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In Search of Non-Random X Inactivation: Studies of Fetal Membranes Heterozygous for Glucose-6-Phosphate Dehydrogenase

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SUMMARY

Extraembryonic membranes and fetal tissues were obtained from 55 specimens of 5–11 weeks conceptual age. The glucose-6-phosphate dehydrogenase (G6PD) electrophoretic phenotype was determined and correlated with that of maternal blood. Fifteen specimens were heterozygous for *G6PD* A, and for nine of these the maternal allele could be determined. In none of these specimens did the isozyme pattern of the membraneous chorion or chorionic villi differ significantly from that of fetal tissue. We have obtained no evidence of non-random inactivation in extraembryonic membranes of human fetal specimens at this stage of development.

INTRODUCTION

Although it seems likely that X inactivation in cells of the mammalian fetus is random with respect to the parental origin of the X chromosome, there is evidence that this is not true for fetal membranes. Using Cattanach's translocation to indicate the parental origin of the allocyclic X chromosome, Takagi and Saseki observed balanced mosaicism in the fetus, but skewing of the pattern in the mouse chorion and yolk sac [1]. The paternal X chromosome was allocyclic in the majority of the cells of these extraembryonic tissues. Unbalanced mosaicism of this type has also been observed in rat membranes [2, 3]. Although it is not clear that the allocyclic behavior observed by Takagi and colleagues can be equated with X inactivation, there is evidence indicating exclusion of a paternal allele. Preferential expression of the maternally derived allele for phosphoglycerate kinase has been observed in the yolk sacs of heterozygous conceptuses irrespective of maternal genotype or that of the uterine environment [4, 5].

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Extending such studies to human extraembryonic membranes, we examined specimens from full term infants heterozygous for the common electrophoretic variants of G6PD. Using cord blood and placental tissues we made the following observations which have been reported in full [6]. There were no novel forms of G6PD in this material. There was no G6PD activity in amnion even in specimens with considerable lactate dehydrogenase activity. The G6PD isozymes in membraneous chorion were phenotypically identical to the maternal G6PD type, even in males, and this was especially notable in specimens which expressed the two G6PD isozymes of the heterozygous mother. The presence of the heterozygous phenotype in specimens of male origin suggests that the membraneous chorion was contaminated with maternal cells. This was confirmed by the phosphoglucomutase phenotype which was a mixture of maternal and fetal genotypes. In contrast, specimens of chorionic villi were always consistent with the fetal *G6PD* genotype, and no maternal contamination was observed. We ascertained 17 newborn females, heterozygous for the variants, and for 12 of these, primarily daughters of non-heterozygous mothers, we were able to determine the maternal allele. In most cases, the chorionic villi expressed equal proportions of the two isozymes, indicating a random distribution of the two cell types. However, in the chorionic villi of three specimens, there was a significant deviation from equality of the two G6PD bands, and in each case the skewing favored the maternal allele. Because this skewing and that reported by Ropers et al. [7] might reflect an underlying non-random pattern, and because the chorionic villi at term may be significantly contaminated with blood vessels of embryonic origin, we examined specimens from earlier stages of embryonic development.

MATERIALS AND METHODS

Subjects

Specimens were from women undergoing suction abortion at the Fertility Control Clinic of the Johns Hopkins Hospital according to an approved protocol and with informed consent.

We obtained samples from 55 fetuses aged 7–13 weeks from the last menstrual period or 5–11 weeks from conception. In each case we obtained a specimen of chorionic villi and maternal blood, and in most cases membraneous chorion and fetal tissue (usually intestine or limb) were also available.

Specimens

Membranes and fetal tissue were treated as follows. Samples of approximately 10 mm diameter were rinsed thoroughly in saline to remove contaminating blood. Chorionic villi were dissected under a microscope and separated from contaminating material. Solid specimens were prepared for electrophoresis by homogenization in water containing triphosphopyridine nucleotide (1 mg/10 ml) followed by lysis in an ultrasonic cleaner or freeze-thawing. The lysates were spun at 12000 *g* for 30 min at 4°C, and the supernatant was used as the enzyme extract. Blood specimens were prepared as previously described [8].

Electrophoresis

All samples were analyzed for G6PD on the day they were obtained. Electrophoresis was performed on cellulose acetate gels as previously described [8], using Tris-glycine-EDTA-sucrose buffer, pH 9.2, at 340 V for 24 min.

RESULTS AND DISCUSSION

Table 1 shows the distribution of G6PD phenotypes in the fetal specimens. Fifteen specimens were heterozygous for the G6PD A variant, and for nine of these, the maternal allele could be determined because the mother was homozygous.

Table 2 presents a summary of the G6PD phenotype in these heterozygous fetal specimens. In seven cases the mother was homozygous for *G6PD* B, while two were homozygous for *G6PD* A. In contrast to newborn specimens, the G6PD phenotype of the membraneous chorion of the first trimester fetus was always consistent with the phenotype of the fetus, and no contamination with maternal cells was observed. As we could find no amnion in these specimens, we do not know if there is more G6PD activity in the amnion during the first trimester than at term. Several specimens of chorion were examined histologically and were found to be essentially pure chorion. The proportion of the two G6PD isozymes in each specimen is presented in Table 2. In all the heterozygous specimens studied, we noted some skewing in the proportion of the two isozymes present. However, there did not seem to be any greater skewing for villi and chorion than for the specimen of fetal tissue.

Table 3 presents a correlation of G6PD phenotype of these heterozygous fetal specimens with the maternal allele. Irrespective of maternal phenotype, there is clearly some skewing towards the G6PD B isozyme in all specimens, attributable in part to the slightly greater specific activity of G6PD B [9, 10]. In the two specimens derived from mothers homozygous for *G6PD* A, the skewing is not in the direction of the *G6PD* A maternal allele but still shows some predominance of the *G6PD* B, the *paternal* allele. Thus we found no indication of non-random inactivation in these extraembryonic membranes.

How can our observations be reconciled with the substantial evidence for non-random patterns observed in rodents, and with the report by Ropers et al. suggesting preferential X inactivation in human placental membranes [7]? With respect to the human specimens, the unbalanced mosaicism was most likely attributable to contamination by maternal cells. As these investigators did not study the phenotype in males, they were unaware of the presence of maternal cells in membraneous chorion at term.

There are several explanations for the fact that the pattern is random in human extraembryonic tissue, but non-random in those of the mouse and rat. First, the rodent specimens studied, although of similar derivation, were at an earlier stage of

TABLE 1

G6PD PHENOTYPES OF FETAL SPECIMENS (5–11 WEEKS)

Specimen	A	B	AB
Complete	4	20	10 (8)*
Without chorion	3	5	3 (1)
Without fetus	1	7	2
Total	8	32	15 (9)

* Maternal allele known.

TABLE 2

SUMMARY OF STUDIES OF G6PD AB FETAL SPECIMENS

Ref. no.	Age in wks from LMP	Maternal blood	Fetus	Villi	Chorion
13	10	B	B >> A	B >> A	A = B
21	13	B	A = B	B > A	A = B
26	13	B	B >> A	B >> A	A = B
29	13	A	B >> A	A = B	B > A
44	13	A^+	B > A	B > A	B > A
47	10	B	B > A	B > A	B ≧ A
53	10	B	A ≧ B	A ≧ B	A ≧ B
66	10	B	B > A	B > A	B > A
10	7	B	A = B	B ≧ A	...

NOTE.—B = A is 1 :1, B ≧ A is 1.3:1, B > A is 2 :1, and B >> A is 3 :1.

development than the earliest human specimens we have obtained. At later stages of development, infiltration of extraembryonic tissues by cells from the fetus may obscure an underlying non-random pattern. However, even with substantial contamination of this kind in mouse yolk sac, West et al. [4] observed a significantly skewed pattern of mosaicism. Unless the phenotype is entirely attributable to contaminating fetal cells, it is unlikely that a non-random pattern could be masked. Because earlier human specimens are not available, it would be interesting to study specimens from rodents at later stages of development.

On the other hand, the non-random pattern may be a peculiarity of rodents and not characteristic of other mammals. The biological basis for preferential expression of maternal X-linked genes is not apparent. Monk has attributed this phenomenon to the timing of the differentiation of the extraembryonic tissues [11], which are the earliest tissues to differentiate. She suggests that differentiation occurs when the paternal X chromosome, inactivated during the process of spermatogenesis, is still inactive. However, the evidence for X inactivation during spermatogenesis comes primarily from cytological observations [12], and needs further substantiation, especially as there is some evidence for a biomodal distribution of G6PD activity in human sperm [13]. In any event, the paternal X chromosome *is* expressed in extraembryonic tissues of X0

TABLE 3

CORRELATION OF G6PD PHENOTYPE OF HETEROZYGOUS FETAL SPECIMENS (5– 11 WEEKS) WITH MATERNAL ALLELE

MATERNAL G6PD ALLELE	SPECIMEN (NO.)	G6PD PHENOTYPE						
		B >> A	B > A	B ≧ A	B = A	B ≦ A	B < A	B << A
A:	Fetus (2)	1	1	...	...	...	...	...
	Villi (2)	...	1	...	1	...	...	...
	Chorion (2)	...	2	...	...	...	...	...
B:	Fetus (7)	2	2	...	2	1	...	...
	Villi (7)	2	3	1	...	1	...	...
	Chorion (6)	...	1	1	3	1	...	...

NOTE.—B = A is 1 :1, B ≧ A is 1.3:1, B > A is 2 :1, and B >> A is 3 :1.

fetal mice (Chapman, personal communication 1979); therefore, the paternal X can function in extraembryonic tissue at this stage of development. Furthermore, it is not clear why expression of the alleles on the paternal X chromosome would be disadvantageous, as paternal alleles specified by autosomes are expressed in these tissues [4].

Alternatively, the discordant results may reflect species specificity in the timing of developmental events in man and rodents. Examples of species variation of this kind include the phenotype of X0 individuals; this karyotype is associated with the premature loss of germ cells in both mouse and man, yet dissimilar timing of germ cell attrition results in significant phenotypic differences with respect to fertility. The definitive explanation for the observed differences in the extraembryonic membrane phenotype requires new insights into early development.

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