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Segregation and Linkage Analyses of von Hippel Lindau Disease among 220 Descendants from One Kindred

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SUMMARY

Von Hippel Lindau disease (vHL), an autosomal dominant precancerous condition, had segregated in a large kindred. Fourteen relatives were known to have been affected; record reviews disclosed features of vHL in 15 previously undiagnosed relatives; presymptomatic evaluations detected vHL in 13 additional members of this kindred. Altogether, among 220 descendants of an ancestral couple, 41 had vHL.

We screened for HLA haplotypes and for polymorphic gene markers at 31 loci in 102 direct descendants and 16 spouses from this kindred, including 23 with vHL. Linkage analyses failed to reveal a significant lod score with any locus tested, or any HLA linkage disequilibrium.

Expression of vHL among the affected relatives was compared with 384 other reported cases of vHL. The age of onset, tissue involvement, and life expectancy in this family were similar to the other reported cases. The sigmoid age-of-onset distribution for vHL most closely matched a square-foot transformation (mean = 26.2^{-2} years; variance = 1.224).

INTRODUCTION

Von Hippel-Lindau disease (vHL) is an autosomal dominant, pleiotropic, precancerous condition, producing cysts and solid tumors in the retina, central nervous

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system, kidneys, pancreas, and elsewhere. It causes retinal, posterior cranial fossa, spinal cord, renal, and pancreatic malignancies [1, 2]. Since 1926, over 384 cases of vHL have been reported in the English-language literature [1–5]. The disease has been found among Africans, Europeans, and Orientals. An individual with the vHL genotype appears to be susceptible to any of its lesions in any combination and in any sequence. The onset of first symptoms is usually between ages 20 and 50, but it has presented in newborn infants and in individuals as old as age 80.

We studied a large kindred that had 14 members known to be affected with vHL, all related via a founder couple, ascertained by us through one proband (fig. 1) [2, 5–11].

Investigations in this family were conducted with the following goals: (1) to determine whether this family had typical vHL; (2) to assess the heterogeneity of vHL within this family; (3) to compare this kindred with published cases for age of onset, tissue involvement, and natural history; and (4) to seek linkage of vHL to a gene marker, for estimating the risk for vHL among presymptomatic relatives.

Clinical aspects to detect affected patients, to screen for presymptomatic subjects with vHL, and to provide timely treatment are presented elsewhere [2, 5, 7–11].

SUBJECTS AND METHODS

The Kindred

The family consisted of 220 individuals in 5 generations (fig. 1), all directly descended from an immigrant Puerto Rican couple, subjects I-1 and I-2, and their two children, II-1 and II-2. A partial pedigree, based on record linkage data, has been reported independently, with some errors [12]. Table 1 lists data for the 42 affected individuals, including the 13 new cases that were detected by screening.

Methods

Pedigree information was obtained from members of the kindred. Patient records, surgical findings, and autopsy histology were examined when available. All surviving direct descendants of subjects I-1 and I-2 who were over age 6 and the spouses of direct descendants were invited to participate in a project that included clinical evaluations, presymptomatic testing for evidence of vHL [2, 5, 7–10], and gene-linkage studies.

Lymphocytes were obtained for HLA serotyping [13]; whole blood, urine, and parotid saliva were shipped promptly on ice to the University of North Carolina for gene-marker studies.

The following autosomal markers were analyzed: ABO, Rhesus (RhC, RhD, RhE), MN, Kell, P, Kidd (Jk), Lutheran (Lu), Duffy (Fy), Km, Gm, pancreatic amylase (AMY2), phosphogluconate dehydrogenase (PGD), adenosine deaminase (ADA), adenylate kinase (AK1), phosphoglucomutase I (PGM1), haptoglobin (HP), group specific protein (GC), orosomucoid (ORM), esterase D (ESD), glyoxalase I (GLO), acid phosphatase I (ACP1), third component of complement (C3), uridine monophosphate kinase (UMPK), properdin factor B (BF), erythrocyte glutamic-pyruvic transaminase (GPT1), Lewis (Le), secretor (Se), hexose-6-phosphate dehydrogenase (H6PD), and double band (DB). These represent 28 loci, known to be localized on at least 11 arms of 10 autosomes (table 2). Techniques used to identify these markers are described elsewhere [14–16].

Segregation analysis using Bernoulli's incompletely dominant genetic model derived the best estimates of genotype for offspring in each sibship. This allowed for uncertainties

in gene assignments [17–20], because many younger family members must have been heterozygous but had not yet become affected. Lod scores were calculated ([21] and R. C. Elston and E. B. Kaplan, GENPED—A general pedigree analysis program, personal communication, 1973) for recombination values from 0 to .45 at .05 increments; lod scores $> +3.0$ were to be considered significant.

Age of onset can be defined as the age at medical intervention, the age of first symptoms, or the age at presymptomatic detection. In our calculations of symptomatic age of onset, the patients we detected presymptomatically were omitted. These data were compared with and then combined with data from the 384 vHL cases described in the English-language literature [2, 7]. Age of symptomatic onset or diagnosis was calculated for a total of 340 published cases with adequate data and for 23 cases from this kindred [2, 10].

RESULTS

Out of this kindred, 102 direct descendants and 16 surviving spouses of descendants, in 43 sibships, participated in the gene-linkage study, including 26 individuals who showed manifestations of vHL (fig. 1). Its most frequent manifestations were retinal hemangioblastoma (affecting 22), renal cysts and carcinoma (affecting 23), and cerebellar or spinal hemangioblastoma (affecting 18).

The diagnosis of vHL had been made prior to our study in 14 patients, six presymptomatically; we made the diagnosis of vHL retrospectively in 15 affected, and detected 13 new cases, including two, subjects IV-49 and IV-66, who developed symptoms after initial screening had been negative. The segregation of affected individuals in this kindred was entirely consistent with a single major autosomal allele, dominant in expression. Both sexes were equally affected, with male-to-male transmission. Seventeen affected parents in generations I–III had 38 affected and 51 unaffected children (discounting eight who had died in infancy). In generation IV, 13 affected parents had 38 children; only three of these have become affected so far. Expression was .77 for presumed heterozygotes, and $< .003$ for probable nonheterozygotes.

The clinical manifestations of vHL in the patients from this kindred were compared with 384 other published cases. Close similarities were found in age of onset, tissues affected, malignant propensity, and life expectancy, except that thorough perusal of medical records and screening failed to detect polycythemia or pheochromocytoma in any patient from this kindred. Patterns of expression were similar in the different branches of this kindred.

None of the lod scores for 27 informative loci was linked to susceptibility for vHL; salivary protein DB was not informative. Negative lod scores, $Z \leq -2.0$, strongly suggested nonlinkage to: Rh, MN, P, Jk, Lu, Fy, PGM1, HP, GC, ORM, ESD, UMPK, BF, GPT1, Le, Se, and H6PD. No linkage disequilibrium was found with any HLA type; this was supported by the negative lod scores for BF, in the major histocompatibility region of chromosome 6p. The highest lod score was +.74 for GPT, at recombination fraction .10 (table 2).

Chromosome-banding studies (by Dr. P. A. Jacobs, University of Hawaii) failed to identify any structural abnormalities or any informative familial segregation of polymorphic markers in key affected and unaffected relatives.

The age-of-onset distributions showed no significant differences between the 23 subjects who presented with symptoms in this family and 340 other published

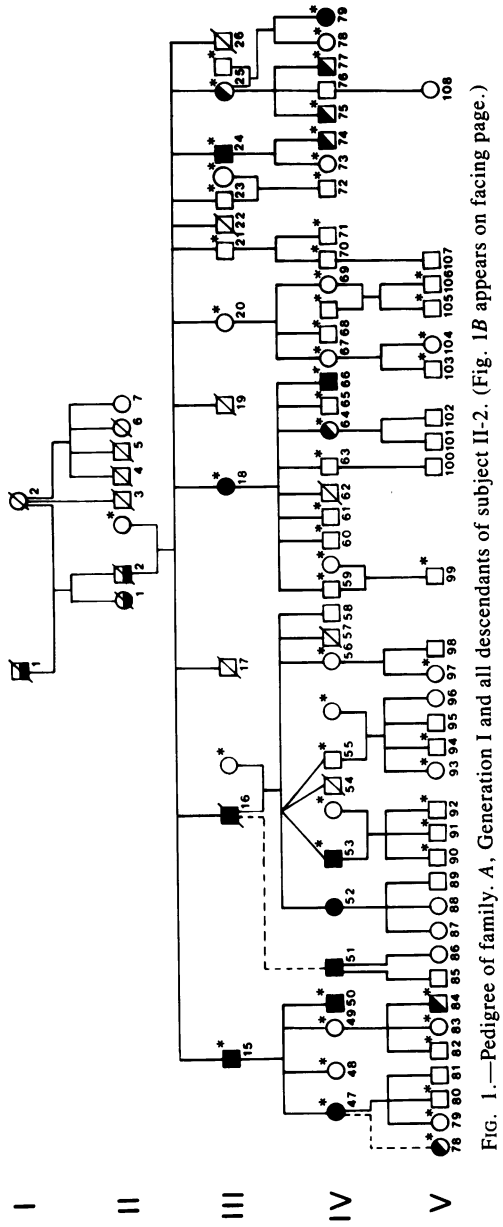

FIG. 1.—Pedigree of family. *A*, Generation I and all descendants of subject II-2. (Fig. 1*B* appears on facing page.)

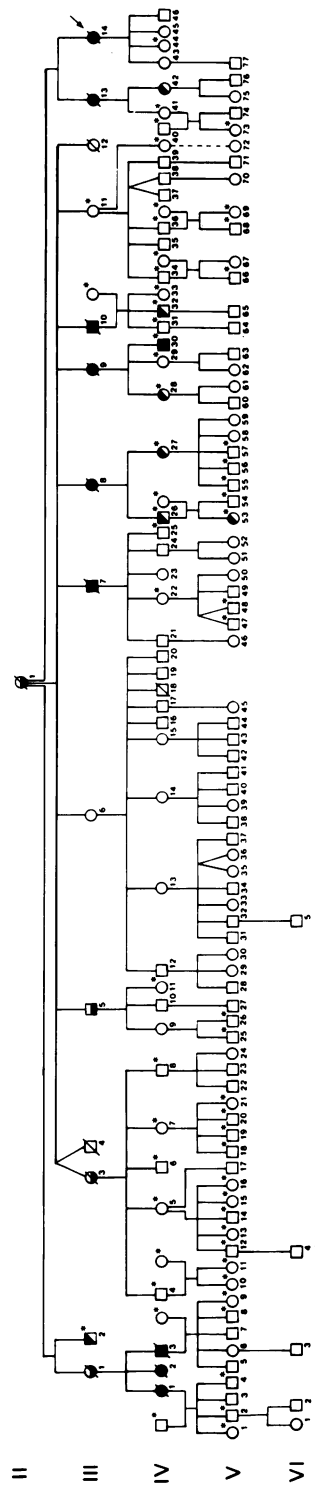

FIG 1.—*B*, All descendants of subject II-1. *Solid symbols* represent affected symptomatic individuals. *Asterisks above pedigree symbols* indicate participants who were tested. *Obliquely half-filled symbols* are affected individuals, detected presymptomatically. *Horizontally half-filled symbols* are probably affected. (Fig. 1*A* appears on facing page.)

TABLE 1

AFFECTED MEMBERS OF THE KINDRED

PEDIGREE NO.	SEX	AGE OF ONSET	AGE AT DEATH	MODE OF PRESENTATION	WHETHER SCREENED	TISSUES AFFECTED				
						Ocular	Neural	Renal	Pancreatic	Epididymal
I-1	M	?	?	I	...	...	...	...	...	...
II-1	F	48	48	M	...	...	...	?R+	...	...
II-2	M	42	42	I	...	?O	...	?R+	...	...
III-1	F	25	25	H,N	...	...	?N	...	...	...
III-2	M	66*	...	P	T	...	...	(R)	...	...
III-3	F	54	54	M	...	...	...	...	P+	....
III-5	M	42	...	H	...	...	N	...	...	...
III-7	M	42	55A	H,N	...	...	N	R+	P	...
III-8	F	25	31A	M	...	...	N	R+	P	...
III-9	F	45	52	H,N,M	...	...	N	R+	P	...
III-10	M	17	33	H	...	...	N	...	...	...
III-13	F	18	35	V	...	O	...	R	P	...
III-14	F	28	44	M	...	...	...	R+	...	...
III-15	M	51	...	M	T	...	N	R+	...	...
III-16	M	35	52	H,N	...	...	N	...	...	...
III-18	F	39*	...	L	T	(O)	...	(R+)	(P+)	...
III-24	M	33	...	V	T	O	N	R+	...	...
III-25	F	38	...	P	T	O	...	(R+)	(P)	...
IV-1	F	38	40	L	...	...	...	R+	P	...
IV-2	F	38	41A	M	...	O	...	R	P+	...
IV-3	M	45	46A	M	...	...	...	R+	...	...

IV-26	M	37*	...	I,P	T	(?O)	...	...	(?P)	...
IV-27	F	35*	...	I,V	T	?O	...	(R)	...	...
IV-28	F	35*	...	P	T	...	...	(R+)	(P)	...
IV-30	M	17	...	H,N	T	(?O)	N	...	(P)	(E)
IV-32	M	22*	...	P	T	(O)	(N)	(R+)	(P)	(E)
IV-42	F	23*	...	P	E	(O)	...	(R+)	(P)	...
IV-47	F	31	...	H,N	T	O	N	...	...	...
IV-49	F	34	...	P,N	T	...	(N)	(R)	...	...
IV-50	M	25	...	N	T	O	N	R+	...	(E)
IV-51	M	31	...	M,N	E	(O)	(?N)	(R+)	P+	...
IV-52	F	32	...	H	E	...	(N)	...	...	...
IV-53	M	29	...	N	T	...	N	...	...	(E)
IV-64	F	25*	...	P	T	(O)	...	...	...	...
IV-66	M	18	...	H	T	...	(N)	...	...	...
IV-74	M	17	...	P	T	(O)	...	...	...	(E)
IV-75	M	20	...	P	T	O	...	(R+)	(P)	(E)
IV-77	M	20	...	P	T	O	...	...	...	(E)
IV-79	F	8	...	V	T	O	...	...	...	...
V-53	F	17*	...	P	T	(O)	...	...	...	...
V-78	F	15	...	P	T	O	...	...	...	...
V-84	M	10*	...	P	T	(O)	...	...	...	...
Total		42 (21 M, 21 F)	15	...	26	22	18	23 18+	14 3+	6

NOTES: Underlined ages of onset were presymptomatic;* = in our study. E = examined; T = tested; A = autopsied. Mode of presentation: I = inferred; M = abdominal mass; H = headache; L = incidental finding at laparotomy; N = neurological (ataxia, paraesthesia); P = presymptomatic; V = visual. Tissues affected: O = ocular; N = neural; R = renal; P = pancreatic; + = malignant; E = epididymal; ( ) = detected by our screening.

TABLE 2

LOD SCORES BETWEEN THE vHL SUSCEPTIBILITY GENE AND 27 MARKER LOCI, WITH PROBABLE CHROMOSOME LOCATIONS [31, 32]

CHROMOSOME		RECOMBINATION FRACTIONS				
Marker	Location	0	.10	.20	.30	.40
ABO	9q34	−0.94	−0.59	−0.37	−0.21	−0.08
RhC	1p36-p32	−8.01	−1.88	−0.84	−0.36	−0.12
RhD	1p36-p32	+0.06	+0.03	0.00	−0.01	−0.01
RhE	1p36-p32	−6.36	−1.58	−0.78	−0.34	−0.01
MN	4q28-q31	−15.58	−3.62	−1.53	−0.61	−0.19
Kell	...	−1.15	−0.58	−0.33	−0.17	−0.07
P	6	−5.99	−2.01	−1.33	−0.81	−0.38
Jk	2	−2.92	−1.41	−0.67	−0.28	−0.08
Lu	...	−10.02	−3.26	−1.60	−0.69	−0.22
Fy	1q13	−13.94	−3.17	−1.36	−0.57	−0.22
Km	...	−3.64	−0.46	+0.37	+0.58	+0.43
Gm	...	−1.28	−0.58	−0.27	−0.11	−0.04
AMY2	1p22.1-q11	−0.08	−0.04	−0.02	−0.01	−0.00
PGD	1pter-p34	−0.40	−0.24	−0.15	−0.07	−0.03
ADA	20q13.2-ter	+0.05	+0.04	+0.03	+0.02	+0.01
AK1	9q34	−0.01	−0.01	0.00	0.00	0.00
PGM1	1p22.1	−7.26	−1.63	−0.82	−0.38	−0.13
HP	16cen-q22	−13.23	−3.36	−1.32	−0.38	−0.02
GC	4q11-q13	−2.87	−1.26	−0.59	−0.27	−0.09
ORM	9q34	−9.08	−2.46	−1.06	−0.36	−0.05
ESD	13q14	−13.36	−3.87	−1.91	−0.92	−0.34
GLO1	6p22-p21	+0.06	+0.47	+0.61	+0.56	+0.35
ACP1	2p23 or p25	−0.89	−3.77	−1.95	−0.92	−0.31
C3	19	+0.19	+0.17	+0.13	+0.09	+0.04
UMPK	1p32	−7.05	−1.68	−0.30	+0.20	+0.27
BF	6p23-p2105	−9.34	−2.33	−1.23	−0.54	−0.20
GPT1	16pter-p11	−2.71	+0.74	+0.67	+0.49	+0.27
Le	...	−2.41	+0.69	+0.65	+0.43	+0.07
Se	19	−2.91	−1.19	−0.58	−0.28	−0.11
H6PD	...	−2.91	−1.19	−0.58	−0.28	−0.11

NOTE: Lod scores at recombination fraction ($\theta_M = \theta_F$).

cases; the combined age-of-onset distribution of these 363 cases better matched a square-root transformation (mean: 26.2^{-2} years; variance: 1.224) than a logarithmic transformation (fig. 2).

DISCUSSION

Virtually all published cases of vHL have had affected relatives [1–4], consistent with autosomal dominant transmission. The two sexes were equally affected, with affected father-son pairs. Occasional skipped generations have been attributed to variable age of onset. The single claim of a family with autosomal recessive inheritance has been retracted because vHL was subsequently expressed in key relatives ([22] and M. H. K. Shokeir, discussion at presentation of [6], Vancouver, October 1978).

Our kindred is the largest known in which the vHL gene has segregated, affecting the most relatives. Subject I-1 had several reportedly unaffected children from previous marriages, and subject I-2 had remarried twice and had borne five other unaffected children (II-3–II-7), yet the only two children born to this couple

were both affected, and in the next generation, among the 20 surviving beyond childhood, 15 were affected. The vHL gene in this kindred followed an autosomal dominant segregation pattern, modified by variable age of symptomatic onset; subject III-2 was asymptomatic until age 64. No obligate heterozygote has failed to express the phenotype; the only apparent exception, subject IV-49, later presented with seizures due to cerebellar hemangioblastomata (fig. 1, table 1). Generations V and VI of our kindred must still contain many heterozygous presymptomatic children and grandchildren of the affected who were undetected by screening because of their youth.

It was disappointing that the diagnosis of vHL had been missed by the physicians caring for many of the affected relatives, and disturbing that even when the diagnosis had been made, relatives were not told, screened, or given genetic counseling. A few immediate relatives of affected members had been screened

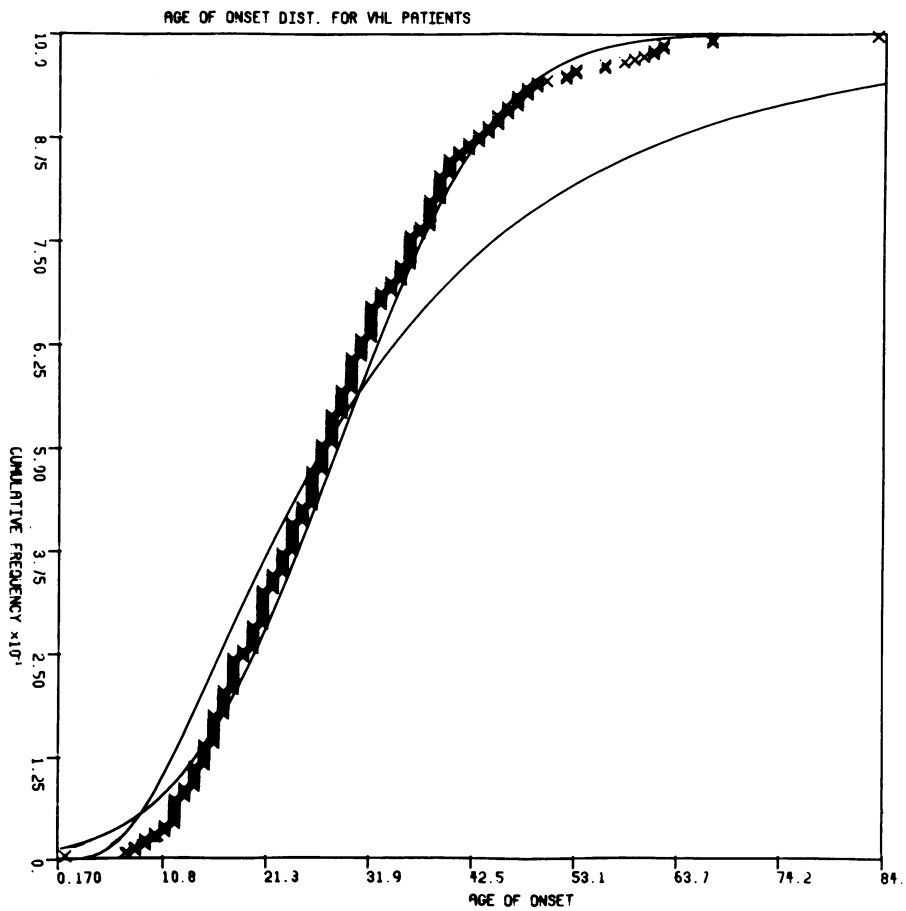

FIG. 2.—Cumulative observed age-of-onset distribution for 363 vHL patients, plotted according to a square-root transformation (*crosses*), compared with the expected cumulative square-root normal (*steeper line*), and expected cumulative normal distributions.

for retinal lesions by ophthalmologists, but only the physicians and geneticists looking after subject III-24 checked thoroughly for vHL lesions in immediate relatives and gave genetic counseling, including advice that his relatives should be screened for manifestations of vHL [23].

Patients from this kindred did not differ substantially from 384 reported vHL cases [2, 10], except for the absence of pheochromocytoma and polycythemia. Polycythemia had been recorded in 10%–20% of vHL patients who had cerebellar, renal, or suprarenal tumors [1, 2]. Pheochromocytoma was reported in 69 of 384 patients with vHL (15 of 88 autopsied cases [2, 24]). These differences could be due to variable pleiotropic expression of a single mutant allele of major dominant effect, or to statistical variability in the small numbers available for comparison.

The sigmoid age-of-onset distribution for vHL (fig. 2) was analogous to that for Huntington disease [25]. This distribution can be useful for calculating heterozygote probabilities for asymptomatic relatives of vHL patients [26]. Although the distribution gave remarkably close fit to a square-root transformation, this fit has no recognized biological significance.

Severity did not correlate with the distribution of lesions, since some vHL patients never developed retinal or neural tumors (e.g., subjects III-2 and IV-3, table 1), and others never had renal involvement, even when they survived to late adulthood (subject III-10 [2, 6]). Small retinal and neural lesions would be much more likely to produce symptoms than renal or pancreatic lesions of a comparable size, while epididymal tumors would rarely cause an individual to seek medical attention, most often being an incidental finding, as in our seven cases.

Analyses for gene-linkage in a single kindred with many informative matings has important advantages over studies on multiple smaller unrelated families. It provides opportunity to study the expression of a common ancestral gene among many descendants, and gene linkages are less likely to be obscured by chance crossings-over, because these would distort linkage associations in only one branch of the family. This was the justification for our gene marker project [27, 28], and the basis for our strategy. Our failure to identify linkage of vHL with a known gene marker was disappointing but not surprising, since major regions of the human genome remain unmapped and not closely linked to known markers. We estimate that the prior probability of finding detectable linkage with the loci we studied is .37 [29–32]. Negative linkage data from this large kindred are still of value, since these lod scores can be incorporated into future studies.

Since an ever-increasing number of restriction endonuclease polymorphic sites are being mapped, studies using DNA polymorphisms would be the next logical step for identifying and localizing the vHL gene [33, 34].

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