypically normal and was ascertained through a nephew with the physical stigmata of the Cornelia de Lange syndrome [9]; case 92/69 was a phenotypically normal woman whose baby boy was noted in the Yale–New Haven newborn survey [10] to have Down's syndrome, but the boy died before chromosome studies were completed; case 99/69 was a phenotypically normal girl whose translocation was detected in the same newborn chromosome survey [10]; and case 102/69 was referred to the Genetics Clinic, University of Oregon Medical School, for possible Turner's syndrome (mild growth retardation, ptosis of the eyelids, and short fourth and fifth metacarpals) but was found, as was her phenotypically normal mother, to have 45,XX,2D–,t(DqDq).

Fourteen of the t(DqGq) cases had Down's syndrome with 46,XX or XY,D–,t(DqGq) and are therefore presumed to have the same chromosomal imbalance, triplication of 21q. One patient (no. 93/69) with t(DqGq) was phenotypically normal, having been detected in a newborn survey [10]. Because this child's family history revealed no cases with Down's syndrome, the nature of the G chromosome component is unknown.

RESULTS

The number of cells scored in each case ranged from 11 to 31 (mean 21.2). Altogether, 382 cells were analyzed in the 19 cases listed in table 1. Of these, 248 (65%) were classified according to the D chromosome stated to be involved in the translocation. Only 13 (3%) were misclassified. The remaining 121 (32%) were consistent with the D classification given, but had intermediate labeling patterns (e.g., missing either no. 14 or no. 15).

The autoradiographic analysis of D chromosomes in Robertsonian rearrangements is presented in table 1 according to their mode of ascertainment. These data are given with comparable data from the literature in table 2. The data on (Dq21q) now comprise 75 cases (table 2, part D): (14q21q) predominates, (15q21q) is less frequent, and (13q21q) is uncommon. There are 34 cases with (DqDq) not ascertained through 13-trisomy syndrome (table 2, part A): the majority have (13q14q), a minority have (14q15q), two have (13q15q), and one, (15q15q). Although only 10 cases with (13qDq) ascertained through 13-trisomy syndrome have been reported (table 2, part B), the majority, six, have (13q14q).

TABLE 2

 AUTORADIOGRAPHIC IDENTITY OF D CHROMOSOMES IN ROBERTSONIAN
 REARRANGEMENTS REPORTED IN THE LITERATURE AND IN THIS STUDY

Translocation	No. of Cases	Reference*
A. Not Ascertained through 13-Trisomy Syndrome		
t(13q14q)	28 (82%)	[2 (three cases); 3; 4 (two cases); 7; 12 (two cases); 16; 21; J. O. van Hemel and J. M. van Brink, personal communication, 1966; 22-24; 25 (two cases); 26; 39-31; 43; 44 (two cases); 45; this report (three cases)]
t(13q15q)	2 (6%)	[27, 43]
t(14q15q)	3 (9%)	[4, 28, this report]
t(15q15q)	1 (3%)	[30]
B. Ascertained through 13-Trisomy Syndrome		
t(13q13q)	2 (20%)	[31, 32]
t(13q14q)	6 (60%)	[2 (two cases), 4, 8, 33, † 34]
t(13q15q)	2 (20%)	[4, 33†]
C. Not Ascertained through Down's Syndrome		
t(14qGq)	1	[this report]
D. Ascertained through Down's Syndrome		
t(13q21q)	2 (3%)	[35, 38]
t(14q21q)	64 (85%)	[2 (three cases); 5 (18 cases); 6 (two cases); 7 (two cases); 35 (seven cases); 36 (five cases); 37 (three cases); A. Valdmanis, J. D. Mann, and D. Johns, personal communication, 1969; M. M. Cohen, personal communication, 1969; J. de Grouchy and I. Emerit, unpublished observation cited in [4]; 39 (two cases); 40; 42; 43 (two cases); 44 (two cases); 45; C. J. Walker, personal communication; this report (11 cases)]
t(15q21q)	9 (12%)	[5 (two cases), 36, 37 (two cases), 43, this report (three cases)]

* Each reference reports one case unless otherwise stated.

† Two translocations found in same patient.

The pattern of involvement in (DqDq), excluding the case of (15q15q) which could be an isochromosome, is nonrandom ($\chi^2 = 37.1$, $P < .001$, 2 df). It also is very highly nonrandom in (Dq21q) ($\chi^2 = 9,224$, $P < .001$, 2 df).

DISCUSSION

We should like here to evaluate some of the possible explanations for the nonrandom composition of Robertsonian translocations: (1) The peculiar compositional patterns might simply reflect differences in the frequency with which chromosomes 13, 14, and 15 break near the centromere. Earlier data showing a lack of t(13q21q) translocations were consistent with this interpretation [5]. The fact that chromosome 13 occurs in the majority of t(DqDq) translocations argues strongly against this hypothesis. (2) Rowley and Pergament [3] have suggested that some types of centric-fusion translocations, once formed, might not survive, perhaps due to their being dicentric. Although this could explain the rarity of t(13q21q), it does not account for the relative commonness of t(13q14q) and of

Robertsonian translocations in general. (3) Selection might act against the survival of individuals with certain translocations, because of the double deletion. This could not account for the deficiency of $t(13q21q)$ in individuals with Down's syndrome, however, since the additional chromosome 21 producing Down's syndrome would supply another 21p segment [3]. (4) Acrocentric chromosomes with breaks might have different tendencies to translocate with other acrocentrics [5]. These translocation preferences might be ascribed to a highly ordered spatial arrangement of acrocentric chromosomes within the nucleus. Chromosome 14 might be regularly located between chromosomes 13 and 21, facilitating $t(13q14q)$ and $t(14q21q)$ translocations and tending to preclude $t(13q21q)$ translocations. The patterns of composition of Robertsonian translocations may thus mirror the chromosomal geography of the nucleus. This hypothesis would not explain the high rate of occurrence of these translocations without the additional postulate that acrocentrics are breakage-prone particularly near the centromeres or that the breakage rate for all chromosomes in man is extraordinarily high, but that acrocentrics, perhaps because of their nuclear geography, tend to translocate onto one another rather than to reunite. (5) Robertsonian translocations may result from meiotic pairing and crossing-over [11]. Rowley and Pergament [3] further suggest that "segments of the short arm of chromosome 14 [might be] homologous with segments near the centromere in the long arm of chromosomes 13 and 21. Pairing of homologous segments and crossing over during meiosis would lead to $t(13q14q)$ or $t(14q21q)$ chromosomes. On the other hand, the union of chromosomes 13 and 21, which would be expected to occur merely by chance, has not been observed." The homologous segment on chromosome 14 would have to be *inverted* with respect to the homologous segments on chromosomes 13 and 21 in order for pairing and exchange to tend to produce $t(13q14q)$ and $t(14q21q)$, but not $t(13q21q)$. The reciprocal product of this type of crossing-over would be a small metacentric chromosome, such as has been occasionally observed [4, 12]. Although there is no direct evidence for homologous segments on acrocentrics, these could have reasonably arisen during evolution as a result of polyploidization and gene duplication, processes which are now generally believed to have occurred in the evolution of the human karyotype. The degree of homology between two acrocentrics might govern the frequency of meiotic pairing and exchange. Conversely, the relative frequencies with which various acrocentrics are found in Robertsonian rearrangements might reflect their degree of homology.

Circumstantial evidence from man and other organisms is also consistent with the concept that Robertsonian chromosomes may result from an orderly, nonrandom process such as meiotic pairing and exchange, rather than from random breakage and fusion. Salient points of evidence include the following:

a) Robertsonian exchanges in man are extraordinarily common compared with other types of translocations [10, 13-15].

b) Robertsonian exchanges in man appear usually to be of spontaneous origin. Rarely, if ever, have they been shown to occur following X-irradiation or exposure to chemicals or other chromosome breakage agents [16].

c) Robertsonian rearrangements in mice have been observed in the offspring of control animals [17, 18] but never in the offspring of mice subjected to irradiation (C. E. Ford, personal communication, 1969).

d) After whole-body irradiation, the resultant marker chromosomes in mice, even when metacentric [19], do not occur in somatic cell clones with a chromosome number reduced from 40 to 39, as would be expected with a Robertsonian rearrangement.

e) In mice and man, somatic chromatid interchanges at the centromere, potentially capable of giving rise to Robertsonian translocations by mitotic segregation, have been observed to occur spontaneously in lymphocyte and fibroblast cultures (C. E. Ford, personal communication, 1969; [20]) but are not inducible.

f) Somatic chromatid interchanges at the centromere have not been observed in material treated with mutagens (e.g., in *Vicia* root-tip mitoses immediately following X-irradiation, or in nitrogen-mustard treatment of *Tradescantia* pollen-grain mitoses after irradiation [C. E. Ford, personal communication, 1969]).

These data from various organisms, including man, indicate that Robertsonian rearrangements differ in several ways from reciprocal translocations. Robertsonian rearrangements are mainly, perhaps exclusively, "spontaneous" rearrangements. They are apparently not produced in somatic or germinal cells by either irradiation or chemical mutagens. Moreover, in man, they appear to be highly nonrandom in their chromosomal composition. The mechanism of formation of Robertsonian translocations may thus be different from that of reciprocal translocations. We favor the proposition that Robertsonian rearrangements are produced by meiotic pairing and crossing-over and that their nonrandom composition reflects this more orderly process.

SUMMARY

This paper reports four new cases of (DqDq) and 15 new cases of (DqGq) translocations with autoradiographic identification of the D chromosomes involved. The (DqDq) rearrangements were (13q14q) in three cases and (14q15q) in one case. The (DqGq) translocations were (14q21q) in 11 cases, (15q21q) in three cases, and (14qGq) in one case where the nature of the G chromosome was not known.

Thirty-four of the 44 reported cases with (DqDq) involve (13q14q), while 65 of the 76 cases with (DqGq) are (14qGq). The explanation for this nonrandomness is not yet known. We favor the concept that it reflects the mechanisms by which (DqDq), (DqGq), and perhaps all Robertsonian rearrangements form, namely, from an orderly, nonrandom process such as meiotic pairing and exchange, rather than from random breakage and fusion.

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