Tertiary Trisomy in a Human Kindred Containing an E/G Translocation

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INTRODUCTION

In the foreseeable future, human cytogenetics will continue to serve the clinical aspects of medicine and the related basic sciences. Occasionally, individual reports provide additional population data and the opportunity to confirm, for man, cytogenetic principles derived from research studies with other organisms. In the study reported here, evidence is presented for the origin, within the known limits of a human pedigree, of an E/G translocation; the involvement of one of the translocation chromosomes in a second type of anomaly (tertiary trisomy); and the use of the information derived from an analysis of the translocation in a prenatal diagnosis.

The proband was brought to the attention of author Macintyre early in 1968. This two-and-a-half-year-old female child (fig. 1) who had been admitted to Babies and Children's Hospital, Cleveland, was suffering from severe growth retardation (below the third percentile), with a bone age of nine months, severe mental retardation (performing at the six-to-nine-month level), and bilateral renal dysplasia resulting in renal rickets. In addition to these major findings, the patient exhibited hypertelorism, an antimongoloid slant of the eyes, hypotonia, and long slender feet and fingers. Despite these clinical stigmata, the child's phenotype was not considered to be consistent with the expectation of a demonstrable chromosome abnormality. However, because the severity of mental retardation and growth retardation could not be explained on the basis of the renal problem, a chromosome analysis was performed. The discovery of an abnormal karyotype in the proband led to a study of various other individuals in four generations of the kindred. The existence of a familial chromosome translocation was ascertained by karyotype analyses, and the anomaly was studied further by autoradiographic

Received March 11, 1970; revised March 22, 1971.

Presented at the meeting of the American Society of Human Genetics, Austin, Texas, October 12, 1968.

Supported by U.S. Public Health Service research grant HD 00549 from the National Institutes of Health and by the Brush Foundation.

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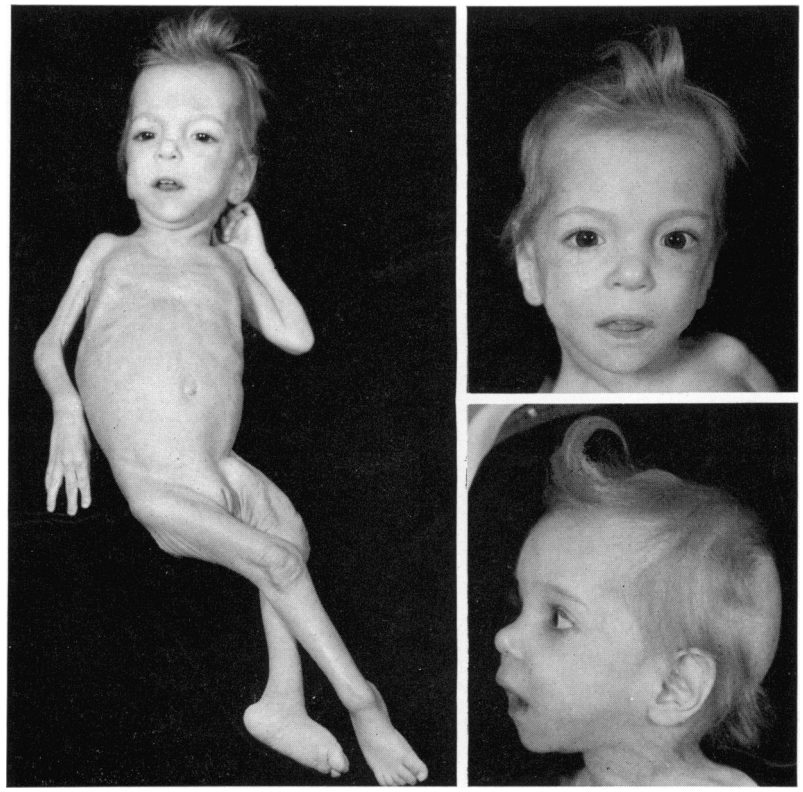

FIG. 1.—Proband DP260665 at age four years and three months

techniques and biometrical analyses. The attendant genetic counseling was complicated by the fact that the proband's mother was pregnant when she was found to be a carrier of the translocation; prenatal karyotypic analysis of the fetus was undertaken.

MATERIALS AND METHODS

Chromosome Preparations

Standard culture and preparatory techniques were utilized in obtaining the lymphocyte chromosome preparations which served as a basis for the karyotypic analysis of all tested individuals except the unborn child. Standard techniques also were used in the autoradiographic studies. Measurements of chromosome arm lengths were made from 20 translocation heterozygote karyotypes which were enlarged (ca. × 10) by projection. The midline of chromatids served as the basis for measurements in each case. Arm ratios and total chromosome lengths were computed for all chromosomes in the E and G groups.

Biometrics

Considering the nonparametric nature of the tests required to document the aberrations suggested in the karyotypes of certain individuals in this study, Wilcoxon rank sum tests were employed. In addition, the range was used to compute the standard deviations appropriate to the samples. According to Wilcoxon and Wilcox [1], the estimated standard

deviation (from ranges) is appropriate for use in Student's t tests. These authors suggest that in comparing two samples of the size employed here (20 or 40), the relative efficiency of the rank sum test compared with the t test would exceed 90%; likewise, the efficiency of the estimate of standard deviation from the range is not less than 85%.

Amniotic Fluid Cell Culture

Amniotic fluid as a source of fetal cells was obtained by transabdominal amniocentesis 15 weeks after the onset of the last menstrual period. The amniocentesis was performed according to the protocol of Stenchever and Cibils [2], except that 40 cm^3 of fluid was withdrawn rather than the 10-cm^3 volume in the published protocol. Half of the amniotic fluid was used to initiate cultures in which the cells were concentrated by centrifugation and removal of the supernatant fluid. The amniotic fluid was replaced by 16 cm^3 of Nutrient Mixture F-12 with glutamine (Grand Island Biological Company), with 20% fetal calf serum and antibiotics added. After resuspending the cells, the mixture was divided equally between two plastic T-30 flasks for culturing. The remaining 20 cm^3 of original amniotic fluid containing suspended cells was divided equally among five plastic T-30 flasks. To each of these five flasks was added 4 cm^3 of the enriched F-12 medium. All flasks were gassed with a mixture of 5% CO_2 in air and incubated at 37° C. Culture medium was replaced after the first 24 hours of incubation to remove nonviable cells. Thereafter, replacement of medium was at three-day intervals.

Cultures were terminated upon the observation of three or four clones of cells, each approximately 5 mm in diameter, or their equivalent. After separation of the cells by trypsinization, the resulting cell suspensions were treated in the same manner as lymphocyte cultures in obtaining chromosome preparations.

RESULTS

The E and G group chromosomes of the proband are shown in figure 2. In all

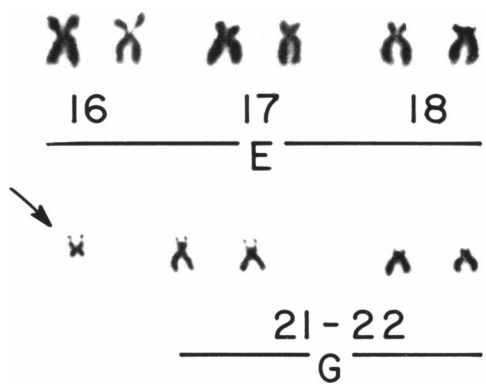

FIG. 2.—Partial karyotype of proband showing E and G group chromosomes and minute chromosome (*arrow*).

cells studied, 47 chromosomes were found, including an apparently normal complement plus a satellited, minute chromosome, that is, 47,XX,mars. Inasmuch as the identity or origin of the minute chromosome could not be inferred from the proband's karyotype, chromosome analyses were performed on her parents and, subsequently, on her siblings and other available relatives.

The father's karytope proved to be normal. The mother's (fig. 3) gives evidence

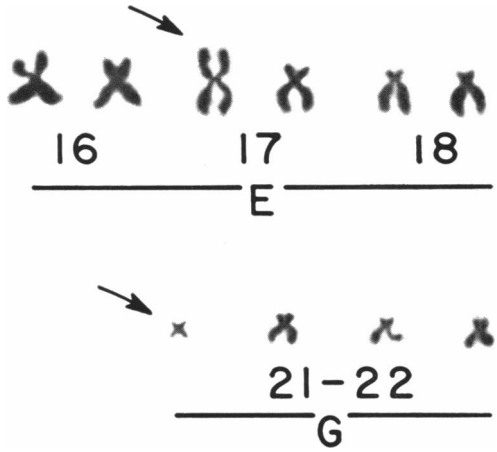

FIG. 3.—Partial karyotype of mother of proband showing E and G group chromosomes and reciprocal exchange (arrows).

of a reciprocal E/G translocation [46,XX,E—,G—,t(Ep+,Gq—)]. The resultant marker G chromosome is equivalent to the satellited, minute chromosome found in the proband's karyotype. The biometrical tests served to confirm these observations. The arm ratios and total lengths of the translocated E and translocated G chromosomes were each significantly different ($P < .005$) from those of their assigned homologues and from the other chromosomes in their respective groups, thus qualifying them as marker chromosomes (table 1). In addition, the normal

TABLE 1

SUMMARY OF CHROMOSOME MEASUREMENTS

	E_1	E_2	E_3	E_4	E_5	E_6	G_1	G_2	G_3	G_4
Arm ratio:										
Mean	1.42	1.42	1.25	2.27	2.53	2.42	2.77	2.30	1.44	2.37
Standard deviation	0.23	0.19	0.22	0.38	0.36	0.26	0.43	0.42	0.29	0.43
Long arm length:										
Mean	2.60	2.52	2.79	2.77	2.71	2.60	1.77	1.73	0.83	1.86
Standard deviation	0.45	0.31	0.32	0.33	0.25	0.29	0.25	0.22	0.17	0.29
Short arm length:										
Mean	1.90	1.82	2.28	1.24	1.08	1.08	0.79	0.76	0.60	0.81
Standard deviation	0.18	0.20	0.31	0.17	0.11	0.14	0.13	0.09	0.10	0.16

no. 17 homologue proved to be unique in the karyotype, contrary to the interpretation of Giannelli and Howlet [3].

In each karyotype, the sum of the lengths of the short arm of the normal E and the long arm of the normal G was compared with the sum of the lengths of the same arms in the two translocated chromosomes. The test of individual differences compiled from all karyotypes demonstrated that neither the sum of all differences nor the average difference was significant.

Grain counts of the no. 17 and no. 18 chromosomes (E_3, E_4, E_5, and E_6) from 15

TABLE 2

SUMMARY OF GRAIN COUNT DATA FOR CHROMOSOMES 17 AND 18 OF INDIVIDUAL III-2

	E_3	E_4*	E_5	E_6
Mean grain count ($N = 15$)	5.6	3.3	12.3	12.0
Standard deviation	3.6	2.4	6.0	6.5

* Marker chromosome.

autoradiographs are summarized in table 2. A t-test value for the difference in the amount of label between the marker 17 chomosome together with its normal homologue (E_3 and E_4) and the members of the uninvolved pair (E_5 and E_6) was highly significant ($P < .01$).

An analysis of the labeling patterns in the G group chromosomes led to the conclusion that it was impossible to make a decisive judgment with respect to the assignment of the marker G chromosome as a no. 21 or 22.

Results of karyotypic analysis of various members of the kindred are shown in the pedigree (fig. 4). The first-born child (IV-1) of the proband's mother died at

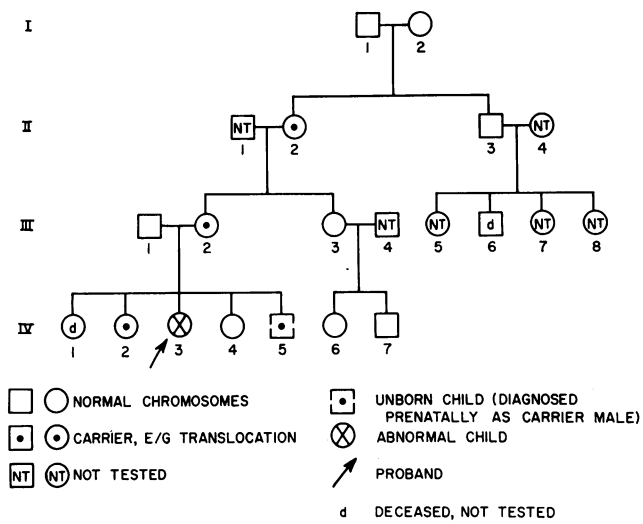

FIG. 4.—Pedigree: maternal blood line of proband

two days of age from subdural and subarachnoid hemorrhage presumed to be the result of birth trauma. An autopsy was performed on this child, and there was no evidence of developmental abnormality. It is assumed that no chromosomal imbalance existed, although chromosome studies were not undertaken. The child shown as IV-5 in the pedigree was unborn at the time of the investigation. However, its karyotype was determined in the eighteenth week of gestation (amenorrhea) from cell cultures derived from amniotic fluid obtained by amniocentesis at the end of the fifteenth week of gestation. The karyotype (fig. 5) is that of a male

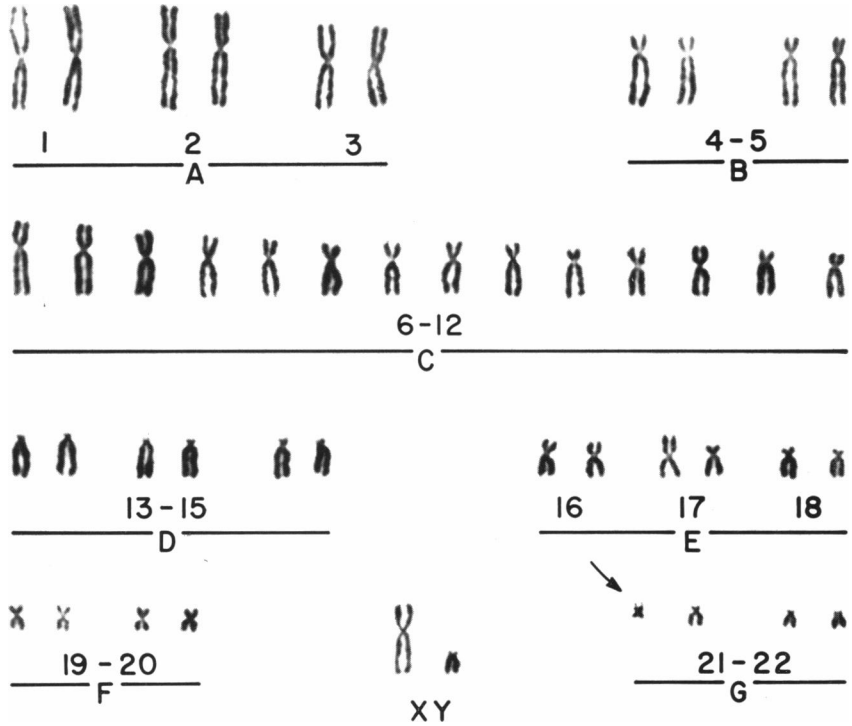

FIG. 5.—Karyotype of unborn sibling of proband obtained at 18 weeks of gestation, indicating a male translocation heterozygote.

translocation heterozygote, 46,XY,17−,G−,t(17p+,Gq−)mat, consistent with the development of a normal phenotype. The pregnancy was not terminated. Subsequently, the birth of IV-5 confirmed the prediction that the child would be a normally developed male. Postnatal karyotypic analysis from leukocyte cultures confirmed the balanced translocation heterozygote condition.

DISCUSSION

The proband in this study does not represent an expected segregant from a balanced translocation parent. The presence of an extra chromosome in a human karyotope is not a unique finding; however, the fact that the extra chromosome is one of those involved in the translocation is of particular interest because only very few cases of apparent tertiary trisomy have been reported in man (e.g., Macintyre et al. [4], Jacobsen et al. [5], Hauschtek et al. [6]).

Belling and Blakeslee [7, 8] and Blakeslee [9] reported on specific examples of trisomies in *Datura,* distinguishing among primary (homologue), secondary (isochromosome), and tertiary trisomy. These authors considered the extra chromosome in tertiary trisomy to be an interchange chromosome, containing more than one arm of one chromosome of the complement and less than one arm of another

chromosome of the complement. Tertiary trisomy has been reported among the progeny of translocation heterozygotes of several plant species, accounting for as much as 2% to 10% of the progeny, according to Burnham [10]. Khush and Rick [11] obtained tertiary trisomies via nondisjunction from tertiary monosomies in *Lycopersicon.*

A difficulty in terminology (nondisjunction versus 3:1 segregation) emerges during a consideration of the origin of a tertiary trisomy. The fact that tertiary trisomies occur exclusively among the progeny of translocation heterozygotes indicates that the pairing of homologous regions may have an effect upon subsequent disjunction. Among the four 3:1 segregational patterns from a translocation heterozygote, it should be noted that when syngamy with a normal gamete occurs, two of the patterns will yield tertiary trisomy and two will yield interchange trisomy. For more extensive discussion of these points and enumeration of the possible segregants from a translocation heterozygote, see Burnham [10], Ford [12], and Ford and Clegg [13].

For the remainder of this discussion, to accommodate all interpretations, we will refer to the origin of the extra chromosome in this case as a nondisjunctional event. Thus, nondisjunction is considered equivalent to 3:1 segregation in the translocation carrier and 2:0 segregation in the nontranslocated parent. It is assumed that the minute chromosome of the proband is identical to the one possessed by the carriers in the pedigree.

Six maternal gametic types are expected from the translocation heterozygote (omitting crossovers and assuming a 2:2 segregation), following the classic concept of one alternate and two adjacent segregational patterns (fig. 6). Recalling that the marker E chromosome does not appear in the karyotype of the proband, it is evident that only gametic types 2, 4, or 6 (fig. 6) could be involved. To account for the karyotype of the proband (47 chromosomes), we assume nondisjunction to have occurred in either the father or the mother. If maternal nondisjunction provided the appropriate chromosome to explain the proband's karyotype, the possibilities include a G^E with a type 2 gamete, a G with a type 4, or an E with a type 6. The possibilities if paternal nondisjunction occurred would be two: nondisjunction of the G (with a type 4 maternal gamete) or nondisjunction of an E (with a type 6 maternal gamete). Thus, the nondisjunctional phenomenon leading to the proband's karyotype could be any of five possibilities, and it is impossible to distinguish the correct one. However, it appears most likely that the basis for the proband's karyotype is maternal, alternate segregation accompanied by nondisjunction of the marker G chromosome (3:1 segregation). This consideration is based on the following points:

1. Alternate segregants are found more frequently in this family's pedigree (fig. 4) and in the pedigrees of most other translocation heterozygotes reported [14].

2. The translocated segments are very small, raising the possibility that occasionally crossing over may fail to occur in the affected arm or that terminalization may occur prematurely. The net result in diakinesis would be the formation of a

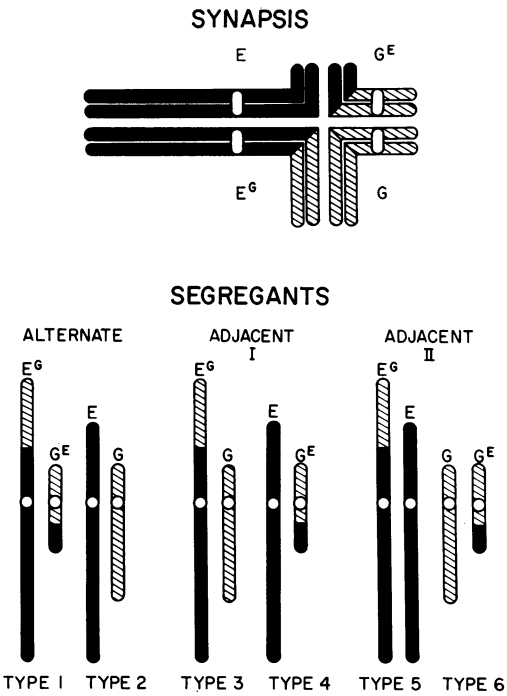

FIG. 6.—Diagram indicating maternal meiotic segregation patterns of the interchange chromosomes and their normal homologues in the translocation heterozygote mother of the proband. Six gametic types (*bottom*) may be derived from alternate, adjacent I, and adjacent II segregation from the association at synapsis (*top*).

chain rather than a ring. Chain formation may enhance the opportunity for alternate segregation accompanied by nondisjunction, that is, formation of a tertiary trisomy [10].

3. Nondisjunction probably is more likely to occur in ovogenesis than in spermatogenesis—for example, the nondisjunction leading to the trisomy in Down's syndrome.

The question remains as to the cytogenetic bases for the proband's stigmata. If we omit consideration of the possible crossover products in the translocation association of four chromosomes at pachynema and other genetic interpretations (e.g., mutant alleles, position effect, etc.), attention is focused on the satellited, minute chromosome which is the cytogenetic anomaly in the proband.

Although the break points of the involved chromosomes are not known, they can be inferred from a study of the minute chromosome. It consists, in part, of a G centromere with its short arm and satellite intact. Since its nonsatellited arm is so short, the break point must have been very close to the centromere. Similarly, the break point on the short arm of the no. 17 chromosome must have been very close to the distal limit of that arm.

If the abnormal phenotype of the proband is the consequence of the cytogenetic

abnormality (the minute chromosome), there are four discrete segments in which the extra genetic information present in the proband may be located: (1) the short arm of G, (2) the long arm segment of G adjacent to the centromere, (3) the centromere of G, and (4) the extreme distal portion of the short arm of the no. 17 chromosome.

Carriers of the D/D, D/G, and G/G translocations in man have been remarkable in their lack of phenotypic abnormalities which could be associated with the evident loss of the short arms and satellite material from the two chromosomes involved in the rearrangement. Whether or not one of the centromeres is also lost in the Robertsonian rearrangement is debatable; we tend to believe that it is. Thus, we assume that the satellites, short arms, and centromeres of satellited chromosomes are genetically inert, or contain so little active material that their loss leads to an undetectable effect on the phenotype. If these assumptions are correct, the active genetic material leading to the phenotypic abnormalities in the case reported here must be located on the remaining segment of the long arm of the involved G chromosome and/or the attached segment of the short arm of chromosome no. 17.

Under most other circumstances, an aberration involving such a relatively small amount of active genetic material would go unobserved. It seems very likely that numerous cases exist in which a small duplication or deficiency of chromosomal material is the basis for phenotypic abnormalities in a patient whose karyotype appears to be entirely normal.

Information derived from both the measurement data (table 1) and the study of labeling patterns (table 2) supports the conclusion that the E chromosome involved in the reciprocal exchange is a no. 17. It is interesting to note from the data presented in table 2 that the marker 17 chromosome (E_3) demonstrates a higher grain count and a larger standard deviation than does its normal homologue (E_4). We are unable to judge whether these differences are due to the greater short arm length of E_3 or to the fact that this increase in length represents material from a G chromosome.

The evidence presented in this case indicates the origin of the aberration within the limits of the pedigree. Presumably, the breakage and rejoining of the interchange segments occurred in the germ line of one of the great-grandparents (I-1 or I-2) or in an early somatic cell of II-2. There was no evidence of mosaicism in the leukocyte cultures of II-2.

The question of prenatal karyotypic evaluation arose with reference to IV-5. The ability to distinguish both marker chromosomes of the interchange aberration indicated that a definite, critical diagnosis of the fetal karyotype could be made. Obviously, the ability to make an unambiguous identification of *both* marker chromosomes of a balanced carrier parent must be demonstrated before a decision to undertake an amniocentesis and fetal karyotype evaluation can be made.

SUMMARY

A patient (the proband) with congenital malformations was found to have an abnormal karyotype consisting of an apparently normal complement plus a satellited, minute chromosome. The proband's mother proved to be a carrier of a

balanced E/G translocation in which the marker G is equivalent to the minute chromosome found in the proband. Evidence that the translocation was a 17/G is presented. One of the proband's two siblings is a carrier of the translocation; the other demonstrates a normal karyotype. The proband's maternal grandmother also is a carrier, but both great-grandparents have normal karyotypes, indicating the origin of the translocation within the known limits of the pedigree.

The proband's karyotype does not represent an expected segregation from a balanced translocation and must have resulted from nondisjunction. Since the extra chromosome is one of those involved in the translocation, this anomaly is considered to be a rare example of tertiary trisomy. The various possible nondisjunctional routes by which the proband's karyotype might be achieved are discussed.

The proband's mother was pregnant at the time she was ascertained as a translocation heterozygote, and a prenatal chromosome analysis was performed. The fetus proved to be a carrier, and the pregnancy was not terminated. Subsequently, the child was born phenotypically normal, as predicted.

ACKNOWLEDGMENTS

The authors wish to acknowledge the contributions made to this study by Warren E. Grupe, Department of Pediatrics, Case Western Reserve University, who provided the clinical evaluation of the proband, and by Albert C. Lammert, Department of Obstetrics and Gynecology, Case Western Reserve University, who performed the amniocentesis on the proband's mother. In addition to their clinical involvement, both of these physicians have assisted in counseling the proband's parents.

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