

Preferential Loss of the Paternal Alleles in the 18q- Syndrome

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Individuals with the 18q- syndrome have variable deletions from the long arm of chromosome 18. They also exhibit a highly variable phenotype. To correlate genotype with phenotype accurately, extensive molecular and phenotypic analyses are needed on each affected individual. As a part of this analysis, we have determined the parental origin of the deleted chromosome in 34 individuals with the 18q- syndrome. We have found that 85% of the de novo deletions are paternal in origin. The percentage of fathers of individuals with paternally derived deletions who were >30 years old was (not significantly) greater than that of the general population. The mothers of individuals with maternally derived deletions were near an average age for childbearing compared to the general population. Individuals with maternally derived terminal deletions had breakpoints as varied as those with paternally derived deletions. These results are consistent with the hypothesis that the reduced incidence of maternally derived deletions is not due to reduced viability, since individuals with large maternally derived deletions of chromosome 18q were found. We hypothesize that the prevalence of paternally derived deletions is due to an increased frequency of chromosome breakage in male germ cells. These results are consistent with results observed in other segmental aneusomies in which there is a high incidence of paternally derived deletions. *Am. J. Med. Genet.* 69:280–286, 1997. © 1997 Wiley-Liss, Inc.

KEY WORDS: chromosome 18; 18q- syndrome; chromosomal abnormalities; parental origin; deletion syndrome

INTRODUCTION

The 18q- syndrome is one of the commoner deletion syndromes, with an estimated frequency of 1/40,000 births. This syndrome is most often caused by a terminal deletion of the long arm of chromosome 18. However, individuals with interstitial deletions have also been identified [Krasikov et al., 1992; Chudley et al., 1992]. Individuals with the 18q- syndrome have a variable phenotype. Intelligence can range from average to profound retardation [Mahr et al., 1996]. Short stature, possibly due to growth hormone insufficiency [Ghidoni et al., 1997], incomplete myelination of the central nervous system [Miller et al., 1990], and hearing impairment are among the more common manifestations [Strathdee et al., 1995]. A list of the physical findings in these individuals shows that many other anomalies are found in fewer than 50% of subjects with the syndrome [Strathdee et al., 1995]. This wide variability in phenotypic presentation can, in part, be attributed to the fact that each subject presents with a unique deletion [Silverman et al., 1995]. Parental origin of the deleted chromosome also may influence phenotype; such biases in parental origin have been observed in chromosomal abnormalities and neoplastic disease [Peterson and Sapienza, 1993].

Parental origin differences may be the result of two factors. First, there can be differences in the rate of formation of the mutation, dependent on the sex of the parent. Parental origin biases in the formation of mutations were reviewed by Chandley [1991]. Chromosome abnormalities in particular have exhibited parent of origin biases. Abnormalities resulting from nondisjunction, such as trisomies 18 and 21, have a maternal parent of origin tendency [Bricarelli et al., 1990; Antonarakis et al., 1993; Fisher et al., 1993; Nothen et al., 1993; Ya-gang et al., 1993]; whereas structural rearrangements such as terminal deletions have an increased paternal origin bias. Mechanistic differences between oogenesis and spermatogenesis may give rise to a parent of origin bias in individuals with chromosomal abnormalities. The paternal bias in structural rearrangements is thought to be due to the greater number of cell divisions during male meiosis, as well as minimal repair capacity in sperm [Chandley, 1991].

A second factor that may account for parental origin

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differences is genomic imprinting, in which expression differences are observed between genes of maternal versus paternal origin. If differences in the severity of the phenotype are significant enough to influence viability, then surviving affected individuals may demonstrate a parental origin bias. Reports of such gene expression differences are numerous. For example, individuals with Albright hereditary osteodystrophy all have identical mutations in the G protein ($Gs\alpha$) gene [Wilson et al., 1994]. However, affected individuals with a maternally transmitted mutation exhibit pseudohypoparathyroidism type I (hormone resistance), whereas those with a paternally inherited mutation have pseudopseudohypoparathyroidism (a normal response to exogenous parathyroid hormone) [Wilson et al., 1994].

Using molecular techniques, we have determined the extent and parental origin of the deletion in 34 individuals with the 18q- syndrome. Our results demonstrate a strong paternal origin, as has been observed with other deletion syndromes. This is in contrast to the findings of Strathdee et al. [1995], who were unable to demonstrate a parental origin bias in 15 patients with the 18q- syndrome.

MATERIALS AND METHODS

Patient Population

Patients with deletions of 18q were referred by their individual physicians or from the Chromosome 18 Registry and Research Society, a support group for families of individuals with chromosome 18 abnormalities. The study was approved by the Institutional Review Board and informed consent was obtained from all subjects.

Cytogenetic Analysis

Cytogenetic studies were performed elsewhere on all patients referred to this study.

Genotypic Analysis

Molecular analysis confirming the loss of material from the long arm of chromosome 18 was performed using polymerase chain reaction (PCR)-based microsatellite markers [Weber and May, 1989]. To obtain DNA for this analysis, blood samples were obtained in ACD (acid citrate dextrose) tubes from the patient and both parents (when available). High molecular weight genomic DNA was extracted from the peripheral blood leukocytes using the methods of Bell et al. [1981]. The DNA from each family was then analyzed using 21 PCR-based markers. All but one of these markers were originally identified by Généthon and have been placed in a linear order on the Généthon Linkage Map [Gyapay et al., 1994]. A highly polymorphic marker for the myelin basic protein (MBP) gene was also utilized in the analysis [Polymeropoulos et al., 1992]. This gene has been mapped to 18q23 [LeBeau et al., 1993].

PCR was performed in a total reaction volume of 10 μ l, using 50 ng of genomic DNA, 50 ng of each primer, 200 mM dNTPs, and 0.5 U Taq polymerase (Perkin Elmer-Cetus, Norwalk, CT). Magnesium levels and annealing temperatures were optimized for each set of

primers. One primer of each pair was end labeled at the 5' end with $\gamma^{32}P$ -dATP. PCR amplification consisted of 30 cycles of 1 min at 95°C, followed by 1 min at the appropriate annealing temperature, and 1 min elongation at 72°C. PCR products were separated on a 7% polyacrylamide gel run at 65 W for 4–6 hr and visualized using Kodak XAR-5 film and intensifying screens,

Lymphoblastoid Cell Lines and Somatic Cell Hybrids

Peripheral blood leucocytes were isolated using LeucoPREP brand cell separation tubes (Becton Dickinson Labware, Lincoln Park, NJ) and were transformed using Epstein-Barr virus (EBV) as described by Anderson and Gusella [1984]. Somatic cell hybrids were constructed in order to separate the deleted chromosome 18 from the nondeleted chromosome. The EBV transformed lymphoblast cells from the affected individual were fused in suspension with Chinese hamster lung (PHL73) cells, a gift from Dr. Robert Nussbaum. The PHL73 cells are deficient for the enzyme thymidylate synthase [Nussbaum et al., 1985], which can be complemented by the human gene on the short arm of chromosome 18. Four $\times 10^6$ PHL73 cells were mixed with 2×10^7 transformed patient lymphoblasts in the presence of 50% (w/v) of polyethylene glycol 1500 (PEG) (Boehringer Mannheim Biochemical, Indianapolis, IN). The cell mixture remained in PEG for 1 min and was then diluted slowly over 3 min with 5 ml of serum-free media. The cells were then placed in fresh serum-free media and incubated for 90 min at 37°C. One-tenth of the cells were placed in each of ten 75 cm² flasks in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal calf serum and FACT (2×10^{-8} M folic acid, 10^{-6} M aminopterin, 1.5×10^{-7} M cyanocobalamin (B_{12}), and 10^{-5} M thymidine). After 48 hr, the cells were washed and refed with minimal media containing DMEM and 10% dialyzed fetal calf serum. Hybrid clones were picked after 2–3 weeks, expanded, and DNA was isolated as described above. Hybrids were then screened with markers found to be informative for that patient's genomic DNA, which allowed us to determine whether the human chromosome 18 present in the hybrid cell line was the deleted or non-deleted chromosome.

Cytogenetic Analysis of Somatic Cell Hybrids

Each hybrid cell line found to carry the deleted human chromosome 18 was also analyzed by fluorescence in situ hybridization (FISH). Metaphase spreads were prepared by treating cells with 0.06 μ g/ml of colcemid for 4 hr, and cells were isolated by mitotic shakeoff (the removal of loosely adherent cells that are in mitosis). The human chromosome 18 was visualized using fluorescently labeled whole chromosome 18 paints (Cambio, Vector Laboratories, Burlingame, CA). This analysis demonstrated that each hybrid carried an intact human chromosome 18 (data not shown) as opposed to small fragments generated by the cell fusion process.

Statistical Analysis

Biostatistical analysis of data were performed with the statistical software packages, BMDP (BMDP Sta-

tistical Software, Los Angeles, CA) and SPSS (Chicago, IL) We tested whether the excess of paternally derived deletions was significant using a one-tailed binomial test by assuming that the proportion of chromosomes with the maternal and paternal deletions was equal (50/50).

The parental age at the time of birth of the affected child was determined and compared to that of the general population [Dept. of Commerce, 1994]. A two tailed t-test was used to compare the percentage of fathers from this study who were >30 years of age with the percentage of new fathers in the population >30 years of age for the years 1987 and 1989.

RESULTS

Paternally and maternally derived deletions were found in 34 individuals with *de novo* deletions of chromosome 18q. Verification of paternity in each case was established using markers in the nondeleted region, in which multiple markers demonstrated inheritance of both maternal and paternal alleles.

The genomic DNA from 34 families was analyzed using the highly polymorphic PCR-based markers developed by Généthon [Gyapay et al., 1994] and one PCR-based marker for the myelin basic protein (MBP) gene [Polymeropoulos et al., 1992]. Figure 1 gives an example of the type of analysis performed. This method was sufficient to establish parental origin in 29 of 30 families in which both parents were available and 2 of 4 families in which only one parent was available. The results from a subset of the markers utilized to determine parental origin are shown in Table I. Parental

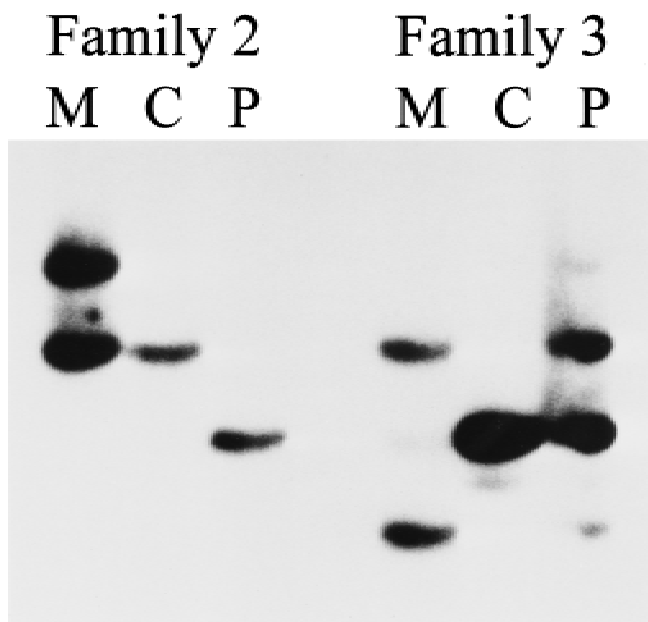


Fig. 1. Example of results of analysis of genomic DNA with polymorphic markers. Lane M, maternal alleles; lane C, child's alleles, and lane P, paternal alleles. Family 2 illustrates a case in which the child has failed to inherit a paternal allele, and family 3 illustrates a case in which the child has failed to inherit a maternal allele.

origin was confirmed in each case with at least three informative markers (all data not shown).

In four cases (families numbered 8, 12, 31, and 38), one parent was not available. However, in two of these families (numbers 8 and 31) specific chromosome 18q alleles from the available parent were shown to be absent in the child. These parent-specific alleles were absent in only a portion of the long arm of chromosome 18 and were present in other areas of the chromosome. These data indicate that the available parent was the biological parent and the deleted chromosome arose from that parent. The results of this analysis are presented in Table I.

In the other two cases in which DNA from only one parent was available (numbers 12 and 38), the available parent's allele was always present in the child's DNA. This would imply that the unavailable parent donated the deleted chromosome. Homozygosity cannot be easily distinguished from hemizyosity using PCR-based markers; therefore, the two chromosomes 18 were separated from each other by constructing somatic cell hybrids from the patient's lymphoblastoid cell line. The results of this analysis are shown in Table II. In family 12, one hybrid contained a copy of human chromosome 18, which is consistent with the mother's alleles. Another hybrid contained a unique chromosome 18, which carried paternally derived alleles and demonstrated the absence of certain markers, indicating that the deleted chromosome was of paternal origin. In family 38, one hybrid contained alleles consistent with the mother and another hybrid contained the paternal alleles, which were deleted at the more distal markers. Therefore, subject 38 had a paternally derived deletion.

Both parents were available in the analysis of patient 24; however, this individual had a small deletion with only one informative locus by the standard analysis. To confirm parental origin, somatic cell hybrids were constructed and analyzed (Table II). One hybrid from this patient demonstrated the paternal allele at one locus, whereas the other loci were deleted. Another hybrid carried the maternal alleles at all loci. Therefore, we concluded that this patient had a deletion of the paternally derived chromosome.

Our results indicate that 29 of the 34 individuals with *de novo* deletions had deletions that were paternally derived ($P = 0.00002$, one tailed binomial test). The fathers of individuals with deletions that were paternal in origin had a mean age of 32.1 yr at the time of birth of the affected child. This is older than the mean age of fathers of newborn infants in the general population, which was 29.7 yr in 1987 (the mean birth year of our population) [Dept. of Commerce, 1994]. In our study, 60.8% of the fathers were >30, whereas that percentage for the general population changed over the years but never exceeded that of our study population. However, the difference was not statistically significant. Our patients were born between 1971 and 1995, with a mean year of 1987 and a median year of 1989. A one-tailed t-test was used to compare our data with data for the general population for the years 1987 and 1989.

In the families in which the deletions were mater-

TABLE I. Genotype Analysis of Genomic DNA From 31 Families With De Novo Deletions of 18q*

Family	D18S477			D18S488			D18S554			D18S461			MBP			Parental origin
	M	C	P	M	C	P	M	C	P	M	C	P	M	C	P	
1	2,2	1,2	1,1	1,2	1,?	1,1	1,3		1,2,3	1,3	3	2,2	1,2	2	1,1	Paternal
2	1,3	3	1,2	1,1	1	2,2	1,1	1,?	1,1	2,2	2	1,1	1,2	1,?	1,1	Paternal
3	1,2	3	1,3	1,1	2	1,2	2,2	1	1,1	1,1	2	2,2	1,2	3	3,3	Maternal
4	1,1	1,?	1,1	1,1	1,?	1,2	2,3	3	1,2	2,2	2,?	1,2	1,2	2	3,3	Paternal
5	1,3	3,?	1,2	3,3	3	1,2	2,2	2	1,1	2,2	2,?	2,2	1,1	1,?	1,1	Paternal
6	1,2	2	3,3	2,4	4	1,3	1,1	2,?	1,2	1,1	1,?	1,1	1,2	1,?	1,2	Paternal
7	2,2	2,?	1,2	1,2	1,2	2,2	1,2	1	2,2	1,2	2	1,1	1,2	1	3,3	Paternal
8	-	1,?	1,2	-	2,?	1,2	-	1,?	1,2	-	3	1,2	-	1,?	1,2	Paternal
9	1,1	1	2,3	2,2	2	1,3	2,2	2,?	1,2	1,1	1	2,2	2,3	3	1,2	Paternal
10	1,2	1,?	1,2	1,2	1	3,3	1,2	2	3,3	2,2	2	1,3	1,2	2	1,3	Paternal
13	1,2	2,?	2,2	1,1	1	2,2	1,2	2,?	1,2	2,3	1,3	1,2	1,2	2,?	2,2	Paternal
14	1,2	1	3,3	1,2	1,?	1,3	2,3	2	1,3	2,2	2,?	1,2	1,2	1	2,2	Paternal
16	1,3	3,?	2,3	1,3	1,?	1,2	1,2	1,?	1,2	2,2	2	1,1	1,1	1	2,3	Paternal
17	1,3	2	1,2	1,1	1,?	1,2	1,2	1,?	1,3	2,2	1	1,1	1,1	1,?	1,2	Maternal
18	1,2	1	2,2	2,2	2	1,1	1,2	2,?	2,2	1,2	2	1,1	1,2	1,?	1,2	Paternal
19	1,1	1,?	1,1	1,3	1,?	1,2	1,3	2	2,2	1,1	1,?	1,1	2,2	1	1,2	Maternal
20	1,3	1	2,2	1,2	2	1,3	1,4	1	2,3	1,1	1,?	1,1	2,3	2,?	1,2	Paternal
21	1,1	1,?	1,1	1,2	1,?	1,1	1,2	2,?	1,2	1,2	2,?	1,2	1,2	1	2,2	Paternal
25	1,3	2	2,3	2,2	2,?	1,2	1,2	2,?	2,3	1,3	3,?	2,3	1,2	2,?	1,2	Maternal
26	1,1	1,?	1,2	1,1	1,?	1,2	1,2	2,?	2,3	2,2	2	1,3	1,1	1,?	1,1	Paternal
27	1,2	1,2	1,1	1,1	1,2	2,2	1,3	3	1,2	2,2	2,?	1,2	1,2	1,?	1,2	Paternal
28	1,3	3	2,2	1,2	1,?	1,1	2,3	2,?	1,2	1,2	2,?	2,2	1,1	1	2,3	Paternal
29	1,2	1,?	1,2	1,1	1	1,1	1,2	1,?	1,2	1,2	2,?	2,3	1,1	1,?	1,1	Paternal
31	2,2	1,2	-	1,1	2	-	1,2	1,?	-	1,3	2	-	1,3	2	-	Maternal
33	3,3	1,3	1,2	1,1	1,?	1,1	1,2	2	3,3	1,2	1	2,3	1,3	1	2,3	Paternal
34	2,3	3	1,2	1,2	2,?	1,2	2,3	2,?	1,2	1,2	2,?	2,2	1,1	1	2,2	Paternal
35	1,3	3	1,2	2,2	2	1,3	1,3	3	1,2	1,1	1,?	1,2	3,3	3	1,2	Paternal
36	2,2	2	1,1	2,2	2	1,3	2,3	3	1,2	2,2	2,?	1,2	3,3	3	1,2	Paternal
37	2,2	2	1,1	1,3	3	2,4	1,2	2,?	2,2	2,2	2	1,1	2,3	3,?	1,3	Paternal
39	1,1	1	2,2	2,3	1,2	1,4	1,3	3	2,4	1,2	2,?	2,3	1,2	2	1,1	Paternal
40	1,2	1	2,2	1,2	2,?	2,2	2,2	2,?	1,3	2,2	2	1,1	1,2	1,?	1,3	Paternal

*M = maternal alleles; C = child's alleles; P = paternal alleles; ? = uninformative; - = individual not available. D18S markers are in proximal to distal order.

nally derived, the mean maternal age was 26.5 yr, and all of these mothers were under 30 yr of age. The mean maternal age in the general population in 1987 was 26.6 yr. Because this represents only five families, the number was too small to draw any meaningful statistical conclusions about an increased or decreased maternal age.

To determine whether the maternally derived deletions were clustered at a specific breakpoint, or if only

deletions of a certain size are viable when they are of maternal origin, we estimated the extent of the deletion from those children with maternally derived deletions. For this analysis we utilized the highly polymorphic PCR-based markers from Génethon [Gyapay et al., 1994]. Figure 2 summarizes the results of this analysis and clearly demonstrates that there are no breakpoint clusters in patients with maternally derived deletions; these breakpoints appear to be as

TABLE II. Analysis of Somatic Cell Hybrids From Three 18q- Patients

Family		D18S65	D18S487	D18S477	D18S488	Parental origin
		Family 12	Mother	1,3	1,3	
	Child	2,3	1,2	1,?	1,?	
	Hybrid 1	2	1	1	1	
	Hybrid 5	3	2	-	-	Paternal
Family 24		D18S477	D18S469	D18S554	D18S497	
	Mother	2,2	2,2	1,2	2,3	
	Child	1,2	2,?	2,?	2	
	Father	1,1	1,2	2,3	1,4	
	Hybrid 1	1	-	-	-	Paternal
	Hybrid 3	2	2	2	2	
Family 38		D18S69	D18S487	D18S477	D18S554	
	Mother	2,2	2,2	1,2	1,2	
	Child	1,2	1,2	1,?	1,?	
	Hybrid 2	1	1	-	-	Paternal
	Hybrid 9	2	2	1	1	

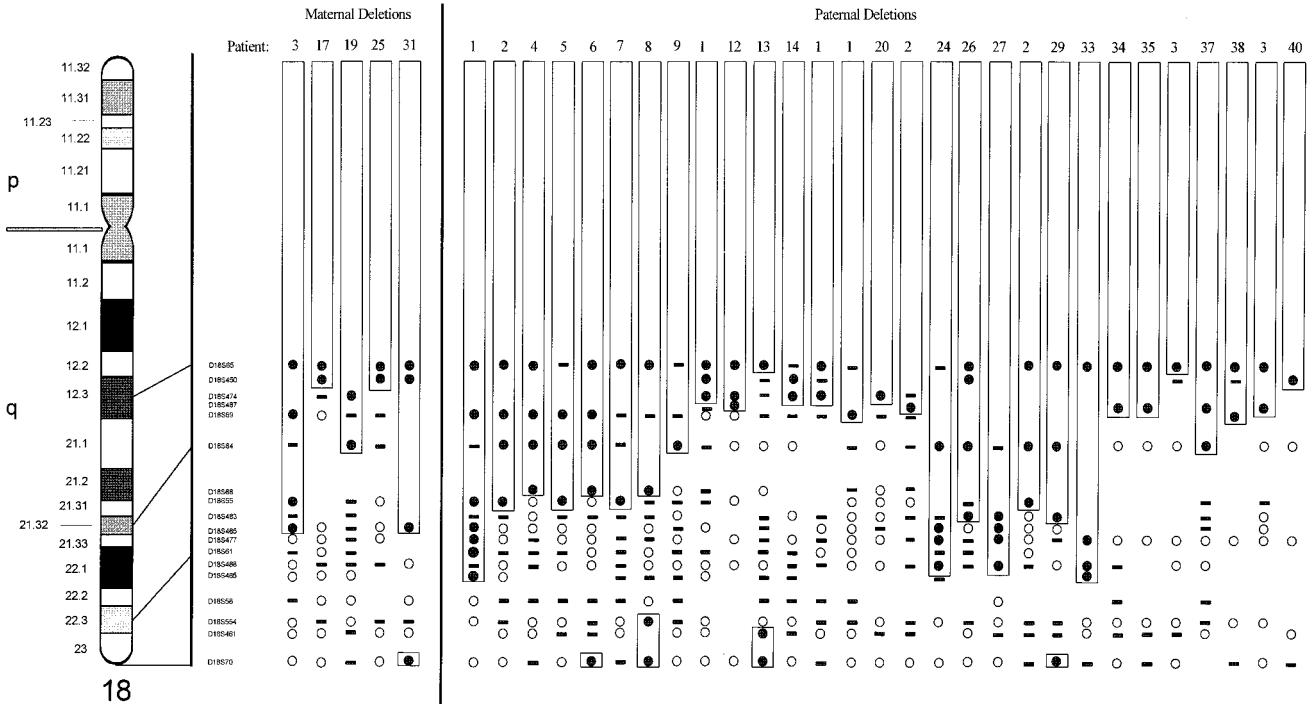


Fig. 2. Molecular analysis of subjects with chromosome 18 deletions using polymorphic markers. Closed circles indicate that the subject had two alleles for that marker. Open circles indicate that the subject had a single allele, and a dash indicates that the markers were uninformative.

widely variable as those seen in individuals with paternally derived deletions.

DISCUSSION

We have shown a parent of origin bias of the chromosome with the deletion in individuals with the 18q-syndrome. These findings are in contrast to the work of Strathdee et al. [1995], who found no parent of origin bias in their study of 15 individuals with 18q-. The apparent contradiction with our data could be due to the smaller number of subjects evaluated by Strathdee et al. If the subjects are combined from all studies, a significant paternal origin bias remains ($P = 0.00015$), as shown in Table III. This analysis is valid if there is no overlap in patient populations in the two studies. However, we are uncertain of the degree of overlap in our patient populations.

Such a parental origin bias can be explained if individuals with maternal deletions are less viable than those with paternal deletions. This differential viability might be caused by imprinting of certain genes in the 18q- region. If imprinting were a factor, the surviving individuals with maternal deletions might have deletions that are restricted to a smaller region because maternal inheritance of imprinted genes would be lethal. To test this possibility, the extent of the deletion was determined in patients with maternally derived deletions (Fig. 2). Individuals with maternally derived deletions have variable deletion sizes, and these deletions are not consistently smaller than deletions of paternal origin. These data do not rule out the possibility of an imprinted gene with influence on viability in the distal 18q- region, which is absent in virtually all sub-

jects with the 18q- syndrome. These results also indicate that there is not a cluster of breakpoints as was seen in the 11q- syndrome [Penny et al., 1995; see discussion below]. Although there is a fragile site at 18q21.3, this fragile site does not appear to be the site of frequent breakage in the 18q- syndrome.

If imprinting is a factor in the phenotype of this condition, then surviving individuals with maternally derived deletions might be expected to be more severely affected than individuals with paternally derived deletions. To date, we have observed no major phenotypic differences related to parental origin of the deleted chromosome; however, our sample of five maternally derived deletions is too small to draw any conclusions.

TABLE III. Parental Origin of Terminal De Novo Chromosomal Deletions

Syndrome	Maternal: paternal	P value*	Reference
4p-	0:5	0.03	Tupler et al., 1992
	2:5	0.22	Dallapiccola et al., 1993
	0:7	0.0078	Quarell et al., 1991
5p-	5:20	0.002	Overhauser et al., 1990
	9p-	0:1	—
11q-	0:1	—	Deroover et al., 1978
	6:8	0.39	Penny et al., 1995
16p-	1:3	0.31	Wilke et al., 1990
18p-	0:2	—	J.M. Fisher, pers. comm.
18q-	0:4	0.06	J.M. Fisher, pers. comm.
	7:6**	0.5	Strathdee et al., 1995
	5:29	0.00002	Present study
18q-	12:39	0.00015	Combined data

*P values were determined using a one-tailed binomial test. Values <0.05 are considered significant.

**Only includes data from individuals with de novo deletions.

There is a single report of imprinting involving genes on chromosome 18. Stine et al. [1995] observed linkage between paternal markers on 18q and bipolar disease, a trait not evaluated in our investigations. Future studies will determine if this or other features in individuals with the 18q- syndrome can be correlated with the parental origin of the deletion, reflecting an imprinting effect. To explore these questions fully, more individuals with maternally derived deletions will need to be identified.

In addition to genomic imprinting, parent of origin biases might be explained by differences in the process of germ cell formation between males and females. Oocytes undergo a limited number of divisions that are completed during fetal development. In contrast, spermatogenesis continues from puberty throughout the life of the individual. This means that the number of cell divisions that must occur prior to the formation of a mature sperm cell increases with age. According to Vogel and Rathenberg [1995], by the age of 25 yr, spermatocytes have undergone 310 cell divisions, whereas oocytes have undergone only 23 cell divisions. The authors speculate that mutations and structural abnormalities occur during cell divisions at a constant frequency. Therefore, the increased number of cell divisions of spermatocytes makes them more vulnerable to structural abnormalities, a vulnerability that increases with age. This increased frequency of structural abnormalities during spermatogenesis may be of particular clinical significance if DNA repair mechanisms are deficient in spermatozoa. In *Drosophila melanogaster*, it has been observed that spermatozoa have little repair capacity, which leads to an increased prevalence of mutations in males [Chandley, 1991]. If spermatocytes are indeed more likely to harbor structural chromosome abnormalities and if DNA repair mechanisms are deficient in human spermatozoa, then most deletions should be paternally derived and older fathers should have a higher frequency of children with deletions. If the number of germ cell divisions is indeed important in increasing the occurrence of chromosome abnormalities, then the frequency of children with maternally derived deletions is independent of the age of the mother, since the number of cell divisions would not change over time and would not increase with age for females. In the present investigation, the percentage of fathers of affected children >age 30 was not significantly different from the general population. However, the paternal bias observed in this study might be accounted for by the increased number of cell divisions during spermatogenesis alone, independent of age.

Our data on parent of origin bias in the 18q- syndrome are consistent with other terminal chromosomal deletion syndromes (Table III). With the exception of the 11q- syndrome (Jacobsen syndrome) [Penny et al., 1995], all terminal deletion syndromes studied to date demonstrate increased frequency of paternal origin of the deleted chromosomes. Patients with the 11q- syndrome may be divided into those with breakpoints near a folate sensitive fragile site, and those with more distal deletions. Of eight 11q- patients with breakpoints near the fragile site, five were maternally derived and three were paternally derived. However, in patients

with more distal breakpoints, one was maternally derived and five were paternally derived. These data suggest that the mechanism at the fragile site of 11q- may differ from that of other breakage events. The 11q- syndrome, like other terminal deletion syndromes, appears to have a paternal parent of origin bias when breakage is unrelated to a fragile site.

The rare fragile site at 11q23.3 has been shown to map to the same region as a (CCG)_n trinucleotide repeat [Jones et al., 1995]. Other cytogenetically detectable rare fragile sites, (FRAXA, FRAXE, and FRA16A) also have been shown to be the result of (CCG)_n expansions [Verkerk et al., 1991; Knight et al., 1993; Nancarrow et al., 1994]. The 18q- region contains one common aphidicolin-sensitive fragile site at 18q21.3 [Hecht et al., 1990]. This fragile site is not known to be caused by a trinucleotide repeat, but a (CTG)_n expansion has been found to be at 18q21 [Sirugo et al., 1995]. Future investigations will determine if this fragile site is a site of breakage. However, Figure 2 demonstrates that this region of the chromosome is not a common site for breakage.

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REFERENCES

- Anderson MA, Gusella JF (1984): Use of cyclosporin A in establishing Epstein-Barr virus transformed human lymphoblastoid cell lines. *In Vitro* 20:856-858.
- Antonarakis SE, Avramopoulos D, Blouin J-L, Talbot CC, Schinzel AA (1993): Mitotic errors in somatic cells cause trisomy 21 in about 4.5% of cases and are not associated with advanced maternal age. *Nature Genet* 3:146-150.
- Bell GI, Karam JH, Rutter WJ (1981): Polymorphic DNA region adjacent to the 5' end of the human insulin gene. *Proc Natl Acad Sci USA* 78:5759-5763.
- Bricarelli FD, Pierluigi M, Grasso M, Strigini P, Perroni L (1990): Origin of extra chromosome 21 in 343 families: cytogenetic and molecular approaches. *Am J Med Genet* 7:129-132.
- Chandley AC (1991): On the parental origin of de novo mutation in man. *J Med Genet* 28:217-223.
- Chudley AE, Kovnats S, Ray M (1992): Recognizable behavioral and somatic phenotype in patients with proximal interstitial 18q deletion: Report on a new affected child and follow-up on the original reported familial cases. *Am J Med Genet* 43:535-538.
- Dallapiccola B, Mandich P, Bellone E, Selicorni A, Mokin V, Ajmar F, Novelli G (1993): Parental origin of chromosome 4p deletion in Wolf-Hirschhorn syndrome. *Am J Med Genet* 47:921-924.
- Deroover J, Fryns JP, Parloir C, Haegeman J, Van Den Berghe H (1978): Partial monosomy of the short arm of chromosome 9. *Hum Genet* 44:195-200.
- Fisher JM, Harvey JF, Lindenbaum RH, Boyd PA, Jacobs PA (1993): Molecular studies of trisomy 18. *Am J Hum Genet* 52:1139-1144.
- Ghidoni PD, Hale DE, Cody JD, Thompson NM, McClure EB, Gay CT, Danney MM, Leach RJ, Kaye CI (1997): Growth hormone deficiency associated with 18q deletion syndrome. *Am J Med Genet* 69:7-12.
- Gyapay G, Morissette J, Vignal A, Dib C, Fizames C, Millasseau P, Marc S, Bernardi G, Lathrop M, Weissenbach J (1994): The 1993-94 Génethon human genetic linkage map. *Nature Genet* 7:246-249.
- Hecht H, Ramesh KH, Lockwood DH (1990): A guide to fragile sites on human chromosomes. *Cancer Genet Cytogenet* 44:37-45.
- Jones C, Penny L, Mattina T, Yu S, Baker E, Voullaire L, Langdon WY, Sutherland GR, Richards RI, Tunnacliffe A (1995): Association of a

- chromosome deletion syndrome with a fragile site within the proto-oncogene CBL2. *Nature* 376:145–149.
- Kleczkowska A, Fryns JP, Lemli L, Van Den Berghe H (1992): Minimal dysmorphic stigmata in 9p deletion of paternal origin. *Genet Counsel* 3:49–52.
- Knight SJL, Flannery AV, Hirst MC, Campbell L, Christodoulou A, Phelps SR, Pointon J, Middleton-Price HR, Barnicoat A, Pembrey ME, Holland J, Oostra, BA, Bobrow M, Davies KE (1993): Trinucleotide repeat amplification and hypermethylation of a CpG island in FRAXE mental retardation. *Cell* 74:127–134.
- Krasikov N, Thompson K, Sekhon GS (1992): Monosomy 18q12.1-21.1: A recognizable aneuploidy syndrome? Report of a patient and review of the literature. *Am J Med Genet* 43:531–534.
- LeBeau MM, Overhauser J, Straub RE, Silverman G, Gilliam TC, Ott J, O'Connell P, Francke U, Gerts van Kessel A (1993): Report of the First International Workshop on Human Chromosome 18 Mapping. *Cyto Cell Genet* 63:78–87.
- Mahr RN, Moberg PJ, Overhauser J, Strathdee G, Kamholz J, Loevner LA, Campbell H, Zackai EH, Reber ME, Mozley DP, Brown L, Turetsky BI, Shapiro RM (1996): Neuropsychiatry of 18q- syndrome. *Am J Med Genet* 67:172–178.
- Miller G, Mowrey PN, Hopper KD, Frankel CA, Ladda RL (1990): Neurological manifestations in 18q- syndrome. *Am J Med Genet* 37:128–132.
- Nancarrow JK, Kremer E, Holman K, Eyre H, Doggett NA, LePaslier D, Callen DC, Sutherland GR, Richards RI (1994): Implications of FRA16A structure for the mechanism of chromosomal fragile site genesis. *Science* 264:1938–1941.
- Nothen MM, Eggermann T, Erdmann J, Eiben B, Hofmann D, Propping P, Schwanz G (1993): Retrospective study of the parental origin of the extra chromosome in trisomy 18 (Edwards syndrome). *Hum Genet* 92:347–349.
- Nussbaum RL, Walmsey RM, Lesko JG, Airhart SD, Ledbetter DH (1985): Thymidylate synthase-deficient Chinese Hamster cells: A selection system for human chromosome 18 and experimental system for study of the thymidylate synthase regulation and fragile X expression. *Am J Hum Genet* 37:1192–1205.
- Overhauser J, McMahon J, Oberlender S, Carlin ME, Niebuhr E, Was-muth JJ, Lee-Chen J (1990): Parental origin of the Chromosome 5 deletions in the Cri-du-chat syndrome. *Am J Med Genet* 37:83–86.
- Penny LA, Dell'Aquila M, Jones MC, Bergoffen J, Cunniff C, Fryns JP, Grace E, Graham JM, Kousseff B, Mattina T, Syme J, Voullaire L, Zelante L, Zenger-Hain J, Jones OW, Evans GA (1995): Clinical and molecular characterization of patients with 11q deletions. *Am J Hum Genet* 56:676–683.
- Peterson K, Sapienza C (1993): Imprinting the genome: Imprinted genes, imprinting genes, and a hypothesis for their interaction. *Ann Rev Genet* 27:7–31.
- Polymeropoulos MH, Xiao H, Merrill CR (1992): Tetranucleotide repeat polymorphism at the human myelin basic protein gene (MBP). *Hum Mol Genet* 1:658.
- Quarell OWJ, Snell RG, Curtis MA, Roberts SH, Harper PS, Shaw DJ (1991): Parental origin of the chromosomal deletion resulting in Wolf-Hirschhorn syndrome. *J Med Genet* 28:256–259.
- Silverman GA, Schneidere SS, Massa HF, Flint A, Lalande M, Leonard JC, Overhauser J, van den Engh G, Trask BJ (1995): The 18q- Syndrome: analysis of chromosomes by bivariate flow karyotyping and the PCR reveals a successive set of deletion breakpoints within 18q21.1-q22.2. *Am J Hum Genet* 56:926–937.
- Sirugo G, Haaf T, Parnas J, Matthyse S, Levy D, Holzman P, Kidd KK (1995): A (CTG)_n expansion detected by RED in a schizophrenia kindred maps to 18q21 by FISH with a CTG/CAG polymer. *Am J Hum Genet* 57:A36.
- Strathdee G, Zackai EH, Shapiro R, Kamholz J, Overhauser J (1995): Analysis of clinical variation seen in patients with 18q terminal deletions. *Am J Med Genet* 59:476–483.
- Stine OC, Xu J, Koskela R, McMahon FJ, Clark CD, Friddle C, Gschwend M, Breschel T, Simpson SG, Botstein D, Marr TG, McInnis MG, Meyers DA, DePaulo JR (1995): Evidence for linkage of bipolar disorder to chromosome 18 with a parent of origin effect. *Am J Hum Genet* 57:A27.
- Tupler R, Bortotto L, Buhler EM, Alkan M, Malik NJ, Jadooa NB, Memo L, Maraschio P (1992): Paternal origin of the de novo deleted chromosome 4 in Wolf-Hirschhorn syndrome. *J Med Genet* 29:53–55.
- US Department of Commerce (1994): "Statistical Abstract of the United States 1994," Tab. 92. Lanham, MD: Bernan Press.
- Verkerk AJMH, Peiretti M, Sutcliffe JS, Fu Y, Kuhl DPA, Pissute A, Reiner O, Richards S, Victoris MF, Zhang F, Eussen BE, van Ommen GB, Blonden LAJ, Riggins GJ, Chastain JL, Kunst CB, Galjaard H, Caskey CT, Nelson DL, Oostra BA, Warren ST (1991): Identification of a gene (FMR-1) containing a CCG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. *Cell* 65:905–914.
- Vogel F, Rathenberg R, (1985): Spontaneous mutation in man. *Adv Hum Genet* 5:223–318.
- Weber JL, May PE (1989): Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. *Am J Hum Genet* 44:388–96.
- Wilke AOM, Buckle VJ, Harris PC, Lamb J, Barton NJ, Reeders ST, Lindenbaum RH, Nicholls RD, Barrow M, Bethlenfalvay NC, Hutz MH, Tolmie JL, Weatherall DJ, Higgs DR (1990): Clinical features and molecular analysis of the α thalassemia/mental retardation syndromes. 1. Cases due to deletions involving chromosome band 16p13.3. *Am J Hum Genet* 46:1112–1126.
- Wilson LC, Oude Luttikhuis MEM, Clayton PT, Fraser WD, Trembath RC (1994): Parental origin of G α gene mutations in Albright's hereditary osteodystrophy. *J Med Genet* 31:835–839.
- Ya-gang X, Robinson WP, Spiegel R, Binkert F, Ruefenacht U, Schinzel AA (1993): Parental origin of the supernumerary chromosome in trisomy 18. *Clin Genet* 44:57–61.