Supplemental data 1

Primers used for sequence analysis

For patients referred to the Reproductive Endocrine Associates of Massachusetts General Hospital, the following primers were used for *TAC3* sequence analysis: 5'-TAAGCCCAAGGGCTGTACC-3' (exon 2 – sense), 5'-CAAGGTGTCAAAGACATGCAG-3' (exon 2 – antisense), 5'-GTTCACTCCTGGAGCACCTA-3' (exon 3&4 – sense), 5'-GTGGGAGCTGGCATATTGTT-3' (exon 3&4 – antisense), 5'-TGGAGGAAGGAAAGGTGAGC-3' (exon 5&6 – sense), 5'-CAAAGCTGGGGACGTTCTCT-3' (exon 5&6 – antisense).

For patients referred to the Reproductive Endocrine Associates of Massachusetts General Hospital, the following primers were used for *TACR3* sequence analysis: 5'-CAGGACGCTCAGTTCTCCA-3' (exon 1a – sense), 5'-ATTTCCCAAAACTGCCACTG-3' (exon 1a – antisense), 5'-ACCAAGCTGGCAACCTCTC-3' (exon 1b – sense), 5'-CCACGGAAGCAGAAACTTGT-3' (exon 1b – antisense), 5'-TTGAACCTAGATCTTCCTGATTCC-3' (exon 2 – sense), 5'-TGACCACACACAAATCATACCA-3' (exon 2 – antisense), 5'-TTGATGGAATTTTGAGATGAATG-3' (exon 3 – sense), 5'-TGTGGACAGCAGCTTATACAAAA-3' (exon 3 – antisense), 5'-GGCAACTGTCCGTATATTGCTT-3' (exon 4 – sense), 5'-AAAGCCTGTGCCTCTCTCAG-3' (exon 4 – antisense), 5'-CTCATTAATGGGAAATTAAGATTCAA-3' (exon 5a – sense), 5'-GATTTGGAATTCCTGCGAGA-3' (exon 5a – antisense), 5'-TCTTTCTGTGGCCTGCTTTT-3' (exon 5b – sense), 5'-CCCATTTTATTTTGGGTGGA-3' (exon 5b – antisense).

For patients referred to the Developmental Endocrinology Unit of the Clinical Hospital in Sao Paolo, Brazil, the following primers were used for *TAC3* sequence analysis: 5'-CAAGCTGCTGGTAATGAATG-3' (exon 2 – sense), 5'-AAATGCCCTCTGACGGAC-3' (exon 2 – antisense), 5'-GATTCAGGATGGGCTCAGG-3' (exon 3&4 - sense), 5'-GGGAGCTGGCATATTGTTTG-3' (exon 3&4 – antisense), 5'-ACAGAGACCAGAAACCCAGTC-3' (exon 5&6 – sense), 5'-TTTAATACCTGTAGCATGGGAG-3' (exon 5&6 – antisense).

For patients referred to the Developmental Endocrinology Unit of the Clinical Hospital in Sao Paolo, Brazil, the following primers were used for *TACR3* sequence analysis: 5'-CAGGGATTGCAGTATCTTTC-3' (exon 1 – sense), 5'-CCTCCTTTCAGCAAAAATTC-3' (exon 1 – antisense), 5'-GCCATGATTACCATTCTACG-3' (exon 2 – sense), 5'-ACTTATTGACCACACACAAATC-3' (exon 2 – antisense), 5'-CAACTGGCAGCATTTGAAAC-3' (exon 3 – sense), 5'-GATTACAGTATGTGGACAGCAGC-3' (exon 3 – antisense), 5'-CTGTCCGTATATTGCTTCACC-3' (exon 4 – sense), 5'-AAGCCTGTGCCTCTCTCAG-3' (exon 4 – antisense), 5'-TGACATAAATTCTAAGAGTCTGG-3' (exon 5 – sense), 5'-CTTTCTCAATTTGACCATAGC-3' (exon 5 – antisense).

Supplemental data 2

Generation of NK3-R mutations through site-directed mutagenesis

All NK3-R mutations were generated by site-directed mutagenesis using the Quik Change II Site-directed Mutagenesis Kit (Stratagene, La Jolla, CA) and a pcDNA 3.1+ expression vector containing full length wild-type (WT) human NK3-R (Missouri S&T cDNA Resource Center, www.cdna.org) as template. The presence of each mutation was confirmed by sequencing.

Functional studies

Forty-eight hours after transfection, media on transfected COS-7 cells were replaced with inositol-free DMEM for 2 hours at 37°C. Subsequently, 2 µCi/ml myo-[2-^{3}H]-inositol (Perkin Elmer, Waltham, MA) was added, followed by the addition of 10 mM LiCl 15 min later. After overnight incubation, inositol phosphate (IP) production was stimulated by the addition of 10^{-7} M neurokinin-B (Genescript, Piscataway, NJ) for 1 hour. Cells were washed once with PBS to remove excess media and lysed with formic acid. The lysates were then neutralized, and protein content was determined by the Bradford method. Supernatants were loaded onto previously equilibrated AGX8 resin anion exchange columns (Fisher Scientific, Pittsburgh, PA). The columns were washed and total IP was eluted. Radioactivity was quantified by liquid scintillation and corrected for protein content. All assay points were performed in triplicate, and each experiment was repeated at least three times in order to ensure accuracy.

LEGENDS:

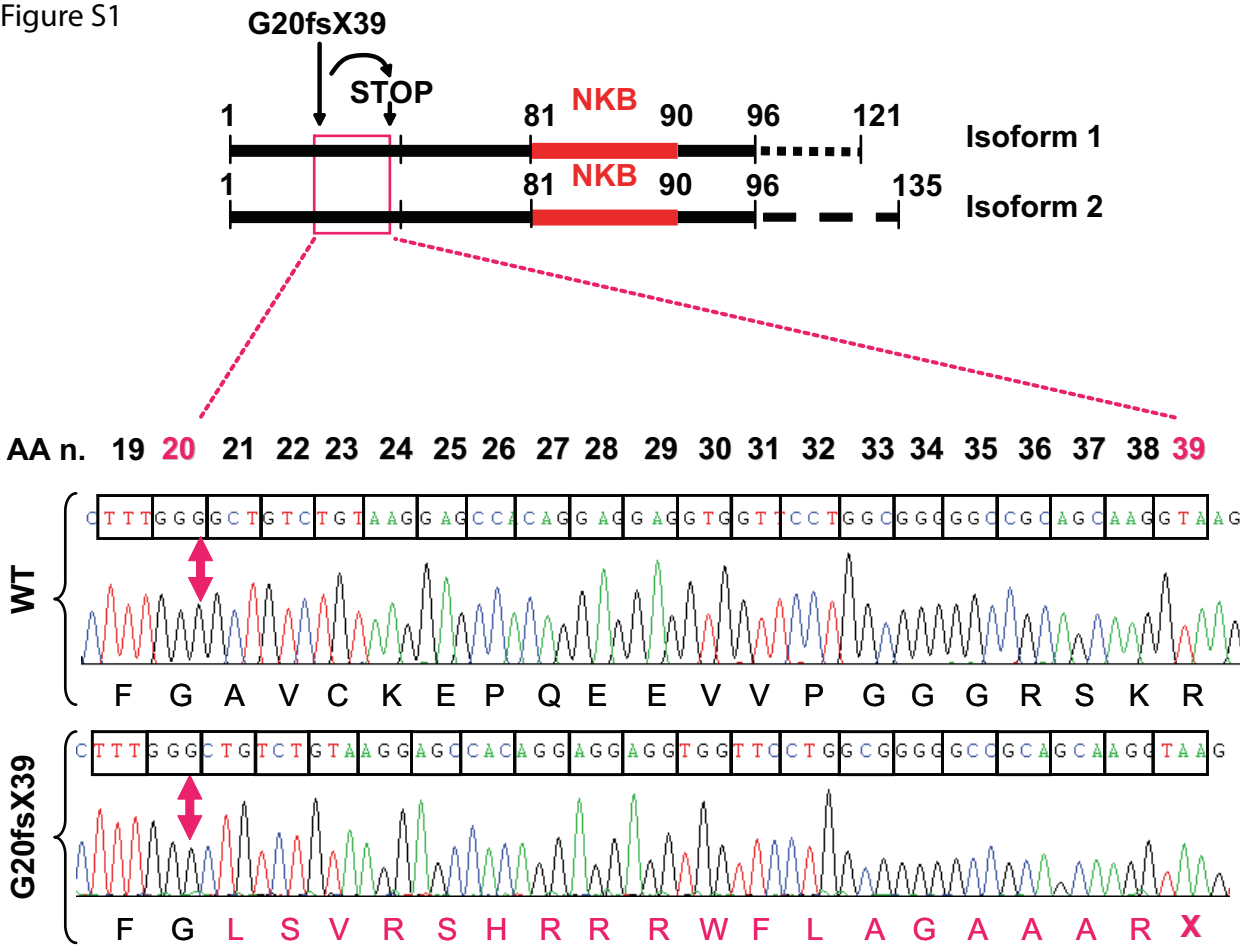
Table 1: Racial distribution of IHH probands screened for rare variants in *TACR3* and *TAC3*.

Figure 1: *TAC3* frameshift mutation.

Upper panel: schematic of NKB precursor isoforms.

Lower panel: [c.G60del] leads to frameshift and premature termination codon at amino acid 39. This homozygous mutation truncates both NKB precursor isoforms, leading to a complete absence of NKB.

Figure S1


G20fsX39
STOP
NKB
1
81
90
96
121
135
Isoform 1
Isoform 2
AA n. 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39
WT
F G A V C K E P Q E E V V P G G G R S K R
G20fsX39
F G L S V R S H R R R W F L A G A A A R X

nIHH probands			
	Probands screened	Probands with rare variant in *TACR3*	Probands with rare variant in *TAC3*
Caucasians	213	10	0
Mixed Europeans	30	0	0
Turkish	37	4	0
Other Europeans (Germany, Greece, Ireland, Italy, Portugal, Spain, United Kingdom: less than 6 each)	18	2	0
Unknown country of origin	128	4	0
Asians	25	2	1
African-Americans	12	1	0
American-Indian/Alaskan natives	1	0	0
Unknown race	94	6	0
Hispanics	11	1	0
Brazilians	60	4	0
Unknown country of origin	23	1	0
Total	345	19	1

Table 1