ping of achromatopsia to chromosome 2 using DNA pooling. Hum Mol Genet 6:689–694
Baraitser M (1997) Microcephaly. In: Motulsky AG, Bobrow M, Harper PS, Scriver C (eds) The genetics of neurological disorders. Oxford University Press, Oxford, pp 17–18
Budowle B, Chakraborty R, Giusti AW, Eisenberg AJ, Allen RC (1991) Analysis of the VNTR locus D1S80 by PCR followed by high-resolution PAGE. Am J Hum Genet 48: 137–144
Cowie V (1960) The genetics and sub-classification of microcephaly. J Ment Defic Res 4:42–47
Dacou-Voutetakis C, Carpathios T, Logothetis N, Constantinidis M, Matsaniotis N (1974) Defective growth hormone secretion in primary microcephaly. J Pediatr 85:498–502
Jackson AP, McHale DP, Campbell DA, Jafri H, Rashid Y, Mannan J, Karbani G, et al (1998) Primary autosomal recessive microcephaly (MCPH1) maps to chromosome 8p22-pter. Am J Hum Genet 63:541–546
Kruglyak L, Daly MJ, Lander ES (1995) Rapid multipoint linkage analysis of recessive traits in nuclear families, including homozygosity mapping. Am J Hum Genet 56: 519–527
Lander ES, Botstein D (1987) Homozygosity mapping: a way to map human recessive traits with the DNA of inbred children. Science 236:1567–1570
Ross JJ, Frias JL (1977) Microcephaly. In: Vinken PJ, Bruyn GW (eds) Congenital malformations of the brain and skull. Vol. 30: Handbook of clinical neurology. Elsevier Holland Biomedical, Amsterdam, pp 507–524
Terwilliger JD, Shannon WD, Lathrop GM, Nolan JP, Goldin LR, Chase GA, Weeks DE (1997) True and false positive peaks in genomewide scans: applications of length-biased sampling to linkage mapping. Am J Hum Genet 61:430–438
Tolmie JL, McNay M, Stephenson JB, Doyle D, Connor JM (1987) Microcephaly: genetic counselling and antenatal diagnosis after the birth of an affected child. Am J Med Genet 27:583–594
Van den Bosche J (1959) Microcephaly in the Netherlands: a clinical and genetical study. Ann Hum Genet 23:91–116
Walsh CA (1999) Genetic malformations of the human cerebral cortex. Neuron 23:19–29

Address for correspondence and reprints: Dr. C. Ruth Jamieson, Laboratory for Medical Genetics, Campus Erasme–ULB, Building C, 808, Lennik, B-1070 Brussels, Belgium. E-mail: rjamieso@ulb.ac.be

0002-9297/1999/6505-0033$02.00

Am. J. Hum. Genet. 65:1469–1473, 1999

Association of *RET* Protooncogene Codon 45 Polymorphism with Hirschsprung Disease

To the Editor:

The *RET* protooncogene (MIM 164761) is expressed in human tissues of neural crest origin and has been recognized as a susceptibility gene for several autosomal inherited diseases, such as Hirschsprung disease (HSCR [MIM 142623]) and multiple endocrine neoplasia type 2 syndromes (MEN 2 [MIM 171400]) comprising medullary thyroid carcinoma (MTC [MIM 155240]) as an obligatory feature (Eng et al. 1997). Of the patients with HSCR, 10%–40% have been reported to harbor germline mutations of the *RET* protooncogene, which are primarily point mutations scattered throughout the extracellular domain and within the intracellular tyrosine kinase domain of *RET* (Edery et al. 1994*b;* Romeo et al. 1994; Angrist et al. 1995; Seri et al. 1997). MEN 2 syndrome germline mutations of the *RET* protooncogene have been found to affect exons 10, 11, and 13–16 (Donis-Keller et al. 1993; Mulligan et al. 1993; Carlson et al. 1994; Eng et al. 1994; Hofstra et al. 1994; Bolino et al. 1995). Functional studies have demonstrated that *RET* mutations that characterize the autosomal dominant–inherited MEN 2 cause activation of the RET-signaling pathway, often in a constitutive manner or by altering the substrate specificity (Borrello et al. 1995; Santoro et al. 1995; Ceccherini et al. 1997; Pasini et al. 1997; Chappuis-Flament et al. 1998). In contrast, *RET* mutations found in HSCR presumably result in either RET-protein truncation or functional inactivation of the molecule. Although loss of one allele in some patients with HSCR suggests haploinsufficiency (Martucciello et al. 1992), the retention of one wild-type allele in patients with HSCR who have an inactivating *RET* mutation seems to explain the presumed autosomal dominant inheritance and implicates a dominant-negative action of the mutated *RET* allele (Badner et al. 1990; Cosma et al. 1998).

Furthermore, several polymorphisms in the coding region of the *RET* protooncogene have been described. A panel of the most frequent polymorphisms has been reported by Mulligan et al. (1993), Ceccherini et al. (1994), and Sáez et al. (1998), comprising those in codons 45, 125, 432, 691, 769, 836, and 904. In the study by Ceccherini et al., the allele frequencies of these polymorphisms were evaluated in a normal control group. These data were confirmed by a study by Gimm et al. (1999), and similar allele frequencies of the codon 45 polymorphism have been described by Edery et al. (1994*a*), who focused on a control population only. All of the investigated polymorphisms are silent mutations, except for the codon 691 polymorphism, which results in a change in the amino acid residue, from glycine to serine. Bugalho et al. (1994) investigated the frequency of the codon 691 polymorphism in a small population of clinically defined sporadic medullary thyroid carcinomas (MTC) and found no significant differences from a normal control population. Elsewhere, Gimm et al. (1999) have investigated all seven *RET* polymorphisms in a population with sporadic MTC and have found an

Table 1

Allele Frequencies of Polymorphic Variants of *RET* in 62 Patients with Sporadic HSCR and in 156 Control Individuals

Exon	Nucleotide Change (Codon)[a]	Restriction Site Changed	Allele Frequency in[b] (%)		Statistic	
			Controls	Patients with HSCR	χ^2	*P*
2	GCG→GCA (A45A)	*Eag*I	76.3	26.6	93.064	<.001
3	GTC→GTA (V125V)	*Mbo*II	98.1	97.6	.108	.742
7	GCG→GCA (A432A)	*Bsm*I	72.4	74.2	.139	.709
11	GGT→AGT (G691S)	*Ban*I	79.8	89.5	5.811	.016
13	CTT→CTG (L769L)	*Taq*I	76.3	57.3	15.556	<.001
14	AGC→AGT (S836S)	*Alu*I	96.4[c]	100	4.575	.032
15	TCC→TCG (S904S)	*Rsa*I	80.1	88.7	4.540	.033

[a] The wild-type allele is underlined.
[b] Of the wild-type allele.
[c] Only 153 control individuals were tested.

overrepresentation of the rare codon 836 polymorphism, compared with the frequency in normal controls. Interestingly, in this study the rare germline codon 836–sequence variant seems to be associated with the presence of a common somatic M918T mutation in the corresponding tumor DNA of patients with sporadic MTC.

To reveal the potential impact that *RET* polymorphisms for etiology have for HSCR in particular, we investigated the genotype distribution of polymorphisms of codons 45, 125, 432, 691, 769, 836, and 904 of the coding region of the *RET* protooncogene in patients with HSCR but without a family history of the disease. The population that we studied comprised 62 individuals with sporadic HSCR who were from two different areas of Germany, around the cities of Dresden (n = 37) and Erlangen (n = 25). The male:female ratio of these individuals was 3.8:1. For inclusion in the study, histopathological criteria of HSCR were (*a*) increased acetylcholinesterase histochemical staining in nerve fibers, in suction biopsies of the rectal submucosa, and (*b*) absence of neuronal ganglia, in operative histochemical and histological evaluation of the aganglionic tract. Patients with additional features or associated diseases were excluded from the study. Anonymous healthy blood donors from each region served as controls (n = 117 for Dresden; n = 39 for Erlangen). Controls were not matched for age or race, although all individuals were white. There was, therefore, a slight potential for population stratification in the patients with HSCR, relative to that in the controls. Genomic DNA was obtained from leukocytes from peripheral venous blood samples isolated by standard protocols. The seven investigated exons were amplified from genomic DNA by use of primers and reaction conditions described by Ceccherini et al. (1994), for exons 2 (codon 45), 3 (codon 125), 11 (codon 691), and 14 (codon 836), and by Mulligan et al. (1994), for exons 7 (codon 432) and 13 (codon 769). To amplify exon 15 (codon 904), we generated a new primer pair (sense, 5′CCCCCGGCCCAGGTCTCAC-3′; antisense, 5′GCTCCACTAATCTTCGGTATCTTT-3′). All analyzed polymorphisms generate or destroy a restriction site of an endonuclease—namely, *Eag*I, *Mbo*II, *Bsm*I, *Ban*I, *Taq*I, *Alu*I, or *Rsa*I (Ceccherini et al. 1994). Genotypes were determined by digestion of the PCR product and electrophoresis on a polyacrylamide gel. In addition, these results from the patient population were confirmed by DNA-sequencing analysis by use of the Thermo Sequenase™ Fluorescent Cycle Sequencing kit (Amersham Pharmacia Biotech), according to the manufacturer's protocol. The sequencing primers were the same as the PCR primers, with an additional Cy5TM labeling, allowing sequence analysis on A.L.F. express devices (Amersham Pharmacia Biotech). Statistical analysis was performed with the Pearson χ^2 test. Written informed consent was obtained from all patients.

Our data revealed that allele frequencies of all polymorphisms in the control population were similar to those reported by Ceccherini et al. (1994), Gimm et al. (1999), and Edery et al. (1994*a*), suggesting that the allele frequency is similar in the German, European, and American populations tested, but the study does not include data of an ethnically diverse, nonwhite population. The genotype distribution for each of the seven polymorphic loci did not deviate significantly from Hardy-Weinberg equilibrium. Although the wild-type allele of the codon 45 polymorphism was detected in 76.3% of 312 control chromosomes, the same allele was found in 26.6% of 124 HSCR chromosomes, an almost inverted relationship (table 1) (for allele frequencies in patients with HSCR vs. those in controls, $\chi^2 = 93.06$, $P < .001$). This highly significant difference between these allele frequencies resulted from a strong overrepresentation of the homozygous codon 45–polymorphism variant in the population with HSCR (34 of 62 patients with HSCR,

vs. 9 of 156 controls). Ceccherini et al. (1994) found the wild-type allele of the codon 45 polymorphism in 71% of 104 chromosomes, the same frequency as later was reported, by Gimm et al. (1999), in an analysis of 96 chromosomes. Furthermore, we found this highly significant association of the codon 45 polymorphism also in the two independent populations with HSCR and in controls from the regions around Erlangen and Dresden (for the allele frequency in Dresden patients with HSCR vs. that in Dresden controls, $\chi^2 = 60.65$, $P < .001$; for the allele frequency in Erlangen patients with HSCR vs. that in Erlangen controls, $\chi^2 = 31.65$, $P < .001$).

Within the population with HSCR, a tendency toward overrepresentation of the codon 769 polymorphism, similar to that of the codon 45 polymorphism, was found, compared with the frequency in the controls (table 1). In addition, we found the codon 769 polymorphism to be associated with HSCR in both populations, compared with what was found in the controls (for the allele frequency of Dresden patients with HSCR vs. that in Dresden controls, $\chi^2 = 9.26$, $P = .002$; for the frequency in Erlangen patients with HSCR vs. that in Erlangen controls, $\chi^2 = 5.72$, $P < .017$).

Although in codons 45 and 769 the polymorphic allele was overrepresented in the population with HSCR, in codons 691, 836, and 904 we found the wild-type allele to be more frequent in the population with HSCR population than in the control group, although the difference was not statistically significant (table 1).

In this study we have demonstrated that the codon 45–polymorphism allele frequency is overrepresented in patients with sporadic HSCR compared with the normal population, a finding that is highly significant statistically. In agreement with our findings, Puffenberger et al. (1994) described a significant excess of this polymorphism (for allele frequencies, $\chi^2 = 12.08$, $P < .001$) on the HSCR haplotype that is transmitted to affected members of Mennonite families with HSCR. However, the predominant mutation identified in this kindred is a founder homozygous W276C *EDNRB* (MIM 131244) gene mutation, which is an interesting association in itself and supports the polygenic, complex inheritance of HSCR. In addition, one patient has been described with both an *EDNRB* mutation and a *RET* mutation that apparently result in aberrant *RET* RNA splicing (Auricchio et al. 1999).

The mechanism by which the silent codon 45 polymorphism may act in HSCR genesis is unknown, but speculations have been made regarding the possible mechanisms. It has, for instance, been proposed that the silent sequence variant could lead to aberrantly spliced products, resulting in a protein with a 21-amino-acid deletion in the extracellular domain, altering a part of the extracellular signal-peptide sequence (Borrego et al. 1998).

In addition, it has been suggested that a seemingly nonfunctional polymorphism may create an unstable downstream sequence, which results in a functional somatic mutation (Gimm et al. 1999). Such a mechanism has been observed in the *APC* (MIM 175100) gene in Ashkenazim with familial colorectal cancer, in which additional somatic mutations were more often found on the allele carrying a conservative amino acid change (I1307K) (Laken et al. 1997).

If no pathogenic effect can be associated with the codon 45 *RET* polymorphism, then the possibility has to be considered that the base substitution is in linkage disequilibrium with an unknown functional variant upstream or downstream. For example, an *Msp*I RFLP of the 3′ end of the human *CYP1A1* (MIM 108330) gene has been shown to be in linkage disequilibrium with an adenine-to-guanine mutation at residue 462 in exon 7. The latter mutation causes an amino acid substitution, which results in increased enzymatic activity of CYP1A1 (Hayashi et al. 1991). Similarly, the silent codon 45 polymorphism may be either closely linked with a functional genetic variant or be functional itself.

Nevertheless, the observed difference in the homozygous genotype of the silent polymorphism—5.8% in the normal population of 156 individuals versus 54.8% in 62 analyzed patients with HSCR—suggests a strong association with the HSCR phenotype.

GUIDO FITZE,[1] MATTHIAS SCHREIBER,[4] EBERHARD KUHLISCH,[3] HANS K. SCHACKERT,[2] AND DIETMAR ROESNER[1]

Departments of [1]Pediatric Surgery and [2]Surgical Research and [3]Institute of Medical Informatics and Biometry, University of Technology Dresden, Dresden; and [4]Department of Pediatric Surgery, University of Erlangen, Erlangen, Germany

Electronic-Database Information

Accession numbers and URL for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for *APC* [MIM 175100], *CYP1A1* [MIM 108330], *EDNRB* [MIM 131244], HSCR [MIM 142623], MEN 2 [MIM 171400], and MTC [MIM 155240], and *RET* [MIM 164761])

References

Angrist M, Bolk S, Thiel B, Puffenberger EG, Hofstra RM, Buys CHC, Cass DT, et al (1995) Mutation analysis of the RET receptor tyrosine kinase in Hirschsprung disease. Hum Mol Genet 4:821–830

Auricchio A, Griseri P, Carpentieri ML, Betsos N, Staiano A, Tozzi A, Priolo M, et al (1999) Double heterozygosity for a *RET* substitution interfering with splicing and an *EDNRB*

missense mutation in Hirschsprung disease. Am J Hum Genet 64:1216–1221

Badner JA, Sieber WK, Garver KL, Chakravarti A (1990) A genetic study of Hirschsprung disease. Am J Hum Genet 46: 568–580

Bolino A, Schuffenecker I, Luo Y, Seri M, Silengo M, Tocco T, Chabrier G, et al (1995) *RET* mutations in exons 13 and 14 of FMTC patients. Oncogene 10:2415–2419

Borrego S, Eng C, Sánchez B, Sáez ME, Navarro E, Antinolo G (1998) Molecular analysis of the ret and GDNF genes in a family with multiple endocrine neoplasia type 2A and Hirschsprung disease. J Clin Endocrinol Metab 83: 3361–3364

Borrello MG, Smith DP, Pasini B, Bongarzone I, Greco A, Lorenzo MJ, Arighi E, et al (1995) RET activation by germline MEN2A and MEN2B mutations. Oncogene 11: 2419–2427

Bugalho MJM, Cote GJ, Khorana S, Schultz PN, Gagel RF (1994) Identification of polymorphism in exon 11 of the *RET* proto-oncogene. Hum Mol Genet 3:2263

Carlson KM, Bracamontes J, Jackson CE, Clark R, Lacroix A, Wells SA, Goodfellow PJ (1994) Parent-of-origin effects in multiple endocrine neoplasia type 2B. Am J Hum Genet 55:1076–1082

Ceccherini I, Hofstra RMW, Luo Y, Stulp RP, Barone V, Stelwagen T, Bocciardi R, et al (1994) DNA polymorphisms and conditions for SSCP analysis of the 20 exons of the *ret* proto-oncogene. Oncogene 9:3025–3029

Ceccherini I, Pasini B, Pacini F, Gullo M, Bongarzone I, Romei C, Santamaria G, et al (1997) Somatic in frame deletions not involving juxtamembranous cysteine residues strongly activate the *RET* proto-oncogene. Oncogene 14:2609–2612

Chappuis-Flament S, Pasini A, DeVita G, Segouffin-Cariou C, Fusco A, Attie T, Lenoir GM, et al (1998) Dual effect on the *RET* receptor of MEN 2 mutations affecting specific extracytoplasmic cysteins. Oncogene 17:2851–2861

Cosma MP, Cardone M, Carlomagno F, Colantuori V (1998) Mutations in the extracellular domain cause RET loss of function by a dominant negative mechanism. Mol Cell Biol 18:3321–3329

Donis-Keller H, Dou S, Chi D, Carlson KM, Toshima K, Lairmore TC, Howe JR, et al (1993) Mutations in the RET proto-oncogene are associated with MEN2A and FMTC. Hum Mol Genet 2:851–856

Edery P, Attie T, Mulligan LM, Pelet A, Eng C, Ponder BAJ, Munnich A, et al (1994*a*) A novel polymorphism in the coding sequence of the human RET proto-oncogene. Hum Genet 94:579–580

Edery P, Lyonnet S, Mulligan LM, Pelet A, Dow E, Abel L, Holder S, et al (1994*b*) Mutations of the *RET* proto-oncogene in Hirschsprung's disease. Nature 367:378–379

Eng C, Mulligan LM (1997) Mutation of the *RET* proto-oncogene in the multiple endocrine neoplasia type 2 syndromes, related sporadic tumours, and Hirschsprung disease. Hum Mutat 9:97–109

Eng C, Smith DP, Mulligan LM, Nagai MA, Healey CS, Ponder MA, Gardner E, et al (1994) Point mutations within the tyrosine kinase domain of the RET proto-oncogene in multiple endocine neoplasia type 2B and related sporadic tumours. Hum Mol Genet 3:237–241

Gimm O, Neuberg DS, Marsh DJ, Dahia PLM, Hoang-Vu C, Raue F, Hinze R, et al (1999) Over-representation of a germline *RET* sequence variant in patients with sporadic medullary thyroid carcinoma and somatic *RET* codon 918 mutation. Oncogene 18:1369–1373

Hayashi S, Watanabe J, Nakachi K, Kawajiri K (1991) Genetic linkage of lung cancer-associated *Msp*I polymorphisms with amino acid replacement in the heme binding region of the human cytochrome *P4501A1* gene. J Biochem 110:407–411

Hofstra RM, Landsvater RM, Ceccherini I, Stulp RP, Stelwagen T, Luo Y, Pasini B, et al (1994) A mutation in the *RET* proto-oncogene associated with multiple endocrine neoplasia type 2B and sporadic medullary thyroid carcinoma. Nature 367:375–376

Laken SJ, Petersen GM, Gruber SB, Oddoux C, Ostrer H, Giardiello FM, Hamilton SR, et al (1997) Familial colorectal cancer in Ashkenazim due to a hypermutable tract in *APC*. Nat Genet 17:79–83

Martucciello G, Bicocchi MP, Dodero P, Lerone M, Silengo Cirillo M, Puliti A, Gimelli G, et al (1992) Total colonic aganglionosis associated with interstitial deletion of the long arm of chromosome 10. Pediatr Surg Int 7:308–310

Mulligan LM, Eng C, Attie T, Lyonnet S, Marsh DJ, Hyland VJ, Robinson BG, et al (1994) Diverse phenotype associated with exon 10 mutations of the *RET* proto-oncogene. Hum Mol Genet 3:2163–2167

Mulligan LM, Kwok JBJ, Healey CS, Elsdon MJ, Eng C, Gardner E, Love DR, et al (1993) Germ-line mutations of the *RET* proto-oncogene in multiple endocrine neoplasia type 2A. Nature 363:458–460

Pasini A, Geneste O, Legrand P, Schlumberger M, Rossel M, Fournier L, Rudkin BB, et al (1997) Oncogenetic activation of RET by two distinct FMTC mutations affecting the tyrosine kinase domain. Oncogene 15:393–402

Puffenberger EG, Hosoda K, Washington SS, Nakao K, deWit D, Yanagisawa M, Chakravarti A (1994) A missense mutation of the endothelin-B receptor gene in multigenic Hirschsprung's disease. Cell 79:1257–1266

Romeo G, Ronchetto P, Yin L, Barone V, Seri M, Ceccherini I, Pasini B, et al (1994) Point mutations affecting the tyrosine kinase domain of the RET proto-oncogene in Hirschsprung's disease. Nature 367:377–378

Sáez ME, Sánchez B, Antinolo G, Borrego S (1998) Identification of a rare polymorphism, S836S, in the tyrosine kinase domain of *RET* proto-oncogene. Hum Mutat 11:416

Santoro M, Carlomagno F, Romano A, Bottaro DP, Dathan NA, Grieco M, Fusco A, et al (1995) Activation of RET as a dominant transforming gene by germline mutations of MEN 2A and MEN 2B. Science 267:381–383

Seri M, Yin L, Barone V, Bolino A, Celli I, Bocciardi R, Pasini B, et al (1997) Frequency of *RET* mutations in long- and short-segment Hirschsprung disease. Hum Mutat 9:243–249

Address for correspondence and reprints: Dr. Guido Fitze, Department of Pediatric Surgery, University of Technology Dresden, Fetscherstrasse 74, D-01307 Dresden, Germany. E-mail: Guido.Fitze@mailbox.tu-dresden.de

0002-9297/1999/6505-0034$02.00

Am. J. Hum. Genet. 65:1473–1475, 1999

The Sex Ratio in Familial Persistent Stuttering

To the Editor:

Stuttering is a speech disorder characterized by involuntary syllable repetitions, syllable prolongations, or interruptions, known as blocks, in the smooth flow of speech (World Health Organization 1992; Bloodstein 1995). Stuttering typically arises in young children, where it affects ⩾15% of children in the age range of 4–6 years (Bloodstein 1995). Stuttering often resolves spontaneously before adolescence, leading to a population prevalence of 1%–2% among adults. Stuttering beyond childhood is characterized by a significant bias toward males, with males outnumbering females by a ratio of 3:1–5:1 (Yairi et al. 1996).

Many studies support the view that inherited factors contribute to stuttering (Howie 1981; Yairi et al. 1996; Felsenfeld and Plomin 1997). As part of a linkage study to identify predisposing loci for this disorder, we assembled >100 small-to-medium–sized unrelated families with multiple cases of persistent stuttering, chosen to represent the typical presentation of familial stuttering in the adult population. In these families, we have observed a male-to-female ratio among the affected individuals that is strikingly different from the generally accepted ratio in the overall adult stuttering population.

Family ascertainment was designed to obtain the most diverse sample possible from the North American population. The NIH families were ascertained under NIH IRB-approved protocol 97-DC-0087, through a broad variety of appeals directed at stuttering interest groups, stuttering support groups, professional speech and language organizations, alumni of stuttering therapy programs—including intensive residential programs and part-time, outpatient programs—and the general public. The enrolled families included whites, African Americans, Hispanics, and Asians, with no evidence for over- or under-representation of any group compared to the general population. Among the identifiable probands in these families, 56% were male and 44% were female. We exhaustively ascertained and evaluated family members aged >8 years according to well-established diagnostic criteria for stuttering (Webster 1978; World Health Organization 1992), using videotaped speech samples and counting the number of stuttering-like dysfluencies, in both conversation and reading. In some cases, audio tape recordings were substituted. The standardized reading passage was 500 words in length and contained balanced numbers of each of the different classes of speech sounds. This tool has been used for >10 years and has well-established performance norms (R. Webster, personal communication; copy available, on request, from corresponding author). For individuals to be classified as affected, a score of ⩾4% dysfluent words (representing the 25th percentile among individuals who present themselves for stuttering therapy) was required in the individual's speech in both conversation and reading. In some cases, videotaped speech samples were not obtainable, and audio recordings of speech were substituted. By these criteria, 224 individuals were classified as affected in our families. Affection status, as determined by professional speech evaluation, was generally in agreement with self-reported affection status. The few discrepancies showed no evidence of bias between males and females. The affected individuals had an age range of 10–86 years, with a mean age of 39.9 years. Among these affected individuals, 137 are male and 87 are female, yielding a male-to-female ratio of 1.57.

To compare this ratio to the male-to-female ratio in the general stuttering population, we examined four different populations of unrelated, persistent stutterers. We chose four different groups of persistent stutterers, because each group was subject to individual ascertainment biases. For example, therapy programs are generally believed to ascertain males preferentially, while support groups are believed to attract more females, frequently affected mothers of affected children. We sought the largest available sources of such populations of stutterers and derived data from the clinical records of two large therapy programs, the Hollins Communications Research Institute (HCRI) and the American Institute for Stuttering (AIS), plus data on two groups, ascertained

Table 1

Numbers of Males and Females in Populations of Unrelated Persistent Stutterers, and χ^2 Analysis of the Differences in Gender Ratios between Groups

	No. of Patients in Population				
Sex	HCRI Alumni	NSP Members	SFA Records	AIS Alumni	Total
Males	810	285	131	826	2052
Females	156	112	52	212	532

Note.—Overall, familial cases versus general stuttering population $\chi^2 = 43$; df 1; $P < .00001$. Familial cases versus cases ascertained via therapy programs $\chi^2 = 63$. $P < .000001$. Familial cases versus cases ascertained without respect to treatment $\chi^2 = 13$. $P < .002$.