RESEARCH ARTICLE

A Genetic Polymorphism in the Coding Region of the Gastric Intrinsic Factor Gene (GIF) Is Associated With Congenital Intrinsic Factor Deficiency

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Communicated by David Rimoin

Congenital intrinsic factor (IF) deficiency is a disorder characterized by megaloblastic anemia due to the absence of gastric IF (GIF, GenBank NM_005142) and GIF antibodies, with probable autosomal recessive inheritance. Most of the reported patients are isolated cases without genetic studies of the parents or siblings. Complete exonic sequences were determined from the PCR products generated from genomic DNA of five affected individuals. All probands had the identical variant (g.68A>G) in the second position of the fifth codon in the coding sequence of the gene that introduces a restriction enzyme site for Msp I and predicts a change in the mature protein from glutamine$_5$ (CAG) to arginine$_5$ (CGG). Three subjects were homozygous for this base exchange and two subjects were heterozygous, one of which was apparently a compound heterozygote at positions 1 and 2 of the fifth codon ([g.67C>G] + [g.68A>G]). The other patient, heterozygous for position 2, had one heterozygous unaffected parent. Most parents were heterozygous for this base exchange, confirming the pattern of autosomal recessive inheritance for congenital IF deficiency. cDNA encoding GIF was mutated at base pair g.68 (A>G) and expressed in COS-7 cells. The apparent size, secretion rate, and sensitivity to pepsin hydrolysis of the expressed IF were similar to native IF. The allelic frequency of g.68A>G was 0.067 and 0.038 in two control populations. This sequence aberration is not the cause of the phenotype, but is associated with the genotype of congenital IF deficiency and could serve as a marker for inheritance of this disorder. Hum Mutat 23:85–91, 2004. © **2003 Wiley-Liss, Inc.**

KEY WORDS: anemia; pernicious anemia; congenital; gastric intrinsic factor; GIF; SNP

DATABASES:
GIF–OMIM: 261000; GenBank: X76562.1, NM_005142.2

INTRODUCTION

Congenital pernicious anemia (IF deficiency) (MIM# 261000) is a rare disorder characterized by the lack of gastric intrinsic factor (GIF, NCBI accession # NM_005142) with normal acid secretion and mucosal cytology [Yang et al., 1985]. These patients have low serum cobalamin (Cbl, or vitamin B12), usually in association with megaloblastic anemia, and do not have autoantibodies against GIF or parietal cells, as occurs in the acquired form of pernicious anemia. The disorder presents in infancy or in early childhood, but patients have been identified in the first and third decades [Carmel, 1983].

Most cases reported have no GIF in the gastric juice, as determined by Cbl-binding activity or immunological reactivity [Carmel, 1983, Miller et al., 1966]. However, low levels of GIF have been found in the gastric juice from some patients, either before or after acid stimulation [Carmel, 1983]. Some patients produce GIF with an abnormal susceptibility to pepsin degradation [Yang et al., 1985], or with reduced affinity for the ileal GIF-Cbl receptor [Katz et al., 1974]. These studies indicate that congenital pernicious anemia may be a consequence of several different mutations of the GIF gene. A study of nine patients with congenital pernicious anemia found low functional GIF in the gastric juice from eight of the patients, consistent with some heterogeneity of the

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Received 19 February 2001; accepted revised manuscript 4 September 2003.

DOI 10.1002/humu.10297
Published online in Wiley InterScience (www.interscience.wiley.com).

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phenotype [Lillibridge et al., 1967]. In a study of archived tissue from these patients, immunoreactive GIF was identified in parietal cells in six of the patients [Levine and Allen, 1985]. Thus, it would appear that the lack of GIF in gastric juice in most of these patients could either be due to a decrease in the rate of synthesis, a decrease in the rate of secretion from parietal cells, or increased degradation within the lumen of the stomach. Yang et al. [1985], however, found no abnormality of GIF secretion in their three patients. The presence of an immunoreactive GIF protein that does not bind Cbl could be due to the fact that even limited proteolysis could eliminate Cbl binding [Gordon et al., 1991], whereas antiserum against GIF may detect fragments of the protein.

In the present study, all the exons of the GIF gene were sequenced from five patients and from the parents of four patients. A single nucleotide substitution in position 2 of codon 5 (of the coding sequence) in one or both copies of the GIF gene was identified in all the subjects (g.68A>G), with additional changes observed in two patients. When COS-7 cells were transfected with plasmids containing either the normal or the mutant cDNA, the secreted GIF proteins had a similar rate of secretion and sensitivity to pepsin degradation.

METHODS

Genomic DNA

In the original analysis of the human GIF gene [Hewitt et al., 1991], the first 90 bp of the 5′ untranslated sequence were not present in the genomic region cloned. Southern blot analysis indicated that this sequence was located in a 6-kb EcoRI fragment >2 kb upstream of the exon containing the signal peptide (designated exon 2). During the course of sequencing the intronic regions flanking the exon/intron junctions, we attempted to find this putative exon 1, using the Human GenomeWalker Kit (Clontech, Palo Alto, CA). Although we sequenced 3 kb 5′ to exon 2, we could not identify another exon. Thus, it would appear that the 90-bp region that we previously reported to be attached to the 5′ end of the human GIF cDNA might be a cloning artifact [Hewitt et al., 1991]. As a result, we have renumbered the exons, starting with the signal peptide–containing exon (now called exon 1), and ending with exon 9.

DNA Sequencing

Coding exons of the GIF gene were amplified by the polymerase chain reaction (PCR) of genomic DNA. The primers used for each exon were intron sequences that are about 100 bp from the 5′ and 3′ ends of each exon. The specific sequences are provided in Table 1. Taq DNA polymerase (Promega, Madison, WI) was used for the amplification.

The product from the PCR reaction was concentrated by ethanol precipitation and analyzed by agarose gel electrophoresis. The DNA band was extracted from the gel with GENECLEAN II (Bio101, Vista, CA), eluted in water, and sequenced using the ABI Prism BigDye Terminator Cycle Sequencing Kit (PE Systems, Foster City, CA). When direct sequencing was not successful, the TA-tagged PCR product was cloned into the TA cloning vector, pCR-TOPO (Invitrogen, Carlsbad, CA) and then sequenced.

Production and Expression of Mutant GIF

The single base pair change (A>G) in codon 5 of the coding sequence of GIF cDNA was generated using the GeneEditor in vitro Site-Directed Mutagenesis System (Promega, Madison, WI) and cloned into the expression vector PSVL (Pharmacia, Biotech, Piscataway, NJ) [Gordon et al., 1992]. The mutant GIF was confirmed by sequence analysis to have complete replacement of the product of codon 5. COS-7 cells were grown to 70% to 80% confluence in 100 mm dishes in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal calf serum (FCS) (GIBCO, Grand Island, NY). Transfection of the cells was performed using FuGENE 6 Transfection Reagent (Roche, Molecular Biochemicals, Mannheim, Germany). Medium and cells were collected at time points covering GIF synthesis for 24–30 and 24–48 hr posttransfection, because GIF production

TABLE 1. Intron/Exon Junction Primers for Sequencing Human Gastric Intrinsic Factor Exons

EXON 1/5′	TAG/GAG/AGG/CTG/GGT/TGG/GGA/CAG/ATT/ATT/
EXON 1/3′	TCA/CAC/TCA/GAC/TTA/CCC/ATT/TTG/GAA/T
EXON 2/5′	TCA/GGA/AGC/TGA/GGC/AGG/AGA/ATC/ACT/TGA
EXON 2/3′	ACA/CAC/ATG/TTT/CTT/TCC/AGG/TAC/CTT/CCA
EXON 3/5′	AAG/ACA/GAT/CAA/AGA/ACT/GTT/AAT/ATT/A
EXON 3/3′	ATT/ATC/AGA/AGG/ACC/AGA/GTG/AAG/GAT/ACA
EXON 4/5′	TGC/TGT/GTC/TCT/CTT/GGG/GGC/AGG/TTT
EXON 4/3′	ACC/ATT/CAG/TTC/ACG/ATG/CCT/CTG/ATG/TTC/CCA
EXON 5/5′	TCA/AAT/GGA/GAT/TGG/CCC/TGC/TGC/TTA/TTT
EXON 5/3′	ACA/TAG/GAA/GGT/AGG/GAG/GAA/TTA/ACA/CTT
EXON 6/5′	TGG/CTT/TGC/ATT/CTG/GGT/GAC/TAC/GTG/A
EXON 6/3′	AGT/CAG/ATG/TAC/TCT/GCC/CAC/ATG/GGA/A
EXON 7/5′	TTT/GTT/AAA/TGG/TAT/GAA/ACT/CAC/TAC/TTT/CT
EXON 7/3′	TGC/TCA/AGC/GCT/ATT/AAA/TAA/AAA/TCT/GTT/AT
EXON 8/5′	TAG/CCT/GGG/TAA/CAA/AGT/CAC/ACC/TTG/TCT/CTA
EXON 8/3′	TAG/AAT/TAT/TGT/ATA/TAC/ACA/TGA/TCT/CA
EXON 9/5′	TCT/CTC/TAG/GAT/GAT/TTT/ACT/CCT/TTA/AAA
EXON 9/3′	TCC/ATG/TTT/TTA/CCA/GGA/ATA/TGG/TAG/CA

following transfection of COS cells does not become linear until the cells have stabilized in culture for 24 hr [Gordon et al., 1991]. After removal of the medium, the culture disk was rinsed three times with medium lacking FCS and the cells were collected by scraping the cell layer. The cells were pelleted and then resuspended in 50 μL of phosphate buffered saline (pH 7.4) containing 1% NP-40, 12 mM sodium deoxycholate, 0.1% SDS, and the 10% Protease Inhibitor Cocktail for Mammalian Cells (Sigma Chemical Corp., St. Louis, MO). The cell pellets were homogenized and then incubated for 1 hr on ice. The samples were then spun at 12,000 *g* for 10 min and the supernatant extract was removed and saved. Samples of medium were mixed with 100 μL of a 50% suspension of washed Cbl-Sepharose beads (Sigma Chemical Corp.) for 1 hr. The agarose was pelleted, washed once, and the GIF bound to the Cbl matrix was extracted with SDS-PAGE buffer, and aliquots of the extract and the medium were analyzed by SDS-PAGE in a 10% gel followed by Western blotting using anti-GIF antiserum [Gordon et al., 1991]. To detect the amounts of GIF in the medium of COS-7 cells during the initial phases of secretion, the medium and cells were harvested 6 hr later and processed as described above. In other experiments, the purified recombinant wild-type and mutated apoGIFs were tested for sensitivity to pepsin, as described previously [Yang et al., 1985]. Each GIF sample was incubated at pH 2.0 for 10 min in the presence of 0.01–0.1 μg of hog pepsin (Sigma Chemical Corp.), neutralized with Tris buffer to pH 8.0, and analyzed by SDS-PAGE and Western blotting.

Case Studies

Details of the five patients are presented in Table 2. Four of the five cases presented with a macrocytic anemia. No antibodies against human GIF were detected in the serum of all five patients. Patient 1 and his family have previously been reported [Remacha et al., 1992]. His father and sister had low levels of GIF in their gastric juice, but neither is known to have clinical symptoms. He was not related to Patients 2 and 3. Patient 2 was a 3-year-old Spanish boy and Patient 3 was his first cousin. Patient 4 presented with macrocytic anemia, and a bone marrow aspirate showed megaloblasts. A diagnosis of systemic lupus erythematosus had been made at age 4 years because of anemia with joint pain and swelling. Patient 5 presented with anemia, anorexia, and diarrhea. Her sister had shown the same symptoms but had died at 21 months of age. Neither parent was anemic. The serum Cbl concentration was 197 pmol/L and 217 pmol/L in the mother and father, respectively, with normal serum methylmalonic acid.

All of the patients responded to Cbl replacement and none had evidence for hemolytic anemia or folate deficiency. Table 2 summarizes the data from these patients.

Population Studies

Genomic DNA samples from two existing screening programs were analyzed for the polymorphism. The Spanish series included samples that were collected from the umbilical cord of all hospital newborns during a 3-month period. Thirty additional samples were selected randomly from the German neonatal screening program. Genomic DNA was extracted from umbilical cord blood leukocytes of newborns by the salting-out method [Miller et al., 1988]. Exon 1, along with the adjacent intronic segment of the IF gene, was amplified using 30 cycles of PCR at 94°C for 30 s, 55°C for 45 s, and 72°C for 30 s, with a final extension of 6 min at 72°C. The primers used were selected from the *Homo sapiens* genomic DNA chromosome 11q sequence as ones that gave very clear results on gels:

GIFF: 5′ GTTGGGGACAGATTATTTAACAAAGG 3′

and

GIFR: 5′ GCACACAATCACACTCAGACTTACCC 3′.

These sequences differed from those in Table 1 that were selected to be near intron/exon junctions. The PCR products were digested with Msp I and size-separated by electrophoresis on 3% Nusieve agarose gel (RMR, Rockland, ME). The size of the undigested fragment observed in the homozygous wild-type samples was 326 bp. Mutated homozygous samples showed two DNA fragments of 176 and 150 bp. Heterozygous carriers displayed a combination of both patterns (326, 176, and 150 bp).

RESULTS

Patient Phenotype

All the patients had findings consistent with congenital IF deficiency; namely, presentation with macrocytic

TABLE 2. Summary of Patients with Congenital Intrinsic Factor Deficiency

No.	Age at onset (yr)	Country	MCV (fl)	Serum [cbl][a] (pmol/L)	Gastric juice IF[b]	Schilling test[c] (%)	αIF Ab[d]
1	2	Spain	116	15	Absent	1→8	Absent
2	3	Spain	107	32	Absent	2→8	Absent
3	2	Spain	110	80	Absent	2→7	Absent
4	11	USA	90	30	<1.5 nmol/L	0.8→11	Absent
5	21/4	Kazachstan	87	40	Absent	0.3→9	Absent

[a]Determined by radioisotope dilution assay, normal >147 pmol/L.
[b]Determined by cobalamin binding after preincubation with cobinamide to saturate haptocorrin (R binder), normal GIF concentration 20–100 nmol/L after maximal stimulation with pentagastrin.
[c]Urinary excretion of [^{57}Co]Cbl in 24 hr after oral dosing without and with (→) oral GIF.
[d]Determined by radioimmunoassay.

anemia at an early age, low serum cobalamin, low or absent GIF in the gastric juice, correction of the Schilling test for cobalamin absorption by the addition of oral GIF, and the absence of GIF antibodies (Table 2).

DNA Sequencing

All the exons were amplified and sequenced from genomic DNA obtained from the five probands. No abnormalities were found in any sequence except in the exon encoding the amino-terminus of the protein (called exon 2 in Hewitt et al. [1991], but herein renamed exon 1). Abnormalities were found in positions 1 and 2 of codon 5 in exon 1, but all five patients had the same change in position 2 of codon 5 (g.68A>G), a guanine for adenine substitution that would code for arginine (CGG) instead of glutamine (CAG), producing the phenotype Q5R. Patients 1, 4, and 5 were homozygous for this base exchange, and Patients 2 and 3 were heterozygous. Figure 1 shows the DNA sequence of codon 5 with Q5R heterozygosity in the parents, and homozygosity in Patient 1 (g.68A>G). Figure 2 shows the sequence for Patient 3 ([g.67C>G] + [g.68A>G]) along with the nucleotide exchanges in the parents that resulted in the two-base substitution in the patient.

Patients 1 and 5 were homozygous for the nucleotide substitution, A>G at position 2 of codon 5/exon 1. In both instances, the parents were heterozygous (Q5/Q5R), and the inheritance appears to be autosomal recessive. Patient 2 has the phenotype of the disease, but is a heterozygote (A/G) at position 2 of codon 5/exon 1 (Fig. 1). The father has the identical GIF gene sequence, but has no clinical evidence of the disorder.

Patient 3 was heterozygous at positions 1 and 2 of codon 5/exon 1, with C/G at position 1 [CAG (glutamine)/GAG (glycine)] and A/G at position 2 [CAG (glutamine)/CGG (arginine)] (Fig. 2). The father was heterozygous at position 1 (C/G) and the mother carried A/G at position 2. The mother of Patient 3 and the father of Patient 2 are siblings, and displayed the same heterozygosity (A/G) at position 2 of codon 5. Therefore, Patient 3 was a compound heterozygote, and the inheritance is autosomal recessive.

Patient 4 is also unique in that he showed homozygosity (G/G) in position 2 of codon 5/exon 1, but in addition, there was a 4-bp deletion downstream in exon 2 (formerly exon 3) (g.183-186delGAAT, base pairs 104–107 in exon 2). The mother was heterozygous for the base exchange in exon 1, and for the deletion in exon 2. DNA from the father was not available. The normal sequence, as well as this deletion in the mother who is phenotypically normal, indicates that the inheritance of this disorder is autosomal recessive. The molecular genetic findings in these five patients are summarized in Table 3.

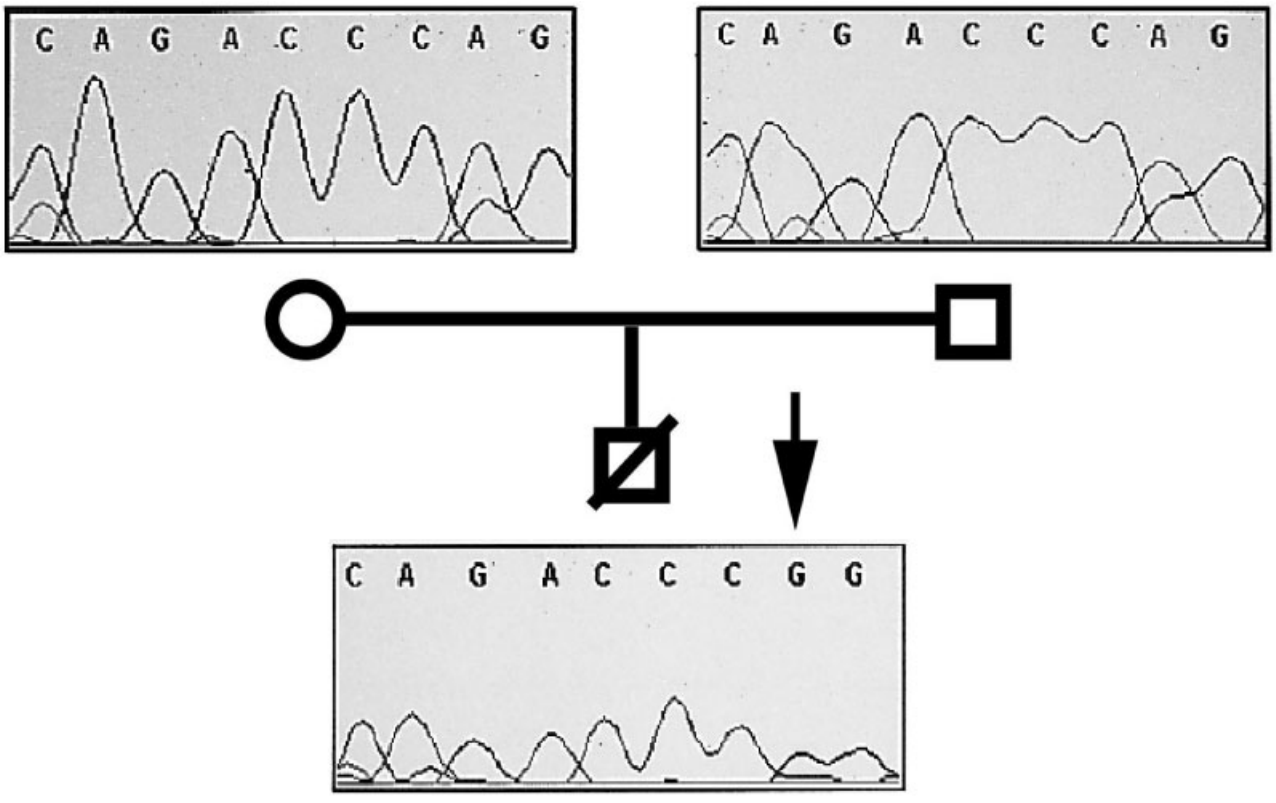

FIGURE 1. **Sequence of base pairs g.61–69 from Case 1 (Patient 1 and parents): both parents demonstrate a dominant peak in base pair g.68 of adenine (A), with a shoulder of guanine (G). Thus, each has a sequence of C(A/G)G at codon 5. In contrast, the patient shows a single peak of G in the same position, and is likely to be homozygous for G, corresponding to CGG (arginine).**

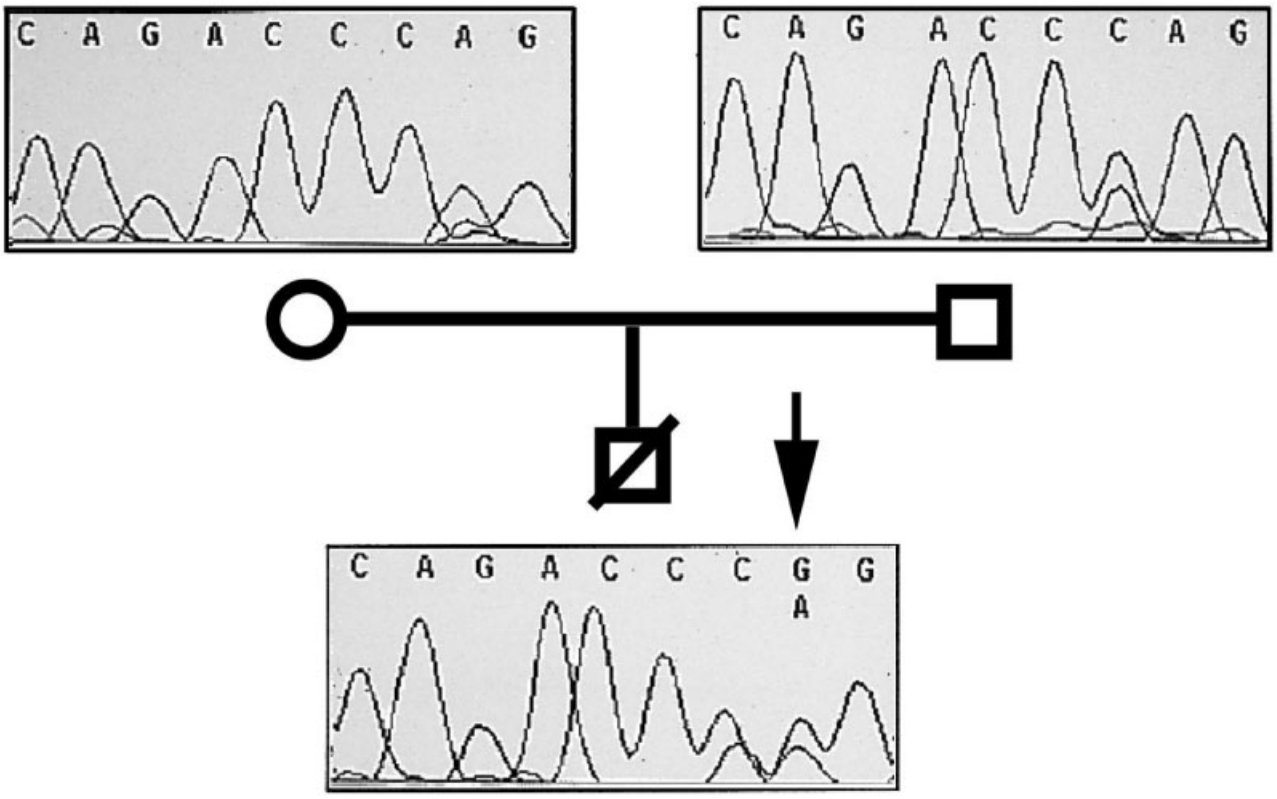

FIGURE 2. **Sequence of base pairs g.61–69 from Case 3 (Patient 3 and parents): the mother shows a dominant cytosine (C) peak and secondary guanine (G) peak at base pair g.67, and only a single adenine (A) peak at base pair g.68, representing (C/G)AG for codon 5. The father shows only a single C peak at base pair g.67, but a dominant A and secondary G peak at base pair g.68, representing C(A/G)G. In contrast, the patient shows two peaks at both base pairs 13 and 14, corresponding to a sequence of (C/G)(A/G)G.**

TABLE 3. Results of Sequencing of the Entire Coding Region of GIF in Patients With Congenital IF Deficiency

	Predicted amino acid substitution in mature IF		
Case	Patient	Father	Mother
1	Q5R/Q5R[a]	Q5R/Q5[b]	Q5R/Q5[b]
2	Q5R/Q5	Q5R/Q5[c]	Q5/Q5
3	Q5G[d]/Q5R	Q5G/Q5	Q5R/Q5[c]
4	Q5R/Q5R 4 bp deletion M43N44[e]	n.a.	Q5R/Q5
5	Q5R/Q5R	Q5R/Q5	Q5R/Q5

[a]Wild-type codon CAG = [5]glutamine; mutant codon CGG = arginine.
[b]Father and mother of case 1 are first cousins.
[c]Father of case 2 and mother of case 3 are siblings.
[d]Wild-type codon CAG = [5]glutamine; mutant codon GAG = glycine.
[e]This deletion occurs in exon 2 (g.183-186delGAAT) and predicts the following effect: wt: ….*GCC*ATG*AAT*CTG. ⇒ …-[60]alanine-[61]methionine-[62]asparagine-….mut: ….*GCC*AT-*—*[CTG*]. ⇒ …-[60]alanine-*frame shift*……[68]stop.
n.a., not available.

Expression of the Mutant IF

The cDNA encoding GIF mutated in codon 5, changing glutamine to arginine (Q R), was transfected into COS-7 cells and the properties compared with that of wild-type GIF transfected into the same cells (Table 4). The size and amount of unlabeled wild-type and mutant GIF secreted into the medium between 24 and 48 hr after transfection was indistinguishable by Western blot. In addition, the amount residing in the cell pellet was very similar. These results suggested similar rates of secretion, as no retention of IF was found in the COS-7 cells. Based on the recovery of GIF from Cbl-Sepharose columns (range about 40–50%), the equal recovery of GIF 24–48 hr after transfection provides support that Cbl binding was not impaired in the mutant protein. We also examined the initial period after linear secretion was established (24–30 hr after transfection) to identify any lag in GIF production by the mutant cDNA, but GIF production during this period was virtually identical to the wild-type cDNA.

Sensitivity to proteolysis is a feature of GIF when the ligand cobalamin is not present [Gordon et al., 1991]. When ~1 ng of the purified recombinant wild-type and mutant apo-GIFs were treated with hog pepsin at three different concentrations (from 0.01 to 0.1 μg/reaction) for 10 min at 37°C and pH 2.0, both GIF proteins were equally sensitive to proteolysis as determined by loss of intact GIF on Western blotting.

Population Studies

Samples were obtained from two neonatal screening programs, 30 samples from Germany and 131 samples from Spain. These were used to calculate the prevalence of the observed base exchange at g.68 in the population. The allelic frequency was 0.067 (95% CI=0.0004–0.13) in the German sample (four out of 30 newborns were heterozygous carriers of the Q5R mutation), and 0.038 (95% CI=0.061–0.15) in the Spanish newborns, including 10 out of 131 heterozygous carriers (see Fig. 3 for identification of typical phenotypes). No samples in the two newborn populations were homozygous for this sequence aberration.

TABLE 4. Properties of Wild-Type and Mutant GIF

GIF property	Wild-type GIF (Q5)	Mutant GIF (Q5R)
Apparent size on Western blot	50 kDa	50 kDa
GIF protein secreted in medium		
Between 24–48 hr after transfection	250 ng	260 ng
Between 24–30 hr after transfection	55 ng	50 ng
GIF protein in cells		
Between 24–48 hr after transfection	25 ng	28 ng
Between 24–30 hr after transfection	3 ng	3 ng
Apo-GIF remaining after pepsin treatment (37°C, 10 min, pH 2.0)		
0.01 μg pepsin/reaction	55%	45%
0.05 μg pepsin/reaction	22%	32%
0.10 μg pepsin/reaction	5%	11%

Production of recombinant GIF and analyses of proteins were performed as described in the Methods section.

DISCUSSION

Too few parents of the probands have been studied in earlier reports to confirm the mode of inheritance of this disorder. In a study of the families of three patients, serum Cbl concentration was normal in all the members of two families. In the third family, the father, but not the mother, had a low serum Cbl concentration [Miller et al., 1966]. The father of another patient with combined GIF and haptocorrin deficiency, had a low serum Cbl concentration [Zittoun et al., 1988]. The mother of this patient was not available for testing. The mother of the family studied by Yang et al. [1985] secreted both normal and abnormal GIF in the gastric juice whereas the proband secreted only abnormal GIF. The father and one sibling secreted normal GIF. These results could be interpreted as the father carrying one normal and one "silent" GIF gene, and the inheritance as autosomal recessive.

All five patients in the present report lacked GIF, had the same mutation in codon 5/exon1, and were either phenotypic homozygotes or heterozygotes. These findings are consistent with autosomal recessive inheritance. The parents were heterozygous for the same sequence alterations and were phenotypically normal. Although the families of the first three patients came from the same region in Spain, the towns are in different provinces, and there is no evidence that these patients have a common ancestry. Consanguinity was present only in the parents of Patient 1.

The consistent finding of a single base change in codon 5 in all of these five patients strongly suggests that it is associated with the phenotype. However in vitro

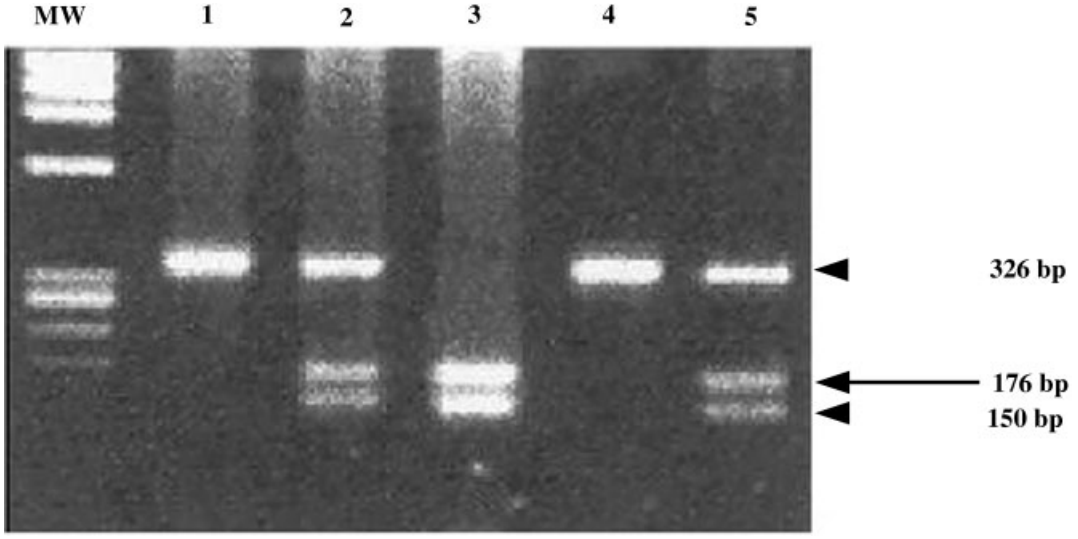

FIGURE 3. Electrophoretic separation of the exon 2 PCR product in a 3% agarose gel. Digestion with Msp I showed in the homozygous wild-type samples, an uncut band of 326 bp (lanes 1 and 4). Patient 1, who is homozygous for the mutation, showed two fragments of 176 bp and 150 bp (lane 3), and in the heterozygous carriers from the control population, a combined pattern was observed (lanes 2 and 5).

mutagenesis studies indicate that this sequence aberration does not significantly alter the protein. In humans, the pH of the gastric contents is about 2.0, except during meals when it rises to about 4.0. The amount of GIF secreted/day ranges from 2.5–300 μg/hr after histamine stimulation [Glass, 1974], compared with 4.3 ± 1.2 mg/hr of pepsin [Lin et al., 1994]. Degradation of GIF occurs less readily when present as the GIF-cbl complex than as apoGIF [Gordon et al., 1995]. Rapid degradation of GIF appears to account for the lack of GIF in a few reported patients [Yang et al., 1985], and might account for low (but not absent) GIF content in the stomach. However, all five patients in this report had absent GIF, and we were not able to demonstrate pepsin sensitivity in vitro of the expressed mutant GIF. Because a second mutation was found within GIF exons in Patients 3 and 4, it seems more likely that complex heterozygosity is required for expression of the full phenotype.

The mutant GIF transfected into COS-7 cells had the same properties as normal IF. In particular, those properties that might explain the phenotype of congenital GIF deficiency were not altered, i.e., there were no apparent abnormalities in the protein size, rate of secretion, or abnormal trafficking within the COS-7 cells, nor was the protein more susceptible to degradation in vitro by pepsin. In addition, as all these assessments used GIF isolated on Cbl-Sepharose beads, the mutant GIF presumably bound Cbl comparably to native GIF. Therefore, the amino acid exchange Q5R is unlikely to be the primary cause of congenital GIF deficiency.

Other rare disorders also show heterogeneity in the functional mutation, but not all cases have mutations within the coding sequence of the gene [Ferec et al., 1999]. The father and sister of Patient 1 had low levels of GIF, but did not have clinical evidence of Cbl deficiency. It seems likely that mutation(s) in the 5′ or other regulatory region of the IF gene must account for the lack of IF in the four patients without a demonstrated mutation, because none of the coding sequences and intron/exon junctions revealed additional sequence alterations. Even though the single base exchange in position 5 of exon 1 does not result in a significantly altered protein or a truncated protein, the consistent association of GIF Q5R with the phenotype provides a genetic marker associated with congenital IF deficiency.

REFERENCES

Carmel R. 1983. Gastric juice in congenital pernicious anemia contains no immunoreactive intrinsic factor molecule: study of three kindreds with variable ages at presentation, including a patient first diagnosed in adulthood. Am J Hum Genet 35: 67–77.

Ferec C, Raguenes O, Salomon R, Roche C, Bernard JP, Guillot M, Quere I, Faure C, Mercier B, Audrezet MP, Guillausseau PJ, Dupont C, Munnich A, Bignon JD, Le Bodic L. 1999. Mutations in the cationic trypsinogen gene and evidence for genetic heterogeneity in hereditary pancreatitis. J Med Genet 36: 228–232.

Glass GBJ. 1974. Gastric intrinsic factor and other vitamin B12 binders: biochemistry, physiology and relation to Vitamin B12 metabolism. Stuttgart: Georg Thieme Publishers. pp 60–63.

Gordon M, Hu C, Chokshi H, Hewitt JE, Alpers DH. 1991. Glycosylation is not required for ligand or receptor binding by expressed rat intrinsic factor. Am J Physiol 260:G736–G742.

Gordon M, Chokshi H, Alpers DH. 1992. In vitro expression and secretion of functional mammalian intrinsic factor using recombinant baculovirus. Biochim Biophys Acta 1132:276–283.

Gordon MM, Howard T, Becich MJ, Alpers DH. 1995. Cathepsin L initiates the intracellular ileal digestion of gastric intrinsic factor. Am J Physiol 268:G33–G40.

Hewitt JE, Gordon MM, Taggard RT, Mohandas TK, Alpers DH. 1991. Human gastric intrinsic factor: characterization of cDNA and genomic clones and localization to human chromosome 11. Genomics 10:432–440.

Katz M, Mehlman CS, Allen RH. 1974. Isolation and characterization of an abnormal intrinsic factor. J Clin Invest 53: 1274–1283.

Levine JS, Allen RH. 1985. Intrinsic factor within parietal cells of patients with juvenile pernicious anemia. Gastroenterology 88:1132–1136.

Lillibridge CB, Brandborg LL, Rubin CE. 1967. Childhood pernicious anemia: gastrointestinal secretory, histological, and electron microscopic aspects. Gastroenterology 52:792–809.

Lin HJ, Perng CL, Lee SD. 1994. The influence of sham feeding on gastric acid and pepsin secretion in the Chinese with duodenal ulcer and liver cirrhosis. Zhonghua Yi Xue Za Zhi (Taipei) 54:389–394.

Miller DR, Bloom GE, Streiff RR, LoBuglio AF, Diamond LK. 1966. Juvenile "congenital" pernicious anemia: clinical and immunological studies. N Eng J Med 275:978–983.

Miller SA, Dykes DD, Polesky HF. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acid Res 16:1216.

Remacha AF, Sambeat MA, Barcelo MJ, Mones J, Garcia-Die J, Gimferrer E. 1992. Congenital intrinsic factor deficiency in a Spanish patient. Ann Hematol 64:202–204.

Yang Y, Ducos R, Rosenberg AJ, Catrou PG, Levine JS, Podell ER, Allen RH. 1985. Cobalamin malabsorption in three siblings due to an abnormal intrinsic factor that is markedly susceptible to acid and proteolysis. J Clin Invest 76:2057–2065.

Zittoun J, Leger J, Marquet J, Carmel R. 1988. Combined congenital deficiencies of intrinsic factor and R binder. Blood 72:940–943.