Polymorphic Detection of a Parthenogenetic Maternal and Double Paternal Contribution to a 46,XX/46,XY Hermaphrodite

Jacques C. Giltay,[1,2] Tibor Brunt,[1] Frits A. Beemer,[1,2] Jan-Maarten Wit,[3,*]
Hans Kristian Ploos van Amstel,[1,2] Peter L. Pearson,[1] and Cisca Wijmenga[1]

[1]Department of Human Genetics, Utrecht University, [2]Clinical Genetics Center Utrecht, and [3]Wilhelmina Children's Hospital, Utrecht, The Netherlands

Summary

True hermaphroditism in humans usually is associated with a 46,XX karyotype or with mosaicism in which admixtures of cells with an XX and an XY karyotype are seen. However, the mechanisms that cause such mosaicisms are poorly understood. To date, with rare exceptions, analyses of hermaphrodites have been limited mostly to cytogenetic investigations. In this report, we describe a 5-year-old patient with true hermaphroditism and a 46,XX/46,XY karyotype (ratio 38:12) in lymphocytes, suggesting involvement of two fertilization events. Microsatellite DNA polymorphisms distributed throughout the genome were analyzed, to investigate the origin of the cell lines concerned. The results are consistent with double paternal and single maternal genetic contributions. Possible mechanisms that would explain these findings are discussed. The most likely mechanism involves a single haploid ovum dividing parthenogenetically into two haploid ova, followed by double fertilization and fusion of the two zygotes into a single individual, at the early embryonic stage.

Introduction

True hermaphroditism in humans is an extremely rare condition, the incidence of which has not been determined precisely (Emery and Rimoin 1996). More than 10% of hermaphroditic patients show mosaicism of the 46,XX/46,XY karyotype (Van Niekerk and Retief 1981). Mosaicism following nondisjunction can explain the existence of 46,XX and 46,XY cell lines in a hermaphrodite (Wit et al. 1987). An alternative mechanism could be the fusion of two different zygotes of opposite sexes. Such a chimera should contain two paternal and two maternal haploid genomes. Support for the mechanism causing 46,XX/46,XY mosaicism can be obtained by comparison of DNA polymorphisms in the patient with the parental alleles.

Previous studies to elucidate the mechanism underlying such mosaicism in hermaphroditic patients used polymorphic blood-group markers and a limited number of DNA polymorphisms near the centromeres or at the short arms of acrocentric chromosomes (Fitzgerald et al. 1979; Minowada et al. 1982; Schoenle et al. 1983; Green et al. 1994; Uehara et al. 1995). In one case, the presence of both a normal and an unbalanced karyotype in the son of a balanced-translocation carrier was regarded as evidence for chimerism (Nyberg et al. 1992). Highly informative DNA markers, such as tri- and tetranucleotide repeats, that provide a much more powerful means of studying the transmission of different alleles from parent to child are now available.

This report describes a true hermaphrodite with a 46,XX/46,XY karyotype. More than 200 DNA short-tandem-repeat markers were analyzed, to study both parents' genetic contributions to the hermaphroditic child and to assess the mode of origin of the mosaicism.

Material and Methods

Case Report

The proband was born, after an uneventful pregnancy, by elective cesarean section, because of his mother's obstetric history. The nonconsanguineous parents have two older, healthy daughters. The first daughter was born by vacuum extraction, and the second was born by cesarean section. Because of secondary infertility after the birth of the second child, the mother had been on clomiphene citrate medication at the time of conception of the proband. On physical examination, the proband was found to have a normal phallus but unpalpable testes. At the age of 2 mo he was hospitalized because of a strangu-

Received November 5, 1997; accepted for publication February 3, 1998; electronically published April 3, 1998.

Address for correspondence and reprints: Dr. Jacques C. Giltay, P.O. Box 18009, 3501 CA Utrecht, The Netherlands. E-mail: Giltay@pobox.ruu.nl

* Present affiliation: University Hospital Leiden, Leiden, The Netherlands.

0002-9297/98/6204-0026$02.00

Table 1

Data from Autosomal Marker Loci

No. of Markers (n = 59)	No. of Distinguishable Alleles			No. of Markers for Which Child Showed Three Alleles	No. of Markers, by Origin of Extra Allele	
	Father	Mother	Total		Paternal	Maternal
29	2	2	4	10	10	0
19	2	1	3	7	7	0
11	1	2	3	0	0	0
Total	...	...	...	17	17	0

lated left inguinal hernia. The inguinal sac appeared to contain a normal testis, with an epididymis on the right side with a deferent duct disappearing into a central Mullerian structure. This structure continued on the left side, into a uterine tube with a small gonad. The other internal organs were normal. The uterine tube and the left gonad were excised. The testis was placed in the scrotum, and the deferent duct was left intact. Histological examination of a biopsy of the right gonad showed normal testicular tissue. The left gonad showed a large number of oocytes, shading off into testicular tissue. At least one primordial follicle was seen. Endocrine studies, including a human chorionic gonadotropin-stimulation test, showed no abnormalities. The patient is now 6 years old and, in other respects, is developing normally.

Cytogenetic Analysis

Metaphase spreads of phytohemagglutinin-stimulated peripheral lymphocytes were prepared in accordance with standard procedures. Chromosomes were analyzed from G-banded preparations.

Molecular Genetic Analysis

DNA was isolated from 20 ml peripheral blood, from the proband and from his parents, in accordance with standard procedures (Miller et al. 1988). A genome screen was performed with 232 markers from Marshfield screening set 6.0 (Marshfield Medical Research Foundation [http://www.marshmed.org/genetics/]). These markers are distributed over all 22 autosomes and the X chromosome. There are an average of 10 markers per chromosome, and the number of markers per chromosome ranges from 1 marker on chromosome 21 to 14 markers on chromosome 2. For every marker, the reverse primer was labeled with either 6-carboxyfluorescein, 4,7,2′,4′,5′,7′-hexachloro-6-carboxyfluorescein, or 4,7,2′,7′-tetrachloro-6-carboxyfluorescein fluorescent dyes (Isogen Bioscience), which allowed separation on an ABI377 DNA sequencer.

DNA was amplified for 30 cycles of 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C, followed by a final

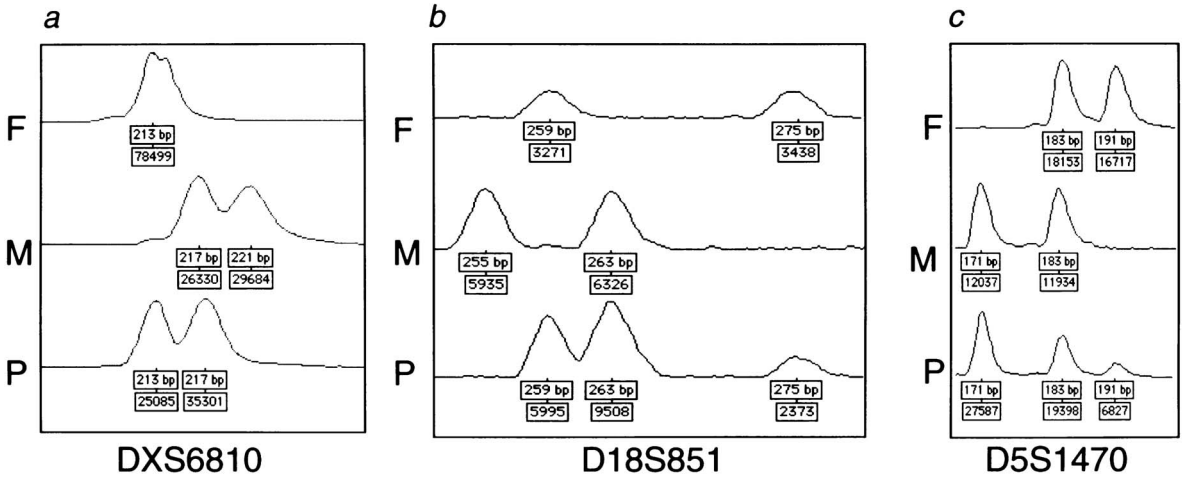

Figure 1 Analysis of completely (*a* and *b*) or partially (*c*) informative markers in the patient (P) and his father (F) and mother (M). The fragment length (in bp) and quantity of the allele are indicated below each peak. *a,* Analysis of X-chromosomal marker DXS6810. In the patient, the amount of the maternally derived allele (217 bp) and that of the paternally derived allele (213 bp) are 35,301 units and 25,085 units, respectively. These relative maternal and paternal contributions reflect the expected ratio of 50:38 (see Results). *b,* Analysis of chromosome 18 marker D18S851. Both paternal alleles (259 bp and 275 bp) and one maternal allele (263 bp) are transmitted to the child. Note that, as expected, the total paternal contribution to the child (5,995 + 2,373 = 8,368) is roughly the same as the maternal contribution (9,508). *c,* Analysis of chromosome 5 marker D5S1470. The mother and father share the 183-bp allele. In the child, the amount of the maternal 171-bp allele (27,587) and the total of the 183-bp and 191-bp alleles (19,398 + 6,827 = 26,225) are roughly the same, indicating that the latter two alleles were derived from the father. Accordingly, the ratio of the paternal alleles (19,398:6,827) reflects the expected 38:12 ratio (see Results).

extension of 10 min at 72°C, on a PE9600 thermocycler. One microliter of the PCR reaction was mixed with an equal volume of formamide, 0.25 μl GS-500 size standard (Perkin-Elmer), and 0.25 μl Dextran Blue (Perkin-Elmer), was loaded directly onto a 5% polyacrylamide gel, and was run on an ABI377 automated DNA sequencer. Gels were analyzed by use of GeneScan 2.0.2 (Perkin Elmer). Allele sizes were determined by use of GenoTyper 1.1 (Perkin Elmer). The peak area of each allele was used to quantify the amount of PCR product. For the few cases in which the parents showed preferential amplification of either allele, the alleles of the proband were normalized according to the ratio observed for the parental alleles.

Results

Cytogenetic Findings

Analyses of GTG-banded chromosomes, in 50 metaphases in peripheral blood lymphocytes of the proband, showed a 46,XX/46,XY karyotype in a ratio of 38:12.

Molecular Genetic Findings

To determine the number and origin of the parental alleles in the proband's lymphocytes, we originally analyzed 232 microsatellite polymorphic markers. A total of 127 marker loci (121 autosomal and 6 X-chromosomal) were fully or partially informative; for 29 autosomal marker loci all four maternal and paternal alleles were distinguishable, and for 2 X-chromosomal marker loci all three maternal and paternal alleles were distinguishable. For an additional 19 autosomal marker loci two paternal alleles were distinguishable from the homozygous maternal allele, and for 11 marker loci two maternal alleles were distinguishable from the homozygous paternal allele. Data on the autosomal marker loci are shown in table 1.

The two completely informative markers located on the X chromosome were used to determine the relative contributions of the parental gametes to the two cell lines in the patient, under the assumption that the cytogenetically determined ratio of these cell lines is reflected by DNA analysis. The X-chromosomal maternal and paternal genetic contributions that can be expected (X_mX_p:X_mY_p = 38:12; $X_{m,total}$:X_p = 50:38 = 1.32 [m = maternal; and p = paternal]), on the basis of chromosomal analysis, were indeed reflected by the quantification of different alleles and imply that this quantification is quite accurate. As an example, the analysis of one of the two markers is shown in figure 1*a*. The results from another four partially informative X-chromosomal markers were consistent with these relative contributions (data not shown).

For 10 of the 29 fully informative autosomal marker loci in both parents, the proband inherited three alleles (table 1). Two of these alleles clearly originated from the father (an example is shown in fig. 1*b*). For 7 of the additional 19 markers informative in the father only, the proband also showed three alleles, with, again, two alleles originating from the father. For a total of 40 informative maternal markers, the proband showed only a single maternal allele. The results from the other 61 partially informative markers were all consistent with double paternal and single maternal genetic contributions. An example of a partially informative marker is shown in figure 1*c*.

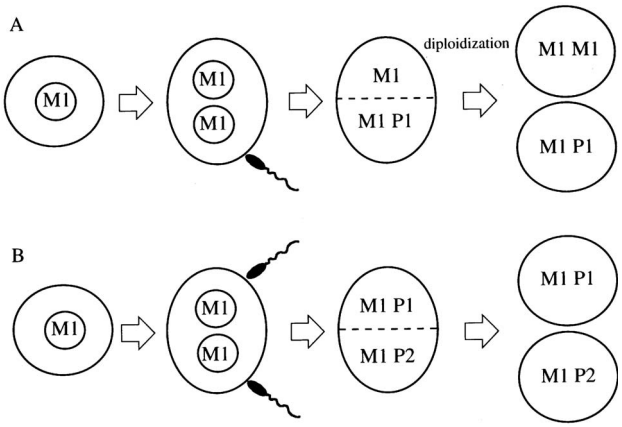

Figure 2 Proposed mechanism to explain early development of the patient described by Strain et al. (1995) (*A*) and the patient described in this report (*B*). In both cases, an ovum (M1) is parthenogenetically activated. In the patient described by Strain et al., one of the two haploid maternal cells is fertilized by a normal sperm (P1), whereas the other is diploidized, either sequentially (as shown) or simultaneously. In the patient described in this report, both (identical) haploid maternal cells are fertilized by a normal sperm (P1 and P2).

Discussion

There is ample evidence to demonstrate that two different spermatozoa contributed to the proband's genome. First, cytogenetic analysis showed that the father contributed an X chromosome to the 46,XX lymphocytes and a Y chromosome to the 46,XY lymphocytes. Second, analysis of 48 fully informative (in the sense that both paternal alleles were distinguishable from the maternal alleles) polymorphic DNA markers clearly demonstrated the inheritance of both paternal alleles, for 17 marker loci. This result is compatible with the involvement of two independent gametes. With respect to the maternal genetic contribution to the proband's genome, there is one single allele present for all 40 informative maternal marker loci. These data strongly suggest a haploid maternal genetic contribution.

The literature describes the following three mecha-

nisms to explain 46,XX/46,XY chimerism in patients with ambiguous genitalia.

1. Postzygotic fusion of two distinct embryos (Green et al. 1994). This mechanism cannot explain the present data, because it requires both a double paternal and a double maternal contribution.

2. Fertilization of an ovum and a second polar body by two different spermatozoa and subsequent fusion of the zygotes (Green et al. 1994). This mechanism can be distinguished from the first by comparison of maternal pericentric markers with distal markers and by evidence of recombination for some distal markers (Bonthron et al. 1997). It could explain the present data only if there were complete or near-complete suppression of meiotic crossing over, involving all chromosomes. However, we are not aware of a precedent for this latter event.

3. Fertilization of a haploid ovum by two different spermatozoa, yielding a 3*n* zygote, followed by a cell division into three 2*n* daughter cells (Plachot and Crozet 1992). This would imply that, on average, one in three cells would have lost the maternal allele. A number of different cell lines would have to be investigated to find an indication for such a loss of maternal alleles. However, results of quantitative analysis of all the maternal and paternal alleles showed equal genetic contributions from each parent, including the results shown in figure 1. Thus, there was no indication of a loss of maternal alleles; therefore, this mechanism appears to be unlikely.

Thus, none of these three mechanisms can satisfactorily explain the data presented in this report. The mechanism we propose is similar, in part, to that described by Strain et al. (1995). They reported a case involving parthenogenetic development in which a haploid nucleus first divided mitotically, resulting in two genetically identical nuclei, followed by fertilization of one nucleus by a spermatozoan and diploidization of the other (fig. 2*A*). The most likely mechanism to explain the genetic data of the case we describe also must involve a parthenogenetic division of a haploid nucleus, to give two identical nuclei, followed by fertilization of both nuclei by separate spermatozoa (fig. 2*B*). Such parthenogenetic activation has been demonstrated in in vitro fertilization (Van Blerkom et al. 1994) and can be induced by agents such as calcium ionofoor A23187 (Taylor and Braude 1994). It is interesting to speculate that clomiphene citrate (which was taken by the mother at the time of conception) could act as such an agent, in vivo. To the best of our knowledge, this is the first documented case of chimerism, in a hermaphrodite, based on a parthenogenetic maternal and a double paternal contribution.

References

Bonthron DT, Strain L, Dean JCS (1997) Amalgamation of *in vitro* fertilized embryos, resulting in birth of a true hermaphrodite chimera. Am J Hum Genet Suppl 61:A147

Emery AEH, Rimoin DL (1996) Principles and practice of medical genetics, 3d ed. Churchill Livingstone, Edinburgh

Fitzgerald PH, Donald RA, Kirk RL (1979) A true hermaphrodite dispermic chimera with 46,XX and 46,XY karyotypes. Clin Genet 15:89–96

Green AJ, Barton DE, Jenks P, Pearson J, Yates JRW (1994) Chimaerism shown by cytogenetics and DNA polymorphism analysis. J Med Genet 31:816–817

Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16:1215

Minowada S, Hara M, Shinohara M, Ishida H, Fukutani K, Isurugi K, Nijima T, et al (1982) Investigation of genetic markers in a true hermaphrodite with chi 46,XX/46,XY. Hum Genet 60:376–378

Nyberg RH, Haapala AK, Simola KOJ (1992) A case of human chimerism detected by unbalanced chromosomal translocation. Clin Genet 42:257–259

Plachot M, Crozet N (1992) Fertilization abnormalities in human in-vitro fertilization. Hum Reprod Suppl 7:89–94

Schoenle E, Schmid W, Schinzel A, Mahler M, Ritter M, Schenker T, Metaxas M, et al (1983) 46,XX/46,XY chimerism in a phenotypically normal man. Hum Genet 64: 86–89

Strain L, Warner JP, Johnston T, Bonthron DT (1995) A human parthenogenetic chimaera. Nat Genet 11:164–169

Taylor AS, Braude PR (1994) The early development and DNA content of activated human oocytes and parthenogenetic human embryos. Hum Reprod 9:2389–2397

Uehara S, Nata M, Nagae M, Sagisaka K, Okamura K, Yajima A (1995) Molecular biologic analyses of tetragametic chimerism in a true hermaphrodite with 46,XX/46,XY. Fertil Steril 63:189–192

Van Blerkom J, Davis PW, Merriam J (1994) A retrospective analysis of unfertilized and presumed parthenogenetically activated human oocytes demonstrates a high frequency of sperm penetration. Hum Reprod 9:2381–2388

Van Niekerk WA, Retief AE (1981) The gonads of human true hermaphrodites. Hum Genet 58:117–122

Wit JM, Quartero AO, Bax NMA, Huber J (1987) A phenotypic male with true hermaphroditism and a 46,XX/46,XY/46,XXY karyotype. Clin Genet 31:243–248