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Half Chromatid Mutations: Transmission in Humans?

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Many mutations must be initiated as single base changes in single DNA strands (half chromatid mutation). If a half chromatid lesion is transmitted in a gamete, then after the first round of DNA replication and cleavage of the zygote, a complete mutation or base pair will be formed in a chromosome of one cell while the sister chromosome in the other cell will carry the parental base pair; that is, the embryo will be a mosaic (fig. 1). A mosaic may also result from a somatic mutation occurring in the early cleavage of an embryo and would be indistinguishable from a mosaic occurring as a result of gametic half chromatid mutation.

Induced mosaic mutations are known in experimental organisms, especially as a result of chemical mutagenesis [1]. On the other hand, X-ray induced mutations result in mosaics much less frequently [1, 2]. Presumably, whether a mutation is expressed as a mosaic or is complete depends on whether DNA replication and/or repair occurs before transmission. This in turn will depend on the nature of the inducing agent and the meiotic stage of the mutagenized cell. If the half chromatid lesion occurs in a germ cell before the final DNA replication period preceding meiosis, only complete mutations should be expected. However, if the initial change takes place after the onset of meiosis, then in the absence of DNA repair a half chromatid mutation may be transmitted (fig. 1). Little is known about the extent of half chromatid lesions among spontaneous germ cell mutations. Evidence from the mouse and *Paramecium* can be interpreted as indicating that a considerable fraction of spontaneous mutations result in mosaicism [2, 3]. There is also one report which suggests that spontaneous somatic mutation in mammalian cell cultures may often result in mosaic colonies [4]. The purpose of this communication is to point out the possibility of determining the significance of half chromatid and early somatic mutation utilizing Lesch-Nyhan disease and to present some preliminary results.

To solve this problem a marker must be used where new mutants represent a significant proportion of the total cases available and where mosaicism can be

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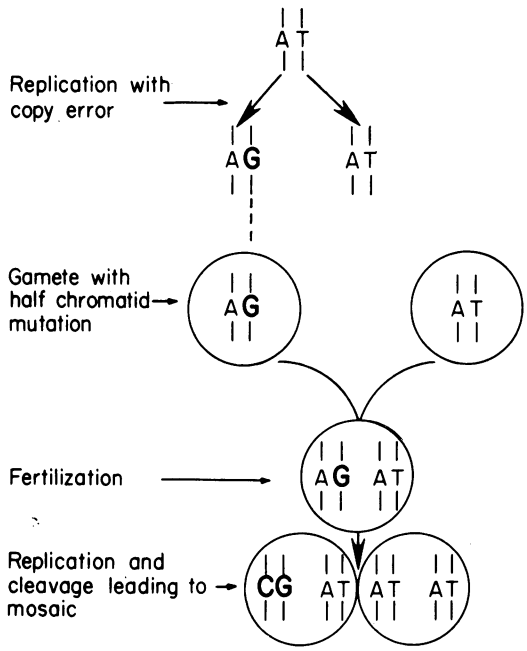

FIG. 1.—Origin of mosaicism following single base change

detected. Lesch-Nyhan disease, which involves a deficiency of hypoxanthine guanine phosphoribosyltransferase (HGPRT) activity, satisfies both of these requirements. It is an X-linked recessive lethal, and one-third of the HGPRT$^-$ alleles at this locus should be lost each generation [5]. This follows from the fact that one-third of the X-linked genes at any locus are in males, and Lesch-Nyhan patients do not reproduce. Therefore at equilibrium one-third of all HGPRT$^-$ genes should be the result of fresh mutation. Only those fresh mutations, half chromatid or complete, which eventually result in Lesch-Nyhan cases can effectively be studied. The marker is cell autonomous, and mosaicism can be detected in cell cultures derived from small skin biopsies and directly in hair follicles [6–11].

The mothers of Lesch-Nyhan cases will be either heterozygous (+/—) or homozygous normal (+/+). In the latter case the Lesch-Nyhan patient would have received a complete mutation from his mother. A half chromatid mutation from his mother would have resulted in mosaicism in the son (+/Y, —/Y). Since all heterozygotes at the HGPRT locus (+/—) are mosaics as a result of X chromosome inactivation and are clinically normal, a mosaic male (+/Y, —/Y) would also appear normal. To our knowledge, no mosaic Lesch-Nyhan patients have been reported. Of the 40 Lesch-Nyhan families recently collected, three cases fall into the category of a homozygous normal mother [12]. According to theory, 13 (1/3) instances of homozygous normal mothers are expected.

In those cases where the mother is a heterozygote, the original mutation may have occurred in the grandparental generation or in still more remote generations.

For practical purposes it is likely that only the grandparents of any particular family would be available for study. They will fall into three classes (fig. 2). In most cases the grandmother will be a heterozygote and the grandfather will

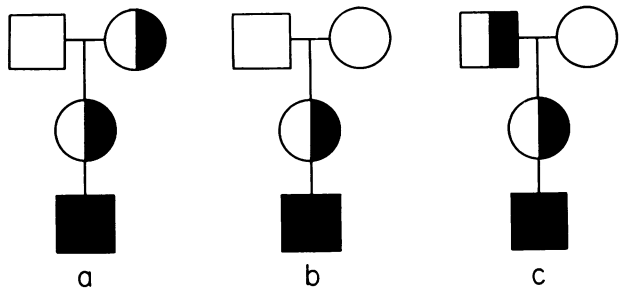

FIG. 2.—Three possible maternal grandparent configurations in Lesch-Nyhan families. □ = +/Y; ○ = +/+; ◑ = +/−; ◨ = +/Y, −/Y; ■ = −/Y.

be normal (fig. 2*a*); such families are noninformative and simply indicate that the segregating mutant gene originated in an earlier generation. In some or possibly all of the remaining cases the grandmother will be homozygous normal and the grandfather normal (fig. 2*b*). Such cases would represent either mutations occurring in the grandmother or the grandfather and transmitted to the daughter, or an early somatic mutation in the mother. Regardless of the type of mutation, the daughter will be a heterozygote and mosaic. The half chromatid and somatic mutant mosaics should have a lower proportion of mutant cells than the full chromatid type, but separation, if at all possible, could only be done on a statistical basis. Finally, if transmission of half chromatid lesions occurs, some cases will have a homozygous normal grandmother and a mosaic grandfather (fig. 2*c*). That is, the grandfather would have been the recipient of a half chromatid mutation (or early somatic mutation) which should result in mosaicism. Clinically he should be normal, since all heterozygotes are mosaics and normal.

We have studied two sets of maternal grandparents where the mother was a proven heterozygote with a single affected son. The mothers and sons and one maternal grandmother were reported in previous publications [8, 13]. Studies included cell culture assays (autoradiography and selection in the presence of 6-thioguanine) and determination of HGPRT activity in hair follicles for two grandmothers and one grandfather, and only hair follicle assays for the other grandfather. All tests were negative for the presence of $HGPRT^-$ cells; thus none of these grandparents was a mosaic. The mutations in these families must either have occurred in one of the grandparents or as an early somatic event in the mother. Only mosaicism in the grandfather would constitute evidence for the possibility of half chromatid mutation. If a series of such cases were all negative, it would have to be concluded that transmission of half chromatid mutations and the occurrence of early somatic mutations were very rare or nonexistent.

To obtain a series of Lesch-Nyhan families where the maternal grandparents

are available will require international cooperation, and one of the purposes of this communication is to call attention to this need. Hair follicles and/or small skin biopsies which are used for assaying mosaicism can be obtained simply and transported easily anywhere in the world. It should be stressed that all Lesch-Nyhan families where grandparents are available for study are of potential value. At first glance it might seem useless to study a family with multiple Lesch-Nyhan first cousins of sibling mothers (fig. 3), since it might be concluded that in such

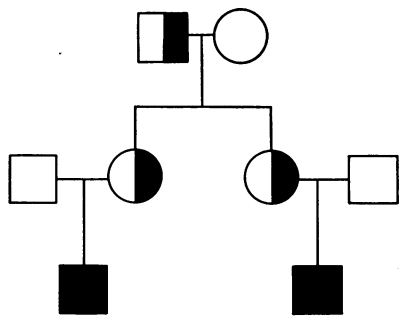

FIG. 3.—Hypothetical Lesch-Nyhan family which could be explained by a mosaic grandfather. See figure 2 for explanation of symbols.

a case the grandmother would be a heterozygote. However, a mosaic grandfather could also have more than one heterozygous daughter.

The concept of mutation producing mosaics bears a possible relationship to Haldane's hypothesis that the mutation rate in males might be much greater than in females [14]. His idea was derived from a statistical analysis of segregation data for sex-linked recessive hemophilia, where he found too few cases of fresh mutation (i.e., affected males of homozygous normal mothers). He pointed out that if mutations occurred primarily in males, very few affected sons appearing as new mutations would be expected in sex-linked recessive disease (i.e., most new mutations would appear as new heterozygotes). The same pattern of appearance of new mutations would be expected if a significant fraction of mutations are transmitted as half chromatid lesions or occur as early somatic events (e.g., a half chromatid mutation occurring in a female would not lead to Lesch-Nyhan disease in the recipient son). Although Haldane's idea has been discussed and reexamined, it is still not completely resolved. An extensive and intensive study of Lesch-Nyhan families could resolve this question as well as that of half chromatid or early somatic mutations.

Are there any other conditions which might be useful in the investigation of the question of transmission of half chromatid mutations or of the occurrence of early somatic mutations? In terms of satisfying the two requirements of high mutation yield and sensitivity of detection of mosaicism, Hunter's syndrome is another possibility. It is also possible that some phenocopies could be explained by mosaic-yielding mutations. For example, in the case of retinoblastoma, a con-

siderable fraction of the sporadic cases appear to be nontransmissible and are therefore phenocopies. Knudson [15] has suggested that these sporadic cases are due to multiple somatic mutations occurring late in development in retinal tissue. While this idea would appear to be the most likely explanation for most sporadic cases, it is possible that some sporadic cases could represent half chromatid or early somatic mutations. Since the condition is a dominant, the resulting mosaicism could lead to significant but less severe clinical effects and not necessarily be transmissible. In the absence of a cellular marker, this speculation cannot be directly tested. There are a number of genetic markers where mosaicism could be detected quite easily (e.g., most of the enzyme and blood group polymorphisms). However, the frequency of new mutants is so low that this type of study is essentially impossible.

Finally we should like to call attention to the possibility of a connection between half chromatid mutation and the apparent inability to demonstrate a radiation effect on mutation induction in humans. Induced mosaic mutations have been frequently observed in experimental organisms; this is especially true of chemical mutagenesis, but mosaics are also observed after irradiation, the proportion of mosaics being inversely related to the dose. Russell's [2] studies with X irradiation in the mouse indicate that mosaics involving point mutations are only rarely found; however his work utilized high doses of irradiation. In humans the only major radiation study has attempted to detect induced gene mutations by looking for mortality effects among the offspring of survivors of the atomic bombing in Japan during World War II. Sex ratio changes were used to detect the possible induction of early acting sex-linked recessive lethals, and general mortality effects were used to detect the induction of autosomal dominants affecting survival. All results to date have been negative [16, 17]. If a significant fraction of radiation-induced mutations in these studies were transmitted as mosaic-producing lesions, then their effect on survival could be considerably less than complete mutations, and a mutagenic effect could go undetected.

SUMMARY

Attention is drawn to the possibility of half chromatid and early somatic mutations and to several implications of these mosaic-yielding events. There is suggestive evidence that spontaneous mutations can result in mosaics. A worldwide cooperative study of Lesch-Nyhan families could determine the extent of half chromatid mutation transmission and early somatic mutation in humans.

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REFERENCES

1. Auerbach C, Kilbey BJ: Mutation in eukaryotes. *Annu Rev Genet* 5:163–218, 1971

2. RUSSELL WL: The nature of the dose-rate effect of radiation on mutation in mice. *Jap J Genet* 40, suppl.: 128–140, 1965
3. KIMBALL RF: Studies on radiation mutagenesis in micro-organisms, in *Genetics Today,* Proceedings 11th International Congress of Genetics, The Hague, edited by GEERTS SJ, vol 2, Oxford, Pergamon Press, 1963, pp 227–234
4. ATKINS JH, GARTLER SM: Development of a nonselective technique for studying 2,6,diaminopurine resistance in an established murine cell line. *Genetics* 60:781–792, 1968
5. HALDANE JBS: The rate of spontaneous mutation of a human gene. *J Genet* 31: 317–326, 1935
6. MIGEON BR, DER KALOUSTIAN VM, NYHAN WL, YOUNG WJ, CHILDS B: X-linked hypoxanthine-guanine phosphoribosyl transferase deficiency: heterozygote has two clonal populations. *Science* 160:425–427, 1968
7. SALZMANN J, DE MARS R, BENKE P: Single-allele expression at an X-linked hyperuricemia locus in heterozygous human cells. *Proc Natl Acad Sci USA* 60:545–552, 1968
8. GARTLER SM, SCOTT RC, GOLDSTEIN JL, CAMPBELL B, SPARKES R: Lesch-Nyhan syndrome: rapid detection of heterozygotes by use of hair follicles. *Science* 172: 572–574, 1971
9. GOLDSTEIN JL, MARKS JF, GARTLER SM: Expression of two X-linked genes in human hair follicles of double heterozygotes. *Proc Natl Acad Sci USA* 68:1425–1427, 1971
10. SILVERS DN, COX RP, BALIS ME, DANCIS J: Detection of the heterozygote in Lesch-Nyhan disease by hair-root analysis. *N Engl J Med* 286:390–395, 1972
11. FRANCKE U, BAKAY B, NYHAN WL: Detection of heterozygous carriers of the Lesch-Nyhan syndrome by electrophoresis of hair root lysates. *J Pediatr* 82:472–478, 1973
12. FRANKE U, GARTLER SM, MIGEON BR, DANCIS J, DE MARS R, SEEGMILLER JE, BAKAY B, NYHAN WL: Mutation rate in the X-linked recessive Lesch-Nyhan disease. In preparation
13. ITIABA K, BANFALVI M, CRAWHALL JC, MONGEAU JG: Family studies of a Lesch-Nyhan patient from an isolated Canadian community. *Am J Hum Genet* 25:134–140, 1973
14. HALDANE JBS: The mutation rate of the gene for hemophilia and its segregation ratio in males and females. *Ann Eugen* 13:262–271, 1947
15. KNUDSON AG: Mutation and cancer: statistical study of retinoblastoma. *Proc Natl Acad Sci USA* 68:820–823, 1971
16. NEEL JV: The detection of increased mutation rates in human populations. *Perspect Biol Med* 14:522–537, 1971
17. NEEL JV, KATO H, SCHULL WJ: Mortality in the children of atomic bomb survivors and controls. *Genetics* 76:311–326, 1974