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Two Progenitor Cells for Human Oogonia Inferred from Pedigree Data and the X-Inactivation Imprinting Model of the Fragile-X Syndrome

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Summary

Laird has proposed that the human fragile-X syndrome is caused by abnormal chromosome imprinting. The analysis presented here supports and extends this proposal. Using published pedigrees that include DNA polymorphism (RFLP) data, we establish that the states of the fragile-X mutation termed "imprinted" and "nonimprinted" usually can be distinguished by the level of cytogenetic expression of the fragile-X chromosome. This information is then used to assess the state of the fragile-X allele in carrier progeny of individual women who inherited a nonimprinted fragile-X chromosome. From this assessment, an estimate is made of the frequency, in individual women, of primary oocytes with an imprinted fragile-X chromosome. The results of this analysis provide additional support for the specific model in which chromosome imprinting occurs in a female in, on average, half of her primary oocytes. This is the expected frequency if X-chromosome inactivation is the initial step in the imprinting of the mutant fragile-X allele. Moreover, this analysis suggests a biological explanation for peculiarities of fragile-X inheritance described by others as "clustering" and the "Sherman paradox." We interpret these peculiarities as consequences of a very small number of oogonial progenitor cells. Two progenitor cells for oogonia is the best integer estimate of the number of such cells at the time of the initial event that leads to chromosome imprinting.

Introduction

The human fragile-X syndrome (also called Martin-Bell, or marker-X, syndrome; catalog no. 30955 in McKusick 1988; also see Martin and Bell 1943; Lubs 1969) is the most common cause of inherited mental retardation. The pattern of inheritance and expression of this syndrome is unusual in that a mutant fragile-X chromosome must first be passed through a female before there is significant cytogenetic or phenotypic expression of the syndrome in her descendants (Pembrey et al. 1985; Sherman et al. 1985*a*; Steinbach 1986; Israel 1987; for reviews, see Sutherland 1985; Sutherland and Hecht 1985; Nussbaum and Ledbetter 1986). One of us has proposed that abnormal chromosome imprinting is the cause of the fragile-X syndrome (Laird 1987). Chromosome imprinting refers to a nonmutational change in a chromosome that predetermines its function or lack of function later in development or in a subsequent generation (Crouse 1960; Chandra and Brown 1975; Reik et al. 1987; Sapienza et al. 1987; Swain et al. 1987). In the case of the fragile-X syndrome, abnormal chromosome imprinting is proposed to result from the fragile-X mutation (Laird 1987).

Pedigree data are consistent with the hypothesis that imprinting occurs in a female when an X chromosome carrying the mutant fragile-X allele goes through a cycle of X-chromosome inactivation and attempted reactivation in the germ-cell lineage (Laird 1987). This inactivation is part of the normal process of dosage compensation in female mammals (Lyon 1961) and occurs in oogonial as well as in somatic cells (Gartler et al. 1975). Prior to meiosis the inactive X chromosome is reactivated (Gartler et al. 1972). Within the frame-

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work of the model, the inheritance and expression of the fragile-X syndrome are unusual because the fragile-X mutation affects a process that occurs only in females (dosage compensation by X-chromosome inactivation) and only in half of her cells (the half in which it was the fragile-X chromosome that was randomly inactivated for dosage compensation). Imprinting thus requires that the mutant fragile-X chromosome be inactivated in a female; the imprint is established only after the attempted reactivation of the fragile-X site in either oogonial cells or primary oocytes prior to meiosis. Progeny who inherit such an imprinted chromosome are at risk for phenotypic manifestations, including mental retardation, because a gene or genes at the imprinted fragile-X site remain transcriptionally inactive. Once imprinted, the fragile-X chromosome remains stably imprinted when transmitted through the mother. We refer to Laird's proposal as the *X-inactivation imprinting model.*

Although there is no direct evidence in support of the X-inactivation imprinting model as an explanation for the fragile-X syndrome, subsequent analyses are consistent with this model. These analyses include estimates of ascertainment biases that exist in fragile-X pedigree data (Sved and Laird 1988); population genetic predictions of the model (Sved and Laird 1990); and assessment of the imprint when an imprinted fragile-X chromosome is transmitted through a male parent (Laird, in press). Nevertheless, the validity of the inference to be presented here is contingent on the correctness of the most general feature of this model, namely, that the fragile-X syndrome results from abnormal chromosome imprinting in which the imprinting event occurs in the germ line of a female who inherits a nonimprinted fragile-X chromosome.

The X-inactivation imprinting model leads to an unusual prediction for a human genetic disorder: females who inherit a nonimprinted fragile-X chromosome potentially will have a mosaic germ line. On average, half of the fragile-X chromosomes in primary oocytes will be imprinted (those in cells in which the fragile-X chromosome was previously inactivated) and the other half will remain nonimprinted (those in cells in which the normal X chromosome was previously inactivated). Previously analyzed pedigree data are consistent with this prediction (Laird 1987). These data, however, were based on average values for inheritance patterns in the fragile-X syndrome, values which depend on assumptions concerning the segregation of the normal and fragile-X chromosomes during meiosis (Sherman et al. 1985*a*, 1985*b*).

More precise information can be obtained by analysis of individual families in which DNA polymorphism (RFLP) data are available. We have analyzed such pedigree data within the context of the X-inactivation imprinting model and use these data to estimate the frequency of chromosome imprinting in individual females who inherited a nonimprinted fragile-X chromosome.

The Data

We have analyzed published pedigrees in which RFLP, cytogenetic, and clinical data are reported. From these pedigrees we have attempted to determine for each individual the presence or absence of a fragile-X chromosome. With RFLP analysis, progeny can usually be classified as having inherited either the mutant fragile-X allele or the normal allele from their mothers. From reported mental status and the frequency of cytogenetic expression of the fragile-X chromosome, we assigned an "imprinted" or "nonimprinted" state to the fragile-X allele in each carrier, using criteria that will be described in the next section. (We use the term *carrier* to refer to any individual who is hemizygous or heterozygous for the mutant fragile-X allele, regardless of the allele state—imprinted or nonimprinted—or the clinical status of the individual.)

We utilized three criteria for including sibships for analysis: (1) mothers of these sibships had inherited a nonimprinted fragile-X chromosome; (2) two or more individuals in the sibships were fragile-X carriers; (3) RFLP, cytogenetic, and clinical data were available, usually including informative RFLP markers that flank the fragile-X site Xq27.3. The first criterion addresses the primary objective of this analysis: in females who inherited a nonimprinted fragile-X chromosome, what is the frequency of oocytes with an imprinted fragile-X chromosome? The second criterion excludes sibships with only one fragile-X carrier, who will usually have an imprinted fragile-X chromosome because ascertainment is usually for affected individuals. The third criterion—of RFLP, clinical, and cytogenetic data—aids in the correct assignment of alleles—normal or fragile—and, if fragile, the state of imprinted or nonimprinted. Flanking RFLP markers usually permit detection of chromosomes that are recombinant near this site. Recombination at or near the fragile-X site makes uncertain the assignment of the nonimprinted state, since recombination could have exchanged the mutant but nonimprinted fragile-X allele for the normal allele. (The imprinted state can be detected even in a recombinant chromosome by the high level of cytogenetic ex-

pression of the imprinted fragile-X allele, as will be discussed in the next section.)

These criteria are met in published data on 39 mothers and their progeny, represented in 18 families that were included in nine publications (table 1). The data lead to an assessment of the frequency with which a fragile-X allele has been transmitted in an imprinted state to progeny of women who inherited a nonimprinted fragile-X chromosome (table 1, last col.).

An example of the analysis that led to entries in table 1 is illustrated in figure 1. Mother II-1 from family 52 of Brown et al. (1987) is represented, along with her parents and four of her 10 progeny. RFLP markers flanking the fragile-X locus are indicated for each chromosome, as are the fragile-X allele and, if mutant, its imprinted or nonimprinted state. Percentage cytogenetic expression of the fragile-X site in lymphocytes is also indicated if data are available in published reports. We conclude that, of the four progeny represented in generation III of figure 1, two are fragile-X carriers (one with an imprinted and one with a nonimprinted fragile-X) and one has the normal allele at the fragile-X locus. It is uncertain whether III-6 has the normal allele or the nonimprinted mutant allele of the fragile-X locus because there was a recombination event at or near the locus. Her fragile-X allele is therefore designated "Q" (questionable). Thus, for mother II-1, the frequency of imprinting among her fragile-X carrier progeny is .5, when the one questionable case is excluded.

The designation of the fragile-X chromosome of III-12 (fig. 1) as imprinted can be made on the basis of the associated mental retardation. We now discuss the basis of assigning an imprinted or nonimprinted state to the fragile-X allele of a mentally normal carrier such as the carrier females represented in figure 1.

Distinguishing Imprinted from Nonimprinted States of the Fragile-X Chromosome by the Level of Cytogenetic Expression

The original application of the concept of chromosome imprinting to the fragile-X syndrome was based on the phenotype of mental retardation: if a carrier male or female was assessed clinically as being mentally impaired, the state of the fragile-X mutant allele was defined as being imprinted (Laird 1987). It was also suggested that the imprinted state of the fragile-X allele could be identified by a high frequency of cytogenetic expression of the fragile site at Xq27 (usually assessed in lymphocytes) and that the nonimprinted state could be identified by a considerably lower (often zero) frequency of expression. A specific model was proposed to explain why cytogenetic expression would increase on chromosome imprinting (Laird et al. 1987).

It is implicit in the published data on nonpenetrant, or transmitting, males and their affected grandsons that nonimprinted fragile-X alleles exhibit lower levels of cytogenetic expression than do imprinted alleles: transmitting males are carriers of a mutant fragile-X allele, they generally express cytogenetically the fragile-X site at a low level, if at all, and they are almost always classified as mentally normal. (Transmitting males, as the name implies, transmit the fragile-X chromosome to their progeny; affected males seldom reproduce.) In contrast, the mentally impaired grandsons of transmitting males express cytogenetically the fragile-X site at high levels. More generally, fragile-X carriers who are classified as mentally retarded usually have moderate to high levels of cytogenetic expression of the fragile-X site; mentally normal carrier males (transmitting males) show low or no cytogenetic expression. Mentally normal carrier females can have either high or low cytogenetic expression (Sherman et al. 1985*a*, 1985*b*), as would be expected if mentally normal female carriers can have either imprinted or nonimprinted states of the fragile-X chromosome (Laird 1987).

If this apparent correlation between the imprinted state and high levels of cytogenetic expression can be verified by quantification, then the level of cytogenetic expression of the fragile-X site in somatic cells of an individual could be used for research purposes to assess the state of his or her fragile-X chromosome independently of clinical assessment of mental function. We now show for the data in table 1 that the levels of cytogenetic expression for imprinted and nonimprinted fragile-X chromosomes, defined by mental function and inheritance pattern, have very different distributions.

Transmitting males, as described above, exhibit low or no cytogenetic expression of the fragile-X chromosome and are classified as mentally normal. Daughters of transmitting males also have low or no cytogenetic expression of the fragile-X chromosome and are classified as mentally normal. On this basis it was concluded that males do not imprint a mutant fragile-X allele (Laird 1987). This conclusion is accommodated by the model of chromosome imprinting, which depends on X-chromosome inactivation for dosage compensation in a female: males do not inactivate an X chromosome for dosage compensation and thus cannot imprint a mutant allele. A nonimprinted fragile-X allele in a male would therefore not act as a block to reactivation of a gene or genes at the fragile-X site, since stable inacti-

vation had not yet occurred at this site. Thus, all daughters of transmitting males should have, in their somatic cells, nonimprinted fragile-X's and should express cytogenetically the fragile site at low (or zero) frequencies. This expectation is generally met for the data set in table 1, which includes data for 16 mothers who are interpreted as being daughters of transmitting males. The average level of cytogenetic expression of their fragile-X chromosomes is 1.9%, with a range from 0% to 9% (14 of 16 express at a level of 6% or less; fig. 2*a*). In contrast, mentally impaired grandprogeny of these transmitting males exhibit levels of cytogenetic expression ranging from 7% to 68%, with a mean of 27.1% (28 of 29 express at levels of 10% or greater; fig. 2*b*). The ratio of average cytogenetic expression in affected grandprogeny compared with daughters of transmitting males is thus ~14:1. The levels of cytogenetic expression form two distributions, with minimal overlap, for individuals whose fragile-X chromosomes are classified, by criteria of chromosome origin or mental retardation, as either imprinted or nonimprinted. For this data set, an expression level of 6% or less is indicative of a nonimprinted fragile-X chromosome; an expression level of 10% or more is indicative of an imprinted fragile-X chromosome. No assignment of chromosome state solely on the basis of cytogenetic data can be made for expression levels between 6% and 10%.

From this result, we now use the level of cytogenetic expression to assess the state of the fragile-X allele in mentally normal carrier grandprogeny of these transmitting males. Two normal carrier grandsons are reported; these males have levels of cytogenetic expression of 0% and 1%; they are thus considered to have nonimprinted fragile-X chromosomes, both by the criterion of normal mental function and by the criterion of low cytogenetic expression (fig. 2*c*, triangles). Eight mentally normal carrier granddaughters are reported; three have levels of cytogenetic expression of 4% or less and are therefore considered to have their fragile-X alleles in the nonimprinted state; five have levels of cytogenetic expression of 15% or greater and are therefore considered to have their fragile-X alleles in the imprinted state (fig. 2*c*).

More generally, the levels of cytogenetic expression for carrier progeny of all mothers considered to have inherited nonimprinted fragile-X chromosomes fall primarily into two groups (fig. 2*d*). Considering first the carrier sons of these mothers, we find that five have cytogenetic expression of 2% or less, with a mean of 0.4%; all these males were classified as mentally normal and hence have nonimprinted fragile-X chromosomes by both cytogenetic and clinical criteria. Thirty-nine sons have cytogenetic expression ranging from 10% to 68%, with a mean of 29.1%; all were classified as mentally retarded and thus have imprinted fragile-X chromosomes by both criteria. Two sons, to be discussed below, have cytogenetic expression between 6% and 10% and could therefore not be assessed as imprinted or nonimprinted solely by cytogenetic data.

Of carrier daughters of these mothers, 18 exhibit cytogenetic expression of 6% or less, with a mean of 1%; all were classified as mentally normal. These daughters are considered to have nonimprinted fragile-X chromosomes by the criterion of low cytogenetic expression. Twenty carrier daughters have levels of cytogenetic expression ranging between 10% and 40%, with a mean of 20.3%; these daughters are considered to have imprinted fragile-X chromosomes by the criterion of high cytogenetic expression. Of these 20 daughters with imprinted fragile-X chromosomes, 12 were classified as mentally normal (although it was noted for five of them that they had learning disabilities), and eight were classified as mentally affected. Thus, in this data set, 47 carrier progeny have imprinted fragile X chromosomes by the criteria of cytogenetics and clinical status; for the 12 carrier progeny who were classified as mentally normal, the level of cytogenetic expression provides an assessment of the state of the fragile-X chromosome that is independent of information on mental function.

As mentioned above, the level of cytogenetic expression of the fragile-X chromosome is not always a reliable indicator of the state of the mutant fragile-X allele. For 115 of the 119 individuals represented in table 1 for whom detailed cytogenetic data are available, a clear assignment of the state of the fragile-X allele could be made solely on the basis of the level of cytogenetic expression. This assignment is corroborated for males and for some females by clinical status of mental normalcy or impairment (fig. 2*d*). There are, however, four data points in figure 2*d* that represent individuals for whom the interpretation of the level of cytogenetic expression is less clear. Two females (IV-46 and IV-49 of Veneema et al. 1987*a*, 1987*b*) express at the level of 9%; yet these females are daughters of a transmitting male and would therefore have nonimprinted fragile-X alleles (Laird 1987). This is an unusually high level of cytogenetic expression for nonimprinted fragile-X alleles (figs. 2*a* and 2*d*). A third unusual datum is for a male classified as retarded (III-4 in family Fx-73 of Thibodeau et al. 1988). His reported level of cytogenetic expression (7%) is below the level of 10% that we

Table 1

Assessment of Imprinting in Carrier Progeny of Women Who Inherited a Nonimprinted Fragile-X Chromosome

Reference	Family	Mother	Maternal X Chromosome in Progeny				f*/(f+f*)	Apparent Imprinting Frequency
				Fragile X				
			+	f*	f	Q		
Arveiler et al. 1988 ...	A	I-2 (U,1) son	3 (II-1m,-4m, -7m)	5 (II-2mr,-3mr, -5fr,-6mr, -8mr)	0	0	5/5	1.0
Data are complete except that ranges rather than specific levels of cytogenetic expression were reported for progeny. No ambiguity is apparent, however, in the assignment of chromosome states. These progeny—and similar ones elsewhere in this table for whom specific levels of cytogenetic expression are not given—are not represented in fig. 2 but are included in families represented in fig. 3a. Additional data on this family A are in Oberlé et al. (1987).								
Brown et al. 1986	F39	I-2 (U,0) son	0	2 (II-1mr,-2mr)	0	0	2/2	1.0
Data are complete except for the reporting of specific levels of cytogenetic expression in progeny. No ambiguity is apparent in the assignment of chromosome states.								
	F50	I-2 (U,0) son	2 (II-2m,-5m)	2 (II-1mr,-4mr)	0	0	2/2	1.0
Data are complete except for the reporting of specific levels of cytogenetic expression in progeny. No ambiguity is apparent in the assignment of chromosome states.								
Brown et al. 1987	20	III-2 (P,0) son	1 (IV-2f0)	3 (IV-1mr, -3mr46, -5mr56)	0	1 (IV-4m0 rec+)	3/3	1.0
Data are complete except for the levels of cytogenetic expression in IV-1 and IV-4. Levels of cytogenetic expression for these individuals were not reported by Brown et al. (1985) but were indicated as being "positive" and "negative," respectively, by Brown et al. (1987).								
		III-3 (P,0) son	0	4 (IV-6fr14, -7mr68, -9mr10, -10mr42)	0	2 (IV-8m0,NR, -11f0,NR)	4/4	1.0
Data are complete except for "questionables"; RFLP data were not reported (NR) for these individuals.								
		III-6 (P,0) son	1 (IV-18f0)	2 (IV-22fd28, -23mr30)	0	6 (IV-19f,NR, -20f0,rec-f, -21f0,rec+, -24m0,rec-f, -25f0,NR, -26f,NR)	2/2	1.0
Data are reasonably complete. The large number of "questionables," however, makes this sibship less informative for the apparent imprinting frequency than it otherwise would be. Retesting of DNA samples for this family with more closely linked RFLP markers would be especially useful in determining which allele is present at the fragile-X site in each of the six progeny listed as questionable.								
		III-10 (P,3) son	0	2 (IV-32mr24, -33mr18)	0	0	2/2	1.0
Data are complete.								
	22	II-1 (U) NI-dau	0	1 (III-3fr)	2 (III-1m0,-5f)	1 (III—4m)NR	1/3	.33
Mother II-1 was reported as deceased; no cytogenetic data were reported. She does not represent a new mutation because her sister, II-3 (see below), also transmitted a fragile-X mutation. We conclude that II-1 inherited a nonimprinted fragile-X chromosome because she passed on a nonimprinted fragile X chromosome to a son (III-1) and to a daughter (III-5). (Once imprinted, a fragile-X chromosome remains imprinted when passed through a female; Laird 1987.) This daughter must have inherited a nonimprinted fragile-X chromosome because she has transmitted a nonimprinted fragile-X chromosome to her daughter, IV-5. Another daughter, III-3, is assumed to have inherited an imprinted fragile-X chromosome because of her reported mental retardation.									

(*continued*)

Table 1 (continued)

Reference	Family	Mother	Maternal X Chromosome in Progeny: +	Fragile X: f*	Fragile X: f	Q	f*/(f+f*)	Apparent Imprinting Frequency
		II-3 (U) NI-dau	0	0	2 (III-9f0, -10m0)	0	0/2	.0
Mother II-3 is assumed to have inherited a nonimprinted fragile-X chromosome because she passed on a nonimprinted fragile-X chromosome to one daughter and one son. See above comments for II-1, who is a sister of II-3, for further explanation.								
		III-9 (M,1) son	0	1 (IV-7mr32)	1 (IV-9fn4)	0	1/2	.5
Data are complete.								
		IV-2 (P,0) neph	0	2 (V-2fn30, -3fn22)	0	1 (V-1f0, possible rec+)	2/2	1.0
Data are complete except for questionable case V-1, who is a possible recombinant. (The distal RFLP marker was not informative.)								
		IV-3 (P,0) son	1 (V-5m0)	2 (V-4mr12, -6mr16)	0	0	2/2	1.0
Data are complete.								
		IV-4 (P,0) son	0	2 (V-7mr14, -8mr26)	0	0	2/2	1.0
Data are complete.								
	52	I-5 (U,0) NI-dau	4 (II-2m0, -4f0,-5m0, -6m0)	0	3 (II-1f0,-3f0, -7m1)	0	0/3	.0
Data are essentially complete even though I-5 was noninformative for distal marker ST14. If the four individuals classified as having the "normal" X chromosome are subsequently shown by the use of an additional RFLP marker to have the mutant fragile-X allele, the effect would be to increase the number of individuals classified as having "nonimprinted" fragile-X chromosomes. The imprinting frequency would thus remain .0 and be increasingly significant. Cytogenetic data not available from Brown et al. (1987) for family 52 were provided by W.T. Brown and E.C. Jenkins (personal communication).								
		II-1 (M,0) NI-dau, son	7 (III-3m0,-5f0, -8m0,-10f0, -13f0,-14f0, -15f0)	1 (III-12mr44)	1 (III-1f0)	1 (III-6f0 rec-f)	1/2	.5
Data are complete.								
		II-3 (M,0) NI-dau	1 (III-37f0)	0	2 (III-35f0, -39f0)	3 (III-31m,NR, -33f,NR, -41f,NR)	0/2	.0
Data are complete except for the possible further testing of progeny classified as "questionable." They cannot yet be classified as having recombinant fragile-X chromosomes, however, and so do not bias the imprinting frequency by their exclusion. (Recombinant fragile-X chromosomes can be said to have the imprinted allele of the fragile-X site if a high level of cytogenetic expression is present; see text. A nonimprinted mutant allele at the fragile-X site cannot, however, be distinguished from the normal allele without the use of genetic data—does an imprinted allele occur in progeny?—or by RFLP data for flanking markers. Thus, the imprinted state of the fragile-X chromosome can be assigned more readily than the nonimprinted state, making recombinant individuals potentially biased toward the former category.)								

(*continued*)

Table 1 (continued)

Reference	Family	Mother	Maternal X Chromosome in Progeny: +	Fragile X: f*	Fragile X: f	Q	f*/(f+f*)	Apparent Imprinting Frequency
		III-39 (M,0) son	1 (IV-30m)	1 (IV-29mr)	1 (IV-31m)	0	1/2	.5
Data are essentially complete. The absence of complete cytogenetic data for fragile-X carrier males is not critical because their clinical classifications permit assignment of fragile-X states.								
Buchanan et al. 1987	XMD101	I-2 (U,0) neph	1 (II-2f0)	2 (II-3fn14, -4fn18)	0	0	2/2	1.0
Data are complete.								
	XMD8	I-1 (U,2) son	1 (II-4m0)	4 (II-1mr28, -2mr52, -3mr24, -6mr44)	0	1 (II-5m,NR)	4/4	1.0
Data are complete except for II-5.								
Camerino et al. 1983	A	II-3 (P,2) son	0	3 (III-3mr36, -4mr56, -5fd34)	0	1 (stillbirth)	3/3	1.0
Data are complete.								
		II-6 (P,0) son	0	2 (III-6fn16, -7mr12)	0	3 (stillbirths)	2/2	1.0
Data are complete.								
		II-8 (P,5) neph	0	1 (III-9fn40)	1 (III-10m6)	2 (stillbirth, III-8m0 rec+)	1/2	.5
Data are complete, although data for III-10 are puzzling. His level of cytogenetic expression (6%) is similar to his mother's 5% and contrasts with his sister's 40%. All other males in this family who have 12% or higher expression of the fragile-X chromosome were classified as mentally retarded rather than as dull or subnormal. The fragile-X chromosome of III-10 has therefore been assigned a nonimprinted state. This male has, however, been classified as mentally "subnormal" (Camerino et al. 1983, table 1) rather than as the mentally "normal" expected for a male with a nonimprinted fragile-X chromosome. He may therefore be mentally subnormal for reasons other than the fragile-X chromosome. This male is represented by the half-filled triangle in fig. 2d. Other data on this family are given by Davies et al. (1985) and Oberlé et al. (1986, 1987).								
Goonewardena et al. 1986	3	II-7 (M,1) son	0	3 (III-12mr12, -13mr30, -14mr18)	0	0	3/3	1.0
Data are complete.								
	2	II-2 (U,0) son	0	2 (III-1mr24, -2mr15)	0	0	2/2	1.0
Data are complete. There is, however, an inconsistency in the reporting of the level of cytogenetic expression of the mother (II-2). She is listed in table 1 of Goonewardena et al. (1986) as having 0/60 cells with a fragile-X chromosome, but in fig. 2 of this reference she is indicated as being positive for the fragile-X chromosome. K. H. Gustavson (personal communication) has indicated that 0% expression is correct, and we therefore have assigned the "nonimprinted" fragile-X state to II-2.								

(*continued*)

Table 1 (continued)

Reference	Family	Mother	Maternal X Chromosome in Progeny: +	Fragile X: f*	Fragile X: f	Q	f*/(f+f*)	Apparent Imprinting Frequency
Oberlé et al. 1986	2	II-2 (U,0) son	0	2 (III-1fn,-2mr)	0	0	2/2	1.0
Detailed cytogenetic data are not reported. III-1 is considered to have an imprinted fragile-X chromosome on the basis of being fragile-X positive (>2%). More detailed cytogenetic data would be useful, as they would increase the confidence with which the state of the fragile-X allele can be assigned.								
	14	II-2 (P,0) son	0	2 (III-1fd, -3mr)	0	1 (III-2f0, possibly rec+)	2/2	1.0
Data are complete, although more precise cytogenetic data would complement the clinical data for III-1 and III-3.								
Thibodeau et al. 1988	Fx-18	I-2 (U,1) grand	0	0	3 (II-2nf3, -4nf0, -5nf0)	1 (II-6fr)	0/3	.0
Data are complete except for II-6, who is a mentally retarded female for whom RFLP data are inconsistent with given parentage (S. N. Thibodeau, personal communication). Because of this uncertainty, and because no cytogenetic data were given for expression of the fragile-X chromosome, she was not included in the analysis described in the Appendix; it is likely that her mental retardation is not caused by an imprinted fragile-X chromosome. It is more likely that II-5 is nonrecombinant than recombinant at the fragile-X site. (Compare II-2, -4, and -5.) Hence she is considered to have a nonimprinted fragile-X chromosome.								
		II-2 (M,3) prog	0	3 (III-1fn17, -2mr24, -3mr26)	0	1 (III-4 stillbirth)	3/3	1.0
Data are complete except for the stillborn (III-4). III-1 was indicated as having IQ within normal range but with a learning disability.								
		II-4 (M,0) prog	1 (III-10f0)	3 (III-7fn10, -8fn14, -9mr25)	0	3 (III-5s,-6s, -11rec+)	3/3	1.0
Data are complete except for the questionable category. III-7 and III-8 were indicated as having IQs in the normal range but with learning disabilities.								
	Fx-28	I-2 (U,0) grand	0	0	4 (II-2nf1, -4fn1, -6nf1, -8fn0)	0	0/4	.0
Data are complete.								
		II-2 (M,1) prog	0	2 (III-2mr29, -3fn12)	0	1 (III-1f0, possibly rec+)	2/2	1.0
Data are complete except for III-1, for whom the distal RFLP marker is noninformative. III-3 was indicated as having an IQ in the normal range but with a learning disability.								
		II-6 (M,1) prog	0	2 (III-6mr18, -7mr43)	0	1 (III-8 stillbirth)	2/2	1.0
Data are complete except for the stillborn.								
	Fx-63	I-2 (U,0) prog	0	1 (II-1mr19)	1 (II-2nf0)	1 (II-3 stillbirth)	1/2	.5
Data are complete except for the stillborn. I-2 is assumed to have inherited a mutant (nonimprinted) fragile-X chromosome rather than as having had a premeiotic mutation in her germ line.								

(*continued*)

Table 1 (continued)

Reference	Family	Mother	Maternal X Chromosome in Progeny: +	Fragile X: f*	Fragile X: f	Q	f*/(f+f*)	Apparent Imprinting Frequency
	Fx-73	II-2 (P,0) prog	0	2 (III-1fn22, -4mr7)	1 (III-3fn0)	0	2/3	.67
Data are complete. Even though III-3 has a recombinant X chromosome, she is interpreted as having a nonimprinted fragile-X chromosome because of the lack of cytogenetic expression of the fragile site and because she has two progeny with imprinted fragile-X chromosomes (see the following entry). III-1 was indicated as having an IQ in the normal range but with a learning disability.								
		III-3 (M,0) son	0	2 (IV-1fn13, -2mr26)	0	0	2/2	1.0
Data are complete.								
Veenema et al. 1987*a*, 1987*b*		IV-46 (P,9) son	0	4 (V-56fr20, -57mr16, -58mr26, -59fr21)	3 (V-53m0, -54f4, -55f4)	0	4/7	.57
Data are complete, although cytogenetic expression in the mother (IV-46) is unusually high for a nonimprinted fragile-X chromosome. She is classified as nonimprinted because she inherited her fragile-X chromosome from her father, who appears to have been a nonpenetrant (transmitting) male.								
		IV-48 (P,2) dau	1 (V-60f0)	2 (V-61fr14, -62fr24)	0	0	2/2	1.0
Data are complete.								
		IV-49 (P,9) son	1 (V-64m0)	2 (V-63mr42, -65fr22)	0	0	2/2	1.0
Data are complete, although the cytogenetic expression in the mother (IV-49) is unusually high for an individual with a nonimprinted fragile-X chromosome (see the above comment for her sister, IV-46).								
		IV-50 (P,0) son	0	2 (V-66mr18, -67mr34)	1 (V-68m1)	0	2/3	.67
Data are complete, although more RFLP markers for V-68 would help confirm (or not confirm) that he received the fragile-X allele instead of the normal allele. The reported RFLP data do not exclude a recombination event proximal to the fragile-X site, although his 1% cytogenetic expression of the fragile-X chromosome is more consistent with a nonimprinted fragile-X allele than with a normal allele. RFLP data for V-66 were not reported, although his level of cytogenetic expression and mental impairment are consistent only with his having an imprinted fragile-X chromosome.								

NOTE.—Individuals indicated in the "Mother" column represent females who inherited a nonimprinted fragile-X chromosome, as assessed by at least one of the following: pedigree data (inheritance of a fragile-X chromosome from a nonpenetrant father), RFLP data, and low cytogenetic expression of the fragile-X chromosome in their lymphocytes, compared with the level of cytogenetic expression in their affected progeny. All such females were classified as mentally normal; those included here have two or more fragile-X carrier progeny. The progeny of each mother are classified with respect to the X chromosome inherited from the mother: normal X(+), fragile-X chromosome imprinted (f*) or nonimprinted (f), and questionable (Q) with respect to whether the allele at fragile site Xq27.3 was derived from the normal chromosome or the mutant chromosome (recombination at or near this region is sometimes represented in these pedigrees; such recombination can make questionable which fragile-X allele is present). The ratio of imprinted to the sum of imprinted plus nonimprinted carrier progeny is indicated [f*/(f+f*)], as is the apparent frequency of imprinting based on this ratio (last col.). The parental source of the mother's fragile-X chromosome is indicated as P (paternal), M (maternal), or U (unknown), followed by the percentage cytogenetic expression of her fragile-X chromosome, if reported, and by her closest affected relative (son; *dau*ghter; *prog*eny; *grand*progeny; *neph*ew; or nonimprinted [NI] *dau*ghter). Progeny are identified by their generation and number, followed by their gender (m or f). The clinical status of progeny with imprinted fragile-X chromosomes is indicated by n (normal), r (retarded), or d (dull). All other progeny were reported as "normal" or were unclassified, unless otherwise noted. Percentage fragile-X expression, where available, is indicated by the number following the designation of clinical status.

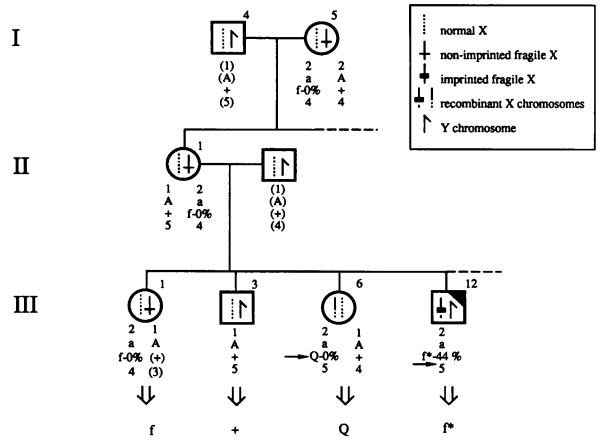

Figure 1 Tracking the fragile-X chromosome with RFLP, clinical, and cytogenetic data. A portion of the pedigree from family 52 of Brown et al. (1987) is illustrated, with interpretations of the state of the fragile-X chromosome. For each individual, RFLP and cytogenetic data are summarized. The shaded area for male III-12 indicates his classification as mentally retarded. RFLP alleles and their respective probes are as follows: 1/2, 52A; a/A, F9; +/f/f*/Q, normal, nonimprinted fragile, imprinted fragile (numbers following these allele designations indicated percentage fragile-X expression), and questionable; and 4/5, ST14. The arrow at RFLP designations indicates sites of recombination between the normal and fragile-X chromosomes.

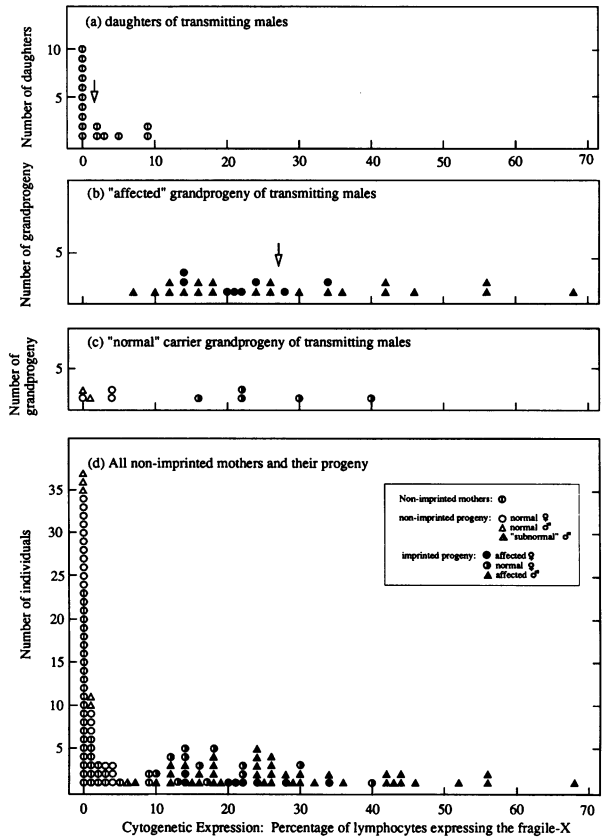

Figure 2 Assessing the state of the fragile-X chromosome with cytogenetic data. Cytogenetic data (percentage of lymphocytes expressing the fragile-X site) for fragile-X carriers from table 1 are grouped according to the origin of their fragile-X chromosome. The number of individuals with various percentages of fragile-X expression are indicated for the following categories: (a), daughters of transmitting males; (b), "affected" grandprogeny of transmitting males (including granddaughters classified as "dull" or "subnormal"); (c), "normal" carrier grandprogeny of transmitting males; and (d), all nonimprinted mothers and their carrier progeny. A key to the symbols is given in (d). Only those individuals for whom precise cytogenetic values are given in table 1 are represented. Arrows in (a) and (b) represent the mean percentage values of cytogenetic expression. The data indicate that for this set of fragile-X carriers the state of the fragile-X chromosome may be assessed in most individuals by the level of cytogenetic expression in lymphocytes.

selected as the cutoff point (see above), but his level of expression is above the 0% expression reported for his mother and normal sibling; we classify him as having an imprinted fragile-X allele. A less clear case is that of male III-10 in family A of Camerino et al. (1983), who expresses the fragile-X site cytogenetically at a level of 6% and who was described as mentally "subnormal." We interpret the state of his fragile-X allele as nonimprinted, for reasons discussed in table 1. The distribution of levels of cytogenetic expression for the data set in table 1 and figure 2 thus appears to provide a good (115/119) but not perfect method to assess the state of a mutant fragile-X allele.

We now use the combination of clinical and cytogenetic methods to estimate the frequency of chromosome imprinting in primary oocytes of individual women, on the basis of the distribution of chromosome states in their carrier children.

Apparent Distribution of Imprinted Fragile-X Chromosomes in Primary Oocytes of Women Who Inherited a Nonimprinted Fragile-X Chromosome

Of the 39 mothers represented in data in table 1, 25 have carrier progeny all of whom have an imprinted fragile-X chromosome (apparent frequency of imprinting = 1.0; fig. 3*a*). Nine mothers have both imprinted and nonimprinted fragile-X progeny, and five mothers have only nonimprinted carrier progeny (apparent imprinting frequency = .0). Thus, 64% of these sibships come from mothers who may have imprinted the fragile-X chromosome in all of their oocytes; 23% appear to have oogonial populations that have imprinted the fragile-X chromosome in only about half of the oocytes; and 13% may have imprinted the fragile-X chromo-

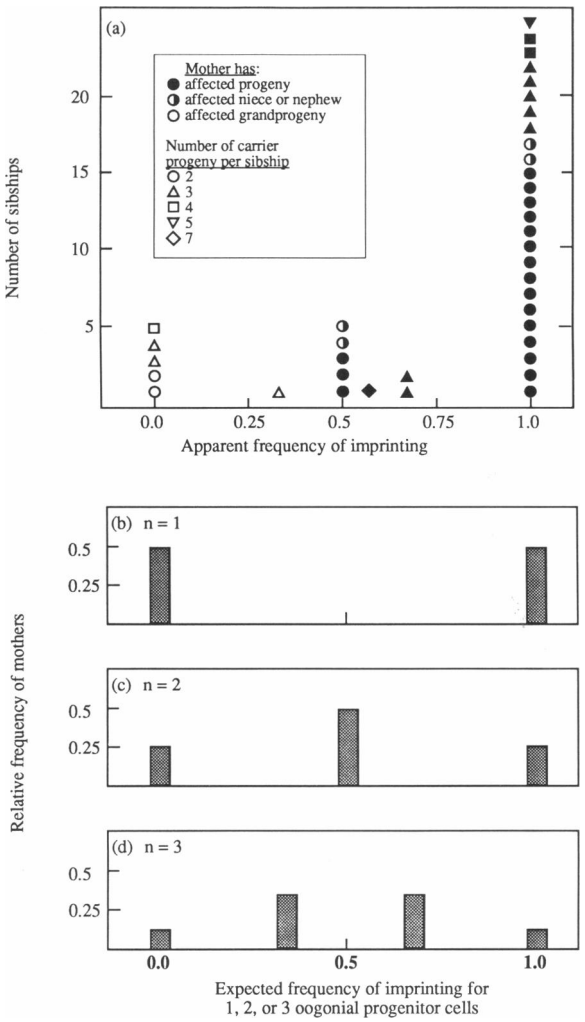

Figure 3 Apparent and expected frequencies of chromosome imprinting in women who inherited a nonimprinted fragile-X chromosome. (a), From published pedigrees indicated in table 1, sibships containing two or more carrier progeny from women who inherited a nonimprinted fragile-X chromosome were analyzed. RFLP data were used to determine whether an individual had inherited the fragile-X chromosome; the level of cytogenetic expression and, in most cases, the classifications of mentally normal or affected were used to assess whether the fragile-X chromosome of that individual was imprinted or nonimprinted. In general, only families that were informative for RFLP markers flanking the fragile-X site are included. A few families with only proximal or only distal RFLP markers were included if no ambiguity existed concerning the nature—normal or fragile—of the X chromosome inherited from the mother. (b)–(d), Expected distribution of imprinting in women who inherited a nonimprinted fragile-X chromosome if there were one (b), two (c), or three (d) progenitor cells for human oogonia. The distributions in (b)–(d) are based on sampling from a large, binomially distributed population with $p = q = .5$. In practice, this sampling of progenitor cells probably occurs from a binomially distributed population initiated from a small number of embryoblasts such that p and q will sometimes vary from .5 (see appendix to Nesbitt 1971). For samplings of a small number of cells, such as the 1, 2, and 3 portrayed in (b)–(d), respectively, the correction for the initial small population size is minor, and it therefore is not included here.

some in none of their oocytes. The distribution of chromosome imprinting appears to be so extreme that some females, rather than having a mosaic population, may have the same state for the mutant fragile-X allele in all of their primary oocytes. We show here that this result is consistent with a very small number of progenitor cells giving rise to human oogonia.

How Many Progenitor Cells for Human Oogonia Would Give the Observed Pattern of Chromosome Imprinting?

As discussed in the Introduction, the imprinting event is assumed to occur with a probability of .5 either within or prior to the establishment of each oogonial progenitor cell. For simplicity, the biological basis of the imprinting event will be assumed to be that previously proposed, i.e., inactivation of the fragile-X chromosome as part of the normal process of dosage compensation in females (Laird 1987). Any basis of stable imprinting that occurred with a probability of .5 in each oogonial progenitor cell would, however, lead to the same quantitative predictions for the distribution of chromosome states—imprinted or nonimprinted—in carrier progeny. It is sufficient to consider models of one, two, or three progenitor cells for human oogonia.

1. *A Single Progenitor Cell*

If there were a single progenitor cell present after the event that leads to chromosome imprinting, then half of the females who inherited a nonimprinted fragile-X chromosome would have a progenitor cell for oogonia in which the fragile-X chromosome remained active; all oocytes of these females would retain the fragile-X chromosome in its nonimprinted state. All carrier progeny from such mothers would consequently have a nonimprinted fragile-X chromosome; all carrier sons and all carrier daughters would be mentally normal and either cytogenetically low expressers or nonexpressers (fig. 3*b*, col. 0.0).

The other half of the females who inherited a nonimprinted fragile-X chromosome would have an oogonial progenitor cell in which the fragile-X chromosome became inactivated; all primary oocytes of these females would have an imprinted fragile-X chromosome (fig. 3*b*, col. 1.0). Hence, all carrier progeny from these mothers would have imprinted fragile-X chromosomes.

Note that the existence of a single progenitor cell after the time of X-chromosome inactivation would preclude any female from having both classes of primary oocytes in her gonad, i.e., some oocytes with imprinted and other oocytes with nonimprinted fragile-X chromosomes (fig. 3*b*). Hence, no mother should have both transmitting and affected sons—or, more generally, both nonimprinted and imprinted progeny. Since published pedigree analyses (Sherman et al. 1985*a*, 1985*b*) and the analysis presented here (fig. 3*a*) indicate that some mothers do in fact have both classes of carrier progeny, we can exclude the possibility that there is always only one oogonial progenitor cell existing after the decision point for X-chromosome inactivation.

2. *Two Progenitor Cells*

If there were two progenitor cells, then oogonia of each female would represent a sampling of two progenitor cells in one of three ways (fig. 3*c*): (1) both cells could have active fragile-X chromosomes, in which case no oocytes would have an imprinted fragile-X chromosome (frequency of imprinting = .0); (2) one cell could have an active fragile-X chromosome and the other cell could have an inactive fragile-X chromosome (if it were assumed that there are no selective cell proliferations for these two classes—to be discussed below—then half of her primary oocytes would have an imprinted fragile-X chromosome and half would have a nonimprinted fragile-X chromosome, for an imprinting frequency of .5); or (3) both cells could have inactive fragile-X chromosomes, a situation that would lead to an imprinting frequency of 1.0 in primary oocytes. The expected distribution of these three classes of females is 1:2:1 (fig. 3*c*).

What kinds of progeny should each class of females have? If we consider sibships of four progeny, two progeny on average will be carriers for the fragile-X chromosome. Only females who imprint at a frequency of .5—and are thus 50:50 gonial mosaics—are potentially able to have both classes of carrier progeny. Because oocytes themselves represent a sampling from a binomially distributed population, only half of the sibships containing two fragile-X carriers from 50:50 gonial mosaic mothers will in fact have one imprinted carrier and one nonimprinted carrier; one quarter of such sibships will have two imprinted carrier progeny; and one quarter will have two nonimprinted carriers (fig. 4*f*). Thus, for sibships that include two carrier progeny, and with the assumption of two progenitor cells after the decision point for X-chromosome inactivation, there should be an observed ratio of 3:2:3 for sibships with, respectively, two nonimprinted carrier progeny, one carrier progeny of each class, and two imprinted carrier progeny (fig. 4*g*).

3. *Three Progenitor Cells*

Three progenitor cells would give four classes of females, with imprinting frequencies of .0, .33, .67, and 1.0, by an argument similar to that in the preceding subsection (fig. 3*d*). Note that with three progenitor cells assumed to have equal contribution to oogonia, no females would be expected to have oogonia with imprinting frequencies of .5. (Apparent imprinting frequencies of .5 would, however, be expected with small sibships, such as those that include two carriers.) In addition, the expected frequency of women with the extreme values of imprinting—.0 and 1.0—would be reduced relative to the intermediate values, in comparison with expectations for a model for two progenitor cells (fig. 3*c*).

Distinguishing among Models of One, Two, and Three Progenitor Cells

1. *Sibships Analyzed by RFLP Identification*

The above analysis for the expected distribution of sibships, given one, two, or three progenitor cells for the oogonia, can be compared with the observed data in figure 3*a*. The data fit qualitatively the model of a small number of progenitor cells in that females with apparently extreme imprinting values of .0 or 1.0, as well as females with intermediate values, are observed. As mentioned in the preceding section, the data do not support the one-progenitor-cell model in its most general form: there are mothers who have both imprinted and nonimprinted progeny, a result ruling out the one-progenitor-cell model for these mothers.

Comparison of the data (fig. 3*a*) with expectations of the two-cell model (fig. 3*c*) indicates a marked excess of mothers whose apparent frequency of imprinting is 1.0. This excess would be expected to derive from biases of ascertainment: most previously analyzed families had at least one affected individual. Females with gonial imprinting of .0 are expected never to have affected progeny; such females are thus not ascertained directly. (See Sved and Laird 1988 for a more complete discussion of ascertainment biases in the fragile-X data.) It will be important to have a complete analysis of large fragile-X families, including especially the progeny of females who inherited a nonimprinted fragile-X chromosome and whose progeny are all classified as clinically normal: which of these progeny are fragile-X car-

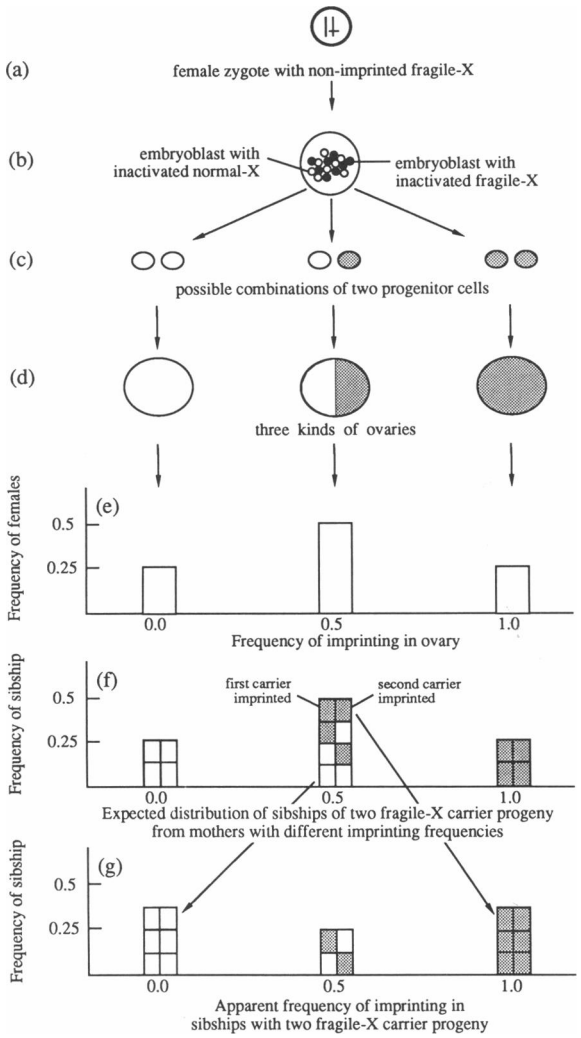

Figure 4 From the zygote of women who inherit a nonimprinted fragile-X chromosome to their progeny. The expected consequences of two progenitor cells for human oogonia are shown for the model of chromosome imprinting in which the probability of chromosome imprinting in each embryonic cell is .5. (a) Female zygote that inherited a nonimprinted fragile-X. After several embryonic cell divisions, a blastocyst is formed containing about 16 embryoblasts in the inner cell mass. Random X-chromosome inactivation is thought to occur at this stage (Fialkow 1973), giving rise, as is shown in (b), to approximately equal proportions of embryoblasts in which the fragile-X or the normal-X is inactivated. If two progenitor cells are chosen at random from the inner cell mass, there are three possible combinations of cells as shown in (c): both cells can have nonimprinted fragile-X's (left, open circles); both can have imprinted fragile-X's (right, shaded circles); or there can be one cell of each (middle). As shown in (d), after progenitor-cell proliferation and differentiation, ovaries from these females will contain primary oocytes that reflect the distribution of inactivated fragile-X's—now imprinted after attempted reactivation—that had been present in progenitor cells (shaded region of ovary). Women with these three classes of ovaries are expected to be binomially distributed about a mean of .5—i.e., 1:2:1—representing oogonia with imprinting frequencies of .0, .5, and 1.0, as shown in (e). The distribution of imprinted fragile-X's in progeny of these women will reflect the frequency of imprinting in the mothers' oogonial cells. Only women with an imprinting frequency of .5 in their oogonial cell population will potentially have both imprinted and nonimprinted carrier progeny; women with all imprinted or with all nonimprinted oogonia will have carrier progeny with similar states of their fragile-X, as shown in (f) for women who have two carrier progeny. In (f) the two carrier progeny are represented by horizontal pairs of boxes, with open and closed boxes representing nonimprinted and imprinted carriers, respectively. Because progeny of women with an imprinting frequency of .5 will represent a binomial sampling from their mothers' oogonia, sibships from these women will have either two nonimprinted carriers (the lower pair at .5), one imprinted and one nonimprinted carrier (the two middle pairs at .5), or two imprinted carriers (the upper pair at .5); these sibships will be distributed 1:2:1, respectively, as shown by middle column of (f). Thus, as shown in (g), the apparent distribution of oogonial imprinting in mothers who inherit a nonimprinted fragile-X, as inferred from sibships with two fragile-X carriers, would be 3:2:3 if there were no ascertainment bias (expanded from data of Laird 1989).

riers, and do these carrier progeny have imprinted or nonimprinted fragile-X alleles?

To analyze more fully the data in table 1, we have used a maximum-likelihood method to obtain the best estimate of progenitor-cell numbers. The best integer estimate of the number of oogonial progenitor cells is two, given the X-inactivation imprinting model and the data in table 1 (fig. 5). Noninteger best estimates range from 1.70 to 1.95, depending on the assumptions made concerning the distribution of family sizes. Best estimates of fewer than two progenitor cells indicate that the distribution in figure 3*a* is more extreme than expected for a model of two progenitor cells. An estimate of 1.75, for example, would imply that three quarters of females had two oogonial progenitor cells and that one quarter had one oogonial progenitor cell. The difference of 2.0 between the ln(likelihood) of the best estimate of oogonial progenitor cell number (1.8) and the ln(likelihood) of a model of three or more oogonial progenitor cells can reasonably be interpreted as sufficient evidence to rule out, with a 95% significance level, the model of three or more oogonial progenitor cells (Mood et al. 1974; fig. 5).

Simulation studies, described in the Appendix, also indicate that the best estimate is two progenitor cells for oogonia but that three progenitor cells cannot be excluded with a high degree of confidence. The simulation studies do rule out four or more progenitor cells with a high degree of confidence. Thus, both the simulation studies and the maximum-likelihood analysis of the data indicate that models of one and of four or more

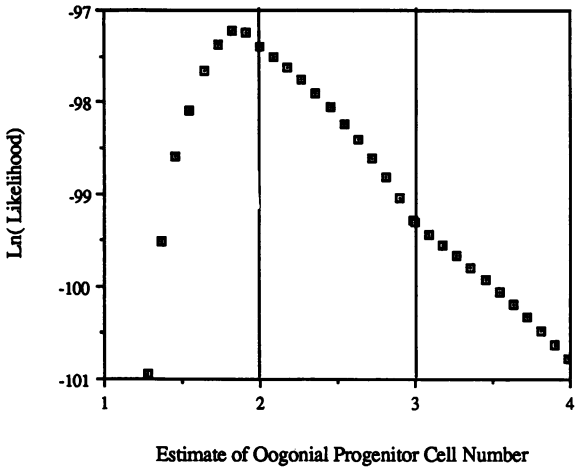

Figure 5 Probability of the data, given models of one through four oogonial progenitor cells. Maximum-likelihood estimate of the number of oogonial progenitor cells was calculated using data in table 1, the X-inactivation imprinting model, and various assumptions concerning family-size distribution and ascertainment parameters (see text and Appendix).

progenitor cells are not compatible with the data in table 1. A two-progenitor-cell model is most consistent with the data; a three-progenitor-cell model cannot be excluded by the data in table 1, although it is considerably less likely than the two-progenitor-cell model.

The conclusion that two is the best estimate of the number of oogonial progenitor cells may also be reached by consideration of the Sherman paradox.

2. *The Sherman Paradox*

The above discussion of chromosome imprinting has been based on data from sibships in which imprinting and fragile-X inheritance are assessed by RFLP analysis to detect a carrier of the fragile-X chromosome and by cytogenetic data to assess the state of chromosome imprinting. Considerable data are also available from more general pedigree analyses. In particular, Sherman et al. (1985*a*, 1985*b*) have calculated the penetrances of the fragile-X syndrome in various pedigree situations. Values of .18 and .74 were reported for the penetrances of the syndrome among carrier brothers of transmitting males and among carrier brothers of affected grandsons of transmitting males, respectively. The "Sherman paradox" (Opitz 1986) refers to these markedly different penetrances observed for males in these two classes of sibships. With standard genetic models, there is no basis for such variable penetrances of a mutation in different generations. In contrast, we show here that the Sherman paradox would be expected from the X-inactivation imprinting model if the number of oogonial progenitor cells were very small.

When the fragile-X syndrome is analyzed as a disorder of chromosome imprinting, these penetrances of .18 and .74 may be considered to be average frequencies of imprinting in sibships containing a transmitting male and in sibships containing an affected grandson of a transmitting male, respectively. Transmitting males and affected grandsons of transmitting males both have mothers who inherited a nonimprinted fragile-X chromosome. But transmitting males, having a nonimprinted fragile-X chromosome, identify sibships in which there is at least one *nonimprinted* fragile-X carrier. Affected grandsons of transmitting males identify sibships in which there is at least one *imprinted* fragile-X carrier. Thus, the ascertainment criteria for these two kinds of sibships were different.

Mothers of transmitting males are expected to be drawn from the classes of females who imprint at a frequency of .0 or .5, if the two-progenitor-cell model is correct; mothers of affected males are expected to be drawn from the classes of females who imprint at a frequency of .5 or 1.0 (figs. 4*d* and 4*e*). With two carrier progeny per sibship, average imprinting frequencies of .20 and .80 would be expected for these two classes of mothers, respectively, without correction for ascertainment bias. This is demonstrated in figure 4*g*, where a ratio of 2:3 is, as noted above, the expected ratio of mothers who appear to imprint at frequencies of .5 and 1.0 and who have at least one imprinted progeny. Such a ratio of mothers would give a 2:8 ratio of nonimprinted to imprinted carrier progeny ("penetrance," or frequency of imprinting = .8), without correction for ascertainment bias. In a similar way, an expected ratio of 8:2 nonimprinted to imprinted carrier progeny ("penetrance," or frequency of imprinting = .20) may be calculated for mothers of two fragile-X progeny when at least one of their progeny is a transmitting male (fig. 4*g*).

In order to analyze the Sherman paradox data from the perspectives of the X-inactivation imprinting model and the number of oogonial progenitor cells, several assumptions are required. These assumptions concern the average family size and the estimated number of carrier brothers of affected grandsons of transmitting males represented by data in Sherman et al. (1985*a*, 1985*b*), as well as the method of ascertainment correction used by these authors. The number of carrier brothers is assumed here to have been 31. (The number was said to be similar to the number of transmitting males [S. L. Sherman, personal communication]; 23/31

= .74.) An average sibship size that includes two fragile-X carriers is a reasonable approximation. (Two carriers would be expected for an average sibship size of 3.4, given that the sibship contained at least one carrier; 3.4 progeny is approximately the sibship size from mentally normal fragile-X carrier females [Sherman et al. 1984].)

The correction for ascertainment bias used by Sherman et al. (1985*a*, 1985*b*) is bounded by the proband method and the simple sib method (S. Sherman, personal communication; J. Sved, personal communication). With the proband method (Vogel and Motulsky 1986), one nonimprinted carrier or one imprinted carrier is subtracted for each ascertained sibship (fig. 4*g*). When this method is used, less extreme frequencies of imprinting would be calculated from a model of two oogonial progenitor cells. Apparent imprinting frequencies of .4 and .6 (apply correction to fig. 4*g*) would be expected for sibships of two carrier progeny that included at least one nonimprinted carrier, or one imprinted carrier, respectively.

When a proband correction method is assumed, the observed values of imprinting (penetrances) of .18 and .74 among brothers and grandsons of transmitting males, respectively, are significantly more extreme than expected for three progenitor cells (table 2). The observed values are not significantly different from those expected for two progenitor cells when data for grandsons of transmitting males are considered ($P < .08$; table 2B) but are significantly different for brothers of transmitting males ($P < .01$). This latter result indicates that the data, with the proband correction method, are more extreme than expected for two progenitor cells and are more compatible with a model in which some females have only one oogonial progenitor cell. The penetrance values reported by Sherman et al. (1985*a*) are not extreme enough for a one-progenitor-cell model for all women, however, where penetrances of .0 and 1.0 would be expected for brothers and grandsons of transmitting males, respectively (table 2).

If the simple sib method is used in analyzing pedigree data (see Vogel and Motulsky 1986), then different expectations are obtained. As can be seen in figure 4*g*, 75% of carrier brothers of affected males would

Table 2

Significance Calculations for Models of Progenitor-Cell Number, Using Sherman Paradox Data

	No. of Progenitor Cells					
	Proband Correction			Simple Sib Correction		
	1	2	3	1	2	3
A. Frequency of imprinting among brothers of transmitting males (n = 32 brothers):						
Expected	.0	.4	.5	.0	.25	.33
No. imprinted, no. nonimprinted	0, 32	12.8, 19.2	16, 16	0, 32	8, 24	10.6, 21.4
"Observed"	⊢	.18	⊣	⊢	.18	⊣
No. imprinted, no. nonimprinted	⊢	6, 26	⊣	⊢	6, 26	⊣
P	.0	<.01	<.001	.0	.28	.059
B. Frequency of imprinting among brothers of affected grandsons of transmitting males (n ~31 brothers):						
Expected	1.0	.6	.5	1.0	.75	.67
No. imprinted, no. nonimprinted	31, 0	18.6, 12.4	15.5, 15.5	0, 31	23.3, 7.7	20.8, 10.4
"Observed"	⊢	.74	⊣	⊢	.74	⊣
No. imprinted, no. nonimprinted	⊢	23, 8	⊣	⊢	23, 8	⊣
P	.0	<.08	<.01	.0	.37	.26

NOTE.—The expected frequencies of imprinting among carrier brothers of transmitting males, compared with carrier brothers of affected grandsons of transmitting males, are based on assumptions illustrated in figs. 3 and 4 for one, two, and three progenitor cells and assume that proband or simple sib correction was carried out as described in the text. Expected frequencies of imprinting for the one- and two-progenitor-cell models are described in the text (also see fig. 4); expected frequencies for a three-progenitor-cell model were calculated similary to those for two progenitor cells (see figs. 4*d*, 4*f*, and 4*g*). These expected frequencies are compared with "observed" values taken from Sherman et al. (1985*a*), where "observed" reflects penetrances based on the number of affected males divided by the expected number of fragile-X carrier males, when regular segregation of the normal X and fragile-X chromosomes in mothers of these males is assumed. Other assumptions concerning these data are discussed in the text. P values are derived from the exact binomial test.

be expected to have imprinted fragile-X chromosomes, compared with only 25% of carrier brothers of transmitting males. Models of two and three oogonial progenitor cells are consistent with the individual Sherman paradox ratios of .18 and .74, when the simple sib method is used (table 2). As with the proband correction method, a model of one oogonial progenitor cell for all females is excluded by the data. We therefore conclude that from the Sherman paradox data, analyzed within the context of the X-inactivation imprinting model, two is the best integer estimate of the number of oogonial progenitor cells.

Two Other Potential Explanations of the Data Are Unlikely

The results of our analysis of RFLP data and the results of Sherman et al. (1985*a*, 1985*b*) using more conventional pedigree analysis are interpreted, within the concept of the imprinting model, as support for an estimate of a very small number of progenitor cells for human oogonia. We now argue that two other potential explanations are not supported by these data.

Could the skewed distribution of apparent imprinting frequencies (fig. 3*a*) result from selective growth rather than from a small number of oogonial progenitor cells? More explicitly, could the data in figure 3*a* be used to argue that all females imprint with a frequency of .5 (such as would be observed if there were a very large number of oogonial progenitor cells) but that selective clonal expansion occurs for one class of oogonial progenitor cells or their descendants? This alternative is made untenable by the following consideration: to explain both the excess of ascertained females who imprint with an apparent frequency of 1.0 (fig. 3*a*) and the imprinting frequency of .74 among carrier brothers of affected grandsons of transmitting males, selective growth of cells with *inactivated* fragile-X chromosomes (the imprinting class) would be required. But to explain the imprinting frequency of .18 among carrier brothers of transmitting males, selective growth would be required of cells with *active* fragile-X chromosomes (the nonimprinting class). Selective growth of the two classes in different females and to the same extent seems very unlikely. Thus, the approximately symmetrical displacement from .5 of imprinting values for sibships containing these two different classes of males strongly argues against selective growth of progenitor cells.

Similarly, it might be argued that the skewed distribution of apparent imprinting frequencies in figure 3*a* is a consequence of females imprinting at an average frequency of .7/oogonial cell rather than the .5 frequency that is explicit in the X-inactivation imprinting model (Laird 1987). This alternative proposal is also made less tenable by the Sherman paradox data (Sherman et al. 1985*a*, 1985*b*), for the reason stated above: the approximately symmetrical displacement of .18 and .74 about .5 would require postulating two classes of women who imprint at different average frequencies of .18 and .74. A biological basis for such a postulate is not apparent, and it is not supported by the data in figure 3*a*. This model therefore seems to be less tenable than a single initial imprinting value of .5 in all females, followed by a random sampling of two oogonial progenitor cells to give three classes of imprinting females.

Other Approaches to Estimating the Number of Oogonial Progenitor Cells in Humans

1. *Glucose-6-Phosphate Dehydrogenase (G6PD) Electrophoretic Variants*

G6PD has been used as a marker for X-chromosome inactivation and reactivation in humans (Gandini et al. 1968). Electrophoretic variants of G6PD, in women heterozygous for a variant enzyme allele, permit estimates of the fractions of cells in which one X, the other X, or both X's are active. There are two reports of G6PD analyses for preparations of human fetal ovaries that were enriched in oogonia. These reports focused on the presence or absence of a hybrid enzyme band that indicated the activity of G6PD alleles on both chromosomes in a single cell (Gartler et al. 1975; Migeon and Jelalian 1977) rather than on the number of progenitor cells for human oogonia. Our interpretation of these published G6PD patterns is that oogonia of five fetuses (samples a, c, and d in Gartler et al. 1975 and samples a and d in Migeon and Jelalian 1977) showed primarily one or the other variant of G6PD and that oogonia of the six other fetuses showed relatively equal levels of the two electrophoretic variants prior to the time of X-chromosome inactivation. (The ratio of the variants expressed in oogonial cells is difficult to calculate precisely because the oogonial preparations are "enriched" rather than purified. Gartler et al. [1975] estimate that their preparations are about 50% pure oogonia [see their table 2]. The densitometric tracing in figure 2 of Migeon and Jelalian [1977] would indicate a similar level of enrichment of oogonia if the authors' explanation ["dilution of the ovarian specimens by non-germ cells"] for the lower-than-expected level of the hybrid enzyme band is correct.) Although the number of fetal

embryos analyzed is small, the distribution of five extreme and six equal distributions of G6PD enzyme variants in oogonial cells is inconsistent with the existence of one oogonial progenitor cell for all women, after the time of X-chromosome inactivation, because, with respect to active X chromosomes, some women have two types of oogonial cells. The data fit well the expected result for two progenitor cells: with two progenitor cells, half of the female fetuses should have had 50:50 mosaic oogonia, and the other half should have had either one electrophoretic variant or the other. The observed values of 6/11 and 5/11, respectively, are close to this expected 1/2.

2. *Genetic Data from Apparent Gonial Mosaics*

There are numerous reports of sibships from phenotypically normal parents in which an apparently dominant or X-linked recessive mutation with high penetrance is expressed in two or more siblings (for review, see Hall 1988). It has been suggested that one of the apparently normal parents is a gonial mosaic in which the mutation occurred very early in a germ-line lineage.

Molecular and chromosomal analysis can establish that a parent has a significant extent of gonial mosaicism for mutant and normal alleles. Such gonial mosaicism would imply that a mutation occurred in the early embryo and that a gonial progenitor cell with the mutation was one of only a few progenitor cells for human gonia (Hall 1985; Gitschier 1986; Bakker et al. 1987; Darras and Francke 1987; Lanman et al. 1987). Although such data on gonial mosaicism for genetic mutations are consistent with the existence of a very small number of gonial progenitor cells, systematic screening of families for gonial mosaics of mutational origin has not been reported. Thus, the well-documented cases reported in the literature could reflect extremes of a distribution that is indicative of a larger number of progenitor cells.

In contrast, the fragile-X data are extensive: each female who inherits a nonimprinted fragile-X chromosome is a potential gonial mosaic for imprinting. The 39 mothers for whom progeny analysis is presented here and the numerous mothers represented by the Sherman paradox data (Sherman et al. 1985*a*, 1985*b*; Opitz 1986) represent a large group of individuals with a potentially mosaic distribution of oogonial cells. Thus the distribution of imprinting observed in progeny of these women should accurately reflect the distribution of imprinting frequencies in oogonia; this distribution in turn is expected to be a direct consequence of the number of oogonial progenitor cells.

Implications for Genetic Counseling

The implications for genetic counseling of individuals who have mosaic gonial cells have been discussed elsewhere (Reed and Falls 1955; David 1972; Fryns et al. 1983; also see references in the preceding section). The risk of subsequent births of affected individuals is a function of the degree of mosaicism, which in turn depends on the number of progenitor cells for human gonia and on the time during development when the mutation occurred (see Hartl 1971).

The conclusions reached here from analysis of the fragile-X syndrome indicate both how extreme this potential mosaicism can be and how significant are the deviations from the normal parameters of genetic counseling. Only one-half of the females who inherit a nonimprinted fragile-X chromosome are expected to be truly mosaic, on the assumption that all women have two progenitor cells for oogonia. One quarter of females who inherit a nonimprinted fragile-X chromosome are expected to have imprinted fragile-X chromosomes in all of their primary oocytes; these females are presumably responsible for the clustering of affected progeny in some sibships. At present, there is no technique to distinguish these females from those who imprint in a mosaic manner (.5) or not at all (.0). If a technique becomes available for distinguishing these three classes of women, then quantitative predictions of their progeny classes may be calculated using table 1 of Laird (1987) and substituting .0 or 1.0, when appropriate, for the average imprinting frequency of .5. For example, the expected frequencies of affected sons from mothers who inherited a nonimprinted fragile-X chromosome are .0, .125, and .25 for women whose oogonia reflect imprinting frequencies of .0, .5, and 1.0, respectively. We consider the wide variation in these expected frequencies to be a consequence of the stochastic nature of cell lineages and random X-chromosome inactivation, which are both normal biological processes.

Prenatal diagnosis, using cytogenetic and RFLP analyses, is reasonably effective at distinguishing fetuses with imprinted (high level of cytogenetic expression) or nonimprinted (low or no cytogenetic expression) fragile-X chromosomes (Shapiro and Wilmot 1986). The conclusions reached here lead to the prediction that information from prenatal diagnosis during successive pregnancies in individual women who have inherited a nonimprinted fragile-X chromosome will result in a distribution of apparent imprinting frequencies at .0 and 1.0—and about 0.5—just as is observed in currently available family data (fig. 3*a*). Such data, however, should not be skewed to the extent shown in figure 3*a*,

if prenatal screening were carried out before birth of any fragile-X carrier. In this case, a woman should be just as likely to have imprinted the fragile-X chromosome with a frequency of .0 as with a frequency of 1.0.

Embryological Significance of Two Progenitor Cells

Estimates of the number of progenitor cells and of the time during embryogenesis at which they are set aside usually rely on patterns of mosaicism that can be observed after differentiation. In placental mammals, for example, the random pattern of X-chromosome inactivation leads to mosaicism that marks cell lineages (Lyon 1961). The number of progenitor cells at the time of cell marking provides information about embryological events and their timing (Gandini et al. 1968). It is thought that X-chromosome inactivation as seen in human somatic cell lineages occurs when there are about 16 cells in the inner cell mass (fig. 4*b*) and that somatic cell lineages are set aside from this common pool after further cell divisions (Gandini et al. 1968; Fialkow 1973). The analysis presented here extends cell lineage information to oogonia in human embryos and indicates a much smaller number of progenitor cells for oogonia than the more than 80 precursor cells that have been estimated for individual somatic cell lineages (Fialkow 1973; the relationship between progenitor and precursor cells for oogonia is not clear; see Searle 1978).

The conclusion presented here—that human oogonial cells are derived from two progenitor cells present at the time of an initial event leading to chromosome imprinting—is based on genetic data and on a model concerning individuals with the fragile-X mutation. It is reasonable, however, to expect that normal human females share this pattern of oogonial cell lineages. This follows because the fragile-X mutation has little or no effect until after chromosome imprinting; the imprinting process is thought to be completed after the attempted reactivation, just prior to meiosis, of an inactivated X chromosome (Laird 1987). The attempted reactivation would occur late in fetal development—after about 10 wk gestation (Gartler et al. 1975; Migeon and Jelalian 1977), a time much later than the embryonic stage at which progenitor cells are established (Van Wagenen and Simpson 1965; Fialkow 1973). Thus the setting aside of progenitor cells for human oogonia occurs before the completion of the process that is proposed to be necessary for imprinting of the fragile-X chromosome.

It has been suggested that progenitor cells for the germ line in animals are unusual in that small numbers of cells are set aside very early in development, perhaps ensuring greater developmental and genetic stability of the germ line than of somatic cells (Weismann 1893). In some animals, a single progenitor cell for the germ line can be identified at the earliest cleavage divisions (Wilson 1937). In *Neurospora,* a variable and small number of germ-line progenitor cells—one and sometimes two—has been inferred from mosaic analysis (Johnson 1976). In nonhuman mammals, estimates of the number of progenitor cells for the germ line have generally been between three and 10 (Russell 1964; Mintz 1974; Searle 1978; Soriano and Jaenisch 1986; however, see McMahon et al. 1983). Thus, for mammals the estimate of two progenitor cells for human oogonia is the smallest and perhaps the most precise estimate yet available of the number of germ-line progenitor cells. Although the timing of establishment of oogonial progenitor cells relative to the establishment of somatic progenitor cells is not known for humans, it is instructive to consider two possibilities. It is plausible that the two oogonial progenitor cells arise from a common pool of embryoblasts after the time of X-chromosome inactivation. Alternatively, two oogonial progenitor cells may be set aside even earlier, perhaps at the initial cleavage divisions. This latter possibility would be consistent with early embryonic determination of germ-cell progenitors, as discussed above, relative to determination of somatic lineages. In this case, X-chromosome inactivation could occur precociously in progenitor cells; alternatively, germ-line progenitor cells may cease division early and only subsequently divide after the general pattern of X-chromosome inactivation occurs at about the 16-embryoblast stage. The results of our analysis are compatible with either possibility.

Implications for the Model of Chromosome Imprinting in the Fragile-X Syndrome

The proposal that the fragile-X syndrome is a disorder resulting from chromosome imprinting (Laird 1987) is extended here to consider what the existence of a small number of oogonial progenitor cells implies for potential mosaicism among primary oocytes. Our analysis identifies females who, within the context of this model, inherited a nonimprinted fragile-X chromosome and who imprinted this chromosome in some but not all of their oocytes. Identifying individual women who have imprinted the fragile-X chromosome at intermediate levels permits the following question to be answered: Have some fragile-X chromosomes truly escaped im-

printing in a mother that is capable of imprinting? It could be argued that a nonimprinted carrier progeny, of a mother who is classified as having an intermediate level of imprinting, had inherited a double recombinant chromosome in which the imprinted fragile-X allele had been removed from the putative nonimprinted chromosome. This cannot be a general explanation: some carrier progeny classified as nonimprinted, from mothers with intermediate levels of imprinting, have had imprinted progeny or grandprogeny, indicating that the mutant fragile-X allele is still present and capable of subsequent imprinting in a female. Examples of such individuals occur in the fragile-X pedigrees analyzed both with and without RFLP data (see III-1 in family 52 of Brown et al. 1987 and IV-5 in family MP1 of Sherman et al. 1985*a*, 1985*b*). Thus, imprinting can occur at intermediate levels in a female, as expected for an event that depends on random X-chromosome inactivation and attempted reactivation.

What can be said, given the analysis presented here, about the nature of the event that leads to imprinting of the fragile-X chromosome? The agreement in estimates of the number of oogonial progenitor cells, estimates based on the fragile-X pedigree data and the analysis of published G6PD data, lends credence to the proposal that X-chromosome inactivation is the necessary event that leads to imprinting. The timing of the event that leads to chromosome imprinting must be coincident, in terms of embryonic cell numbers, with the timing of X-chromosome inactivation in embryonic progenitor cells of oogonia. Thus, the detailed analyses of fragile-X pedigrees containing RFLP data, of G6PD data from fetal oogonial cells, and of the clustering and Sherman paradox phenomena identified by Sherman et al. (1985*a*) are compatible with predictions of the X-inactivation imprinting model.

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Appendix

The Ascertainment Model

The key to the analysis of the data in table 1 is a correct model of ascertainment. A rigorously correct model cannot be obtained because complete ascertainment information is not readily available for these data. The general nature of this ascertainment process, however, can be inferred. Most fragile-X pedigrees initially come to attention because they contain one or more affected individuals. On identification of a person with fragile-X syndrome, cytogenetic data are obtained from relatives of the affected individual. The next, or sometimes concurrent, step in the collection of data is RFLP analysis. Although it is reasonable to expect that the factors that convince a researcher to invest time and money on RFLP analysis include the structure of the pedigree combined with cytogenetic data, the precise factors that lead to RFLP analysis are unknown. The final step in the ascertainment of the data in table 1 was our own. From published pedigrees, we chose only those sibships whose mothers were interpreted as having a nonimprinted fragile-X chromosome (see text). Sibships were included only if at least two members of the sibship appeared to be carriers of a fragile-X chromosome in either its imprinted or nonimprinted state.

The sibships in table 1 are usually gleaned from larger pedigrees. As stated above, the ascertainment process favors the presence of affected individuals in the pedigrees. This bias will be reduced for large pedigrees. For very large pedigrees, this bias should be negligible. We have chosen to ignore this bias and to assume that the probability that an individual becomes a proband depends on whether the individual has inherited an imprinted fragile-X chromosome. Although the sibships in table 1 do not contain probands per se, in our ascertainment model we assume that a sibship will be ascertained if and only if it contains at least one proband. The distinction between "proband" and "ascertainment" should be noted: all probands have been ascertained, but not all ascertained individuals are probands. The probability of an individual becoming a proband is assumed to be independent of the proband status of its siblings. One parameter in the model (r^*) represents the probability that an imprinted fragile-X carrier becomes a proband. Another parameter (r) represents the probability that an individual who is not a carrier of an imprinted fragile-X chromosome becomes a proband.

In table 1, individuals were categorized according to their maternally inherited X chromosome. There are four categories: normal, imprinted fragile-X chromosome, nonimprinted fragile-X chromosome, and questionable. In most cases, cytogenetic data and clinical (mental status) data allow detection of imprinted individuals. RFLP analysis allows separation of normal from nonimprinted fragile-X individuals. When a recombination event makes RFLP analysis uninformative, a mentally normal individual is classified as questionable. In addition, when results of RFLP analysis were not reported for a fragile-X negative individual, the presence of the fragile-X allele is denoted questionable.

Represented in table 1 are a small number of females, included as questionable, who are mentally normal but for whom cytogenetic data are not published. Because imprinted fragile-X carrier females are classified as mentally normal about 50% of the time (see Laird 1987, for interpretation of the data summarized by Sherman et al. 1985*a*, 1985*b*), the knowledge that a female is mentally normal affects the probability that she inherited an imprinted chromosome. Ideally, our model would have a parameter that represents the probability that a mentally normal female is not cytogenetically tested. The extra complication of adding such a parameter did not seem to be worthwhile in light of the fact that only 4% (6 of 148) of the individuals considered in this analysis are mentally normal females for whom no cytogenetic data are available. Instead of including such a parameter, we examined the data in three separate ways:

1. We ignored the presence of these females.
2. We ignored an entire sibship if it contained at least one of these females.
3. We placed these females into the questionable category.

The best estimate of oocyte progenitor cell number was not significantly affected by which of these three approaches was employed. In this appendix, only the results of the third approach are discussed.

In our model, there is a parameter (q) that represents the probability that an individual who is not an imprinted fragile-X carrier is classified as questionable. We do not allow the value of the parameter to vary from sibship to sibship. In reality, the value of the parameter does vary from sibship to sibship. The probability of classifying a fragile-X-negative individual as questionable depends on how closely linked the RFLP markers are to the fragile-X site. It also depends on completeness of the cytogenetic data for a family. Both the amount of information from RFLP markers and the completeness of the cytogenetic data vary from sibship to sibship. The extent to which this variation affects the accuracy of our model is unknown.

More formally, let

M = total number of sibships,
q = probability that an individual who is not an imprinted fragile-X carrier is classified as questionable,
S_i = the i^{th} sibship in table 1,
W_i = total number of offspring in S_i who can be shown to have inherited a normal fragile-X chromosome,
I_i = total number of offspring in S_i who can be shown to have inherited an imprinted fragile-X chromosome,
N_i = total number of offspring in S_i who can be shown to have inherited a nonimprinted fragile-X chromosome,
Q_i = total number of offspring in S_i who are classified as "questionable,"
T_i = $W_i + I_i + N_i + Q_i$ = total number of offspring in S_i,
r^* = probability that an offspring who inherited an imprinted fragile-X chromosome becomes a proband,
r = probability that an offspring who was placed into the normal category, the nonimprinted fragile-X category, or the questionable category becomes a proband
A_i = event that S_i is ascertained,
H_i = [0 if $N_i + I_i < 2$
1 if $N_i + I_i \geq 2$].

Because $P(A_i/S_i) = 1 - P(S_i \text{ is not ascertained})$, we have

$$P(A_i/S_i) = H_i[1 - (1 - r^*)^{I_i}(1 - r)^{(W_i + N_i + Q_i)}] .$$

Imagine that all females have either i or $i + 1$ oogonial progenitor cells. Let

$R_{(i),i+1}$ = probability that a female has i oogonial progenitor cells
$R_{i,(i+1)} = 1 - R_{(i),i+1}$ = probability that a female has $i + 1$ oogonial progenitor cells.

We have considered these cases:

1. All females have either one or two progenitor cells.
2. All females have either two or three progenitor cells.
3. All females have either three or four progenitor cells.

The fact that some females have both imprinted and nonimprinted carrier offsprings means that some females must have more than one oogonial progenitor cell. If all females have two oogonial progenitor cells, then our estimate of $R_{1,(2)}$ should be close to 1.0 while the estimates of $R_{2,(3)}$ and $R_{3,(4)}$ should be close to .0. If all females have three progenitor cells, then our estimates of $R_{(1),2}$ and $R_{(2),3}$ should be close to .0 while our estimate of $R_{(3),4}$ should be close to 1.0. If all females have four or more progenitor cells, then our estimates of $R_{1,(2)}$, $R_{2,(3)}$, and $R_{3,(4)}$ should be close to 1.0. Because the estimates of $R_{1,(2)}$, $R_{2,(3)}$, and $R_{3,(4)}$ are related, we call the best estimate of $R_{i,(i+1)}$ the estimate in this group with the highest likelihood score.

Let $L(R_{i,(i+1)}, q,r^*,r)$ be the probability of the data given ascertainment, the X-inactivation/imprinting model, and knowledge of the sibship sizes to be included in the data set before collection of the data (*i.r.*, T_1, $T_2, \ldots, T_M$). Let $\hat{L}(R_{i,(i+1)}, q,r^*,r)$ be the maximum value of $L(R_{i,(i+1)}, q,r^*,r)$. Without the assumption that the sizes of sibships to be included in the data set were known a priori, it would be necessary to know the fragile-X family-size distribution to compute the likelihood of the data (i.e., it would be necessary to know the probability that $T_i = t$ for all nonnegative integer values of t). Accurate estimates of the fragile-X family-size distribution are not available. Exploratory studies were performed to determine the effects of this assumption. These exploratory studies indicated that choice of sibship-size distribution did not significantly alter the best estimate of oogonial progenitor cell number. A sample of arbitrary sibship-size distribution (e.g., a sibship-size distribution that was Poisson with mean 3.25 was examined) all yielded values between 1.70 and 1.95 oogonial progenitor cells as being the best estimate.

So,

$$L(R_{i,(i+1)}, q,r^*,r) = P(\text{all data/ascertainment of the data and } T_1,T_2, \ldots, T_M) = \prod_{i=1}^{M} P(S_i/A_i,T_i)$$

$$P(S_i/A_i) = P(S_i,A_i)/P(A_i) = P(A_i/S_i)P(S_i)/P(A_i)$$
$$P(S_i) = P(S_i,T_i) = P(S_i/T_i)\,P(T_i)$$
$$P(S_i/A_i,T_i) = P(S_i,A_i/T_i)/P(A_i/T_i)$$
$$= P(A_i/S_i,T_i)P(S_i/T_i)P(A_i/T_i)$$
$$P(A_i) = \sum_{S_i} P(A_i/S_i)P(S_i)$$
$$P(A_i/T_i) = \sum_{S_i} P(A_i/S_i,T_i)P(S_i/T_i)$$

Let $K_{v/w}$ = probability that a female with w oogonial progenitor cells who inherits a nonimprinted fragile-X chromosome imprints the fragile-X chromosome in exactly v of the w progenitor cells. According to the X-inactivation/imprinting model, the number of oogonial progenitor cells in which the fragile-X chromosome is imprinted (v) is a binomial random variable with parameter .5 and sample size equal to the total number of oogonial progenitor cells (w). In other words, since X inactivation is random, $R_{v/w}$ can be found analytically.

If a female with w oogonial progenitor cells who inherits a nonimprinted fragile-X chromosome imprints the fragile-X chromosome in exactly v of the w oogonial progenitor cells, then

1. the probability that a child of hers is categorized as normal is $.5(1.0-q)$;
2. the probability that a child of hers is categorized as imprinted is $.5(v/w)$;
3. the probability that a child of hers is categorized as a nonimprinted fragile-X individual is $.5\,[1.0-(v/w)](1.0-q)$;
4. the probability that a child of hers is categorized as questionable is $.5q[2.0-(v/w)]$.

So,

$$P(S_i/T_i) = (T_i!/W_i!N_i!I_i!Q_i!)0.5^{T_i}\left\{R_{j,(j+1)}\sum_{v=0}^{j+1}\left[K_{v/(j+1)}(1-q)^{W_i}[v/(j+1)]^{I_i}\left(\{1-[v/(j+1)]\}(1-q)\right)^{N_i}\left(q\{2-[v/(j+1)]\}\right)^{Q_i}\right] + R_{(j),j+1}\sum_{v=0}^{j}K_{v/j}(1-q)^{W_i}(v/j)^{I_i}\left\{[1-(v/j)](1-q)\right\}^{N_i}\left\{q[2-(v/j)]\right\}^{Q_i}\right\}.$$

The parameters pertinent to likelihood estimation (i.e., $R_{i(i+1)}$, q,r^*,r) all have values that must be between .0 and 1.0. We divided the range of each parameter into increments of size .09. Specifically, a computer program was written that allowed parameters to assume all values of the form $(.009 + .09B)$, where $B \in (0,1,2, \ldots, 11)$. Therefore, the calculated values of $\hat{L}(R_{i,(i+1)}, q,r^*,r)$ are each the maximum of the 12^4 cases tested.

The best estimate of $R_{i,(i+1)}$ for the data set in table 1 is $R_{1,(2)} = .819$. This corresponds to our best estimate of oogonial progenitor cell number being 1.819. The likelihood score for $R_{1,(2)} = 1.0$ was found to be higher than the likelihood score for $R_{2,(3)} = 1.0$ or for $R_{3,(4)} = 1.0$. Therefore, two is our best integer estimate of oogonial cell number (fig. 5). Additional support for this conclusion comes from simulations.

Data sets with sibship-size distribution identical to that of table 1 were constructed. As in table 1, each simulated data set had 39 sibships: 11 of size two, 15 of size three, etc. To be included in a simulated data set, a sibship had to be ascertained. The probability of ascertainment was calculated using our ascertainment model. These simulated data sets allowed assessment of the performance of the parameter-estimation program. The values of the parameters used to construct a simulated data set can be compared with the estimates of these parameters that are obtained when the simulated data set is analyzed.

Three groups of simulated data sets were contrasted. The first group of data sets was simulated by assuming that all females have two progenitor cells. At $R_{2,(3)} = .0$, our best joint estimate of (q,r^*,r) is $q = .369$, $r^* = .369$, $r = .099$. Therefore, the values of the parameters used in generating the first group of simulated data sets were $q = .369$, $r^* = .369$, $r = .099$.

The second group of data sets was simulated by assuming that all females have three progenitor cells. At $R_{2,(3)} = 1.0$, our best joint estimate of (q,r^*,r) is $q = .369$, $r^* = .279$, and $r = .099$. Therefore, the values of the parameters used in generating the second group of simulated data sets were $q = .369$, $r^* = .279$, $r = .099$.

The third group of data sets was simulated by assuming that all females have four progenitor cells. At $R_{3,(4)} = 1.0$, our best joint estimate of (q,r^*,r) is $q = .369$, $r^* = .279$, and $r = .099$. Therefore, the values of the parameters used in generating the third group of simulated data sets were $q = .369$, $r^* = .279$, $r = .099$.

The simulation results indicate that a best estimate of 1.819 for oogonial progenitor cell number might be obtained if all mothers in the data set from table 1 actually have two oogonial progenitor cells or, less frequently, if all mothers in the data set from table 1 actually have three oogonial progenitor cells. A best estimate of 1.819 or fewer oogonial progenitor cells was obtained for 15 of 25 simulated data sets constructed from mothers with two oogonial progenitor cells. A best estimate of 1.819 or fewer oogonial progenitor cells was obtained for two of 25 simulated data sets constructed from mothers with three oogonial progenitor cells. A best estimate of 1.819 for oogonial progenitor cell number would be unusual if all of the mothers in the data set from table 1 actually have four oogonial progenitor cells. Of 25 simulated data sets constructed from mothers with four oogonial progenitor cells, the best estimate of oogonial progenitor cell number was always greater than 1.819. Thus, the simulations are consistent with all females having two oogonial progenitor cells or all females having three oogonial progenitor cells; two is a somewhat better estimate than three.

The maximum-likelihood score for the data in table 1 is extremely low when compared with the maximum-likelihood scores of simulated data sets. This large disparity indicates that our ascertainment model is unrealistic. A priori, we were aware that our ascertainment model was artificially simplistic. The degree of mental impairment of imprinted fragile-X carriers is highly variable. Although the degree of mental impairment should affect ascertainment frequency, our model does not take this into account. Our ascertainment model allows only the presence or absence of an imprinted fragile-X chromosome to affect ascertainment frequency. Furthermore, as previously mentioned, individuals classified as questionable tend to be clustered. Our ascertainment model ignores this property of the ascertainment process. The flaws in the ascertainment model may or may not introduce a bias into our best estimate of oogonial progenitor cell number. We have no indication that such a bias exists, but we cannot eliminate the possibility. An alternative analysis of some of the data in table 1, using different assumptions concerning the ascertainment process, also leads to a best estimate of two progenitor cells for oogonia (M. H. Israel, personal communication).

In conclusion, maximum-likelihood estimates from the data in table 1 support the conclusion that there are fewer than four oogonial progenitor cells in humans. Two oogonial progenitor cells is a better estimate than three oogonial progenitor cells, but more data are needed to draw a firm conclusion. The data rule out the model that all females have one oogonial progenitor cell but cannot rule out the model that some females have only one progenitor cell. In isolation, the large disparity between the maximum-likelihood score from the real data set and the maximum-likelihood scores from simulated data sets casts doubt on both the reality of our ascertainment model and the precision of the conclusions drawn from it. When the results of this analysis are considered in conjunction with the other observations listed in the present paper, however, two is seen to be the most reasonable estimate of the number of oogonial progenitor cells.

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