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Research Article

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Molecular Basis of Selective IgG2 Deficiency

The Mutated Membrane-bound Form of γ2 Heavy Chain Caused Complete IgG2 Deficiency in Two Japanese Siblings

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Abstract

Patients with IgG2 deficiency have recurrent sinopulmonary infections caused by *Pneumococcus* and *Hemophilus*. Hereditary and selective IgG2 deficiency was suspected in two Japanese siblings whose serum IgG2 levels were under detection limits, while other serum levels of immunoglobulin subclasses were within normal ranges. Expression level of spontaneous germline Cγ2 transcript was normal, but that of the spontaneous mature Cγ2 transcript was greatly decreased in the patients' PBMCs, suggesting the presence of a defect at or after the class switch to Cγ2. We sequenced the Cγ2 gene region, and in both patients a homozygous one-base insertion (1793insG) was present in exon 4 of the Cγ2 gene, just upstream from the alternative splice site for M exons. The mutant membrane-bound γ2 heavy chain loses the transmembrane domain and the evolutionarily conserved cytoplasmic domain. Considering several lines of evidence showing that intact expression of the membrane-bound heavy chain is essential for a normal response of B cells and production of secreted immunoglobulin in mice, we concluded that 1793insG is responsible for selective and complete IgG2 deficiency in these two siblings. This is the first documentation of a mutation in human selective IgG2 deficiency. (*J. Clin Invest.* 1998. 101:677–681.) Key words: immunodeficiency • mutation • alternative splicing • M exon • IgG subclass

Introduction

The B cell antigen receptor (BCR)[1] initially expressed on B cells consists of membrane-bound immunoglobulin made up of μ heavy (H) chains plus light chains associated with the Ig-α-Ig-β heterodimer (1). When B cells are stimulated with antigen, they often undergo isotype switching leading to expression of H chains of other classes. These other H chains are present in secreted antibodies, and can also be expressed as a component of the BCR on the cell surface (2). The membrane-bound forms of γ, ε, and α H chains differ from those of μ and δ in that they possess cytoplasmic tails of 28 (γ, ε) and 14 (α) amino acids, which have been highly conserved throughout evolution. Membrane-bound immunoglobulin expression is essential for generation of efficient primary and secondary immunoglobulin responses. The primary immunoglobulin response as well as the expansion, maintenance, or both, of immunoglobulin-bearing memory B cells, depends strongly on the cytoplasmic tail of the heavy chain in mice (3–5).

IgG subclass deficiency is an immunodeficiency associated with the absence or severe reduction in the level of one or two subclass(es), but with normal or increased levels of other subclass(es). Only a homozygous deletion of the Ig H chain constant region genes has been found in some patients with IgG subclass deficiencies (6). The molecular basis of selective IgG2 deficiency has not been defined. We investigated molecular events of selective and complete IgG2 deficiency in two Japanese siblings in whom serum IgG2 levels were retained under the detection limit for at least 4 yr; some transient IgG2-deficient children show normalization without therapy (7). We identified a homozygous 1793insG mutation in Cγ2 exon 4, which resulted in loss of the transmembrane domain and the evolutionarily conserved cytoplasmic domain in a membrane-bound γ2 heavy chain (mγ2HC).

Methods

Case presentation. The proband (5-yr-old boy) and his elder brother (10 yr old), both Japanese, were examined. The parents were not consanguineous. Otitis media and respiratory infection such as pneumonia had occurred repeatedly in both patients. Serum IgG2 was undetectable (< 2.7 mg/dl), and the levels of other immunoglobulins, including other IgG subclasses, were practically normal in both patients. The specific IgG2 antibody to *Streptococcus pneumoniae* was also undetectable. The IgG2 level in the mother was within a normal range. Samples from the father were not available.

Serum IgG subclass level. The serum IgG subclass levels were measured, using ELISA as described (8). We used highly purified monoclonal antibodies against each of the four human IgG subclasses (HP6012 for IgG1, HP6014 for IgG2, HP6050 for IgG3, and HP6011 for IgG4, as recommended by the World Health Organization).

RNA preparation and reverse transcription. PBMCs were isolated from heparinized blood by gradient centrifugation in Ficoll-Paque (Pharmacia Fine Chemicals, Uppsala, Sweden). Total cellular RNA was extracted from the 1.0×10^6 PBMCs using Isogen (Nippon Gene, Tokyo, Japan). Total RNA was reverse transcribed at 37°C for 60 min with 200 U of MMLV reverse transcriptase (GIBCO-BRL, Gaithersburg, MD), and 30 ng of oligo d(T) primer in 20 μl of 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 10 mM DTT, 1 mM dNTPs, and 20 U of RNase inhibitor (Pharmacia Fine Chemcials).

Preparation of a Sty I fragment for competitor DNA. PCR amplification of interleukin-12 receptor cDNA (9) was carried out with flanking primers and cycling conditions as follows: forward, 5′-^{13}tcgcaggtggcagagaggct-3′; reverse, 5′-^{1296}TCCTGCCCCATTGCC-

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1. *Abbreviations used in this paper:* BCR, B cell antigen receptor; H chain, heavy chain; mγ2HC, membrane-bound γ2 heavy chain; RT, reverse transcription; sγ2HC, secreted form of γ2 H chain.

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Table I. Oligonucleotide Primers

Name	Direction	Sequence (5′ to 3′)	Position	Target	Reference
IS1	sense	^{954}tctcagccaggaccaaggac	Iγ 2	germline Cγ 2 transcript	10
HA1	antisense	^{922}ACTCGACACAACATTTGCG	Cγ 2 exon2 (hinge)	germline and mature Cγ 2 transcripts	11
JHS	sense	^{1940}CCTGGTCACCGTCTCCTCA	JH region	mature Cγ 2 transcript	12
HS	sense	^{892}ctctctgcagAGCGCAAATGTT-GTGTCGAG	Cγ 2 exon 2 (hinge)	Cγ 2 fragment (for Sma I digestion)	11
MA	antisense	^{217}ggccctggacaccccgcagagggtgn*	common M exon sequence	γ 2 M exons	14, 15
CS1	sense	ggggg<u>aatt^{17}c</u>cgggcctgactttggcttt	upstream from Cγ 2 exon1	Cγ 2	11
CA	antisense	tc<u>agaat^{1976}tc</u>agactcggcctgacccac	downstream from Cγ 2 exon4	Cγ 2	11
CS2	sense	^{1929}cccctgcgagactgtgatggttctttccgt	downstream from Cγ 2 exon4	γ 2 M exons	11
IS2	sense	^{740}ggggcttccaagccaacagggcaggaca	Iγ 2	lγ 2-Cγ 2 fragment (first PCR)	10
HA2	antisense	^{933}GCACGGTGGGCACTCG-ACACAACATTTGCG	Cγ 2 exon2 (hinge)	lγ 2-Cγ 2 fragment (first PCR)	11
SS	sense	^{1350}gacgggaggatctgggagctcac	downstream from 3′ Iγ 2 border	Sγ 2 (second PCR)	10
SA	antisense	^{72}ctcagtgggatgggctcacactcccttcct	inverted repeat of 5′ Sγ 2 segment	Sγ 2 (second PCR)	10

Oligonucleotide sequences for γ 2-specific amplification. de novo Eco RI site was introduced CS1 and CA primers for cloning (*underlined*). *n = A, G, C, or T.

CCAGA-3′; 95°C for 1 min, 60°C for 1 min 30 s, and 72°C for 3 min for 35 cycles. The product is 1284 bp in length and yields the expected 113 bp, 191 bp, 233 bp, 348 bp, and 399 bp fragments after Sty I digestion. The 233-bp fragment was separated by polyacrylamide-gel electrophoresis, and purified.

Oligomers used in following experiments. Fig. 1 schematically shows the locations of oligomers in the regions of JH, Iγ2, Sγ2, Cγ2, and M exons. Table I shows sequences of oligomers and some information. The nucleotide numbers on oligomers are given in the references. Capital letters were used in cases of coding sequences, and small letters were used in cases of noncoding sequences.

Quantitative PCR for germline Cγ2 transcript. PCR amplification of germline Cγ2 transcript was carried out with IS1 and HA1 primers and cycling conditions (10, 11) as follows: 94°C for 45 s, 60°C for 1 min, and 72°C for 1 min 30 s for 35 cycles. The plasmid containing 341 bp cDNA from germline Cγ2 transcript was partially substituted with the 233-bp Sty I fragment from interleukin-12 receptor cDNA, and was used as a competitor DNA. The PCR product of the wild-type was 341 bp, and that of the competitor was 287 bp. Each template contained the same amounts of cDNA from RNA extracted from 7.3×10^3 PBMCs, and one of threefold dilutions of germline Cγ2 transcript competitor from 1.0×10^{-1} attomole to 1.4×10^{-4} attomole. The equivalent point was determined by polyacrylamide-gel electrophoresis.

Quantitative PCR for mature Cγ2 transcript. To amplify the mature Cγ2 transcript specifically, we prepared a common 5′ primer for JH1, JH4, and JH5 genes, JHS (12). PCR amplification of the mature Cγ2 transcript was carried out with JHS and HA1 primers, and cycling conditions were as follows: 94°C for 1 min, 56°C for 1 min, and 72°C for 2 min, for 35 cycles. The competitor DNA for mature Cγ2 transcript was generated as follows: the plasmid containing 335-bp cDNA from the mature Cγ2 transcript was partially substituted with the 233-bp Sty I fragment from interleukin-12 receptor cDNA, and was used as a competitor DNA. The PCR product of the wild-type is 335 bp, and that of the competitor is 301 bp. Each template contained the same amounts of cDNA from RNA extracted from 7.3×10^3 PBMCs and one of several tenfold dilutions of mature Cγ2 transcript competitor from 1.0×10^1 –1.0×10^{-5} attomole. The equivalent point was determined by polyacrylamide gel electrophoresis.

Amplification of the genomic Sγ2 region. Genomic DNA was purified from a polynuclear cell fraction with a Sepa Gene (Sanko Jyunyaku, Tokyo, Japan) according to the manufacturer's instruction. Nested PCR amplification of genomic region for Sγ2 was carried out with IS2 and HA2 primers (first PCR), SS and SA primers (second PCR), and cycling conditions as follows: 94°C for 1 min, 65°C for 2 min, 72°C for 6 min for 40 cycles (first PCR), 94°C for 1 min, 65°C for 2 min, and 72°C for 4 min for 40 cycles (second PCR).

Amplification of the genomic Cγ2 region. PCR amplification of genomic region for Cγ2 was carried out with CS1 and CA primers and cycling conditions as follows: 94°C for 1 min, 60°C for 1 min, 72°C for 5 min for 5 cycles; and 94°C for 1 min, 60°C for 2 min, and 72°C for 2 min for 40 cycles.

Cloning of M exons for mγ2HC. Human Cγ2 gene cloned by a Charon 4A phage was kindly donated by Dr. T. Honjo (Kyoto University; 13). We subcloned a 6.4-kb Hind III fragment including Cγ2 exons to pTZ18U (United States Biochemical Corp., Cleveland, OH) from the phage clone. We prepared the primers on exon 4 of Cγ2 and the highly conserved sequence of human γ M2 exons (MA; 14, 15). Cycle conditions were as follows: 94°C for 1 min, 65°C for 2 min, and 72°C for 5 min for 40 cycles. The fragment was cloned into pT7Blue T-vector (Novagen, Inc., Madison, WI) and analyzed.

Amplification of cDNA for mγ2HC. Complementary DNA for mγ2HC was amplified with HS and MA primers and cycling conditions as follows: 94°C for 1 min, 65°C for 2 min, and 72°C for 5 min for 40 cycles.

Results

Quantitative reverse transcription (RT)-PCR for germline Cγ2 transcript. Expression of germline transcripts of each constant H region, except for Cδ, is apparently essential for class switch recombination (16, 17). The germline Cγ2 transcript was reported to be expressed spontaneously in PBMCs (18). We prepared 5′ Iγ2 and 3′ Cγ2 specific primers (Fig. 1), and quantitative RT-PCR for germline Cγ2 transcript was carried out. The relative quantities of germline Cγ2 transcript were assayed by the equivalent points of competitive PCR in the polyacrylamide-gel electrophoresis patterns (Fig. 2 *A*). Proband's target cDNAs and competitor were almost equivalent at 3.3×10^{-2} attomole, and those of a control were also equivalent at 3.3×10^{-2} attomole. As shown in Fig. 2 *C*, the relative quantity of germline Cγ2 transcript, determined at the equivalent points

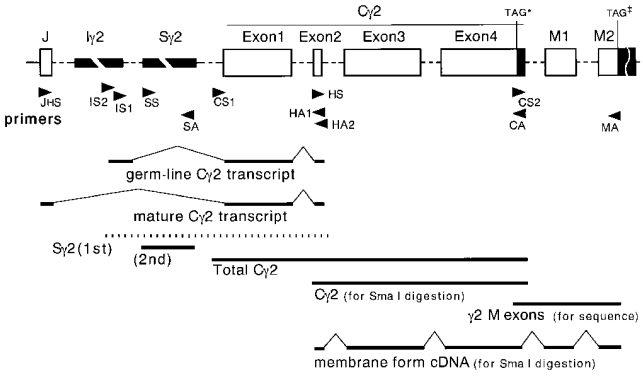


Figure 1. Schematic diagram of PCR strategies. Iγ2 and Sγ2 regions are indicated by *thick bars.* Coding exons are indicated by *open boxes*. 3′ Noncoding regions corresponding to secreted and membrane-bound forms of Cγ2 cDNAs are indicated by *solid boxes*. Termination codons of the secreted form (*) and membrane form (‡) are marked. Primers are indicated by *arrowheads*, and their names and sequences are given in Table I. PCR fragments amplified from genomic DNA are indicated by *thick lines*. The PCR fragment amplified by the first step of nested PCR of Sγ2 is indicated by *broken thick lines*. PCR fragments amplified from cDNA are indicated by *fragmented thick lines*.

in this competitive PCR, showed no apparent difference between patients and controls.

Quantitative RT-PCR for mature Cγ2 transcript. Since spontaneous IgG2 production in PBMCs can occur (19), spontaneous expression of mature Cγ2 transcript can also be expected. To amplify the mature Cγ2 transcript specifically, we set a sense primer on the conserved sequence for JH1, JH4, and JH5 genes (12), and an antisense primer on the hinge region of Cγ2 gene (Fig. 1) and performed quantitative PCR as shown in Fig. 2 *B*. Proband's target cDNAs and competitor were almost equivalent between 1.0×10^{-4} and 1.0×10^{-5} attomole, and those of a control were almost equivalent between 1.0×10^{-1} and 1.0×10^{-2} attomole (Fig. 2 *B*). The mature transcript levels in both patients were reduced to about one-hundredth or one-thousandth of the level of the controls (Fig. 2 *C*).

Taken together with the normal expression level of germline transcript, the defect was thought to lie at or after the class switch recombination step. We also amplified the genomic Sγ2 region specifically from the proband and from controls (Fig. 1), however, no apparent size difference was detected in the amplified fragments (data not shown).

Amplification and sequencing of genomic Cγ2 region. Because of the high degree of homology between the human Cγ1, Cγ2, Cγ3, and Cγ4 genes (the percentage of homology in the coding regions is 95.3–97%, with a homology of 93.3–95% in noncoding areas; 20), we designed a pair of primers that would specifically amplify the Cγ2 gene (Fig. 1). The product of the proband's Cγ2 region, which includes exons 1–4 for the Cγ2 gene, was entirely sequenced.

When the nucleotide is numbered according to the database EMBL J00230, the termination codon of the secreted form of γ2 H chain (sγ2HC) is ^{1800}TGA. We identified one-base replacement from T to C (1790T/C), and one-base insertion of G between nucleotide numbers 1792 and 1793 (1793insG) in the proband (Fig. 3 *A*). Sequence analysis revealed that both 1790T/C and 1793insG were detected homozygously in both the patients' DNAs, heterozygously in their mother's DNA, but not detected in 28 healthy controls. These two mutations, which had only a 3-bp distance from each other, were linked in the alleles tested. Therefore, using 1790T/C, which creates a new Sma I site, we screened genomic DNAs of 65 independent normal controls (130 alleles), but detected no 1790T/C mutant allele (Fig. 3 *B*). 1790T/C causes no amino acid change, and 1793insG causes only a deletion of the last lysine residue in sγ2HC by a frame shift (Fig. 4 *A*).

Cloning and sequencing M exons for mγ2HC. To assess the effects of these mutations on mγ2HC, we cloned and sequenced the two M exons for mγ2HC. Sequences of γ2 M

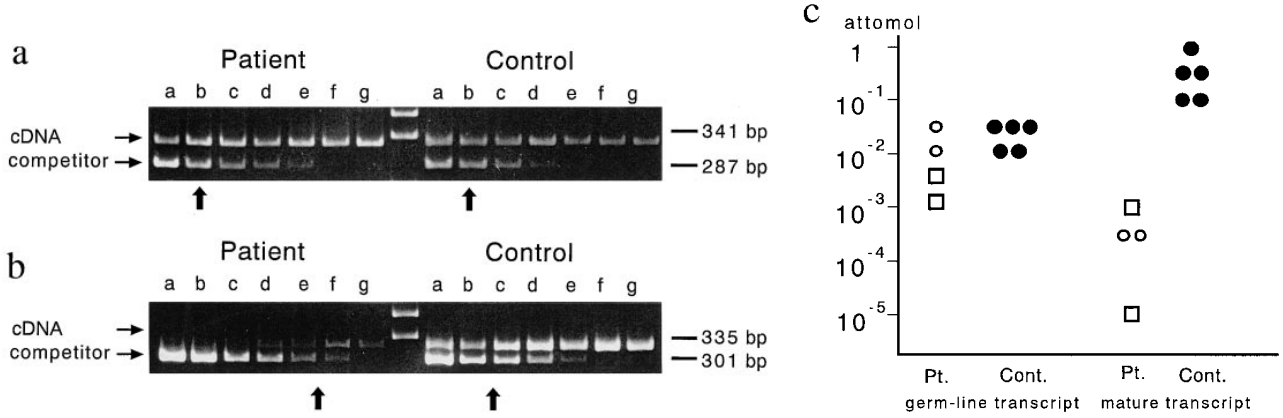


Figure 2. (*a*) Competitive PCR for the germline Cγ2 transcript. Each template contained the same amounts of cDNA from RNA extracted from 7.3×10^3 PBMCs and one of threefold dilutions of germline Cγ2 transcript competitor from 1.0×10^{-1}–$1.4\ 10^{-4}$ attomole (lanes *a–g*). Target cDNA and competitor were almost equivalent at 3.3×10^{-2} attomole (lane *b*) in both the proband and a control, as indicated by *arrows*. (*b*) Competitive PCR for mature Cγ2 transcript. Each template contained the same amounts of cDNA from RNA extracted from 7.3×10^3 PBMCs and one of tenfold dilutions of mature Cγ2 transcript competitor from 1.0×10^1–1.0×10^{-5} attomole (lanes *a–g*). The probands' target cDNAs and competitor were almost equivalent between 1.0×10^{-4} (lane *e*) and 1.0×10^{-5} attomole (lane *f*), and those of a control were almost equivalent between 1.0×10^{-1} (lane *b*) and 1.0×10^{-2} attomole (lane *c*) as indicated by *arrows*. (*c*) The expression levels of germline and mature Cγ2 transcripts were plotted logarithmically. ○, patient 1; □, patient 2; ●, controls.

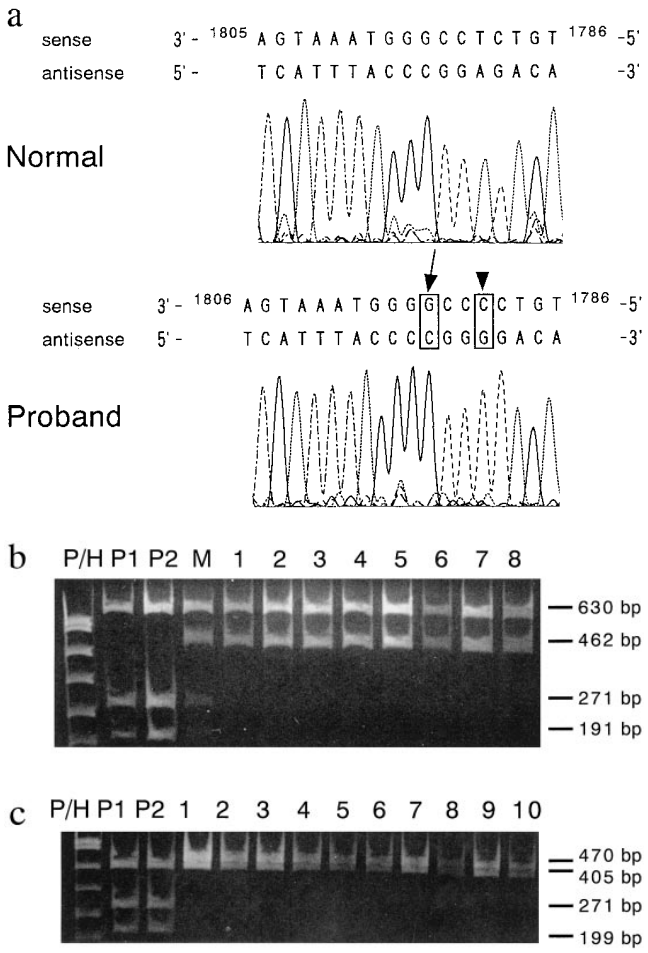


Figure 3. (*a*) Sequence analysis of the Cγ2 gene (antisense patterns). The proband sequence had a ^{1790}T to C substitution (1790T/C; *arrowhead*) and a ^{1793}G insertion (1793insG; *arrow*). (*b* and *c*) Sma I-digested PCR products of Cγ2 region from genomic DNA (*b*) and cDNA for mγ2HC (*c*). *P/H*, size marker; *P1* and *P2*, patients; *M*, their mother; 1−10, controls. The PCR product from genomic DNA is 1092 bp long and yields the expected 630- and 462-bp fragments (normal) or 630-, 271-, and 191-bp fragments (mutant) after Sma I digestion. The PCR product from cDNA is 875 bp long and yields the expected 470- and 405-bp fragments (normal) or 405-, 271-, and 199-bp fragments (mutant) after Sma I digestion. In case of the cDNA, both1790T/C and 1793insG are required for Sma I digestion (see *a*).

exons (DDBJ, EMBL, and GenBank accession numbers AB006775 and AB006776) were highly homologous to those of other γ M exons (14, 15). The γ2 M1 exon was identified ~1.1 kbp downstream from the termination codon for sγ2HC. The M2 exon started 447 bp downstream from the last base of M1 exon.

Amplification of cDNA for mγ2HC. Thereafter, we designed a pair of primers so that the cDNA for mγ2HC was specifically amplified (Fig. 1). The cDNAs were successfully amplified in controls and in both patients. The alternative splice site was found at ^{1794}G/GT in the Cγ2 exon 4 just downstream from the mutation site (Fig. 4 *A*). Hence, 1793insG resulted in a frame shift of mRNA for mγ2HC, causing complete changes of amino acid sequence encoded by M1 and M2 exons and generated an abnormal 117 amino acid sequence (Fig. 4, *A* and *B*). The cDNAs for mγ2HC were then subjected to Sma I restriction assay. As expected, the mutations were homozygous in both patients' cDNAs, but the mutations were nil in 20 healthy controls' cDNAs (Fig. 3 *C*).

Discussion

We cloned M1 and M2 exons for mγ2HC, identified the alternative splice site in Cγ2 exon 4 for M exons, and showed that 1793insG in Cγ2 exon 4 caused a frame shift in mγ2HC that resulted in complete changes of amino acid sequence encoded by M1 and M2 exons. We propose that 1793insG is the cause of complete and selective IgG2 deficiency in these two Japanese siblings. Since the heterozygous mother had a normal IgG2 level, this mutation is unlikely to account for the large number of individuals with a low IgG2 level.

There are at least two possibilities that would explain the mechanism of IgG2 deficiency with the frame shift of mγ2HC. One is that this mutation results in complete loss of function and structure as a BCR, and the mutant mγ2HC never appears on the B cell surface. The other is that the mutant mγ2HC, which lost the conserved motif in the cytoplasmic tail, could be expressed on the B cell surface; however, it could not complete the signal transduction or antigen processing. It was conceivable that the first case was the counterpart of mice lacking the transmembrane and cytoplasmic domains of mγ1HC or mεHC, and the latter case was mice lacking most of the cytoplasmic tail of mγ1HC or mεHC, respectively (3, 4). The levels of serum IgG1 and IgE were reduced to a lesser extent in mice lacking the transmembrane domains than in mice lacking partial cytoplasmic domains. Because of a complete deficiency of serum IgG2, it seems likely that the patient's B cells could not express mγ2HC on the surface. Hydropathy profile of normal and mutant sequences encoded by M exons was investigated according to Kyte and Doolittle (21). The average hydropathy of 19-residue segments of normal mγ2HC transmembrane domain was $> +2$, satisfying a condition of membrane-spanning sequences. On the other hand, most of the mutant sequence encoded by M exons was hydrophilic, and the average hydropathy of any 19-residue segment of the mutant sequence was $< +1.2$, which strongly suggests that there is no membrane-spanning sequence in the mutant sequence (data not shown).

Weiser et al. reported that transformed B cells expressing an IgG2a BCR require the cytoplasmic tail of γ2a, and specifically a tyrosine-based motif in this structure (5) that is also present in human mγ2HC for efficient presentation of antigen to T cells after surface immunoglobulin–mediated internalization. The frame shift mutation lost the tyrosine-based motif (5). Based on these results in mice, we conclude that the frame shift of mγ2HC caused by 1793insG resulted in a complete and selective IgG2 subclass deficiency.

The above cases including our case were those of a selective immunoglobulin deficiency. Cases of defects in the μ H chain were also noted both in mice and humans. In a model mouse with a disrupted M exon of the μ H chain gene, a complete absence of B cell production and profound hypogammaglobulinemia were evident (22). Mutations in the μ H chain gene were also identified in patients with agammaglobulinemia who did not have mutations in Bruton's tyrosine kinase (23). These observations mean that an intact membrane-bound μ H chain, which mediates signals into pro-B cells via

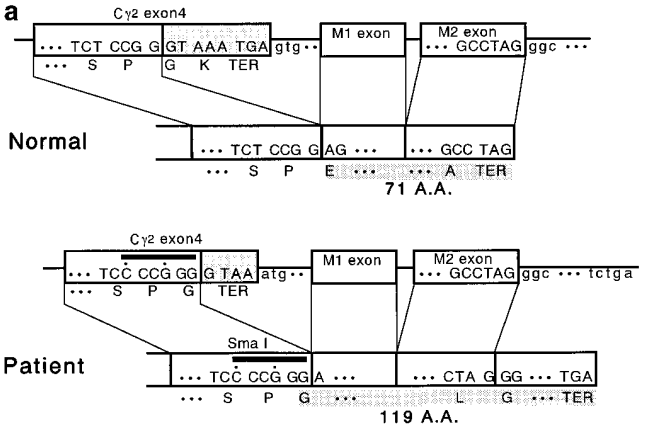
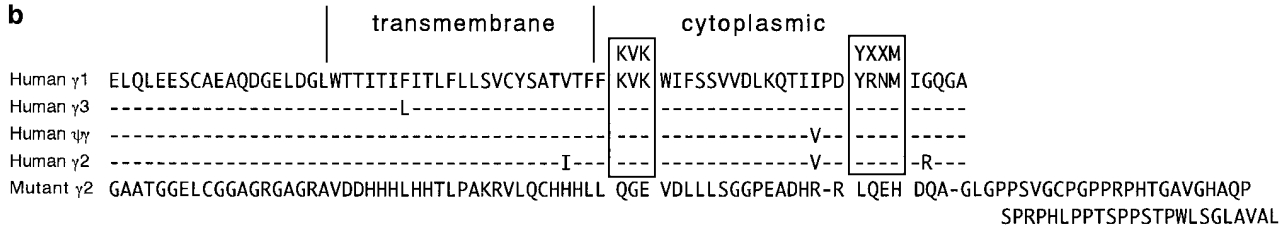


Figure 4. (*A*) Diagram of the last exon (exon 4) and M exons for mγ2HC. The COOH terminal of sγ2HC is marked by a *shadowed box,* and that of mγ2HC is marked by *shadowed amino acid sequences.* The1790T/C and 1793insG are shown by *dotted letters*. Just before the alternative splice site, the new Sma I site was introduced in the mutant (*black bars*). (*B*) Amino acid sequences encoded by M exons for human γ H chains. The one base insertion results in a frame shift of mγ2HC, and generates a completely different amino acid sequence of the transmembrane and intracellular portion. The hydrophobic amino acids cluster and conserved cytoplasmic tail residues (Lys-Val-Lys motif and Tyr-X-X-Met motif) are absent in the mutant sequence. Nucleotide sequences around M1 and M2 exons for mγ2HC will appear in the DDBJ, EMBL, and GenBank nucleotide sequence databases with accession numbers AB006775 and AB006776.



BCR, is essential for B cell development in both mice and humans. The efficient primary and secondary immunoglobulin responses depend on the appropriate expression of the membrane-bound form of the H chain in humans as well as mice.

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