A human parthenogenetic chimaera

Lisa Strain, Jon P. Warner, Thomas Johnston & David T. Bonthron

In mice, parthenogenetic embryos die at the early postimplantation stage as a result of developmental requirements for paternally imprinted genes, particularly for formation of extraembryonic tissues. Chimaeric parthenogenetic↔normal mice are viable, however, due to non-random differences in distribution of their two cell types. Species differences in imprinting patterns in embryo and extra-embryonic tissues mean that there are uncertainties in extrapolating these experimental studies to humans. Here, however, we demonstrate that parthenogenetic chimaerism can indeed result in viable human offspring, and suggest possible mechanisms of origin for this presumably rare event.

University of Edinburgh, Human Genetics Unit, Department of Medicine, Western General Hospital, Edinburgh EH4 2XU, UK

Correspondence should be addressed to D.T.B.

Normal mammalian development requires functionally distinct genetic contributions from male and female gametes. Though recent studies of differential gene expression from paternal and maternal genomes provide molecular evidence for the phenomenon of genomic imprinting, its existence was originally inferred from embryological studies of the abnormal fate of spontaneously or experimentally induced parthenogenetic or androgenetic embryos, particularly in the mouse. Some mouse strains show high rates of spontaneous parthenogenetic oocyte activation, and in humans, such naturally occurring parthenogenetic development is also well documented, through the study of benign ovarian teratomas. In at least some cases, these originate by development of gametogenic cells which have completed the first meiotic division[1]. However, parthenogenetic or gynogenetic development has not previously been reported in viable human pregnancies. The inability of parthenogenetic mouse embryos to develop beyond the early postimplantation stage results in large part from the need for a paternally imprinted genome for correct formation of extraembryonic tissues[2,3]. In chimaeric normal↔parthenogenetic mice, however, parthenogenetic cells can contribute to many mature tissues, and can even be transmitted through the germline[4-7]. In these chimaeras, there is often selection against the parthenogenetic cells during embryogenesis. This is a non-random phenomenon which occurs particularly in certain tissues (for example virtually eliminating parthenogenetic cells from skeletal muscle), and may reflect the need for tissue-specific imprinted differentiation genes[6-11]. These mouse studies raise the

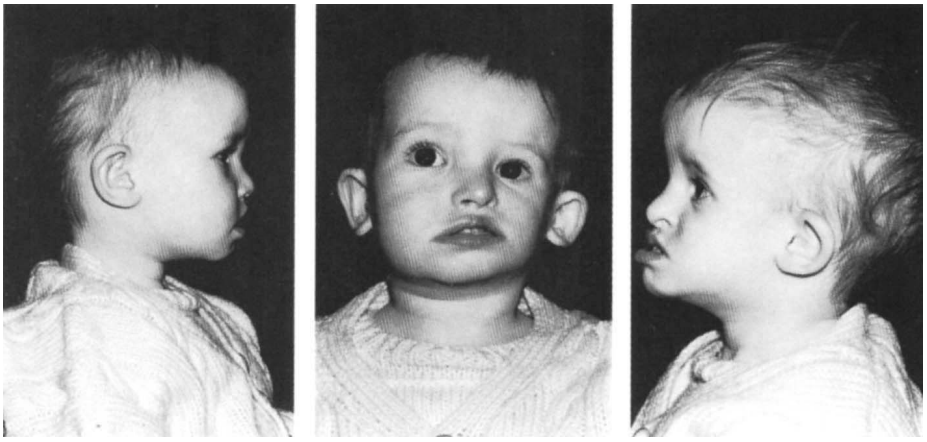

Fig. 1 FD aged 1.2 years. The facial profile from the right is essentially normal, with all the visible abnormalities confined to the left.

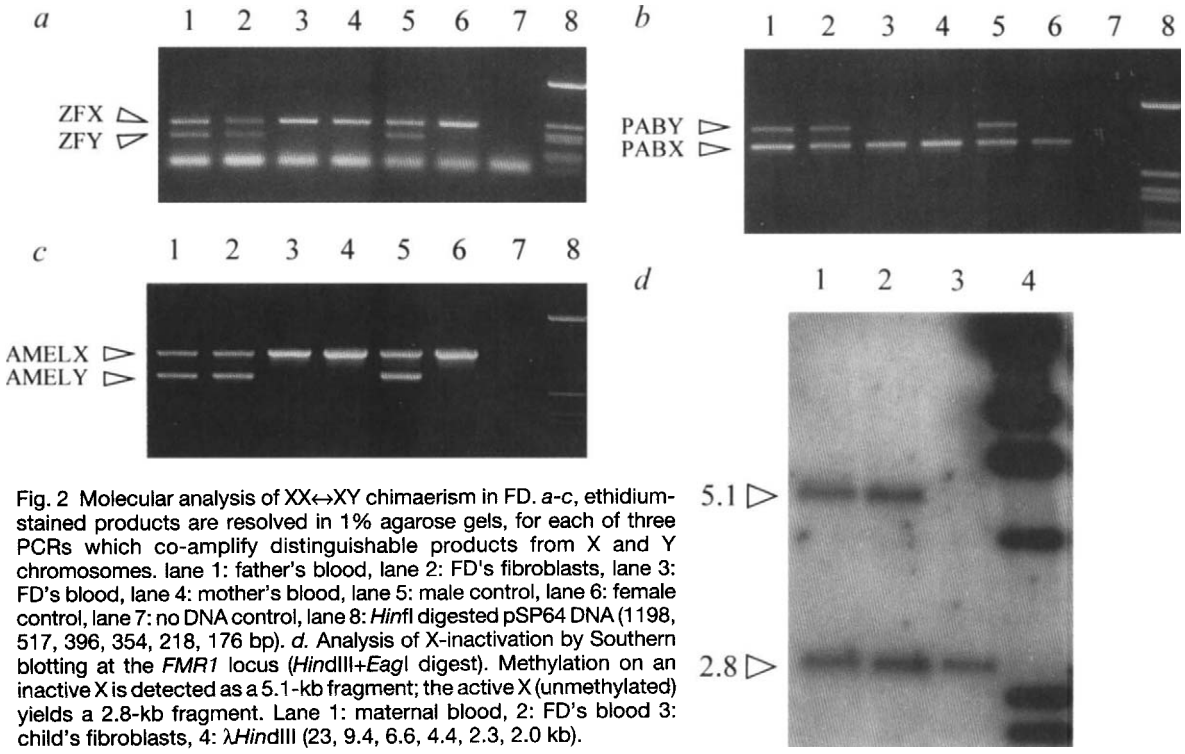

Fig. 2 Molecular analysis of XX↔XY chimaerism in FD. *a-c*, ethidium-stained products are resolved in 1% agarose gels, for each of three PCRs which co-amplify distinguishable products from X and Y chromosomes. lane 1: father's blood, lane 2: FD's fibroblasts, lane 3: FD's blood, lane 4: mother's blood, lane 5: male control, lane 6: female control, lane 7: no DNA control, lane 8: *Hin*fI digested pSP64 DNA (1198, 517, 396, 354, 218, 176 bp). *d*. Analysis of X-inactivation by Southern blotting at the *FMR1* locus (*Hin*dIII+*Eag*I digest). Methylation on an inactive X is detected as a 5.1-kb fragment; the active X (unmethylated) yields a 2.8-kb fragment. Lane 1: maternal blood, 2: FD's blood 3: child's fibroblasts, 4: λ*Hin*dIII (23, 9.4, 6.6, 4.4, 2.3, 2.0 kb).

possibility that mosaic or chimaeric parthenogenetic development could occur in humans. In support of this concept, we present here a molecular genetic analysis of a mildly developmentally-delayed child, whose peripheral blood leukocytes are entirely parthenogenetic.

Facial asymmetry and sex reversal

The patient FD (Fig. 1) was referred as a case of apparent sex reversal. He was the 3.36 kg product of a full-term pregnancy. A phenotypic male, his blood karyotype had been examined in the neonatal period because of left-sided hemifacial microsomia, unexpectedly revealing 46,XX in all cells. His only other obvious physical abnormality was small testes. A bifid uvula and minimal submucous cleft palate were also later noted during ENT examination under anaesthesia. Mild learning difficulty and intermittent aggressive behaviour became apparent in early childhood. He is left-handed.

To define the basis for FD's sex reversal, we performed an analysis of Y chromosome STS DNA markers on peripheral blood DNA. All the PCRs failed to yield a Y-specific product, despite reliable amplification of control male samples. In particular, PCRs for *ZFY/ZFX*, *PABY/PABX* and *AMELY/AMELX*, which simultaneously amplify X- and Y-specific fragments of different sizes, showed only X-specific bands (Fig. 2*a-c*). Since *ZFY* and *PABY* flank the testis-determining gene *Sry*, and are <200 kb apart, these two results alone virtually excluded the presence of *Sry* in blood DNA. A reliable PCR for *Sry* itself was also negative, though when the products were blotted and probed with high-specific activity ^{32}P-labelled *Sry* product from a normal male, a faint band was detected in FD's blood DNA (not shown).

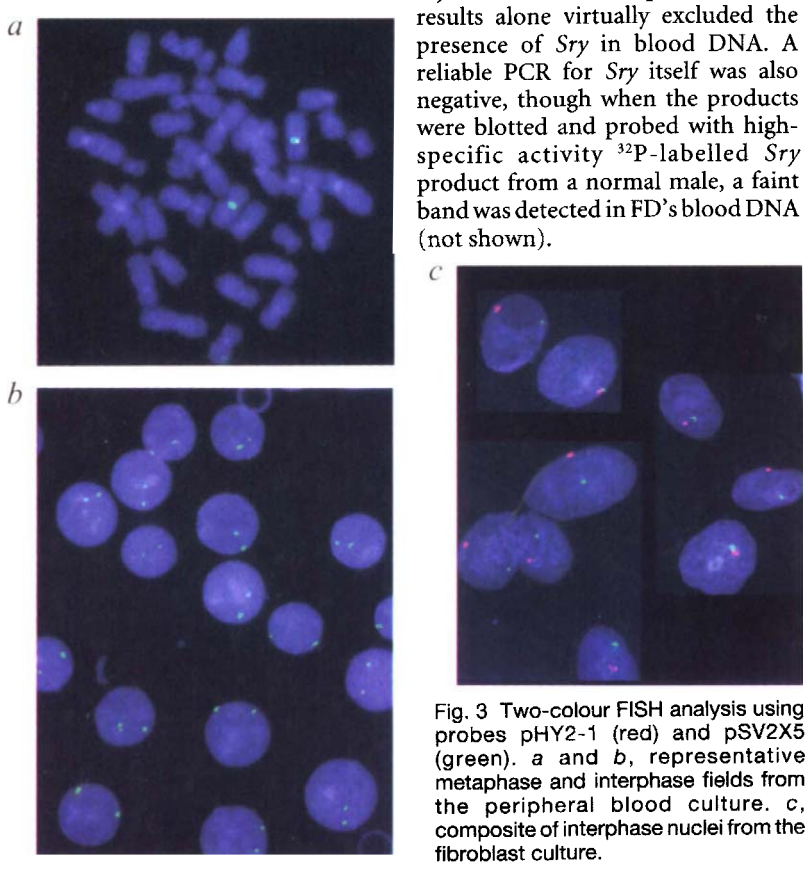

Fig. 3 Two-colour FISH analysis using probes pHY2-1 (red) and pSV2X5 (green). *a* and *b*, representative metaphase and interphase fields from the peripheral blood culture. *c*, composite of interphase nuclei from the fibroblast culture.

Table 1 Results of typing informative X-linked microsatellite markers, and their cytogenetic locations (where known)

Locus	Location	P:father's blood	F:FD's fibroblasts	B:FD's blood	M:mother's blood	
DXS1060	Xp22.33	3	1	1	1,2	I
DXS996	Xp22.33	3	2	2	1,2	I
DXS237	Xp22.32	2		1	1,1	(I)
DXS987	Xp22.2	1	2	2	2,3	I
DXS207	Xp22.2	1	1	1	1,2	
DXS1053	Xp22.2	1		2	1,2	(I)
DXS1223	Xp22.2	3	1	1	1,2	I
DXS1195	Xp22.13	2	1	1	1,2	(I)
DXS418	Xp22.13	3	2	2	1,2	I
DXS999	Xp22.13	2	3	3	1,3	I
DXS443	Xp22.13	2		1	1,1	(I)
DXS1229	Xp22.13	1		2	2,2	(I)
DXS365	Xp22.13	3	1	1	1,2	I
DXS989	Xp22.12	3	2	2	1,2	I
DXS451	Xp22.12	2	1	1	1,3	I
DXS1048		2	3	3	1,3	I
DXS1234	Xp21	1		2	2,2	(I)
DXS997	Xp21	2		1	1,1	(I)
DYSI	Xp21.1	2		1	1,2	(I)
DXS538	Xp21.1	1		2	2,2	(I)
DXS1110	Xp21.1	2		1	1,1	(I)
DXS1058	Xp11.4	1		2	2,3	I
DXS993	Xp11.4	2		1	1,1	(I)
DXS1201	Xp11.4	3	2	2	1,2	I
DXS228I	Xp11.4	1		1	1,2	
DXS228II	Xp11.4	1		2	2,2	(I)
MAOA	Xp11.3	2		2	1,2	
MAOB	Xp11.3	1		2	2,2	(I)
DXS1055	Xp11.3	2	2	2	1,2	
ARAF1	Xp11.3	2		2	1,2	
DXS426	Xp11.3	2		1	1,1	(I)
DXS1039	Xp11.23	2		1	1,1	(I)
DXS988	Xp11.22	1	2	2	1,2	(I)
DXS1204	Xp11.22	2		1	1,3	I
DXS991	Xp11.21	3	2	2	1,2	I
DXS1216		2		1	1,2	(I)
AR	Xq11.2	2	3	3	1,3	I
DXS135	Xq12	2		1	1,1	(I)
DXS983	Xq13.1	2		1	1,1	(I)
DXS566	Xq13.3	1		2	1,2	(I)
DXS986	Xq21.1	2	2	2	1,2	
DXS1002	Xq21.31	2		1	1,1	(I)
DXYS1X	Xq21.31	2		1	1,2	(I)
DXS1203	Xq21.33	2	2	2	1,2	
DXS1217		1		2	1,2	(I)
DXS3	Xq21.33	1	1	1	1,2	
DXS990	Xq21.33	2	2	2	1,2	
DXS178	Xq22.1	2	2	2	1,2	
COL4A5A	Xq22.3	2		1	1,1	(I)
DXS994	Xq24	2		1	1,2	(I)
DXS1047	Xq25	2		1	1,2	(I)
DXS1114	Xq26.1	1		2	2,2	(I)
HPRT	Xq26.1	2	1	1	1,2	(I)
DXS1192	Xq26.3	3	2	2	1,2	I
DXS1227	Xq27.1	2	3	3	1,3	I
DXS691	Xq27.3	1	2	2	1,2	(I)
DXS548	Xq27.3	1		1	1,2	
FRAXA	Xq27.3	2		3	1,3	I
FRAXE	Xq28	2		2	1,2	
DXS1113	Xq28	2		1	1,2	(I)
p26	Xq28	2	3	3	1,3	I
DXS1108	Xq28	3	1	1	1,2	I

Alleles are numbered arbitrarily, 1 representing in each case the largest allele seen in the family. In the last column, informativeness of each marker is summarized as follows: I: marker demonstrates maternal uniparental isodisomy, (I): marker demonstrates maternal uniparental disomy.

Together with the clinical picture of an asymmetrical developmental abnormality, the last result suggested the possibility of mosaicism for a Y-bearing line. However, fluorescent *in situ* hybridization (FISH) with the Y-specific probe pHY2-1 (*DYZ2*)[12] showed signals on less than 1 in 3,000 peripheral blood nuclei (not shown). Two colour metaphase and interphase FISH with pHY2-1 and the X-alphoid repeat probe pSV2X5 (ref. 13) showed that >98% of PBL nuclei had two X chromosomes (the same proportion as seen using this probe in control females), and again, no Y signals were seen in >3000 PBL nuclei (Fig. 3*a*,*b*).

PCR of a urine sediment using the *PABY/PABX* primers produced the first confirmation of a Y-specific product, though amplification was poor. A skin biopsy from the right ankle was therefore cultured and analysed. In this material, in contrast to the results from blood, *PABY*, *ZFY* and *AMELY*-specific PCR products were all seen (Fig. 2*a-c*, lane 2). A 46,XY karyotype was confirmed cytogenetically, and FISH with pHY2-1 showed Y signals in >95% of 500 interphase nuclei. Typical two-colour FISH images using pHY2-1 and pSV2X5 are shown in Fig. 3*c*. No XX nuclei were identified; >95% had a single X signal, the same percentage as in control male preparations. (Twin X signals were seen in some postreplicative nuclei, which also had twin Y spots.) From these studies, FD appeared to be a 46,XY/46,XX mosaic, but with extreme segregation of the two lines between different tissues.

Complete maternal isodisomy of the X

The parental origin of the two X chromosomes in FD's blood was next determined, using microsatellite polymorphisms. Sixty-two multi-allelic X markers were fully or partially informative (Table 1). For 50 markers, a paternal allele is clearly absent (maternal uniparental disomy). At 33 of these 50 loci (including 20 at which all three parental alleles were distinguishable) maternal uniparental isodisomy is present (examples in Fig. 4*a*). At the other 17 of these 50 loci, it is not possible to distinguish between maternal isodisomy and hetero-disomy. At the final 12 of the 62 loci, FD is homozygous and his mother heterozygous, but the parental origin of FD's allele cannot be assigned. FD is thus homozygous at all 62 loci. The

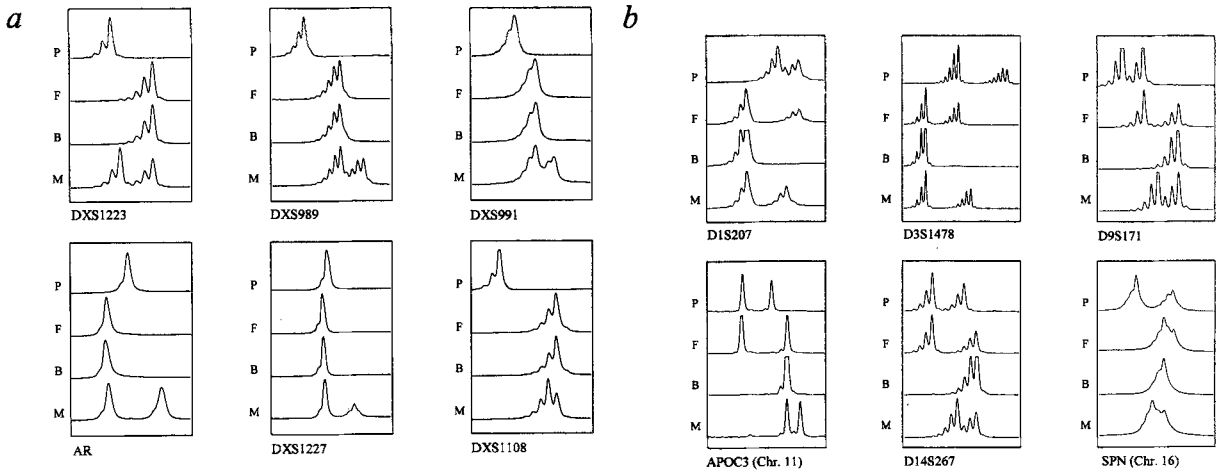

Fig. 4 *a*, Examples of the segregation of fully informative X-chromosome markers, determined by laser fluorescence detection of PCR products resolved on denaturing polyacrylamide gels. Cytogenetic locations of each marker are given in Table 1. Samples are identified as follows: paternal blood (P), FD's skin fibroblasts (F), FD's blood (B), maternal blood (M). *b*, Examples of fully informative autosomal markers.

results demonstrate complete maternal isodisomy of the X chromosome, with no indication of segmental heterodisomy as may result from meiotic segregation errors. For 27 of the fully or partially informative X markers, the genotyping was confirmed on DNA from two independent blood samples, taken at ages 2.9 and 3.6 years. Finally, apparently normal inactivation of one X chromosome in blood was shown by analysis of methylation at the *FMR1* locus (probe Ox1.9, *Hin*dIII+*Eag*I digest), while the single X in FD's fibroblasts was unmethylated (Fig. 2*d*).

Maternal uniparental disomy for all 22 autosomes

In view of the isodisomy of the X chromosome, the parental origin of the autosomes was examined. By typing multiple microsatellites, at least one informative marker was identified for each autosome (Table 2). At each of these loci, in FD's blood only a single maternal allele was seen. His skin fibroblasts, in contrast, were heterozygous at all these loci, with alleles in all cases consistent with normal biparental codominant inheritance (Fig. 4*b*). Taken in conjunction with the more detailed analysis of the X chromosome, these results indicate maternal isodisomy for all 23 chromosome pairs, that is, FD's blood leukocytes are completely gyno- or parthenogenetic. Finally (and importantly for considering possible mechanisms), comparison of the maternal alleles transmitted to the blood and to the fibroblasts showed that for all maternally informative markers (representing all 22 autosomes and 45 X-linked loci) the same maternal allele was present in both of these tissues (Tables 1 and 2).

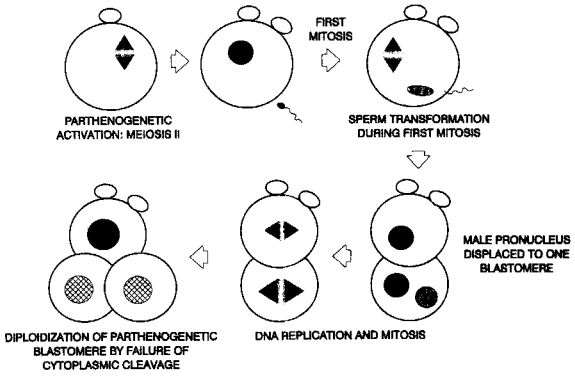

Fig. 5 Proposed mechanism for early development of FD. A parthenogenetically activated embryo was fertilized by a normal sperm, allowing decondensation of the sperm head during the first mitotic cycle, and formation of a male pronucleus (light grey). The first mitotic division was completed independently of fertilization, and one of the resulting haploid parthenogenetic nuclei (dark grey) accompanied the male pronucleus into one blastomere. The second mitotic division may or may not have occurred synchronously in the normal and parthenogenetic blastomeres, as shown. However, at some stage, following the second or later round of replication, cytoplasmic cleavage would have failed in a parthenogenetic blastomere, allowing a diploid chromosome number to result. This model is based on the experimental studies of Maleszewski and Bielak[16].

Discussion

Using the terms precisely, our patient FD is neither a mosaic nor a chimaera. Since the maternal allele in the abnormal XX cell line is in every case the same as that in the normal XY cell line, both lines are derived from the same oocyte (suggesting 'mosaicism'). However, they do not derive from a common zygote (the usual definition of mosaicism), and we therefore favour the term chimaerism, as usual in the literature on parthenogenesis in the mouse, despite the origin of both lines from one oocyte. What genetic mechanism could then account for our observations? Deductions can be made by considering the expected products of crossing over between maternal homologues in oogenesis.

Mosaicism/chimaerism for a gynogenetic line might originate by equal cytoplasmic cleavage after meiosis I of oogenesis, followed in one of the two cleavage products by normal meiosis II and fertilization, and in the other by failure of cytoplasmic cleavage causing persistence of diploidy. However, this model is incompatible both (a) with the observed isodisomy (no heterodisomy) for all maternal markers in blood, and (b) with the identical maternal genetic contributions to both skin and blood lineages.

Table 2 Informative or partially informative autosomal markers

Locus/probe	P:father's blood	F:FD's fibroblasts	B:FD's blood	M:mother's blood	
D1S207	1,3	1,4	4,4	2,4	I
D1S216	2,3	1,3	1,1	1,4	I
KHK/CA1 [2]	2,3	1,3	1,1	1,3	(I)
D3S1265	2,3	1,3	1,1	1,2	I
D3S1478	1,3	3,4	4,4	2,4	I
FABP2/PCR2.1-2.2 [4]	1,2	1,3	3,3	1,3	(I)
D4S413	2,2	1,2	1,1	1,2	(I)
D5S407	1,3	1,2	2,2	1,2	(I)
HLA/82I [6]	2,2	1,2	1,1	1,2	(I)
HLA/D3A [6]	2,2	2,3	3,3	1,3	I
D6S305	1,2	1,2	1,1	1,2	
D6S314	1,4	1,3	3,3	2,3	I
D7S629	1,1	1,2	2,2	2,2	(I)
D7S484	2,2	1,2	1,1	1,2	(I)
D8S273	2,2	1,2	1,1	1,2	(I)
D9S171	3,4	1,3	1,1	1,2	I
D9S175	1,3	2,3	2,2	2,4	I
D10S197	1,1	1,2	2,2	1,2	(I)
D11S922	1,2	1,3	3,3	3,4	I
APOC3/PCR2.1-2.2 [11]	3,4	2,4	2,2	1,2	I
D12S77	1,4	3,4	3,3	2,3	I
D12S99	2,4	2,3	3,3	1,3	I
D12S368	1,2	1,2	2,2	2,3	
D13S122	1,3	2,3	2,2	2,3	(I)
D13S153	3,3	1,3	1,1	1,2	I
D13S158	1,2	1,2	1,1	1,2	(I)
D14S80	1,1	1,2	2,2	1,2	(I)
D14S267	2,4	1,4	1,1	1,3	I
FBN1/MTS1S-1AS [15]	1,3	2,3	2,2	2,3	(I)
FBN1/MTS2S-2AS [15]	1,2	2,3	3,3	1,3	I
SPN/SI1-2 [16]	1,4	1,2	2,2	2,3	I
D16S515	3,3	2,3	2,2	1,2	I
D17S789	3,3	1,3	1,1	1,2	I
D18S71	1,3	2,3	2,2	1,2	I
D19S220	2,3	1,2	1,1	1,1	(I)
D19S221	1,2	1,2	1,1	1,1	
D19S225	1,1	1,2	2,2	2,3	I
D20S107	2,2	1,2	1,1	1,3	I
D20S186	1,3	1,3	1,1	1,2	(I)
D21S65	1,2	1,3	3,3	3,4	I
D22S264	1,2	1,4	4,4	3,4	I
D22S343	1,2	2,3	3,3	3,3	I

Square brackets enclose chromosome numbers for those loci designated by gene symbols rather than D numbers. Informativeness of each marker is designated in the last column as follows: (I): Marker demonstrates maternal uniparental isodisomy in FD's blood. I: Marker demonstrates both maternal uniparental isodisomy in FD's blood and biparental contributions to FD's fibroblasts. Alleles are arbitrarily numbered as in Table 1.

A second possibility is that diploidization and further development of the second polar body, in parallel with the normally fertilized oocyte, occurred. However, the gynogenetic and normal cell lines should then have opposite maternal alleles for any marker distal to an odd number of chiasmata. Since FD's blood and skin have the same maternal allele at *all* markers typed, this mechanism could only apply if there had been extreme suppression of recombination in meiosis I. Such suppression of crossing over is indeed observed in some meioses in which chromosomal non-disjunction later occurs, both meiosis I and meiosis II errors[14,15]. However, there is much less data in relation to parthenogenetic ovarian teratomas. Though some of these clearly result from failure to complete meiosis II (or from second polar body reincorporation), Parrington *et al.* observed a relative lack of heterozygosity for distal loci in those teratomas which showed reduction to homozygosity at the centromere[16]. This implies either suppression of recombination in meiosis I, or, as these authors suggested, that most of these teratomas originate by doubling of a haploid chromosome set after completion of meiosis II. In our case, it has unfortunately not been possible to obtain grandparental material, examination of which could have distinguished with certainty between failure of meiosis II with no crossing over and a post-meiotic event (the model which we invoke below).

The simplest interpretation of our genetic data is that duplication of the haploid set of maternal chromosomes in the oocyte, and a third cleavage division, occurred *after* completion of meiosis II, but *before* male-female syngamy. Theoretically, this could have occurred (a) at the one-cell stage, if a cytoplasmic cleavage then separated one female pronucleus from the other two (male and female) pronuclei. Alternatively, (b) parthenogenetic activation and one or more cleavage divisions of the oocyte, followed by fertilization of only one of the resulting haploid blastomeres, may have occurred. These two mechanisms are equivalent in outcome, and cannot be distinguished by genetic analysis. In the mouse, there is experimental support for the ability of sperm to fertilize parthenogenetically activated embryos; parthenogenotes do not develop a plasma membrane block to sperm until the 8-cell stage[17]. However, there is also some evidence that nuclei of sperm which fertilize parthenogenetic embryos during or after second mitosis do not activate and form pronuclei[18]. In contrast, after insemination of a parthenogenetic embryo during its first mitotic cycle, decondensation, recondensation, and passive displacement of the male nucleus to one or other blastomere at cleavage do occur[18]. Sperm-derived nuclei activated in this way synthesize DNA and form mitotic chromosomes in the following cycle. We are therefore inclined to favour model (a) above, and suggest that in FD's mother, parthenogenetic activation of an oocyte was followed by second polar body extrusion, initiation of mitosis, fertilization during the first mitotic cycle, and production at first cleavage of one blastomere containing a female pronucleus and one containing male and female pronuclei. a female pronucleus and one containing male and female pronuclei. This model is shown in Fig. 5, though we admit that the timing of the various events depicted, including that of sperm head decondensation, is speculative. Other models, invoking for example second polar body development, also cannot be absolutely excluded, as discussed above.

In the mouse, chimaeric parthenogenetic↔normal or gynogenetic↔normal embryos can survive to term[4,5],

and parthenogenetic cells can contribute to most mature tissues, including the female germline[6,7]. However, early in development, parthenogenetic cells are eliminated from the trophoblast and then from the yolk sac mesoderm and endoderm[8-10]. Many parthenogenetic–normal chimaeras show growth retardation, and there is general selection against parthenogenetic cells in all tissues as embryogenesis progresses[7,9,11]. FD's hemifacial microsomia may therefore be the result of retarded growth of a spatially restricted parthenogenetic cell line. His behavioural problems and left-handedness might similarly relate to selective impairment of left sided cortical development; it is known in mouse chimaeras that parthenogenetic cells contribute to the brain in higher numbers than to most other tissues[6,7]. However, the exact tissue distribution of FD's parthenogenetic cells is likely to remain unknown. We are particularly surprised that the virtually 100% parthenogenetic composition of the blood leukocytes has been maintained to the age of 3.5 years. Some tissues of parthenogenetic–normal chimaeric mice, such as skeletal muscle and liver, exclude or eliminate parthenogenetic cells[6,7]. Gradual selection against parthenogenetic cells has also been invoked to explain their absence from rapidly self-renewing tissues, including blood[6]. However, parthenogenetic cells appear not to be systematically excluded from blood, since in another study, blood was one of the most frequent tissues to contain parthenogenetic cells (averaging 10% over all chimaeras examined)[7]. Nonetheless, in view of the elimination of parthenogenetic cells from yolk sac in mouse chimaeras, it worth recalling that there is ongoing debate as to whether definitive postnatal haematopoietic tissue derives from yolk sac cells which colonize liver and other definitive embryonic sites of haematopoiesis, or instead derives from a fresh wave of stem cells which differentiate *de novo* in their embryonic sites. This is actively being addressed by molecular methods[19]. If the elimination of parthenogenetic cells from yolk sac is a lineage specific effect, rather than one determined by anatomical site, then FD's 100% parthenogenetic blood may argue in favour of *de novo* origin of definitive haematopoietic tissue in man.

Most XX↔XY mouse chimaeras develop as phenotypic males[20]. Only XY cells participate in spermatogenesis in such chimaeras, but fertility is certainly possible. In male parthenogenetic↔normal chimaeras, the parthenogenetic cell line may contribute to the testis[7], though again of course only the normal cell line is represented in the gametes. We therefore predict normal offspring for FD should he prove fertile.

Methods

X- and Y- specific markers and X inactivation. *ZFY/ZFX* were simultaneously amplified using primers 5'–ATTTGTTCTAAGTC-GCCATATTCTCT–3' (common), 5'–CATCAGCTGAAGCTTGT-AGACACACT–3' (*ZFY*) and 5'–AGACACACTACTGAGCAAAAT-GTATA–3' (*ZFX*) according to standard conditions (10 mM Tris-HCl pH 8.3, 1.5 mM $MgCl_2$, 200 μM each dNTP, 0.25 μM each primer, 250 ng DNA) with 35 cycles of 94 °C for 60 s, 65 °C for 60 s and 72 °C for 90 s (10 min in last cycle). *PABY/PABX* amplification was carried out using the primers described[21]; conditions were as above except that the annealing temperature was 54 °C. *AMELY/AMELX* amplification was carried out using primers 5'–CTCTGAT-GGTTGGCCTCAAGCCTGT–3' and 5'–CACTGTCCCTCATCC-TAGAAACACA–3'; other conditions were as above. Southern analy-sis of *FMR1* was performed as described[22].

Fluorescent *in situ* hybridization. This was performed essentially as described[23], except that the biotin labelled first probe (pHY2-1) was detected using Texas red-avidin DCS (Vector Laboratories A-2016), biotinylated goat anti-avidin D (Vector Laboratories BA-0300) and a second layer of Texas red-avidin DCS. The second probe (pSV2X5) was labelled by nick translation with digoxigenin-11-dUTP and hybridized and detected simultaneously, using anti-digoxigenin monoclonal antibody (Boehringer Mannheim 1333 062) and fluorescein-conjugated horse anti-mouse IgG (Vector Laboratories FI-2000).

Microsatellite genotyping. Primer sequences for each marker were as described in the Genome Database (GDB) except for those used for typing CA dinucleotide repeats at the following loci: *KHK* (chromosome 2p23, B.E. Hayward and D.T.B., unpublished) and *HLA* (R.D. Campbell, Oxford University, personal communication). One member of each primer pair was 5'-end-labelled with fluorescein during synthesis. PCR was performed under standard conditions using 250 ng genomic DNA, and products analysed on 6% polyacrylamide, 7 M urea gels, on an A.L.F. DNA sequencer (Pharmacia LKB).

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