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A Genetic Study of Hirschsprung Disease

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Summary

Hirschsprung disease, or congenital aganglionic megacolon, is commonly assumed to be a sex-modified multifactorial trait. To test this hypothesis, complex segregation analysis was performed on data on 487 probands and their families. Demographic information on probands and the recurrence risk to relatives of probands are presented. An increased sex ratio (3.9♂:♀) and an elevated risk to sibs (4%), as compared with the population incidence (0.02%), are observed, with the sex ratio decreasing and the recurrence risk to sibs increasing as the aganglionosis becomes more extensive. Down syndrome was found at an increased frequency among affected individuals but not among their unaffected sibs, and the increase was not associated with maternal age. Complex segregation analysis was performed on these family data. The families were classified into separate categories by extent of aganglionosis. For cases with aganglionosis beyond the sigmoid colon, the mode of inheritance is compatible with a dominant gene with incomplete penetrance, while for cases with aganglionosis extending no farther than the sigmoid colon, the inheritance pattern is equally likely to be either multifactorial or due to a recessive gene with very low penetrance. A model of gene action with random effects during morphogenesis is compatible with our observations.

Introduction

Hirschsprung disease (HRSD), or aganglionic megacolon, is associated with a lack of intrinsic ganglion cells in the myenteric (Auerbach) and submucosal (Meissner) plexuses in the gastrointestinal tract. HRSD has an estimated population incidence of approximately 1/5,000 live births, with males being 3.5–4.0 times more likely to be affected than females (Bodian and Carter 1963; Passarge 1967, 1972; Goldberg 1984; Garver et al. 1985; Spouge and Baird 1985). However, as the aganglionosis becomes more extensive, the sex ratio decreases, suggesting etiologic heterogeneity. HRSD also shows an elevated incidence among Down syndrome individuals (Spouge and Baird 1985).

HRSD has been noted to be familial, with risks to relatives being much higher than the incidence in the general population (Bodian and Carter 1963; Passarge 1967; Garver et al. 1985). This suggests that there are genetic components to its etiology, but the pattern of inheritance does not appear to be due to a single gene in all families. Thus, HRSD has been assumed to be a multifactorial disorder, with multiple genes and the environment playing a role in the development of the clinical phenotype (Bodian and Carter 1963; Passarge 1967; Garver et al. 1985). Furthermore, the recurrence risk to relatives is dependent on the sex of the proband as well as on the sex of the relative, an observation that is compatible with a sex-modified multifactorial inheritance model.

HRSD is not purely due to multifactorial inheritance, since several large pedigrees with HRSD have been reported. These pedigrees do not appear to include other anomalies, e.g., Waardenburg syndrome. Bodian and Carter (1963) described four families in which HRSD appears to segregate as an incompletely penetrant autosomal dominant trait; Passarge (1967, 1972) has reported on two additional families. Kindreds in which HRSD appears in multiple sibships and multiple generations have also been reported by Verdy et al. (1892), Jarmas et al. (1983), and Lipson and Harvey (1987). In all of these pedigrees, the parents of the HRSD cases are almost never affected, although they appear to trans-

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mit a gene for HRSD. Other familial cases include a family with two affected brothers and two affected maternal uncles (Reynolds et al. 1983), parent-offspring transmission (Prier and Hochberger 1981; Carmi et al. 1982), multiple affected sibs with unaffected parents (Laurence et al. 1975; Prier and Hochberger 1981; al-Gazali et al. 1988; Santos et al. 1988), and affected half-sibs with a common unaffected father (Hamilton and Bodurtha 1989).

Further evidence of genetic etiologies of HRSD is provided by chromosomal abnormalities in HRSD patients, of which trisomy 21 (Down syndrome) is probably the most frequent. Partial trisomy of 22q11 and 11q23 (Beedgen et al. 1986), and deletion of 2p22 in combination with the reciprocal translocation (3;7)(p21;q22) (Webb et al. 1988), have all been associated with single cases of HRSD. Sparkes et al. (1984) describe a remarkable patient with retinoblastoma and long-segment HRSD with a constitutional deletion of 13q14.1-q22.3. Recently, Lamont et al. (1989) have described two patients with short-segment HRSD and deletions between 13q22.1-q32.1; these two patients also had other intestinal anomalies. These latter findings suggest that there may be a gene for HRSD on the long arm of human chromosome 13. The phenotypic features of these three HRSD cases suggest that a family of genes regulating gut development may reside on chromosome 13q.

There are several animal models for HRSD—in the mouse, rat, and the horse—and, in each of these cases, a single-gene mutation is the cause of congenital megacolon. In the mouse, three independent loci can each cause aganglionosis. Two of these mutations, piebald-lethal (s^l) and lethal-spotting (*ls*), are autosomal recessive (Lane 1966), and one mutation, dom spotting (*Dom*), is an autosomal semidominant (Lane 1984). Biological studies of these mutant mice suggest failure of the migration of neural crest cells in the piebald-lethal mouse (Webster 1973). This cell migration defect may be secondary to either a cell adhesion defect or a deficiency in the number of neural crest cells. Failure of the neural crest cells to colonize the gut, as in the lethal-spotted mouse (Jacobs-Cohen et al. 1987), is also a possible etiology. The cause for this may be an environment unfavorable to neural crest cell development or a failure in the maturation of neural crest cells. The *Dom* mutation has an unknown etiology.

No formal pedigree analysis has ever been performed on HRSD to elucidate the mode of inheritance. A recurrence risk to siblings of 1.5%–17.6% (odds ratio 75–880) depending on the extent of aganglionosis (Bodian and Carter 1963; Passarge 1967; Garver et al. 1985), relative to the population incidence of 0.02%, appears too high to be explained by multifactorial inheritance, while a single-gene model remains probable at least for some cases. The presence of single genes leading to megacolon in the mouse also suggests the possibility that their single-gene homologues may cause HRSD in humans. Since single genes can be identified through linkage studies, this would lead to improved genetic counseling and possible determination of the molecular etiology of HRSD.

In the present study, we present empirical information on both the sex ratio among probands and the recurrence risk to sibs, by the extent of aganglionosis. Inheritance of HRSD was studied by analyzing the family histories of probands. Pedigree analysis using well-established methods of segregation analysis was performed on the family data. This analysis indicated genetic heterogeneity by the extent of aganglionosis. These results are presented, as is the recurrence risk to various relatives as calculated under the inferred genetic model. Estimates of the expected prevalence of Down syndrome in this HRSD population are calculated using the observed maternal age distribution and are compared with the observed prevalence.

Material and Methods

Data on 477 families (487 probands) were collected from several sources. In all cases, families were ascertained through at least one proband with histologically proved aganglionosis. Information on 212 families (218 probands) was obtained from patients treated at the Children's Hospital of Pittsburgh between 1950 and 1977. Data on the first-degree relatives of probands were obtained through questionnaires filled out by the families and/or through medical records. In families with information from both sources, the information was found to be consistent. Data on 134 of these families were previously used in a study to estimate the empirical risk to sibs of HRSD probands (Garver et al. 1985).

Family histories on 203 additional families (207 probands) were culled from the surveys of HRSD by Bodian and Carter (1963) and Carter et al. (1981). These probands were treated at the Hospital for Sick Children in London between 1948 and 1959. Bodian and Carter (1963) interviewed parents of patients with regard to family histories and included parents, siblings, aunts, uncles, and first cousins of the index case. Complete information on first-degree relatives was available; information on other relatives was available only if at least one of them was affected. The study by Carter

et al. (1981) analyzed the incidence of HRSD in the children of the probands in their 1963 study.

In addition, information on 62 families (62 probands) was found in publications by Passarge (1967, 1972). These data were obtained from medical and pathological records at various hospitals in Cincinnati, primarily at the Children's Hospital, and from the family or family physician. All probands were examined between 1948 and 1966. All families included in the present study had information regarding affection with HRSD on all first-degree relatives. In some families, information on more distant relatives was available if at least one was affected.

The total family data were subdivided by considering all cases wtih aganglionosis at or beyond the transverse colon to be *long segment* and by considering the rest of the cases to be *short segment.* This division was made because the embryonic midgut is the precursor of the gastrointestinal tract from the duodenum to the proximal two-thirds of the transverse colon, while the embryonic hindgut is the precursor of the distal one-third of the transverse colon to the rectum. This difference in embryonic origin is responsible for differences in vascular supply and extrinsic nerve supply (Langman 1981). However, other authors have defined *long segment* as aganglionosis extending to or beyond the descending colon (Bodian and Carter 1963; Garver et al. 1985). Preliminary calculations of recurrence risk from the total data (see table 1) indicated that there is increased familial risk in cases with aganglionosis extending beyond the sigmoid colon. Therefore, short-segment HRSD was further divided into (*a*) recto-sigmoid-segment HRSD, with aganglionosis extending from the rectum up to the sigmoid colon, and (*b*) colonic-segment HRSD, with aganglionosis extending from the rectum to the descending colon or splenic flexure. Because Passarge (1967, 1972) did not subdivide the short-segment HRSD data further in the Cincinnati sample, that data set could not be used for this part of the analysis. A family was classified according to the extent of aganglionosis of its most severely affected member. All families with probands affected with HRSD and Down syndrome were excluded from the calculation of empirical risks and the segregation analyses.

Complex segregation analysis was performed on the family data by using both the mixed model of inheritance (Morton and MacLean 1974) and the method of pointers (Lalouel and Morton 1981) (see fig. 1). For a dichotomous trait, the mixed model assumes that familial aggregation of a trait is due to an underlying, but unobservable, liability scale (y) to which Mendelian inheritance of a single gene (l), mulifactorial transmission (c), and random environmental effects (e) contribute additively and independently: $y = l + c + e$. Affection status is defined by a threshold Z on the liability scale, such that all individuals with a liability value above Z are defined as affected. For a dichotomous trait, l is assumed to have mean 0 and variance L. The multifactorial transmissible component and the environmental component are assumed to be normally distributed as $N(0,C)$ and as $N(0,E)$, respectively. Thus, the phenotypic variance is the sum of the three variance components, i.e., $V = L + C + E = 1$. The major locus has two alleles, G and g, with the disease al-

Table 1

Sex Ratio and Recurrence Risks to Sibs for HRSD, by Segment Affected

Segment Affected	No. of Cases	Sex Ratio (♂:♀)	No. of Sibs	Risk to Sibs (%)
Rectum	63	5.3	109	0
Rectosigmoid	98	7.2	227	2
Sigmoid	166	3.9	308	5
Descending colon	24	2.0	57	2
Splenic flexure	21	1.3	31	11
Transverse colon	5	.2	8	55
Hepatic flexure	3	∞	6	17
Ascending colon	9	.4	15	13
Terminal ileum	17	2.4	24	16
Ileum and above	10	2.3	27	17
Short	424	4.4	847	3
Long	49	1.9	97	17
All cases	487	3.9	979	4

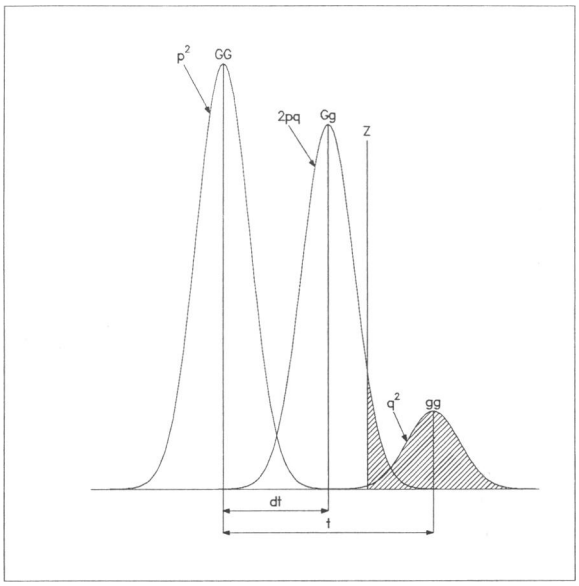

Figure 1 Mixed model of inheritance, representing the phenotypic distributions of the genotypes of the major gene. G = normal allele; p = frequency of G; dt = displacement between the mean of the normal homozygote and the mean of the heterozygote; Z = liability threshold for being affected. All other terms are as defined in the text.

lele (g) having a frequency q. The difference, in units of SD, between the means of the liability distribution of the two homozygous classes is t, and the larger the numerical value of t the greater the effect of the mutant gene on the phenotype. The degree of dominance is d, so that t multiplied by d is the difference between the mean of the homozygous normal class and the mean of the heterozygous class. When $d = 1$, the heterozygous liability distribution is the same as the disease-allele homozygous distribution; therefore, the disease is dominant. When $d = 0$, there is no displacement between the first homozygous class and the heterozygous class; therefore, the disease is recessive. For major genes with additive effects, $d = 1/2$, and the heterozygous distribution is centered between the two homozygous distributions. The effects of new mutation and selection against gene carriers, when the gene is dominant, is considered through the parameter x, which is defined as the proportion of cases due to new mutations; x is also equivalent to the reduction of reproductive fitness for heterozygotes for the disease allele. Variation around each major genotype is normally distributed with variance due to both multifactorial transmission, C, and random nonfamilial environmental effects, E. Heritability, h, is defined as C/V and is the proportion of familial phenotypic variance due to multifactorial effects. C—and consequently h—may be numerically different in adults and children, possibly because of the effects of a common sibling environment, so these h values are calculated separately. The ratio of childhood h to adulthood h is z. Phenotypic variance which cannot be explained by either a major gene or multifactorial transmission is assumed to be the residual environmental variance, E.

Ascertainment values were estimated from the distribution of the probands in our own data and were calculated separately for long- and short-segment HRSD and for each subset of the data. For short-segment HRSD, probability of ascertainment was calculated as .65, .60, and .01 for the data from Pittsburgh, England, and Cincinnati, respectively. For long-segment HRSD, these values were .80, .01, and .01, respectively. For the extended pedigrees published by Bodian and Carter (1963) and Passarge (1967, 1972), an additional ascertainment correction was necessary, since these families were ascertained only if there was at least one affected person who was not a first-degree relative. This correction was made by arbitrarily labeling one affected person outside the nuclear family as a proband. Thus, these families were analyzed as if they were ascertained through two affected individuals within the family. All pedigrees were divided into their constituent nuclear families by using pointers, as outlined by Lalouel and Morton (1981).

The segregation analysis incorporated the different liabilities to affection for different groups of individuals. The overall incidence was assumed to be 0.02%, of which the risks to long-segment HRSD, colonic-segment HRSD, and rectosigmoid-segment HRSD were in the proportions .10:.11:.79, as estimated from the frequencies of these classes among all 487 probands. Sex differences in the risks were incorporated such that, as observed, 35%, 38%, and 17% of females were affected among all probands with long-segment, colonic-segment, and rectosigmoid-segment HRSD, respectively. To determine which mode of inheritance provides the best fit to the family data, analyses were conducted using the computer program POINTER (Lalouel and Morton 1981), which calculates the likelihood of a *particular* genetic model (sporadic, multifactorial, recessive, or dominant) and compares these with the *general* or best-fitting model incorporating a single gene, multifactorial inheritance, and random environmental effects. Conditional likelihoods were used for each genetic model, and parameters were estimated by the maximum-likelihood method. For the multifactorial model,

the parameters were h and z, and for the major locus model, the parameters were d, t, q, and x (for dominant models only). The relative likelihood of each model was tested by calculating a likelihood-ratio χ^2 (Rao 1965). If a particular model of inheritance was not significantly different from the general model of inheritance, it was taken as the most parsimonious model of inheritance. The most parsimonious model was chosen because it explains the data most efficiently with the least number of parameters.

Results

Sex Ratio, Recurrence Risk to Sibs, and h

The sex ratio and risk to sibs were calculated for each colonic or ileal segment (table 1). Clearly, the sex ratio tends to decrease—and the sibling risk tends to increase—with increasing extent of aganglionosis, a result suggesting etiologic heterogeneity. The sample sizes are small in some categories, so overall trends cannot be definitively stated; however, the sex ratio (χ^2 = 5.13, df = 1, $P < .025$) and sibling risk (χ^2 = 13.16, df = 1, $P < .0005$) are significantly different between long-segment and short-segment HRSD. Table 2 presents the risk to siblings, by sex of the proband and of the sib, for both short-segment and long-segment HRSD. In order to assess the genetic contribution to HRSD, we have estimated h by using the method of Falconer (1965). As can be observed, all of the estimates are high, and none are significantly different from one. High h values do not prove that the disease is inherited as a single gene. However, high h values would be expected if a single gene was primarily responsible for the inheritance of HRSD, since there would be strong familial phenotypic resemblance. Multiple genes with strong effects are also probable. All parents of the probands were unaffected. This would appear to weigh against an autosomal dominant gene leading to HRSD. Since, until recently, HRSD cases would not have survived to reproduce, an incompletely penetrant dominant gene is still compatible with the family data. Among the offspring of HRSD cases in the present study, one of the 111 offspring of short-segment HRSD probands and both of the two offspring of long-segment HRSD probands were affected. Both of the latter two offspring came from Carter's survey of the offspring of the HRSD probands in his original study (Carter et al. 1981). While it is striking that both would be affected, that study gives no evidence that affected offspring were specifically selected for. In fact, of the 103 offspring of HRSD patients studied by Carter et al. (1981), only three were found to be affected: two from long-segment families and one from a short-segment family.

Segregation Analysis

Table 3 presents the results of the segregation analysis for long-segment HRSD. The parameters d, t, q, h, z, and x are as described above. P is the probability that each model explains the data as well as the general model, and it is calculated from the likelihood-ratio χ^2 value. While it is conventional to take a P value less than .05 as indicative of a significant difference, the multiple comparisons being made in the present study may lead to significant differences by chance alone. Therefore, although the convention of stating significance at $P < .05$ will be followed, the models that are marginally significant should not be ruled out.

The general or mixed model shows a dominant gene with new mutation, high h among children and low h among adults. This difference in h values between children and adults may be due to the effects of an envi-

Table 2

Risks to Sibs and *h* of HRSD, by Sex and Segment Affected

Segment Affected	Sex of Proband	Sex of Sib	Risk to Sibs (%)	$h \pm$ SD
Short	Male	Male	5	.97 ± .06
		Female	1	.81 ± .12
	Female	Male	5	.87 ± .12
		Female	3	.92 ± .15
Long	Male	Male	17	1.00[a] ± .11
		Female	13	1.00[a] ± .14
	Female	Male	33	1.00[a] ± .15
		Female	9	1.00[a] ± .24

[a] Values set to a boundary value.

Table 3

Segregation Analysis of Long-Segment HRSD

Model	d	t	q	h	z	x	χ^2	P
Sporadic							334.8	$<1 \times 10^{-5}$
Multifactorial				1.00[a]	1.00[a]		78.2	$<1 \times 10^{-5}$
Major locus								
Recessive	(.00)[b]	8.22	3.8×10^{-3}				35.7	$<1 \times 10^{-5}$
Additive	(.50)[b]	9.30	1.2×10^{-5}				6.5	<.17
Dominant	(1.00)[b]	4.65	1.2×10^{-5}			(.00)[b]	6.5	<.17
Dominant	(1.00)[b]	7.56	1.2×10^{-5}			.19	2.8	.42
General	1.00[a]	7.51	9.6×10^{-6}	1.00[a]	.01	.15		

[a] Parameter went to bound.
[b] Parameter was set and not iterated.

ronment common to sibs, i.e., a cohort effect. This difference may also be caused by parents being unaffected because of selection. The sporadic model is strongly rejected ($\chi^2 = 334.8$, df = 6, $P < 10^{-5}$), and thus there is clear evidence of familial resemblance for long-segment HRSD. The multifactorial model is also rejected ($\chi^2 = 78.2$, df = 4, $P < 10^{-5}$). It is of interest that both adult and childhood h values approached 100%. In spite of the fact that no parents were affected, the recessive model is clearly rejected ($\chi^2 = 35.7$, df = 4, $P < 10^{-5}$). Neither the additive model ($\chi^2 = 6.5$, df = 4, $P < .17$), nor the dominant model without mutation ($\chi^2 = 6.5$, df = 4, $P < .17$), nor the dominant model with mutation ($\chi^2 = 2.8$, df = 3, $P < .42$) can be rejected. The only difference between the model incorporating a dominant gene without mutation and the additive model is the phenotype of individuals who are homozygous for the disease allele of the major gene. Since these individuals would have been very unlikely to have appeared in this data set given the low incidence, these two models cannot be distinguished from each other. The dominant model with mutation is not significantly different from the dominant model without mutation ($\chi^2 = 3.7$, df = 1, $P < .06$). The data were also subdivided by the geographical source (Pittsburgh, England, or Cincinnati) and were analyzed using the multifactorial model, which is a measure of familial aggregation. No heterogeneity was found ($\chi^2 = 0.2$, df = 2, $P < .90$).

For short-segment HRSD, the results of the segregation analysis are demonstrated in table 4. Mutation was found not to be a factor in the initial analyses, so x was not taken into consideration. The general model shows a dominant gene with high h among children and with low h among adults, a result similar to that of the general model for long-segment HRSD. The sporadic model is clearly rejected ($\chi^2 = 178.7$, df = 5, $P < 10^{-5}$), and the multifactorial model ($\chi^2 = 10.9$, df = 3, $P < .025$) and recessive model ($\chi^2 = 13.8$, df = 3, $P < .005$) fit the data poorly. Neither the additive model nor the dominant model is significantly different from the general model ($\chi^2 = 7.6$, df = 3, $P < .10$). As with long-segment HRSD, the dominant model is the most parsimonious model in this case. Also, be-

Table 4

Segregation Analysis of Short-Segment HRSD

Model	d	t	q	h	z	χ^2	P
Sporadic						178.7	$<1 \times 10^{-5}$
Multifactorial				1.00[a]	1.00[a]	10.9	<.025
Major locus							
Recessive	(.00)[b]	3.02	2.5×10^{-2}			13.8	<.005
Additive	(.50)[b]	5.33	2.3×10^{-4}			7.6	<.10
Dominant	(1.00)[b]	2.67	2.2×10^{-4}			7.6	<.10
General	1.00[a]	3.04	1.9×10^{-5}	.83	.02		

[a] Parameter went to bound.
[b] Parameter was set and not iterated.

Table 5

Segregation Analysis of Colonic-Segment HRSD

Model	d	t	q	h	z	χ^2	P
Sporadic						117.5	$<1 \times 10^{-5}$
Multifactorial				1.00[a]	.98	21.0	$<1 \times 10^{-4}$
Major locus							
Recessive	(.00)[b]	5.77	7.6×10^{-3}			20.3	$<1 \times 10^{-4}$
Additive	(.50)[b]	7.85	2.2×10^{-5}			.2	<1.00
Dominant	(1.00)[b]	3.92	2.2×10^{-5}			.2	<1.00
General	1.00[a]	3.85	2.3×10^{-5}	.32	1.00		

[a] Parameter went to bound.
[b] Parameter was set and not iterated.

tween the different geographical sources of data, there was no heterogeneity in familial aggregation ($\chi^2 = 1.7$, df = 2, $P < .43$).

To determine whether the extent of aganglionosis was associated with genetic heterogeneity among the short-segment HRSD cases, the data were further subdivided into rectosigmoid-segment HRSD and colonic-segment HRSD. Table 5 presents the results of the segregation analysis for colonic-segment HRSD. Initial analysis showed that mutation was not a factor, so x was not considered. The general model shows a dominant gene with high h among adults and children. The most parsimonious model is the dominant model ($\chi^2 = 0.2$, df = 3, $P < 1.00$). As with long-segment HRSD, the additive model is very similar to the dominant model. The sporadic model ($\chi^2 = 226.1$, df = 3, $P < 10^{-5}$), multifactorial model ($\chi^2 = 21.0$, df = 3, $P < 10^{-4}$), and recessive model ($\chi^2 = 20.3$, df = 3, $P < 10^{-4}$) can be rejected. Thus, colonic-segment HRSD appears to be inherited in a manner similar to that for long-segment HRSD.

The results of the segregation analysis for rectosigmoid-segment HRSD are presented in table 6. The general model is a pure multifactorial trait. However, this was not significantly better than a recessive model ($\chi^2 = 4.5$, df = 3, $P < .25$). The sporadic model ($\chi^2 = 113.5$, df = 3, $P < 10^{-5}$) and dominant model ($\chi^2 = 10.5$, df = 3, $P < .025$) can be rejected. The additive model ($\chi^2 = 4.5$, df = 3, $P < .25$) is similar to the recessive model in that it shows very low penetrance among heterozygotes (data not shown). In the multifactorial model, the high h value among children and the low h value among adults indicates that there is a high phenotypic correlation between siblings but not between parents and offspring. This is what would be observed in a recessive model. For these data, it cannot be established whether the true genetic model is multifactorial or recessive, since both models fit the data equally well.

Table 7 presents the penetrance and proportion of sporadics for each of the most parsimonious models for the three different forms of HRSD. For the recessive model in rectosigmoid-segment HRSD, the penetrance is low in males and very low in females, with a low rate of sporadics among males. For both colonic-segment and long-segment HRSD, the dominant model demonstrates an incompletely penetrant gene and a

Table 6

Segregation Analysis of Rectosigmoid-Segment HRSD

Model	d	t	q	h	z	χ^2	P
Sporadic						113.5	$<1 \times 10^{-5}$
Multifactorial				.87	.01	.00	1.00
Major locus							
Recessive	(.00)[b]	3.23	3.8×10^{-2}			4.5	<.25
Additive	(.50)[b]	4.48	3.8×10^{-2}			4.5	<.25
Dominant	(1.00)[b]	3.72	1.8×10^{-3}			10.5	<.025
General	.00[a]	.00[a]	.00[a]	.87	.01		

[a] Parameter went to bound.
[b] Parameter was set and not iterated.

Table 7

Penetrance and Proportion of Sporadics for Different Forms of HRSD

Parameter	Rectosigmoid-Segment HRSD[a]	Colonic-Segment HRSD	Long-Segment HRSD
Penetrance (%):			
Male	17	37	66
Female	4	29	51
Sporadics (%):			
Male	4	39	41
Female	0	21	13

[a] Penetrance and sporadics are calculated for the recessive model.

significant number of sporadics. Males have a higher penetrance and a higher rate of sporadic cases. Colonic-segment HRSD is less penetrant and has a higher percentage of sporadics than does long-segment HRSD.

The most parsimonious genetic model allows prediction of the recurrence risk to all relatives, the advantage of this being that risks to individuals in families with structures that are infrequent in the general population (e.g., families with multiple affected individuals) can be estimated. Table 8 presents these risks for the different forms of HRSD. For rectosigmoid-segment HRSD, the risk to sibs is significant, with males having a 4%–6% risk and females having a 1%–2% risk. The risk to offspring and second-degree relatives is negligible under the multifactorial model and is small under the recessive model. In families with multiple affecteds, the risk is elevated over the expectation when only one person is affected. The risk for males is 4%–10%, and that for females is 1%–4%. The range in risk is dependent on the genetic model used and on the relationship and gender of the persons affected. Relatives of colonic-segment HRSD cases had increased risks of HRSD vis-à-vis relatives of rectosigmoid-segment HRSD cases. Male sibs had an 8%–12% risk, and females had a 6%–10% risk, the range in risk being dependent on the sex of the affected individual. The risk to offspring was slightly elevated over that to affected sibs; second-degree relatives had a nontrivial risk (4%–6% for males and 3%–4% for females). If two relatives are affected, the risk is increased to 20% and 15% for males and females, respectively. For long-segment HRSD, the risk to male sibs of affected males is 9%–12%, and that for male sibs of affected females is 21%–24%. For sibs of affected females, the risk is 21%–24% and 17%–19% for males and females, respectively. For offspring the risk for the various parent-child combinations are as follows: male-male 16%–19%, male-female 12%–14%, female-male 27%–29%, and female-female, 21%–22%. The risk to second-degree relatives is similar to that calculated for colonic-segment HRSD. If multiple relatives are affected, the estimated risk is 33% and 25% for males and females, respectively.

Table 8

Risk (in %) to Relatives, as Calculated under the Most Parsimonious Genetic Model for the Different Forms of HRSD

	Rectosigmoid HRSD				Colonic-Segment HRSD Dominant		Long-Segment HRSD Dominant	
	Multifactorial		Recessive					
Parameter	Male	Female	Male	Female	Male	Female	Male	Female
Sibs of affected males	4	1	4–5	1	9–10	7	9–12	7–9
Sibs of affected females	6	2	5	1	12–13	10	21–24	17–19
Offspring of affected males	~0	~0	1	<1	10–11	8–9	16–19	12–14
Offspring of affected females	~0	~0	1	<1	14–15	11	27–29	21–22
Risk to second-degree relatives	~0	~0	<1	~0	4–6	3–5	4–9	3–7
Risk in multiplex families[a]	5–10	2–4	5–9	1–2	19	14	33	25

[a] Two sibs, a parent and sib, or a second-degree relative and sib affected.

Anomalies in HRSD

In the current compiled data, the most common anomaly in the 506 affected individuals is Down syndrome, which was present in 4.5% of the affecteds vis-à-vis 0.3% of 960 unaffected sibs of affected probands. To determine whether this increase is statistically significant, the expected number of Down syndrome cases among affecteds and unaffecteds was calculated from the observed maternal age distribution at the birth of each individual. The prevalence of Down syndrome for each year of maternal age was obtained from Hook and Chambers (1977). For affecteds, the expected number of Down syndrome cases was 0.70 ± 0.84, as compared with 23 observed. For unaffecteds, expected and observed cases were 0.99 ± 0.99 and 1, respectively. The increase of Down syndrome among affecteds but not among unaffecteds suggests that there is an increased risk of HRSD to Down syndrome cases—rather than a common factor, such as maternal age, leading to an increase in both. All but one case had short-segment HRSD; one had unknown extent of aganglionosis. The sex ratio was 10.5 ♂:♀, which is markedly higher than both the 3.9 observed for HRSD overall and the 4.4 observed for short-segment HRSD. Congenital heart defects occur in 29%–39% of all Down syndrome cases (Fabia and Drolette 1970; Pueschel 1983) and have also been found to occur more frequently than expected in HRSD patients (Spouge and Baird 1985). In this sample, 22% of the Down syndrome cases with HRSD were reported to have a congenital heart defect. Although this is not much lower than expected, this may be an underestimate, since the presence of a congenital heart defect was not consistently reported in the data.

A second defect that was common among affecteds was congenital heart disease not associated with Down syndrome. This affected 4.8% of the cases, as compared with 0.5% of the unaffected siblings. Of the 14 cases with congenital heart disease, five had septal defects, one had aortic stenosis, three had murmurs, and five were of unknown type. If only septal defects are studied, they have an incidence of 1.7% in this HRSD population, whereas the population incidence of septal defects is 0.13%–0.37% (Ferencz et al. 1985; Spouge and Baird 1985). The finding of increased prevalence of septal defects among HRSD patients is compatible with previous findings (Spouge and Baird 1985). However, it may not be appropriate to make comparisons between a clinical survey and a population survey, since there may have been both different diagnostic criteria and differences in clinical surveillance. Therefore, the increase of septal defects among HRSD cases may not be as great as suggested by these numbers. No other anomaly occurred in more than 1% of the cases in this sample.

Discussion

The evidence presented here indicates that a dominant gene or several different dominant genes play a role in the etiology of HRSD with aganglionosis extending beyond the sigmoid colon. This is compatible with reports, in the literature, of families in which HRSD appears to segregate as an incompletely penetrant dominant trait (Verdy et al. 1982; Jarmas et al. 1983; Lipson and Harvey 1987). In affected relatives of probands with long-segment HRSD, the majority of the former also had long-segment HRSD, although a significant number were affected with short-segment HRSD. This indicates that expression of this gene is not limited to long-segment HRSD.

It is difficult to determine what role new mutation plays in this model, as the only way to separate sporadics from new mutation is to study the offspring of affecteds. Since few of the affected cases had reproduced, this was not feasible. The fact that females are less likely to be affected than males could simulate an X-linked recessive trait if transmission is from the maternal lineage. Parents are rarely affected, since, until fairly recently, patients with HRSD had low reproductive fitness. Therefore, if only first-degree relatives of HRSD cases are studied, an incorrect genetic model may be inferred.

For rectosigmoid-segment HRSD, which comprises ~80% of HRSD cases, two different modes of inheritance are equally plausible. Information in this data set was primarily on nuclear families. If data on relatives outside of the nuclear family were present, it would have been easier to distinguish between these genetic models. Furthermore, there is evidence of curtailment of childbearing after the birth of a child with HRSD (Badner 1988). Thus, families with multiple affected sibs would be underrepresented and would weaken the evidence for any genetic models. Therefore, in any future studies of HRSD, it would be important to collect information on relatives outside the nuclear family, regardless of their affection status.

The estimated risk to relatives increases with increasing extent of aganglionosis. For rectosigmoid-segment HRSD, there is, under both the multifactorial model and the recessive model, a small but significant risk to sibs, which is increased when there are multiple affected individuals. For other relatives, the risk is very low. The similarity, in risk figures, between these two genetic

models demonstrates the difficulty in distinguishing between these models. For colonic-segment HRSD, there is a significant risk to all first-degree relatives and a lesser risk to second-degree relatives. These risks increase significantly when there are multiple affected individuals. For long-segment HRSD, the risks are elevated over those for colonic-segment HRSD. For colonic-segment and long-segment HRSD, there is a relatively high risk to individuals if both a sib and a second-degree relative are affected, as compared with the risk when only a sib is affected. This demonstrates the importance of taking a careful family history when counseling a family that has HRSD.

HRSD has been associated with Waardenburg syndrome (Omenn and McKusick 1979; Mahakrishnan and Srinivasan 1980; Shah et al. 1981; Cohen and Gadd 1982), and it is thought that this association is due to a common neural crest cell anomaly. Waardenburg syndrome is a complex consisting of a wide nasal bridge secondary to a large intercanthal distance, pigmentary abnormalities of the hair and irises, and cochlear deafness. An individual may be mildly affected with only some of these features or be severely affected with multiple anomalies. HRSD in association with Waardenburg syndrome can be inherited as an incompletely penetrant autosomal dominant disorder (Badner and Chakravarti, in press); however, the gene for HRSD in this case may be different from the gene for HRSD not associated with Waardenburg syndrome. Waardenburg syndrome was not known to occur in any of the families in this data set. However, there exists the possibility that the presence of Waardenburg syndrome was not studied or reported for some families in this data set. If this were the case, some of the evidence for a dominant gene may have come from these families. However, there are families in the literature which do not appear to have any manifestations of Waardenburg syndrome but in which HRSD appears to be inherited as a dominant trait (Verdy et al. 1982; Jarmas et al. 1983; Lipson and Harvey 1987). In addition, the gene associated with HRSD with Waardenburg syndrome and the gene associated with HRSD without Waardenburg syndrome may not be distinct from each other but may be associated with pleiotropic manifestations or may be different alleles of the same gene. Therefore, even if cases with Waardenburg syndrome were included, the inclusion of these cases need not lead to an erroneous conclusion.

Other syndromes demonstrating associations with HRSD are metyphyseal chondrodysplasia, McKusick type (McKusick et al. 1965), an autosomal recessive form of dwarfism, and Smith-Lemli-Opitz syndrome type II (Curry et al. 1987), a lethal syndrome with multiple anomalies involving the heart, lungs, kidneys, polydactyly, and sex reversal and an elevated risk of the disease among sibs. HRSD may occur with other congenital anomalies and be inherited as a single phenotype, i.e., HRSD, congenital heart disease, broad big toes, and ulnar polydactyly (Laurence et al. 1975) or HRSD with type D brachydactyly (Reynolds et al. 1983). As with Waardenburg syndrome, none of these syndromes were known to be included in this data set. It is possible that these syndromes were included unknowingly, particularly from the older data sets (Bodian and Carter 1963; Passarge 1967, 1972). Since these synromes are rare, they would appear to have little effect on this genetic analysis.

The above discussion suggests reasons why HRSD has been considered as a multifactorial disorder. Multifactorial inheritance has traditionally been defined as being determined by a combination of genetic and environmental factors. The genetic influence is assumed to be polygenic in nature, with a large number of genes, each with a small effect, acting additively. Frequently a trait will be defined as multifactorial simply because it appears to be familial but does not fit any of the single-gene models. Recently, Kurnit et al. (1987) described how the effects of a single gene with random or stochastic variation during morphogenesis can lead to variable outcomes and appear multifactorial. Their model demonstrates that the expression of a single gene may lead to a normal phenotype, a slightly altered phenotype, or a greatly altered phenotype, all under apparently identical environments. They argue that stochastic factors in gene expression and morphogenesis may lead to tremendous variation in the phenotype of an individual. When recurrence risks are calculated under Kurnit et al.'s model, they are very similar to those expected for multifactorial inheritance. The risks increase with the number of affected relatives and with the severity of the abnormality and are dependent on whether the individual is in a group (e.g., male or female) which tends to be less likely to be affected. All these factors are traditionally associated with multifactorial inheritance and are observed in HRSD (Bodian and Carter 1963; Passarge 1972; Garver et al. 1985). Given the great variability in the expression of this trait, we postulate this model for the effects of the gene for HRSD. The susceptibility allele of this gene may lead to an individual being normal, having short-segment HRSD, or having long-segment HRSD. In addition, risk to relatives of HRSD patients may appear artificially

low because many affected relatives may die prior to diagnosis.

A primary motivation for proposing multifactorial inheritance for HRSD is the large number of isolated cases. However, Kurnit et al.'s model provides some support for the observed pattern of inheritance being explained by stochastic gene effects. A final answer to the mode of inheritance can only be provided by molecular analysis, since segregation analysis cannot distinguish between sporadic and isolated genetic cases and provides no clues as to whether sporadic and familial cases are due to mutations in the same gene. Retinoblastoma provides an attractive model for the occurrence of both somatic and genetic mutations at the same locus leading to the same phenotype. For HRSD, somatic mutation in the gut or in the enteric ganglia may lead to aganglionosis, and the somatic mutations could occur at the same locus as does the genetic defect in familial cases. Alternatively, some of the familial cases could be due to germinal mosaicism, the importance of which is only recently being recognized (Hall 1988). Families with this mechanism would have unaffected parents and multiple affected offspring and would appear "recessive." All of the above-mentioned factors could be due to mutations at single genes and yet appear multifactorial. Cytogenetic studies of HRSD patients have identified multiple chromosome aberrations, and this suggests genetic heterogeneity, not multifactorial inheritance, for HRSD.

A further complication in the genetics of HRSD may be an effect of genomic imprinting on expression. Of the 14 cases in the literature where there is parental-offspring transmission, 11 are transmitted maternally (Prier and Hochberger 1981; Carmi et al. 1982; Jarmas et al. 1983; Lipson and Harvey 1987). Of the 17 cases where an unaffected parent is presumed to be transmitting the gene because of an affected relative, 10 are transmitted maternally (Bodian and Carter 1963; Passarge 1967, 1972; Verdy et al. 1982; Lipson and Harvey 1987; Hamilton and Bodurtha 1989). A parsimonious explanation of both situations would be genomic imprinting leading to increased expression of the HRSD gene if inherited maternally.

This data set showed an increased prevalence of Down syndrome among HRSD cases, an increase which may be due to the increased cellular adhesion of the ganglion cells in Down syndrome cases, since increased adhesion has been demonstrated in fibroblasts of Down syndrome patients (Wright et al. 1984). It is thus possible that there is a gene on chromosome 21 which may lead to HRSD. This may be similar to the "locus" which predisposes Down syndrome cases to congenital heart disease, since this latter symptom is also increased among HRSD cases. However, the association of congenital heart disease with HRSD may be due to a shared defect in neural crest cells, since ablation of specific neural crest cells in chick embryos is associated with cardiac defects (Besson et al. 1986).

Several studies have demonstrated an association between HRSD and coloboma (Mahboubi and Templeton 1984; Hurst et al. 1988; Webb et al. 1988). One of these cases had a deletion of 2p22 (Webb et al. 1988). Coloboma and the possibly related aniridia trait have been mapped to the short arm of chromosome 2 (Ferrell et al. 1980; Arias et al. 1984), and coloboma has also been found in trisomy 22q (Reiss et al. 1985; Magenis et al. 1988), as has HRSD (Beedgen et al. 1986). HRSD has also been associated with polydactyly, syndactyly, or brachydactyly (Laurence et al. 1975; Reynolds et al. 1983; Santos et al. 1988). Lewandowski and Yunis (1977) demonstrate that trisomy of 13q31-q34 leads to postaxial polydactyly and that deletion of this region leads to agenesis of the thumb and the first metacarpal and to syndactyly of the fourth and fifth metacarpals and metatarsals. HRSD is also associated with deletions in 13q (Sparkes et al. 1984; Lamont et al. 1989). These findings suggest that cases of HRSD in association with other defects may be due to chromosomal anomalies (either deletions or insertions) or to contiguous gene defects. The size of the chromosomal anomaly affects what defects are involved as well as how easily detectable the anomaly would be on cytogenetic analysis. A very small anomaly, limited to one or a few genes, may lead to HRSD alone. The anomaly may be a somatic mutation or a germinal mutation or may segregate as a single gene. The size of the chromosomal anomaly may also affect the severity of the phenotype (e.g., the extent of aganglionosis).

In summary, evidence suggesting that HRSD has a single-gene etiology includes (1) isolated pedigrees in which it segregates as an incompletely penetrant dominant, (2) mouse models in which single gene mutations lead to megacolon, (3) chromosomal abnormalities associated with HRSD, and (4) the results of this segregation analysis. The high proportion of sporadics and the appearance of multifactorial inheritance may be explained by one or more of the following hypotheses: (1) Kurnit et al.'s model of single gene action with stochastic effects during morphogenesis, (2) genomic imprinting leading to differing penetrance in the offspring, depending on the sex of the parent transmitting the allele, (3) somatic or germinal mosaicism, (4)

selection against affecteds, leading to a higher proportion of those transmitting the gene to be unaffected, and (5) curtailment of childbearing after the birth of an affected child.

Now that evidence for a single dominant gene for the more extensive forms of HRSD has been demonstrated, linkage studies can be performed on families with multiple affected members. Since Down syndrome is associated with HRSD, genetic markers on chromosome 21 may be suitable candidates for a linkage analysis. The long arm of chromosome 13 also would provide candidate genes, as would the short arm of chromosome 2 and the long arm of chromosome 22. Information on chromosomal locations of mouse megacolon mutations may lead to suitable markers as well, since there are many homologous segments between the mouse and human genomes (Searle et al. 1987). Esterase D and retinoblastoma, both of which have been mapped to human 13q, have been mapped to chromosome 14 of the mouse (Searle et al. 1987); this chromosome is also the site of the murine s^l mutation. This suggests that the putative gene for HRSD on 13q may be homologous to the spotted lethal mutation in the mouse. If a marker linked to HRSD is found, then this marker can aid in the detection of genetic heterogeneity both within HRSD and between HRSD expressed alone and in combination with other traits.

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