that they are not due to the alterations in the amounts of estrogen and progesterone during the cycle.

GUY M. HARBERT, JR.
GEORGE W. CORNELL
W. NORMAN THORNTON, JR.
Department of Obstetrics and Gynecology, University of Virginia School of Medicine, Charlottesville 22901

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5. Supported in part by PHS research grant HD-02798.

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Heritable Fragile Site on Chromosome 16: Probable Localization of Haptoglobin Locus in Man

Abstract. *We have found recurrent chromosome breaks at a site (the "fragile site") on the long arm of chromosome 16. This site segregates in simple Mendelian dominant fashion in a large family. The distal portion of the chromosome sometimes shows selective endoreduplication. Preliminary linkage results reveal only 3 recombinants in 33 opportunities for recombination between the fragile site and the alpha locus of haptoglobin, an indication that the* α-Hp *gene is located near this region on chromosome 16.*

Frequent spontaneous breaks may occur at a specific site in a human chromosome (*1–3*). Most reports (*1, 2*) of this phenomenon have involved sporadic cases. However, Lejeune *et al.* (*3*) found a chromosome 2 with a fragile region in a mother and her daughter, and Shaw observed a similar chromosome 2 in an unspecified number of individuals from two unrelated families. We wish to report a large family with simple Mendelian transmission of a fragile region on chromosome 16.

The family was ascertained through an 18-year-old boy, who had recurrent cold urticaria and immunoglobulin A (IgA) deficiency. Chromosome analysis of his lymphocytes (*4*) showed 8 of 33 metaphases figures with an isochromatid break in the long arm of a chromosome 16. The breaks were consistently at the junction of the middle and distal thirds of the long arm (Fig. 1). This region will be designated the "fragile site."

Lymphocyte cultures from the patient showed a variable proportion of cells with breaks at the fragile site. Two cultures labeled with tritiated thymidine (*5*) showed a general increase in both single and isochromatid breaks, but no increase in breaks at the fragile site, compared to unlabeled cultures. A direct preparation of bone marrow chromosomes (*6*) showed 2 of 41 metaphases with an isochromatid break at the fragile site. None of 165 fibroblast metaphases (*7*) showed breaks at that site, and none of the other cases in which fibroblasts were cultured (*2*) revealed a fragile site in fibroblasts. The fragile site on chromosome 16 might conceivably be manifest only in lymphocytes and their precursors in the patient and be related to his IgA deficiency. However, his mother had IgA deficiency with no evidence for the fragile site, whereas his father had normal amounts of IgA and manifested the fragile site.

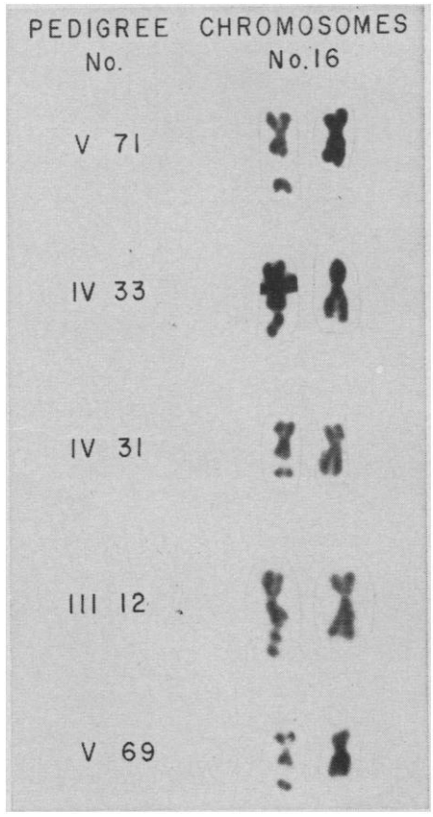

Fig. 1. Composite showing the fragile site on chromosome 16 in the patient and relatives.

Family studies led to the construction of a pedigree containing 238 individuals; lymphocyte cultures from 127 living members have been studied (Fig. 2). The criteria (*8*) for determining the presence or absence of the fragile site in an individual were as follows: (i) a minimum of two metaphases in 60 should show the fragile site; (ii) at least 60 metaphases should be examined before the individual can be said not to have the fragile site; (iii) if one among the first 60 metaphases appears to have the fragile site, another 60 metaphases should be examined, and so on. A minimum of 12 metaphases were also photographed, and karyotypes of at least two metaphases were prepared from each person.

In addition to the father of the patient, 29 other paternal relatives were found to have the fragile site (Figs. 1 and 2). Fifty persons in the family had a parent carrying the fragile site, if it is assumed that III-5 and IV-26 were affected (Fig. 2). Twenty-eight of the 50 had the fragile site; 22 did not. This is not significantly different from the 1:1 ratio expected for simple Mendelian dominant transmission ($\chi^2 = 0.72$; $P > .30$, d.f. = 1). Every person with the fragile site, whose parents were alive, had an affected parent. Unaffected persons had only unaffected children. Thus, the fragile site appears to be fully penetrant.

The cause of the fragility is not known. It is probably due to the particular structure of the fragile site, since there was no generalized increase in chromosome breakage.

Two to 12 copies of the distal fragment (Fig. 3) were observed in some cells from the affected persons. This suggests that the control of replication for the distal portion of this chromosome is not the same as for the remainder of the chromosome. The possibility that the distal fragment undergoes recurrent nondisjunction seems unlikely, since cells were not found with the fragment or the centromeric portion alone, as would be expected with nondisjunction. Lejeune *et al.* and Shaw (*3*) made similar observations in families with a fragile site in chromosome 2. They attributed it to selective endoreduplication, a conclusion in which we concur.

Heritable fragile sites provide an additional tool for use in gene localization. Since the location of these sites can be determined with precision, they

Table 1. Frequency of breaks at fragile site in lymphocytes from patient.

Date of culture (1969)	Length of culture (hour)	Total cells examined (No.)	Cells with break in chromosome 16 (No.)
3 February	72	33	8 (24 %)
20 May	48	17	3 (18 %)
3 June	72*	76	7 (9 %)
21 August	72*	100	11 (11 %)
23 December	72	85	11 (13 %)

*[^{3}H]Thymidine (1 μc/ml) was added 6 hours before harvest for autoradiography.

can potentially provide complementary information to that obtained from experiments with somatic cell hybridization, which can indicate that certain genes are on a particular chromosome (*9*), and studies of heritable translocations, which can indicate that certain genes are on a particular chromosome arm (*10*). Heritable fragile sites are minor chromosome variations, in some ways comparable to the uncoiled region on one arm of chromosome 1 to which the Duffy blood group locus is probably linked (*11*).

We have begun linkage studies in this family; we have tested various genetic polymorphisms, all of which appear to segregate independently and not to be linked to the fragile site, except for haptoglobin (Hp). This serum protein is composed of two types of polypeptide chains, α and β, determined by separate gene loci, the common variations involving the α locus (*12*). The preliminary results show 33 informative persons born to individuals with the fragile site who are heterozygous at the *α-Hp* locus. Of the 33, 30 are apparent nonrecombinants. The remaining three show recombination between the fragile site and the *α-Hp* locus (*13*). The lod (logarithm of the odds) scores have been calculated (*14*) by one of us (E.W.L.). The maximum lod score is 5 for a recombination fraction of 0.1, indicating that the odds are 10^5 in favor of linkage at 10 map units. Therefore, the *α-Hp* gene can probably be localized to a discrete segment of the chromosome near the junction of the middle and distal thirds of the long arm of chromosome 16.

Robson *et al.* (*10*), using heritable translocations involving chromosome

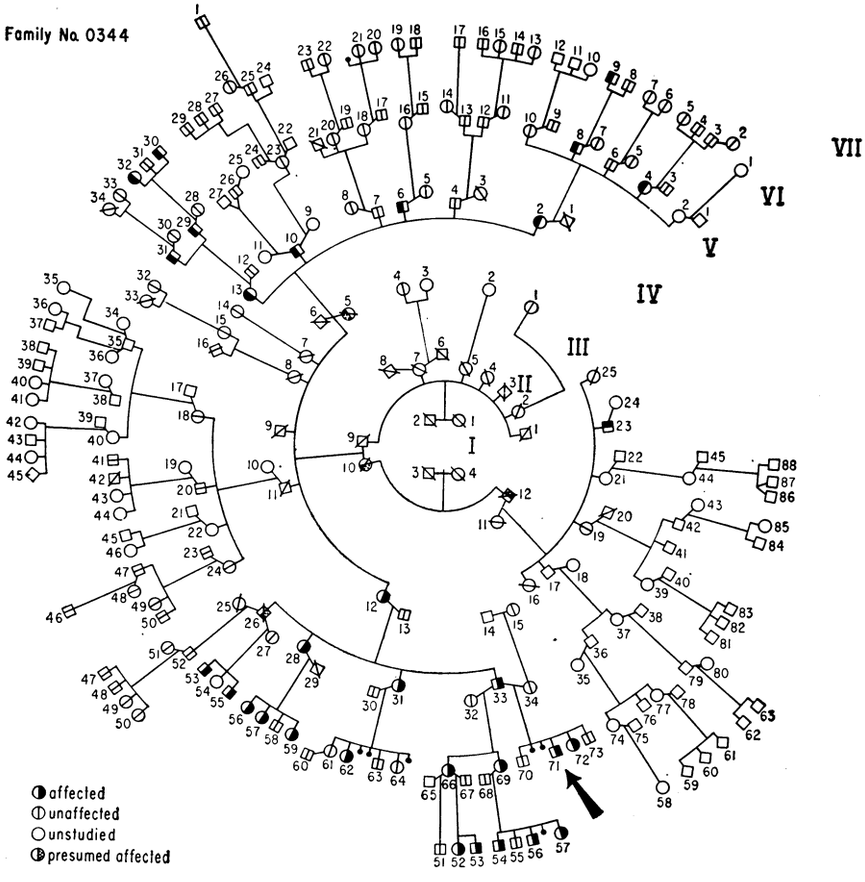

Fig. 2. Pedigree. The squares represent males, the circles females, and the diamonds individuals of unspecified sex. The diamond with a line indicates pregnancy (VI-45); the small solid circle indicates abortion. Diagonal slash means dead. Arrow points to propositus.

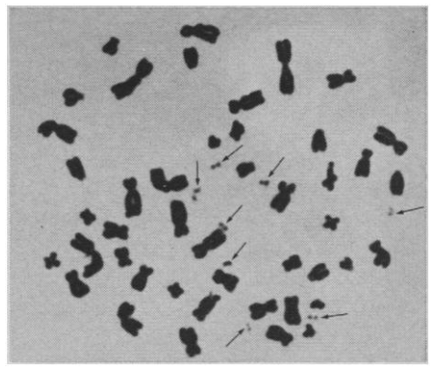

Fig. 3. Lymphocyte metaphase spread. Arrows point to endoreduplicated distal portions of the long arm of chromosome 16.

16, recently assigned the *α-Hp* locus a probability of 0.97 of being somewhere on the long arm of chromosome 16. Our results are consistent with their data and provide more precise information as to the location of the *α-Hp* locus.

Note added in proof: Preliminary data from Amos *et al.* (*15*) suggested linkage between *Hp* and the HL-A histocompatability locus. On 28 August 1970 we received a communication describing recent work (*16*) from J. H. Renwick reading: "Extensive data from an Amish isolate suggest a recombination fraction of 0.25 between *Hp* and the HL-A histocompatability region with a peak lod of 4.0 and 95 percent probability limits of 0.19 and 0.33. There is a probability of 0.98 that HL-A is syntenic [*17*] with *Hp* and therefore also on chromosome 16." We propose further that the HL-A region is on the long arm of chromosome 16 near the fragile site, the order being HL-A/fragile site/*α-Hp* or fragile site/*α-Hp*/HL-A.

R. Ellen Magenis
Frederick Hecht
Everett W. Lovrien
Division of Medical Genetics, Department of Pediatrics, and *Genetics Clinic, Crippled Children's Division, University of Oregon Medical School, Portland 97201*

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7. Fibroblasts were grown from a skin explant and harvested after 52 and 106 days in culture [T. Kajii, R. L. Neu, L. I. Gardner, *Lancet* **1967-I**, 897 (1967)].
8. The criteria for determining the minimum number of cells to be scored were derived from sequential analysis with α and $\beta = 0.05$. Previously, we had found that only 2 of 2800 metaphases from 100 persons unrelated to the present family had breaks in the long arm of chromosome 16. Thus, if 2 of 60 metaphases from a person showed isochromatid breaks at the fragile site, the score was sufficient to reject the null hypothesis that the person did not have the fragile site.
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18. We thank Dr. K. M. Taylor for lymphocyte cultures of several family members living near San Diego; Dr. E. Bardana for referring the patient; Dr. W. Kimberling for statistical consultation; Dr. J. Higgins, S. Underwood, and P. Evans for help with family studies; S. Ditewig, C. MacDonald, B. McCaw, T. Ryan, M. Case, D. Worthington, C. Trombly, and H. Wyandt, for helping R.E.M. and F.H. with chromosome studies; and S. Rowe, J. Beck, and S. Hazard for helping E.W.L. with gene marker studies. We especially thank the family for their interest and cooperation. R.E.M. is a postdoctoral fellow on NIH genetics training grant 5-T01-HD00165. Work was supported by NIH grant AM-13173 and by a grant from the Children's Bureau for a Genetics Program.

2 July 1970

Potent Sex Attractant of the Gypsy Moth: Its Isolation, Identification, and Synthesis

Abstract. *The sex attractant emitted by the female gypsy moth has been identified as* cis-*7,8-epoxy-2-methyloctadecane. The structure was verified by spectral, gas chromatographic, and biological comparisons with the synthesized compound. Nine closely related isomers were considerably less effective.*

The gypsy moth *Porthetria dispar* (L.) is a serious despoiler of forest and shade trees in the northeastern part of the United States. Crude extracts of the abdominal tips of virgin females containing the sex attractant are used in traps to determine the occurrence and abundance of the moth (*1*); control measures are then applied to prevent excessive defoliation and the migration of the moth into new territory. Since the application of insecticide is made only where needed, residues are held to a minimum.

Jacobson and co-workers (*2*) identified the gypsy moth sex attractant as *cis*-7-hexadecene-1,10-diol 10-acetate (gyptol) and reported that its homolog, gyplure (*cis*-9-octadecene-1,12-diol 12-acetate), was also a highly active gypsy moth sex attractant. Preparations of these compounds by other researchers were reported as inactive (*3*); a reinvestigation by Jacobson *et al.* (*4*) confirmed that the compounds were inactive and indicated that the original gyptol preparation was active because of the presence of traces of another substance with "extraordinarily high biological activity."

We have isolated the gypsy moth sex attractant, and it has been identified as *cis*-7,8-epoxy-2-methyloctadecane, for which the name disparlure is proposed. The synthesized compound was active in laboratory and field tests at concentrations of 2×10^{-12} and 1×10^{-9} g.

The sex attractant was extracted from 78,000 tips (last two abdominal segments of female moths) collected in Spain and concentrated essentially as described previously (*5*). The concentrate was refluxed under nitrogen with ethanolic potassium hydroxide to saponify fats (*6*), and the neutral fraction, which contained the activity (*7*), was chromatographed on Florisil (*8*). The hydrocarbons were eluted with hexane and the active material with 2 to 6 percent ether in hexane. The active material was chromatographed again on Florisil and purified further by silica-gel thin-layer chromatography (TLC) with a double development with 20 percent CH_2Cl_2 in cyclohexane and another double development of the eluted active portion with 40 percent CH_2Cl_2 in cyclohexane (R_F, 0.50).

A portion of the partially purified material was analyzed to obtain information on the nature of the active substance; the activity was determined by laboratory bioassay (*7*). Chromatographic mobility (R_F) on silica-gel TLC plates was consistent with an aliphatic ketone (but not a methyl ketone), aliphatic epoxide, methyl alkyl ether, or an alkyl ester other than an acetate, an indication that the compound was monofunctional. Gas-chromatographic retention indices (*9*) indicated that the compound contained 18 to 20 carbon atoms (*10*). Because ozonolysis of the compound did not diminish activity or affect the R_F value on silica-gel TLC, the presence of a double bond was excluded. The material on a TLC plate was overspotted with aqueous semicarbazide hydrochloride solution and the plate developed; elution of the area active with an untreated sample indicated that the active component was not an aldehyde or ketone. Similar spotting of the sample with phosphoric acid, which reacts with epoxides and retains them at the origin, eliminated activity. Both powdered lithium aluminum hydride and dry, powdered periodic acid destroyed the activity.

Reaction gas-chromatographic trials with subtraction loops (*11*) mounted after the column were also used, and male moths were used to monitor the effluent to determine whether the active compound survived reaction. Boric acid (subtracts alcohols) and *o*-dianisidine (subtracts aldehydes) loops did not affect activity. A benzidine loop (subtracts aldehydes, ketones, and some epoxides) diminished activity, while a loop containing phosphoric acid (subtracts epoxdes) totally eliminated activity.

These results led us to postulate that the natural attractant is a C_{18} to C_{20} alkyl epoxide. Unfortunately the amount of pure attractant in the 78,000 tips was considered insufficient for adequate characterization of the attractant.

We speculated that the insect might contain an olefin precursor from which the attractive epoxide could be formed, and that this olefin might be used to generate more of the sex attractant. Treatment of the original neutral fraction with *m*-chloroperbenzoic acid, which epoxidizes olefins, caused a tenfold enhancement of the activity of the