Corrections and Retraction

CORRECTIONS

MEDICAL SCIENCES. For the article “Ex vivo cell labeling with 64Cu–pyruvaldehyde-bis(N4-methylthiosemicarbazone) for imaging cell trafficking in mice with positron-emission tomography,” by Nora Adonai, Khoi N. Nguyen, Joseph Walsh, M. Iyer, Tatsushi Toyokuni, Michael E. Phelps, Timothy McCarthy, Deborah W. McCarthy, and Sanjiv Sam Gambhir, which appeared in issue 5, March 5, 2002, of Proc. Natl. Acad. Sci. USA (99, 3030–3035; first published February 26, 2002; 10.1073/pnas.052709599), the author name Nora Adonai should have appeared as Nona Adonai. The corrected author line appears below. The online version has been corrected.

Nona Adonai, Khoi N. Nguyen, Joseph Walsh, M. Iyer, Tatsushi Toyokuni, Michael E. Phelps, Timothy McCarthy, Deborah W. McCarthy, and Sanjiv Sam Gambhir

www.pnas.org/cgi/doi/10.1073/pnas.0604177103

MEDICAL SCIENCES. For the article “Familial hypercatabolic hypoproteinemia caused by deficiency of the neonatal Fc receptor, FcRn, due to a mutant β2-microglobulin gene,” by Manzoor A. Wani, Lynn D. Haynes, Jonghan Kim, C. L. Bronson, Chaity Chaudhury, Sudhasri Mohanty, Thomas A. Waldmann, John M. Robinson, and Clark L. Anderson, which appeared in issue 13, March 28, 2006, of Proc. Natl. Acad. Sci. USA (103, 5084–5089; first published March 20, 2006; 10.1073/pnas.0600548103), the authors note that on page 5087, the first full sentence in the left column appears incorrectly, due to a printer’s error. “Most of these mutations have been cytoplasmic tail (CT) deletions in the leader sequence that result in reading frame shifts and downstream nonsense mutations” should read: “Most of these mutations have been CT deletions in the leader sequence that result in reading frame shifts and downstream nonsense mutations.” This error does not affect the conclusions of the article.

www.pnas.org/cgi/doi/10.1073/pnas.0604086103

FIG. 5. AMS detection of 14C in human plasma. Measurements were performed on 30 μl of plasma; the entire sample set consumed <1 ml of whole blood.

www.pnas.org/cgi/doi/10.1073/pnas.0604332103

APPLIED BIOLOGICAL SCIENCES. For the article “Human vitamin B12 absorption measurement by accelerator mass spectrometry using specifically labeled 14C-cobalamin,” by Colleen Carkeet, Stephen R. Dueker, Jozsef Lango, Bruce A. Buchholz, Joshua W. Miller, Ralph Green, Bruce D. Hammock, John R. Roth, and Peter J. Anderson, which appeared in issue 15, April 11, 2006, of Proc. Natl. Acad. Sci. USA (103, 5694–5699; first published April 3, 2006; 10.1073/pnas.0601251103), the authors note an ambiguity in the units of Fig. 5, which contradicted one of the axes. The figure and its corrected legend appear below.
For the article “Consistent simulations of multiple proxy responses to an abrupt climate change event,” by A. N. LeGrande, G. A. Schmidt, D. T. Shindell, C. V. Field, R. L. Miller, D. M. Koch, G. Faluvegi, and G. Hoffmann, which appeared in issue 4, January 24, 2006, of Proc. Natl. Acad. Sci. USA (103, 837–842; first published January 13, 2006; 10.1073/pnas.0510095103), the authors note that the color bar label for Fig. 2a is inverted. The corrected figure and its legend appear below. This error does not affect the conclusions of the article.

![Corrected Figure 2a](image_url)

Fig. 2. Proxy validation for model simulations indicates the relationship between $\delta^{18}O$ and temperature in the atmosphere and ocean. (a) The temporal gradient in SAT vs. $\delta^{18}O_{\text{precip}}$ (‰/°C), the relationship crucial for interpreting many atmospheric paleoclimate records, is steepest and most highly correlated at high latitudes and is very shallow or reversed with lower correlation in the subtropics and tropics. (b) The correlation of SST vs. $\delta^{18}O_{\text{calcite}}$, the relationship crucial for interpreting many sediment core paleoclimate records, breaks down in most of the northern North Atlantic, with the exception of areas along the northern Greenland, Iceland, and Norwegian (GIN) Seas.
Familial hypercatabolic hypoproteinemia caused by deficiency of the neonatal Fc receptor, FcRn, due to a mutant β2-microglobulin gene


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Contributed by Thomas A. Waldmann, January 20, 2006

Two siblings, products of a consanguineous marriage, were markedly deficient in both albumin and IgG because of rapid degradation of these proteins, suggesting a lack of the neonatal Fc receptor, FcRn. FcRn is a heterodimeric receptor composed of a nonclassical MHC class I α-chain and β2-microglobulin (β2m) that binds two ligands, IgG and albumin, and extends the catabolic half-lives of both. Eight relatives of the siblings were moderately IgG-deficient. From sera archived for 35 years, we sequenced the two siblings’ genes for the heterodimeric FcRn. We found that, although the α-chain gene sequences of the siblings were normal, the β2m genes contained a single nucleotide transversion that would mutate a conserved alanine to proline at the midpoint of the signal sequence. Concentrations of soluble β2m and HLA in the siblings’ sera were <1% of normal. Transfection assays of β2m-deficient cultured cells with β2m cDNA indicated that the mutant β2m supported <20% of normal expression of β2m, MHC class I, and FcRn proteins. We concluded that a β2m gene mutation underlies the hypercatabolism and reduced serum levels of albumin and IgG in the two siblings with familial hypercatabolic hypoproteinemia. This experiment of nature affirms our hypothesis that FcRn binds IgG and albumin, salvages both from a degradative fate, and maintains their physiologic concentrations.

albumin | IgG | MHC class I | hypoproteinemia | hypogammaglobulinemia

The neonatal Fc receptor, FcRn, is a heterodimer of a nonclassical MHC class I (MHC I) α-chain and β2-microglobulin (β2m) that binds the two most abundant serum proteins, IgG (1) and albumin (2), after their constitutive uptake by many cells of the body. FcRn binds both ligands with high affinity at the low pH of acid endosomes and releases them at the physiologic pH of the cell surface, where they are free to circulate, thus diverting them from lysosomal degradation (2–5). Such FcRn-mediated recycling explains the uniquely long half-lives and the direct concentration–catabolism effect of IgG and albumin. The capacity of this salvage mechanism is astonishing; without FcRn, mice would need dramatically increased albumin and IgG synthetic rates to maintain normal concentrations of these proteins (6).

In accordance with this hypothesis, we have found that mice lacking FcRn because of defective genes for the α-chain or β2m show low serum concentrations and rapid degradation rates of both proteins (7). Searching for analogous FcRn-deficient humans, we reexamined two siblings, born of a consanguineous marriage, whose disorder, familial hypercatabolic hypoproteinemia (FHH), had been thoroughly studied in the late 1960s (8, **). Both siblings manifested hypercatabolic albumin and IgG deficiencies for which no known cause could be found at the time of investigation. Recognizing that these two individuals might have been homozygous for a defect in one or the other of the two genes encoding the heterodimeric FcRn, we analyzed their archived sera, the subjects themselves being no longer available. We now ascribe their defect to a severe FcRn deficiency resulting from a β2m gene mutation.

Results

Analyzing serum samples by ELISA, we found soluble β2m (sβ2m) concentrations for the two siblings, S1 and S2, to be <10 ng/ml (Table 1). These values were 100-fold less than those for our three control groups, which included normal sera (N1, N2, and N3) analyzed simultaneously, eight National Institutes of Health (NIH)-archived sera analyzed simultaneously, and assay normal values. The serum concentrations of soluble HLA (sHLA) were likewise very low, being <0.2% of the normal mean (Table 1). Values for serum iron, total iron-binding capacity (TIBC), percent transferrin saturation with iron (%TS), and ferritin, determined either in our laboratories or at NIH in the 1960s, were normal (Table 1).

We PCR-amplified and sequenced all exons of both the β2m and the FcRn α-chain genes from DNA purified from sera of the two siblings and three normal individuals. Whereas gene sequences in both directions of β2m exons 2, 3, and 4 of all individuals were normal, the sequences of exon 1 of β2m in the two siblings alone showed a single nucleotide transversion (G913C) (Fig. 1a). This mutation was confirmed by digesting the amplified exon 1 DNA with a restriction enzyme that uniquely distinguishes between the normal and the mutant nucleotide at this site (Fig. 1c). The pattern of bands of digested DNA from the two siblings was distinctly different from those of the normal individuals (Fig. 1b). The G913C transversion would be predicted to cause a mutation from alanine to proline at amino acid 11 at the midpoint of the signal sequence (Fig. 1d). We estimated the frequency of this G913C mutation in an ancestry-matched population by assessing a collection of 100 DNA samples with the restriction enzyme digestion assay described in Fig. 1c and found no other instances of such a mutation. Therefore, the gene frequency of this mutation in the sampled population is <0.5%. The sequences of the gene for the FcRn α-chain in the two siblings were normal.

To test the prediction that the mutant β2m signal sequence would hinder cellular expression of β2m protein, we transfected a β2m-lacking human cell line with a plasmid containing mutant β2m cDNA bearing the single nucleotide transversion (mut).
with wild-type (wt) β2m cDNA, and with the empty plasmid vector (p). We then assessed cell surface expression of β2m protein by flow cytometry by using a fluorescent antibody specific for β2m. The fluorescence profiles shown in Fig. 2a indicate that cells transfected with wt β2m cDNA were on average brighter [mean fluorescence intensity (MFI) = 109] than control cells (MFI = 4), and that a broad shoulder of very bright cells was apparent (peak of 20% of mut transfected cells at a fluorescence intensity of ~450). Cells transfected with empty plasmid were not distinguishable from cells incubated with an irrelevant antibody of the same isotype as the anti-β2m antibody (Fig. 2c), which was consistent with the cells being β2m-deficient. Cells transfected with mut β2m and stained with anti-β2m antibody (MFI = 15) were only 10% as bright as cells transfected with wt β2m cDNA. An equivalent conclusion was reached if only cells brighter than the vector-transfected cells were analyzed; i.e., mut β2m-transfected cells were 6% as bright as wt β2m-transfected cells.

Anticipating that β2m expression would rescue the surface expression of MHC I in this β2m-deficient cell line, we also

### Table 1. Serum components of the two FHH siblings and controls

<table>
<thead>
<tr>
<th>Serum component</th>
<th>sβ2m, μg/liter</th>
<th>sHLA, μg/liter</th>
<th>Iron, μg/liter</th>
<th>TIBC, μg/liter</th>
<th>%TS</th>
<th>Ferritin, μg/liter</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FHH siblings</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S1</td>
<td>9</td>
<td>16</td>
<td>8, 8*</td>
<td>40, 27*</td>
<td>21, 29*</td>
<td>5</td>
</tr>
<tr>
<td>S2</td>
<td>7</td>
<td>2</td>
<td>7, 4*</td>
<td>32, 28*</td>
<td>13, 15*</td>
<td>6</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal human sera</td>
<td>1,180 ± 126</td>
<td>838 ± 536</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Archived sera</td>
<td>1,300 ± 1,000</td>
<td>788 ± 793</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Laboratory standard values</td>
<td>800–2,000</td>
<td>1,600 ± 1,000</td>
<td>7–18</td>
<td>25–45</td>
<td>26–38</td>
<td>3–30</td>
</tr>
</tbody>
</table>

Sera from the two siblings, S1 and S2, along with three normal human sera and eight sera archived at NIH since the 1960s, were tested for sβ2m, sHLA, serum iron, TIBC, %TS, and ferritin, and they were compared with laboratory standard values for each assay, given as either normal range or mean ± SD. The sβ2m and sHLA values for S1 and S2 are the means of two determinations, with deviations from the mean being <10%. S2 values have been corrected as described in Materials and Methods. ND, not determined.

*Values extracted from NIH medical records from the 1960s.

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measured the binding of an MHC I-specific antibody in parallel flow cytometric experiments (Fig. 2 b and d). Cells transfected with wt β2m cDNA showed high levels of MHC I expression (MFI = 3,176); those transfected with empty vector (MFI = 156) showed no expression beyond background levels; and those transfected with mut β2m cDNA (MFI = 738) showed levels of expression <20% of that seen in the wt (duplicate experiments, 19% and 13%). Equivalent results were seen when only those cells brighter than vector-transfected cells were analyzed; i.e., mut β2m-transfected cells were 20% as bright as wt β2m-transfected cells.

To measure total cellular, rather than simply surface, expression of mut β2m, we subjected these same transfected cell populations to an alternative analysis, namely, immunoblotting of detergent lysates of cells. Analyzing cell lysates directly, we first immunoadsorbing with anti-FcRn antibody (H9251), we noted that very little FcRn expression have been described in humans, some cancer cell lines bear mutations in signal sequences have been described in specific diseases (12–15).

We also measured the capacity of mutant β2m to enhance the expression of the FcRn heterodimer. Assessing cell lysates by immunoblot with anti-FcRn α-chain antibody, we noted that very little FcRn α-chain was expressed, even after transfection with wt β2m (Fig. 3a, lane 2). We therefore transfected the cells with FcRn α-chain cDNA and noted α-chain protein expression (Fig. 3a, lane 4) in the absence of β2m, as others have reported (9). Cotransfecting these cells with β2m cDNA, we noted that mut β2m was only 22% as effective as wt β2m in enhancing the amount of FcRn α-chain seen in immunoblots (average of two experiments, 14% and 30%) (Fig. 3a, lanes 5 and 6 vs. 4). Determination of whether diminished expression of mut β2m protein resulted from defective transcription or translation was beyond the scope of the present study.

**Discussion**

Our analysis of the archived serum from these two consanguineous siblings with FHH indicated that both were homozygous for a single nucleotide transversion of the β2m gene that encoded a mutation from an evolutionarily conserved alanine to a proline in the center of the hydrophobic core of the β2m signal sequence α-helix. A proline at this site would be expected to disrupt targeting of the nascent polypeptide to the endoplasmic reticulum (10). Indeed, the serum β2m concentrations in the FHH siblings were very low, and the mutant β2m cDNA was able to support only 10–20% of wt β2m expression in a cell transfection assay.

It is clear that a proline mutant might compromise signal peptide function, depending on where it is situated in the sequence. The mutation of alanine to proline does not affect the net charge or hydrophobicity of the α-helix but presumably disturbs the secondary structure as a result of its helix-breaking properties, thus leading to compromised interaction with the signal recognition protein and abnormal insertion into the endoplasmic reticulum membrane (11). Similar proline mutations in signal sequences have been described in specific diseases (12–15).

Although no β2m gene mutations abrogating protein expression have been described in humans, some cancer cell lines bear...
null $\beta_2m$ genes (16). Most of these mutations have been cytoplasmic tail (CT) deletions in the leader sequence that result in reading frame shifts and downstream nonsense mutations. The only known substitution mutation is in the Daudi B cell line, where methionine is replaced by isoleucine as the N-terminal amino acid, abolishing $\beta_2m$ expression.

$\beta_2m$ is generally known to be required for efficient expression and function of several classical and nonclassical members of the MHC I family of proteins, which are heterodimers consisting of $\beta_2m$ and a distinguishing $\alpha$-chain of about 43 kDa. The family members include FcRn, CD1, hemochromatosis protein (HFE), and MHC I itself. Considering the data available on the two siblings studied, we can conclude with virtual certainty that they were FcRn-deficient. Their phenotype, which features low serum IgG and albumin concentrations and hypercatabolism of both proteins, is mirrored by the $\beta_2m$ and FcRn $\alpha$-chain knockout mouse strains (2). Unfortunately, we have no means of assessing FcRn expression directly in these siblings because we have no available neither tissue nor a satisfactory assay of serum-soluble FcRn. However, the mutant $\beta_2m$ cDNA, transfected into $\beta_2m$-deficient cells, was severely deficient in supporting the expression of FcRn. Moreover, SHLA concentrations in serum were very low in both siblings, and in a cell transfection assay, mut $\beta_2m$ cDNA was able to rescue only $\sim$20% of normal surface expression of MHC I protein. Thus, these siblings were likely severely MHC I-deficient. Although they were probably also HFE-deficient, they showed no increase in saturation of transferrin with iron, perhaps because of their relative youth (34 and 17 years of age) or the low penetrance of the type 1 hemochromatosis genotype.

Concluding that the two FHH siblings were homozygous for a defective $\beta_2m$ allele, we then reassessed the remainder of the pedigree for whom serum IgG concentrations were available (table 1 in ref. 8). On the basis of the $\beta_2m$ genotype of the two siblings, we inferred that six of eight of the studied relatives were definitely heterozygous, and two were likely heterozygous, for the $\beta_2m$ gene (patients 5 and 6, the unaffected brother and sister of S1 and S2, had a 66% chance of being heterozygous). We noted that the IgG concentrations of these eight relatives (mean $\pm$ SD = 9.2 $\pm$ 1.4 mg/ml; $P < 0.001$) were distinctly lower than the normal value of 12.4 $\pm$ 2.2 mg/ml. Although we know nothing about the albumin concentrations in the eight relatives, the IgA, IgM, IgD, and $\kappa$-chain concentrations were not different from normal. A single normal $\beta_2m$ allele thus may confer a moderate IgG deficiency. It is unclear whether mice expressing a single $\beta_2m$ allele are IgG-deficient, although the one large published study of heterozygous $\beta_2m$ knockout mice concluded that there were no gross differences in IgG concentrations between wt and heterozygotes (17). Nevertheless, $\beta_2m$ heterozygote mice expressed reduced levels of splenocyte MHC I and had diminished numbers of CD8-expressing thymocytes (18).

On the basis of our analysis of 35-year-old archived sera, we conclude that FcRn deficiency caused by a mutant $\beta_2m$ gene underlies the hypercatabolism of both IgG and albumin seen in FHH.

**Materials and Methods**

**Cell Line.** The $\beta_2m$-lacking human melanoma cell line FO-1 (19), kindly provided by William Carson (Ohio State University, Columbus) was maintained in RPMI 1640 medium (Invitrogen) supplemented with 10% heat-inactivated FCS in a 5% CO$_2$-humidified atmosphere at 37°C.

**Serum Samples.** Sera from S1 and S2 and from eight anonymous NIH patients had been archived in NIH freezers since the late 1960s. Neither the patients nor tissue were available. The S2 serum used for protein determinations was found to have been concentrated 1.6-fold (calculated by measurement of electrolytes), likely because of sublimation in storage. The results given in Table 1 have, therefore, been corrected by dividing the observed values by 1.6. Additional sera were collected from three healthy adult individuals.

**$\beta_2m$ Serum Concentrations.** The $\beta_2m$ concentrations of the serum samples were determined by using a sandwich ELISA ($\beta_2m$ ELISA Kit; Alpha Diagnostic, San Antonio, TX). Briefly, 100 µl of diluted serum of the normal individuals (1:100 dilution) and the two FHH patients (1:5 dilution) were loaded in duplicate wells in a 96-well plate coated with anti-human $\beta_2m$ (h$\beta_2m$) monoclonal antibody and incubated at room temperature for 30 min. The wells were washed five times with wash buffer, and 100 µl of horseradish peroxidase-conjugated rabbit polyclonal anti-$\beta_2m$ antibody was added into each well. The plate was further incubated at room temperature for 30 min. After five washes with wash buffer, 200 µl of substrate solution mix was added into the wells. The reaction was stopped by adding 50 µl of stop solution (0.5 M sulfuric acid), and the yellow color developed was read within 30 min at 450 nm by using an EL$\_x$ 808 ELISA plate reader (Biotek Instruments, Luton, U.K.). A reference $\beta_2m$ standard ranging from 1.25 to 50 ng/ml was run in parallel with each experiment. Normal values for this assay, according to the supplier, are given in Table 1.

**Measurement of Iron, Ferritin, and Transferrin.** Serum iron and TIBC were measured in the clinical laboratory of the Columbus Children’s Hospital by using the pyridyl azo dye ascorbic acid method for iron, a competitive immunoassay for ferritin, and the alumina adsorption method for TIBC. %TS was calculated as 100 $\times$ serum iron concentration/TIBC. Normal values in Table 1 are from the same laboratory. Values at the time of the FHH siblings’ NIH evaluation were taken from the hospital record.
ELISA for sHLA. ELISA for sHLA was performed as described (20). Briefly, mAb TF25–99 (a kind gift from SangStat Medical Corporation, Menlo Park, CA), which recognizes both β2m-free and associated HLA (21), was used as the capture antibody; rabbit anti-human β2m (Accurate Chemical & Scientific, Westbury, NY) and horseradish peroxidase-coupled goat anti-rabbit IgG (Sigma) were used as the detection antibodies. A standard curve was generated with purified HLA-B7 diluted serially to concentrations between 20 and 0.3125 ng/ml. Tetramethylbenzidine microplate substrate and stop solution (Kirkegaard & Perry Laboratories) were used. The plates were read at 450 nm by using the Bio-Tek ELx800 reader, and data were analyzed with KC3 software (Biotek Instruments). Values for sera of 27 normal individuals and the 8 archived NIH sera are given in Table 1.

Serum DNA Extraction, PCR Amplification, and Mutational Analysis. DNA was extracted from 400 μl of serum by adsorption to silica-gel membrane (QIAamp DNA Blood Mini Kit; Qiagen, Valencia, CA) and quantified with the PicoGreen dsDNA quantitation method by fluorometry (Molecular Probes). PCR was performed in 50-μl reactions containing 2.5 units of TaqDNA polymerase (Qiagen), 1× buffer and solution Q, 2 mM MgCl₂, 0.4 μM dNTP mix, 200 nM of each primer, and 25 ng of serum DNA as template. Samples were subjected to PCR conditions as follows: initial denaturation at 95°C for 4 min, followed by 35 cycles of 94°C for 1 min, annealing at 57°C for 1 min, extension at 72°C for 1 min, and a final extension cycle at 72°C for 10 min. A positive cellular DNA and a negative template-free control were also run in parallel with each PCR run. After amplification, the DNA was resolved by electrophoresis through 1.5% agarose gels containing 0.5 μg/ml ethidium bromide, and was visualized on a UV transilluminator and compared with molecular weight markers run in parallel. Single specific bands observed in all PCRs were excised from the agarose gel and purified on Qiaex II gel extraction beads (Qiagen), eluted in water, and sequenced with a 3730 DNA Analyzer (Applied Biosystems). All exonic fragments were amplified individually and sequenced one at a time. Primer pairs were chosen from the sequence at least 100 bp upstream of the exon start and at least 100 bp downstream from the end of exon, to ensure analysis of the junctions. Although normal, the sequences of S2 and one normal control individual (N3) contained a single copy of a known allotypic polymorphism, a synonymous C2585T (22) change encoding arginine-171 like the normal allele (23) (GenBank accession no. AF200219; data not shown).

Plasmids and Site-Directed Mutagenesis of hβ2m cDNA. We used expression vectors pCMV-Sport6 (cDNA clone MGC:45276; Open Biosystems, Huntsville, AL) containing full-length hβ2m cDNA (24) (GenBank accession no. BC032589), and pREP9 (Invitrogen) containing full-length human FcRn α-chain cDNA (23) (GenBank accession no. U12255). FH8 mutant cDNA was prepared by site-directed mutagenesis (Quik Change Kit; Stratagene), with pCMV-Sport6 β2m cDNA as the template. The upper oligonucleotide, 5′-GCCTTAATGTCGTCTCCTCCGCC- TACTCTCTTCTTG-3′, and the lower oligonucleotide, 5′-CAGAAGAGAGATGCAGGCGAGCACTAAGGCC-3′, were used for mutation of alanine-11 to proline in the exon 1 signal sequence of the β2m cDNA (base substitutions italicized). The generated mutant FH8 cDNA was sequenced in both directions to ensure fidelity.

Cell Transfection. FO-1 cells were transfected with pCMV-Sport6 containing full-length human β2m cDNA, FH8 mutant β2m cDNA, and pREP9 (Invitrogen) containing full-length FcRn α-chain cDNA by using Lipofectamine 2000 (Invitrogen) in accordance with instructions from the supplier. Briefly, cells were plated on 10-cm dishes (Corning) overnight in antibiotic-free medium. The next day, 90% confluent cells were washed twice with serum-free medium and overlayed with 3 ml of serum-free and antibiotic-free medium containing 20 μg of each plasmid and 60 μl Lipofectamine, and then with 7 additional ml of medium. After 5 h of culture at 37°C, an additional 10 ml of antibiotic-free medium containing 20% FCS (10% final) was added. Cells were harvested at 24 h after transfection.

Flow Cytometry. Cells were detached from the 10-cm dishes by adding 1 ml of cell dissociation solution (Sigma), and were centrifuged at 400 × g for 5 min at 4°C. Cells (1 × 10⁶) were incubated for 1 h at 4°C with 5 μg/ml of primary antibodies, mAb W6/32 for MHC I expression, or BBM.1 for β2m expression (in 200 μl of PBS containing 0.2% BSA and 0.1% sodium azide). Cells were washed twice and incubated for 1 h with FITC-tagged goat anti-mouse IgG (Caltag, South San Francisco, CA). Cells were then washed three times and resuspended in 0.5 ml of PBS. Control staining with isotype-matched IgG2a and IgG2b proteins was performed for each analysis. Samples were analyzed on a FACS Caliber flow cytometer (Becton Dickinson). The MFI values for all cells stained with each antibody were compared directly. The expression efficiency (E%) of the mutant β2m compared with the wt β2m was calculated by subtracting the MFI of the cells transfected with empty vector (e) from the MFI of cells transfected with β2m and expressing the quotient of specific mut β2m MFI (m) over specific wt β2m MFI (w) as a percentage; that is, E% = 100 (m - e) / (w - e).
European descent and from serum genomic DNA of the two affected siblings of British ancestry by using the forward primer 5'-TGAAGTCCTAGAATGACGCCCTGTTGTCCCG-3' and reverse primer 5'-CGGCCTGAACCTTTGTCGCCG-3', in accordance with the conditions described above. The 488-bp PCR-amplified fragment was purified by using a Montage PCR centrifugal filter (Millipore) and eluted in 20 μl of water. DNA was digested at 37°C overnight with 10 units of restriction enzyme HinP1I (New England Biolabs) that recognizes five GCGC sequences within the 488-bp PCR-amplified fragment, one of which is the site of the FHH mutation. The digested DNA was resolved on a 2% ethidium bromide-stained agarose gel and visualized on a UV transilluminator.

We thank Arthur Burghes for valuable advice and for the donation of the DNA samples, Daniel Lui for developing the serum FcRn assay, and Qianzheng Zhu for helpful comments. This work was supported in part by National Institutes of Health (NIH) Grants HD38764, CA88053, and AI57530 and by the Intramural Research Program of the National Cancer Institute (NIH).