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Linkage of Atypical Vitelliform Macular Dystrophy (*VMD-1*) to the Soluble Glutamate Pyruvate Transaminase (*GPT1*) Locus

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SUMMARY

One hundred twenty-eight blood samples were drawn from members of a single family with atypical vitelliform macular dystrophy (*VMD-1*) characterized by variable expressivity in affected members of at least 5 generations. Because of the late onset of detectable retinal lesions in most family members, phenotype data from only 93 individuals who were at least 14 years of age were analyzed for linkage. Phenotype data from the remaining 35 members of the family who were under age 14 were excluded from the analysis. Maximum-likelihood analysis for linkage between *VMD-1* and 13 biochemical and serological markers in the family demonstrated linkage between *VMD-1* and the soluble glutamate pyruvate transaminase (*GPT1*) locus, which has been tentatively assigned to the short arm of chromosome 16. A maximum lod score of $Z = 4.34$ (odds favoring linkage of approximately 22,000 to 1) was obtained at a recombination fraction of $\theta = .05$.

INTRODUCTION

A large family with atypical vitelliform macular dystrophy (*VMD-1*) with variable expressivity was recently described by Hittner et al. [1]. In this family, fluorescein angiography was found to be more helpful than electrooculography in ascertaining affected individuals. Specifically, minimal fluorescein angiographic changes in the macula and peripapillary region, and small yellow lesions in the macula and

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periphery, were recognized as early signs of *VMD-1*. Moderate accumulations of the yellow material in the central and peripheral retina, and advanced depigmented lesions of the central and peripheral retina and peripapillary region, were also documented in family members. These findings are similar to those described in typical vitelliform macular dystrophy, which is known to involve the retinal pigment epithelium and invariably has an abnormal EOG [2–4].

Our study reports the results of a linkage study between *VMD-1* and 13 polymorphic biochemical and serological markers in that kindred. Linkage was established between *VMD-1* and the soluble glutamate pyruvate transaminase (*GPT1*) locus, which has been tentatively assigned to the short arm of chromosome 16.

MATERIALS AND METHODS

Venous samples of whole blood were drawn in ACD vacutainers. Erythrocytes were typed for antigens of the *ABO, Rh, MNS,* Kell, Duffy (*Fy*), P, and Kidd (*JK*) blood-group systems, and for the erythrocyte enzymes adenylate kinase, adenosine deaminase, acid phosphatase-1 (*ACP1*), esterase D (*ESD*), phosphoglucomutase-1 (*PGM1*), phosphoglucomutase-2, glyoxalase-1 (*GLO1*), glutamate pyruvate transaminase-1 (*GPT1*), peptidases A, B, C, and D, superoxide dismutase-1, phosphoglycolate phosphatase (*PGP*), phosphoglucose isomerase, 6-phosphogluconate dehydrogenase, and hemoglobin. Plasmas were typed for the proteins haptoglobin alpha (*HPA*) and transferrin. Sample preparation and typing were essentially as described by Ferrell et al. [5].

Lod scores, as defined by Morton [6], were obtained using the computer program LIPED [7]. The *Rh* locus was treated by using the convention suggested by Ott [8] for analyzing human leukocyte antigen typing data in order to include all segregating haplotypes. Lod scores at various values of recombination [$Z(\theta)$s] are presented, using the convention of Keats et al. [9].

The family was initially identified in 1974; however, at that time, linkage analysis was not performed. In 1981, the family was re-examined ophthalmologically, and 105 blood samples were drawn for a linkage study. These ophthalmologic examinations consisted of visual acuities best corrected, external, motility, slit lamp (hand-held instrument), and direct and indirect ophthalmoscopic examinations, and fundus photography (hand-held camera). Based on these examinations, each individual in generations II, III, and IV was assigned a tentative phenotype with respect to *VMD-1*. Individuals in generation V were excluded because the majority were too young to express the disease clinically.

A preliminary linkage analysis suggested linkage between *VMD-1* and the erythrocyte enzyme *GPT1* ($Z = 1.35$, $\theta = .10$). Given this suggestion of linkage, all available family members over age 14 who were potentially informative were examined using fluorescein angiography. This included most members of generation IV whose affected (or possibly affected) parent had a 2-1 *GPT1* phenotype. Thus, a special effort was made to study all 27 children of five members of generation III (III_9, III_{14}, III_{15}, III_{17}, and III_{18}), since these members of generation IV would contain most of the genetic information regarding linkage in this family [10]. Only two (IV_{23} and IV_{43}) of these 27 members of generation IV could not be studied to determine their disease status and *GPT1* phenotype. The two children of III_{27} were not studied because they were not over age 14. It was not deemed necessary to perform fluorescein angiograms on all 42 children of the nine members of generation III who had a 1-1 *GPT1* phenotype since these members of generation IV would not contribute significantly to the lod score for linkage [10]. After performing the indicated fluorescein angiograms, a disease phenotype was assigned by studying the angiograms blinded with regard to the *GPT1* genotype. At the same time that these studies were obtained, some other members of generations II–IV who were desirous of clarifying their disease status were studied, and 23 more blood samples were obtained from family members and their spouses.

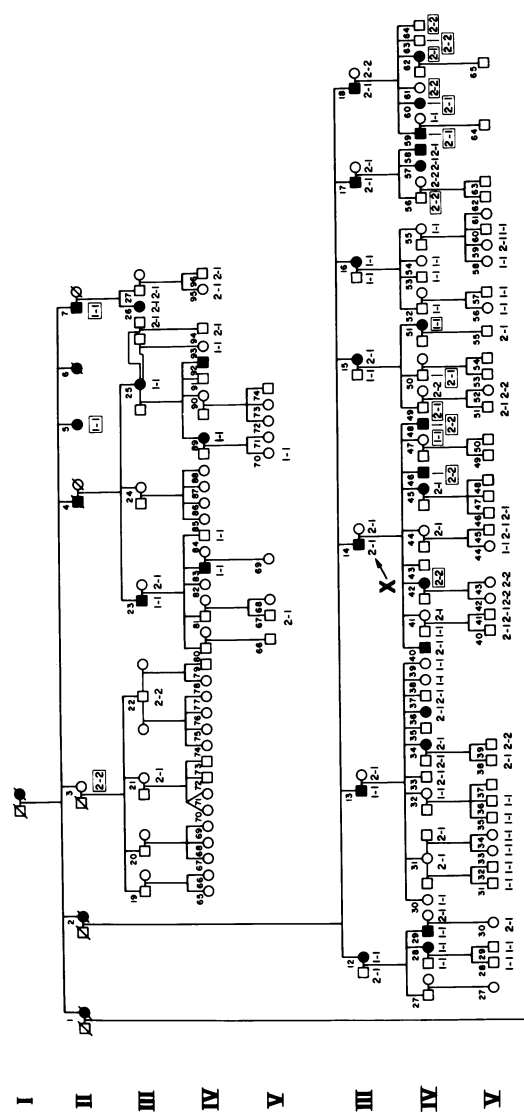

FIG. 1.—Pedigree of family with VMD-1, showing disease phenotype (*filled* or *empty symbols*) and GPT1 phenotypes. *Slash*, decreased; *boxes*, GPT1 phenotypes of informative individuals; *X*, recombinant. Phenotypes with respect to all other polymorphic loci are available from R. E. F. upon request.

RESULTS AND DISCUSSION

In figure 1, the phenotype of each individual over age 14 is designated with respect to disease status (44 affected), and the *GPT1* phenotype of each individual from whom blood was obtained (128 samples) is indicated. Disease status of deceased individuals was determined by review of ophthalmic photographs. The clinical characteristics of *VDM-1* in members of this family have been described by Hittner et al. [1]. The extreme variability of the disorder in this family has been emphasized (see fig. 2). The disease status of all family members was based on severe retinal lesions seen ophthalmoscopically (with or without fluorescein angiography) and on minimal or moderate retinal lesions that may or may not have

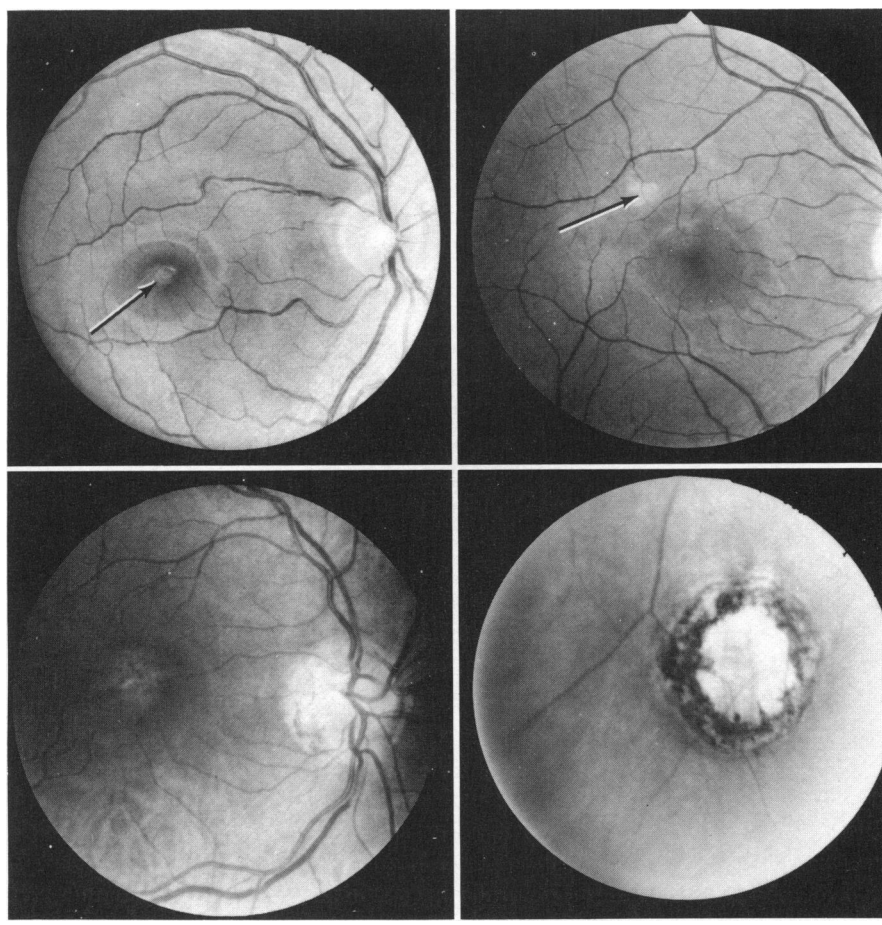

FIG. 2.—Retinal lesions of family members with VMD-1. *A (top left)*, Patient IV_{92} showing moderate central lesion (*arrow*) and temporal peripapillary change; *B (top right)*, patient IV_{45} showing moderate peripheral lesion (*arrow*) and temporal peripapillary change; *C (bottom left)*, patient II_5 showing severe central lesion and advanced peripapillary change; *D, (bottom right)*, patient III_{12} showing severe peripheral lesion.

been seen ophthalmoscopically but which were identified or substantiated by fluorescein angiography. In this particular family, variability of expression, combined with the difficulties in obtaining good fluorescein angiographic studies in small children, makes diagnosis prior to the early teen-age years difficult. Therefore, we decided to eliminate all family members under age 14 from this analysis. This excludes all members of generation V except individuals V_{19-21} and excludes three members of generation IV (IV_{94-96}). Because the early changes observed (macular and peripapillary fluorescein changes and small macular and peripheral yellow lesions) were clearly detectable in children with no clinically significant findings, an arbitrary age truncation seemed reasonable. The analysis performed essentially eliminated the youngest generation, but because of the size of the family, it was not thought that this would substantially bias the lod scores obtained. Since most studies of VMD used diagnostic procedures that fail to detect the minimal lesions identified in members of this family, there are no data to calculate an age-dependent penetrance function. Examination of individuals under age 14 may show the subtle abnormalities that are present quite early in younger members of this family, and this possibility is being explored. If so, careful examination including fluorescein angiography that specifically demonstrates the foveal avascular zone may be useful in the study of other families with hereditary macular degeneration.

In figure 1, the *GPT1* phenotypes of informative individuals are enclosed in boxes. These include three members of generation II (both members of generation I must have had 2–1 *GPT1* genotypes), and 18 members of generation IV. In this family, *VMD-1* behaves as an autosomal dominant trait with complete penetrance and seven instances of male to male transmission. It is, of course, possible that some children of affected persons with the 1–1 *GPT1* genotype who were not studied by fluorescein angiography will be found to be affected in the future. They will not significantly alter the results of this linkage study, only the incidence of the disease. At the present time, 43 of 101 (43%) of those at risk over age 14 are definitely affected.

The results of LIPED analysis for linkage between *VMD-1* and 13 segregating biochemical serological markers is shown in table 1. A significant positive lod score was obtained only for the polymorphic erythrocyte enzyme *GPT1*. A maximum lod score $Z = 4.34$ (odds favoring linkage 22,000 to 1) at a recombination fraction $\theta = .05$ was obtained. Using the convention of Morton [11], we propose that the mutation leading to the phenotype of *VMD-1* is definitely linked to the locus coding for *GPT1* in humans. Visual inspection of the pedigree suggests that, in this family, the *VMD-1* mutation occurs on a chromosome bearing the *GPT1*1* allele and that individual III_{14} represents the only recombinant (see fig. 1, "X"). The recombinant chromosome was clearly inherited in three of his nine offspring. While most of the matings are intercrosses at the *GPT1* locus, this seems to be the most conservative interpretation given the lod scores obtained.

While the information derived from this family establishes linkage between *VMD-1* and *GPT1*, the specific chromosomal assignment of the *VMD-1* gene is still not possible. The locus for *GPT1* has been provisionally assigned to the short arm

TABLE 1

LIPED ANALYSIS FOR LINKAGE BETWEEN VMD-1 AND 13 BIOCHEMICAL AND SEROLOGICAL MARKERS

				Z(θ)				
L-1	L-2	Z	θ	.05	.1	.2	.3	.4
VMD-1	*GPT1*.....	*4.34*	*.05*	*4.34*	*3.90*	*2.66*	*1.39*	*0.41*
VMD-1	ABO	0.00	.50	−6.88	−3.59	−0.96	−0.02	−0.21
	ACP1	0.00	.50	−4.22	−2.24	−0.73	−0.23	−0.04
	ESD.....	0.36	.25	−1.07	−0.19	0.33	0.31	0.12
	Fy	0.00	.50	−6.60	−3.96	−1.66	−0.61	−0.13
	GLO1.....	0.00	.50	−0.57	−0.24	−0.14	−0.19	−0.13
	HPA	0.10	.35	−1.77	−0.82	−0.12	0.08	0.09
	JK	0.72	.20	0.07	0.53	0.72	0.55	0.22
	MNS	0.00	.50	−5.92	−3.26	−1.21	−0.47	−0.15
	P.....	0.00	.50	−2.75	−1.38	−0.35	−0.04	0.00
	PGM1.....	0.00	.50	−5.63	−3.49	−1.56	−0.60	−0.12
	PGP.....	0.00	.50	−5.64	−3.22	−1.14	−0.26	−0.06
	Rh.....	0.00	.50	−6.97	−3.79	−1.29	−0.30	−0.07

L-1 = locus 1; L-2 = locus 2; Z = maximum lod score; θ = recombination fraction at Z; Z(θ) = lod score (Z) at various values of recombination (θ). Only family members at least 14 years old were included in the LIPED analysis.

of chromosome 16, and more precisely to the p12-p13 region [12]. This family was also segregating for two additional markers that have been assigned to chromosome 16. Haptoglobin has been assigned to 16q21 based on its proximity to the 16q22.00 fragile site [13]. The *PGP* locus has been assigned to the short arm of chromosome 16 [14]. Significant positive lod scores for linkage were not observed for these additional chromosome 16 markers nor for any other segregating markers in this family with *VMD-1*.

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