

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Lofrano-Porto A, Barra GB, Giacomini LA, et al. Luteinizing hormone beta mutation and hypogonadism in men and women. *N Engl J Med* 2007;357:897-904.

ONLINE APPENDIX

Table S1. Hormonal data of six consanguineous family members heterozygotes for the IVS2+1G>C mutation in the luteinizing hormone beta-subunit gene

Subjects	Sex	Age (yr)	LH (IU/L)		FSH (IU/L)		E ₂ (pg/mL)	T (ng/dL)	Free α (ng/L)	In B (pg/mL)	Phenotype
			Baseline	Peak ^a	Baseline	Peak ^a					
I-1	M	76	5.0		4.9		27.4*	567	456.8	185	Fertile
I-2	F	66	6.4	20.0	33.9	49.9	< 13	43.8*	575.5	< 10	Fertile/ menopause
II-2	F	49	17.6		31.5		56	31.3*	1486.3	48	Fertile/ climateric
II-7 ^b	F	36	<0.6	23.6	4.8	24.7					Fertile
II-14	M	25	4.2	15.2	6.0	10.6	43.3*	542	447.4	62	Fertile
III-3	M	28	1.44*		2.26*		32.9*	577*			Fertile
Normal range:											
M			1.0-8.4	12-29.7	0.6-10.5	2.9-7.8	<35	271-965	120-790	80-300	
F:											
follicular			2.2-6.8	7.6-31.7	2.4-9.3	4.6-11.7	22-215		80-604	15-90	
menopause			15-64		31-134				340-400		

Luteinizing hormone (LH), Follicle-stimulating hormone (FSH), Estradiol (E₂), Testosterone (T) and Free alpha-subunit (Free α) were measured by an immunofluorimetric assay (autoDELFLIA); Inhibin B (In B) was measured by an enzyme immunoassay (Active Inhibin B ELISA, Diagnostics).

*Values marked with an asterisk were determined by a chemiluminescent immunoassay (IMMULITE).

a. LH and FSH levels at peak after GnRH stimulation.

b. Subject II-7 was pregnant at the time of the first evaluation. Basal and GnRH-stimulated LH and FSH levels were obtained after five month of childbirth, when she was still nursing.

Figure S1. Nucleotides and predicted amino acid sequence of wild type (wt) and mutant *LHB* exon 2, intron 2 and exon 3. Exonic sequences are underlined and intron 2 sequence is in italic. The *LHB* gene NCBI reference sequence ref[NC_000019.8|NC_000019:c54212159-54211049] was identical to the mutant

gene amplified fragment, except for the mutated nucleotide (shown in red). Intron 2 retention resulted in the insertion of 79 amino acids and in exon 3 frameshift. The predicted aberrant protein would lack important regions encoded by exon 3, such as the seat belt region, formed by amino acids 90-110 (light gray), and also some cysteine residues at positions 57, 88 and 90 (yellow), essential for the cysteine knot formation. Numbers above each series refer to amino acid positions in the *LHB* wt mature protein. (GenBank accession number for *LHB* IVS2+1G>C is EF621778).

1	G L L L L L L L L L S M G G A W A S R	WT
	<u>GGGCTGCTGC TGTTGCTGCT GCTGAGCATG GGCGGGGCAT GGGCATCCAG</u>	
	G L L L L L L L L L S M G G A W A S R	Mutant
10	E P L R P W C H P I N A I L A V E	WT
	<u>GGAGCCGCTT CGGCCATGGT GCCACCCCAT CAATGCCATC CTGGCTGTCTG</u>	
	E P L R P W C H P I N A I L A V E	Mutant
20	30	
	K E G C P V C I T V N T T I C A	WT
	<u>AGAAGGAGGG CTGCCAGTG TGCATCACCG TCAACACCAC CATCTGTGCC</u>	
	K E G C P V C I T V N T T I C A	Mutant
40	G Y C P T M	WT
	<u>GGCTACTGCC CCACCATG</u> T <u>GAGCTGCCTG GGGCCAGGGG CAGATGCTGC</u>	
	G Y C P T M L S C L G P G A D A A	Mutant
	WT
	<u>CACCTCAGGG CCAGACCCAC AGAGGCAGCG GGGGAGGAAG GGTGGTCTGC</u>	
	T S G P D P Q R Q R G R K G G L P	Mutant
	WT
	<u>CTCTCTGGCC TGCGGTTGGG GAATGGGGTG TGGGAAGGCA GGAACAGAGG</u>	
	L W P A V G E W G V G R Q E Q R	Mutant
	WT
	<u>GCTTCCTGGG CTCCTGAGTC CAGGACCTGT GGGGTCAGCT TGGGAGCTCA</u>	
	A S W A P E S R T C G V S L G A Q	Mutant
	WT
	<u>GCTGAGGCGC TGGCCTCAGG CACATGCTCA TTCCCCACT CACACGGCCT</u>	
	L R R W P Q A H A H S P T H T A S	Mutant
50M R V L Q A V L P P L P Q V V C	WT
	<u>CCAGATGCGC GTGCTGCAGG CGGTCCTGCC GCCCCTGCCT CAGGTGGTGT</u>	
	R C A C C R R S C R P C L R W C	Mutant

60	70	
T Y R	D V R	F E S I R L P G C P
<u>GCACCTACCG TGATGTGCGC TTCGAGTCCA TCCGGCTCCC TGGCTGCCCG</u>		
A P T V	M C A	S S P S G S L A A R
		WT
		Mutant

80	90	
R G V D	P V V	S F P V A L S C R C
<u>CGTGGTGTGG ACCCCGTGGT CTCCTTCCCT GTGGCTCTCA GCTGTCGCTG</u>		
V V W	T P W S	P S L W L S A V A V
		WT
		Mutant

100	
G P C R R S T	S D C G G P K D H P
<u>TGGACCCTGC CGCCGAGCA CCTCTGACTG TGGGGTCCC AAAGACCACC</u>	
D P A	A A A P L T V G V P K T T
	WT
	Mutant

110	120	
L T C	D H P Q L S G L L F L *	
<u>CCTTGACCTG TGACCACCC CAACTCTCAG GCCTCCTCTT CCTCTAAAGA</u>		
P *		
	WT	
	Mutant	

CCCTCCCCGC AGCCTTCCAA GTCCATCCCG ACTCC