Neurokinin B: A Novel Regulator of Reproductive Functions

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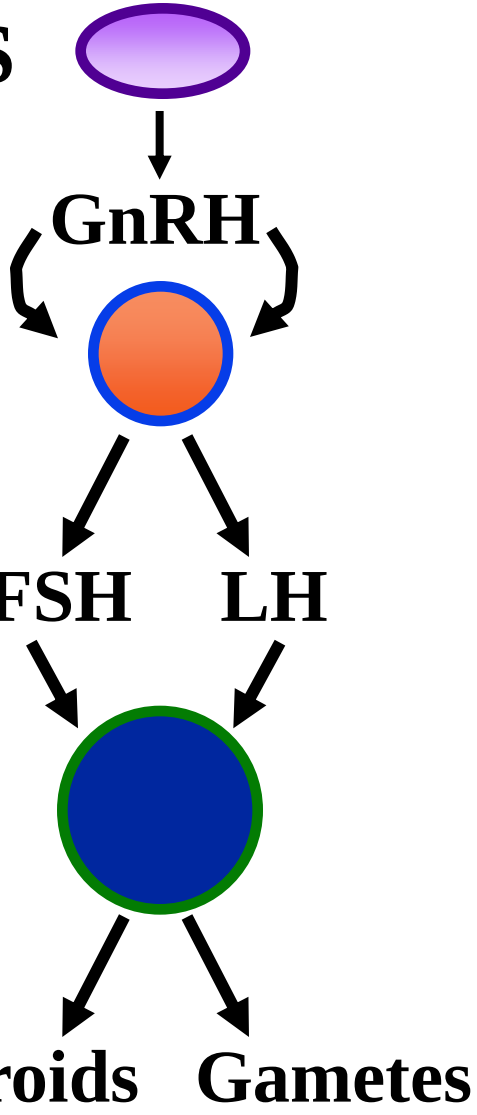
Normal H-P-G Axis

HYPOTHALAMUS

PITUITARY

GONAD



“GNRH pulse generator”

- a functionally interconnected and synchronized network of GNRH neurons
- inhibited throughout the childhood following a period of pubertal level activity during fetal life and early infancy
- release of this inhibition at the early years of the second decade marks the beginning of puberty

What triggers puberty?

Nutrition--including that received in utero--seems to help set this mysterious biological clock, but no one knows exactly what forces childhood to end.

HYPOTHESIS

Gene(s) taking role in initiating human puberty may be identified via autozygosity mapping in consangious human families with two or more affected siblings with Normosmic Idiopathic Hypogonadotropic Hypogonadism

TÜBİTAK proje no: 106S276

NORMOSMİK İDİOPATİK HİPOGONADOTROPİK HİPOGONADİZMLİ OLGULARDA MOLEKÜLER GENETİK ANALİZLER YOLUYLA İNSANDA PÜBERTE SÜRECİNDE ROL ALAN YENİ GENLERİN TANIMLANMASI.

Doç.Dr. Kemal Topaloğlu, Proje Yürütücüsü
Prof. Dr. Bilgin Yüksel, Araştırmacı
Doç.Dr. Neslihan Ö Mungan, Araştırmacı

Çukurova Ü. Tıp Fakültesi
Çocuk Endokrinoloji ve Metabolizma BD
Adana

Inclusion criteria

- Male >14 Female>13 y
- Bone age>11.5y
- Tanner stage 1 breast in girls
- Testicular volume <4 ml in boys
- Prepubertal levels of sex steroids and FSH/LH
- Prepubertaal response to LHRH stimulation
- Normal hypothalamo-pituitary anatomy on MRI
- Normal olfactory bulbus and sulci on MRI

Exclusion criteria

- Anosmia/hyposmia (Kallmann syndrome)
- Inflammation, infection, tumor etc at the hypothalamus, pituitary
- Mutiple pituitary hormone def (e.g. PROP1, HESX1)
- Chronic sysytemic diseases e.g. uremia, diabetes, IBD etc
- Extreme thinnes , athletes, anorexia nervosa, malnutrition
- Obesity (Leptin, Leptin receptor def)
- Syndromes e.g P. Wili, Bardet Biedl etc

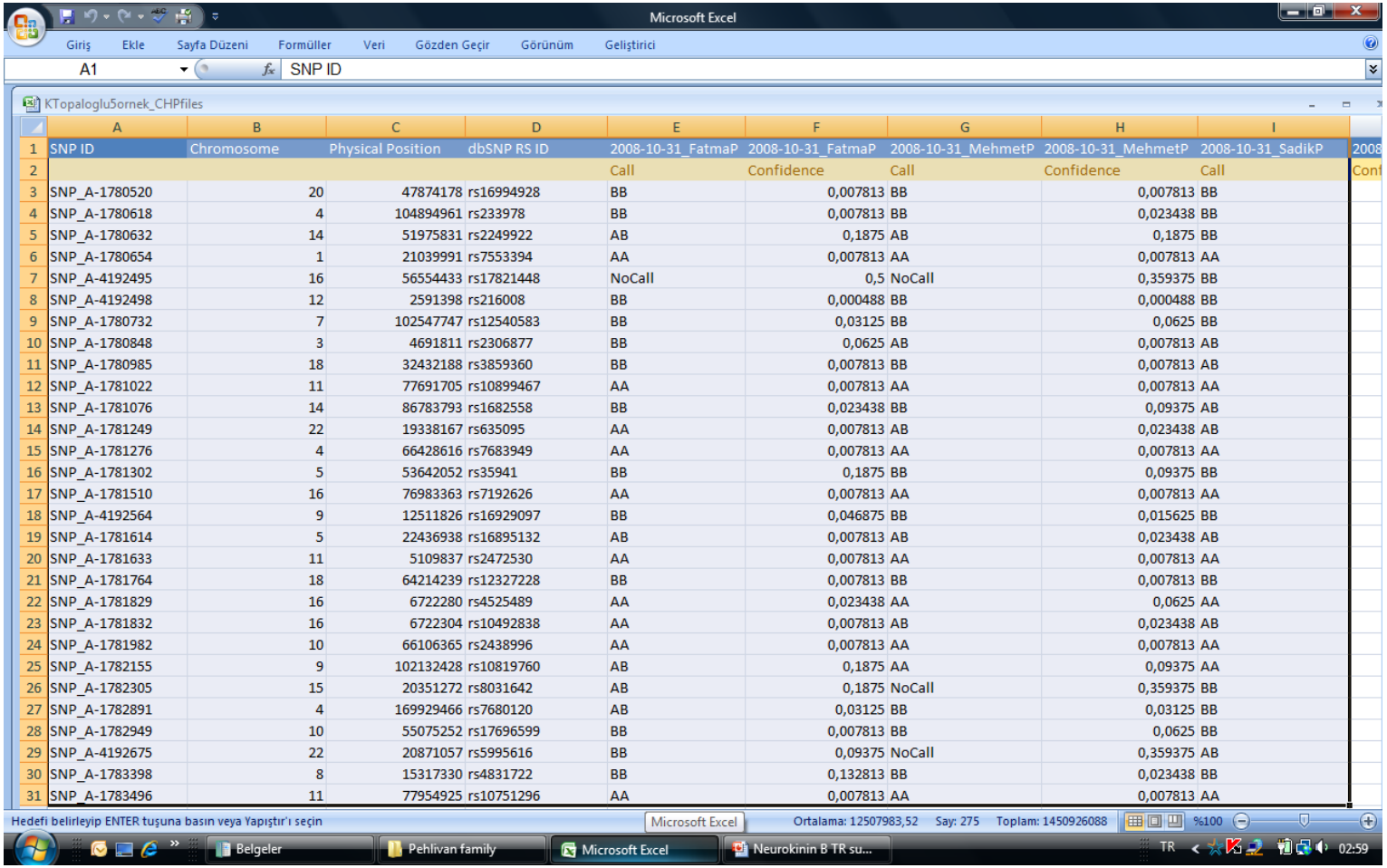
Study cohort

- 9 consangious families with at least 2 affected sibs

Genes cleared

- KAL1
- FGFR1
- GNRHR
- GNRH1
- GPR54
- KISS1
- PROK2
- PROK2R
- NELF

A genome-wide 250K NspI Affymetrix SNP microarray



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2					Call	Confidence	Call	Confidence	Call
3	SNP_A-1780520	20	47874178	rs16994928	BB	0,007813	BB	0,007813	BB
4	SNP_A-1780618	4	104894961	rs233978	BB	0,007813	BB	0,023438	BB
5	SNP_A-1780632	14	51975831	rs2249922	AB	0,1875	AB	0,1875	BB
6	SNP_A-1780654	1	21039991	rs7553394	AA	0,007813	AA	0,007813	AA
7	SNP_A-4192495	16	56554433	rs17821448	NoCall	0,5	NoCall	0,359375	BB
8	SNP_A-4192498	12	2591398	rs216008	BB	0,000488	BB	0,000488	BB
9	SNP_A-1780732	7	102547747	rs12540583	BB	0,03125	BB	0,0625	BB
10	SNP_A-1780848	3	4691811	rs2306877	BB	0,0625	AB	0,007813	AB
11	SNP_A-1780985	18	32432188	rs3859360	BB	0,007813	BB	0,007813	AB
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14	SNP_A-1781249	22	19338167	rs635095	AA	0,007813	AB	0,023438	AB
15	SNP_A-1781276	4	66428616	rs7683949	AA	0,007813	AA	0,007813	AA
16	SNP_A-1781302	5	53642052	rs35941	BB	0,1875	BB	0,09375	BB
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18	SNP_A-4192564	9	12511826	rs16929097	BB	0,046875	BB	0,015625	BB
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22	SNP_A-1781829	16	6722280	rs4525489	AA	0,023438	AA	0,0625	AA
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25	SNP_A-1782155	9	102132428	rs10819760	AB	0,1875	AA	0,09375	AA
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27	SNP_A-1782891	4	169929466	rs7680120	AB	0,03125	BB	0,03125	BB
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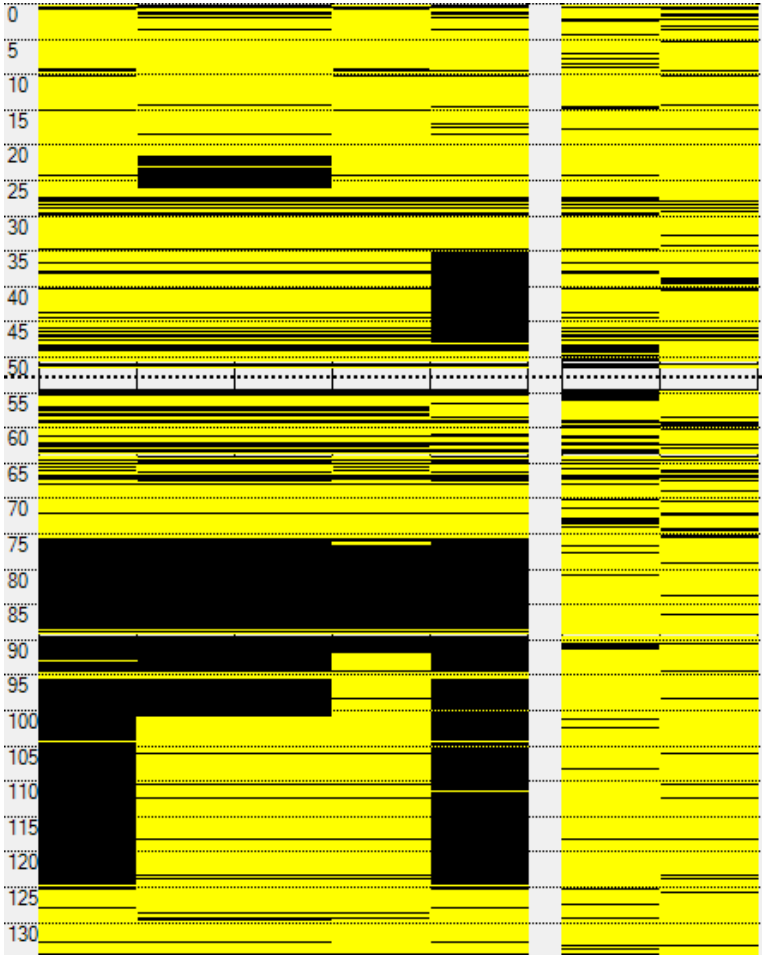
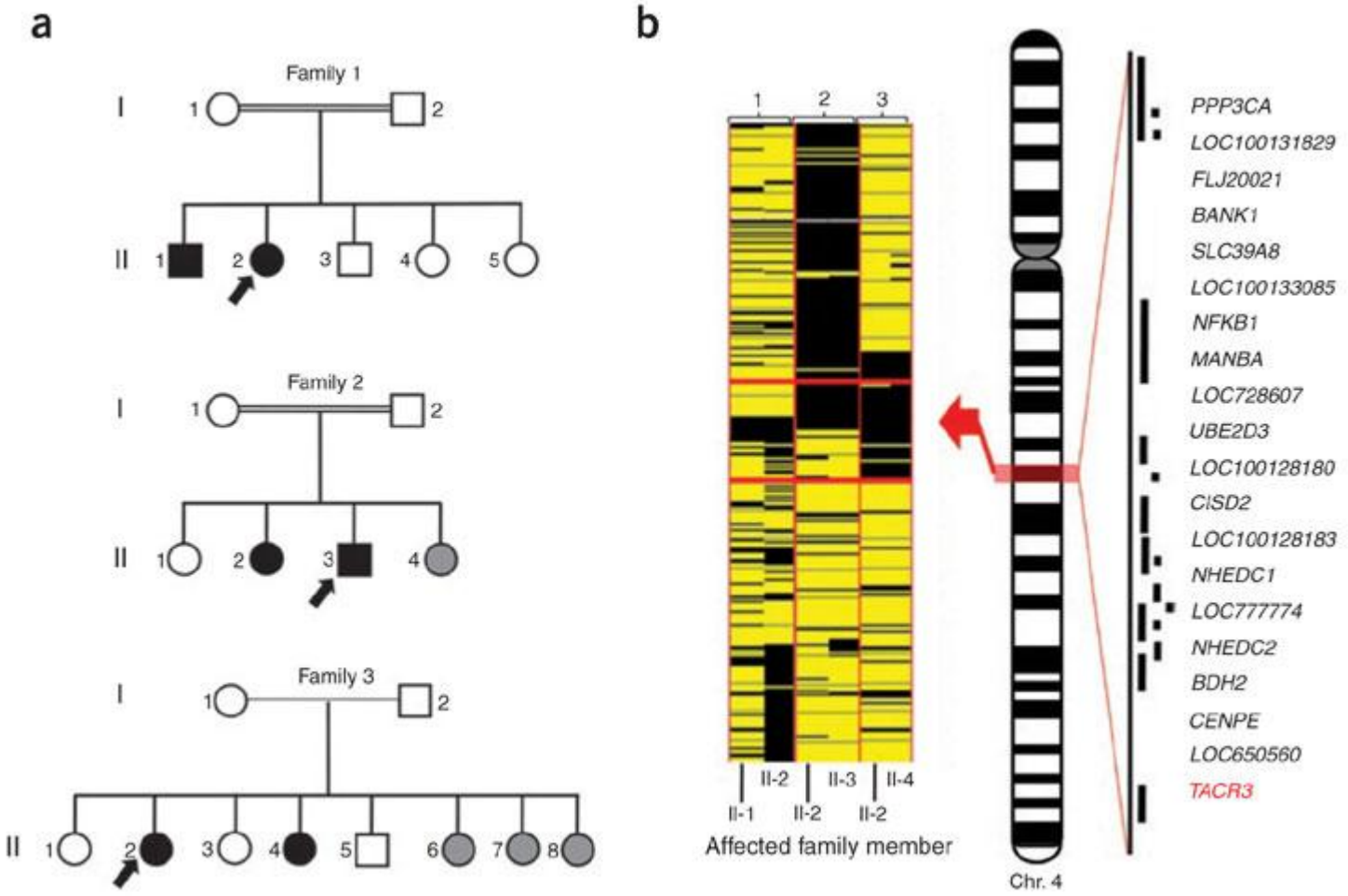
30	SNP_A-1783398	8	15317330	rs4831722	BB	0,132813	BB	0,023438	BB
31	SNP_A-1783496	11	77954925	rs10751296	AA	0,007813	AA	0,007813	AA

SNP microarray gene chip data analyzed by AutoSNPa software (http://dna.leeds.ac.uk/autosnpa/)

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I
1
2
II
1
2
3
4
5
Family 2
I
1
2
II
1
2
3
4
Family 3
I
1
2
II
1
2
3
4
5
6
7
8
b
1
2
3
II-1
II-2
II-2
II-3
II-2
II-4
Affected family member
Chr. 4
PPP3CA
LOC100131829
FLJ20021
BANK1
SLC39A8
LOC100133085
NFKB1
MANBA
LOC728607
UBE2D3
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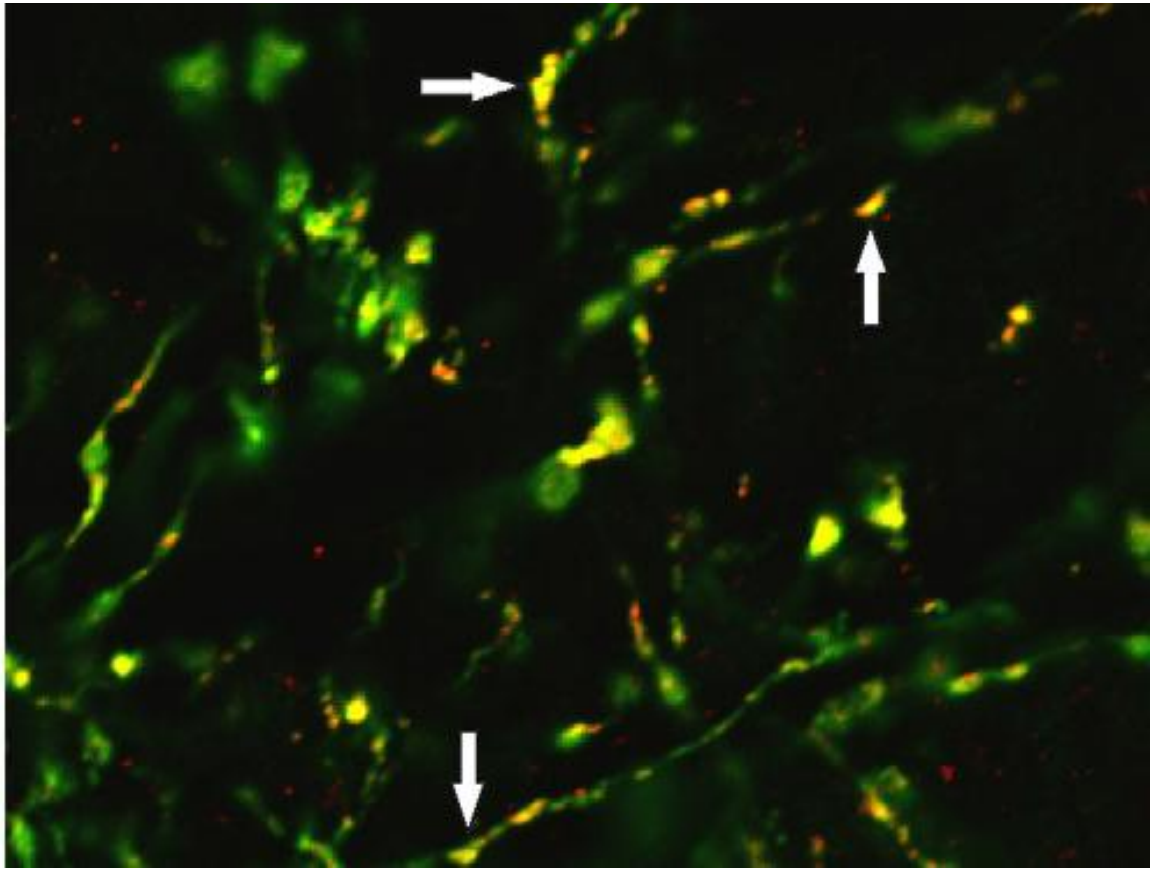
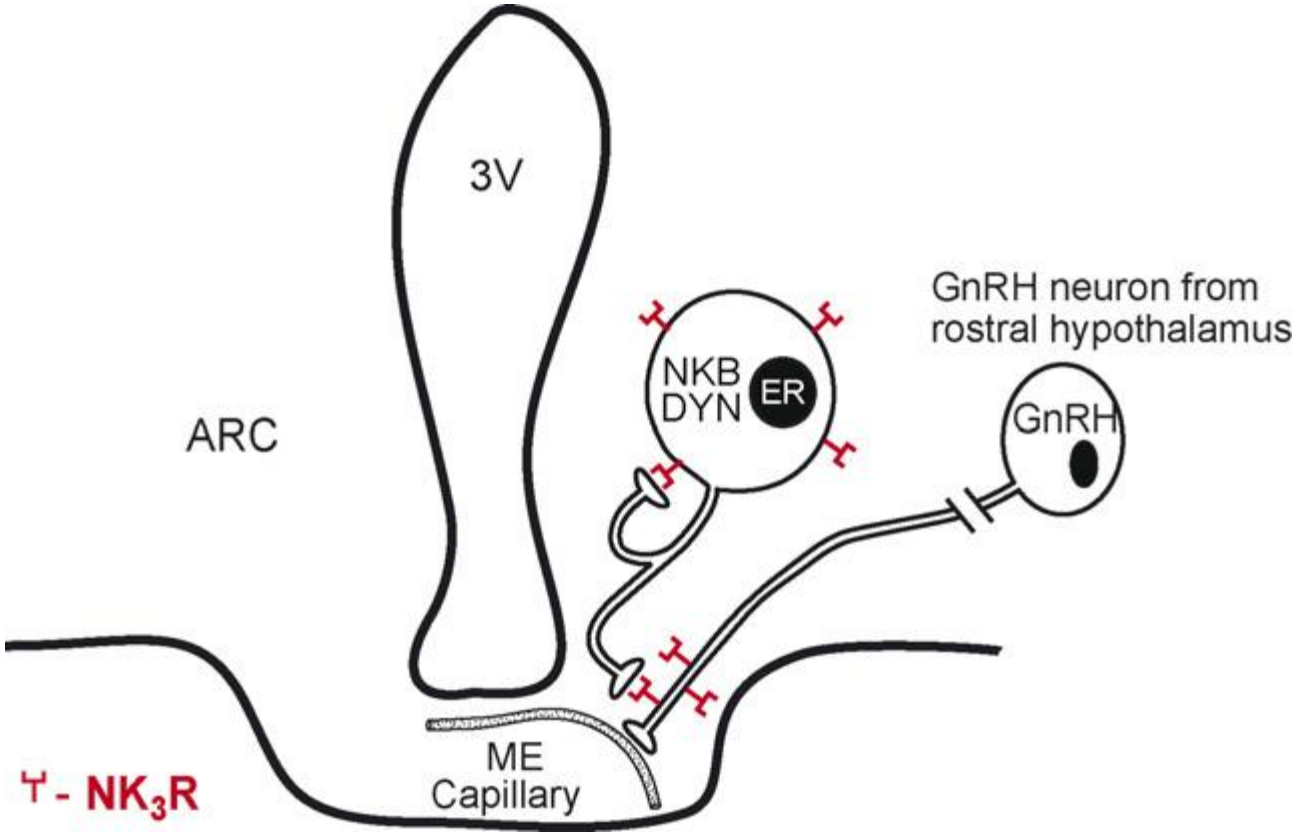
CISD2
LOC100128183
NHEDC1
LOC777774
NHEDC2
BDH2
CENPE
LOC650560
TACR3



Combined images of GnRH (green) and NK3R (red)-immunofluorescence show punctate colocalization of NK3R on GnRH fibers (yellow, arrows). (Krajewski J Comp Neurol 2005)

Schematic diagram of relationship between Neurokinin B and ER and GNRH (Rance Peptides 2008)



1: Endocrinology. 2007 Dec;148(12):5752-60. Epub 2007 Sep 6.

Kisspeptin neurons in the arcuate nucleus of the ewe express both dynorphin A and neurokinin B.

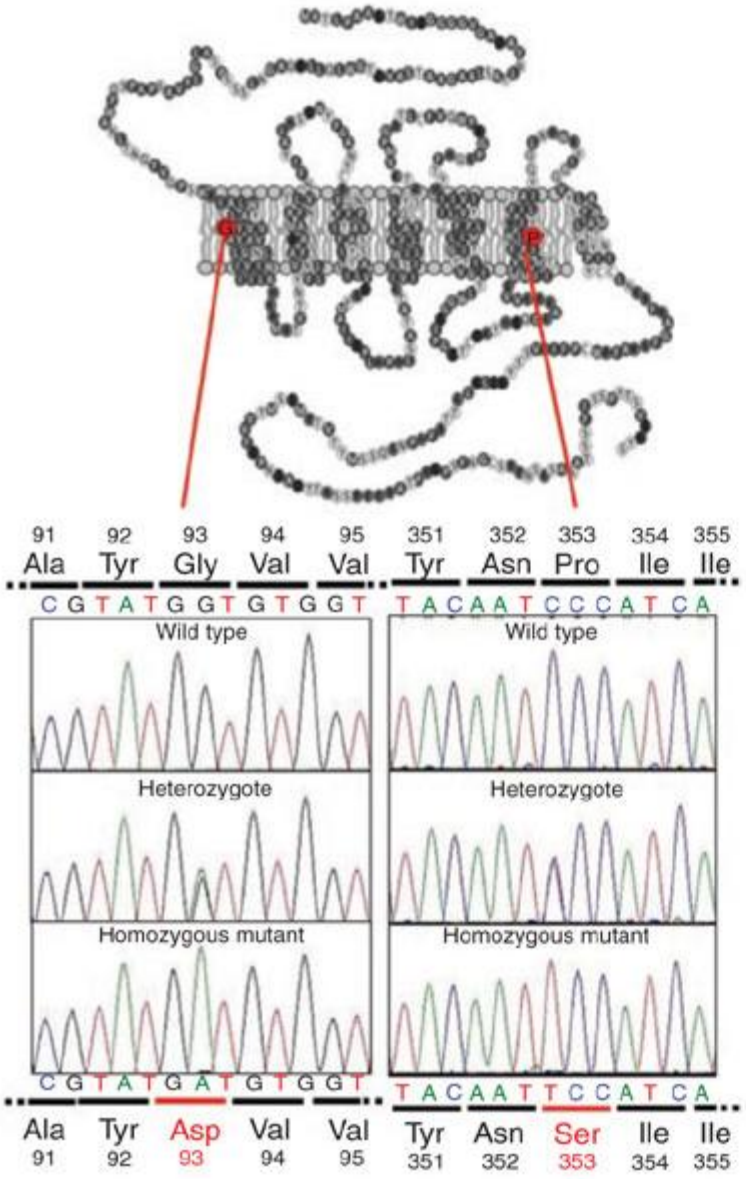
Goodman RL, Lehman MN, Smith JT, Coolen LM, de Oliveira CV, Jafarzadehshirazi MR, Pereira A, Iqbal J, Caraty A, Ciofi P, Clarke IJ.

Department of Physiology and Pharmacology, West Virginia University, Morgantown, West Virginia, USA. bgoodman@hsc.wvu.edu

Kisspeptin is a potent stimulator of GnRH secretion that has been implicated in the feedback actions of ovarian steroids. In ewes, the majority of hypothalamic kisspeptin neurons are found in the arcuate nucleus (ARC), with a smaller population located in the preoptic area. Most arcuate kisspeptin neurons express estrogen receptor-alpha, as do a set of arcuate neurons that contain both dynorphin and neurokinin B (NKB), suggesting that all three neuropeptides are colocalized in the same cells. In this study we tested this hypothesis using dual immunocytochemistry and also determined if kisspeptin neurons contain MSH or agouti-related peptide. To assess colocalization of kisspeptin and dynorphin, we used paraformaldehyde-fixed tissue from estrogen-treated ovariectomized ewes in the breeding season (n = 5). Almost all ARC, but no preoptic area, kisspeptin neurons contained dynorphin. Similarly, almost all ARC dynorphin neurons contained kisspeptin. In experiment 2 we examined colocalization of kisspeptin and NKB in picric-acid fixed tissue collected from ovary intact ewes (n = 9). Over three quarters of ARC kisspeptin neurons also expressed NKB, and a similar percentage of NKB neurons contained kisspeptin. In contrast, no kisspeptin neurons stained for MSH or agouti-related peptide. These data demonstrate that, in the ewe, a high percentage of ARC kisspeptin neurons also produce dynorphin and NKB, and we propose that a single subpopulation of ARC neurons contains all three neuropeptides. Because virtually all of these neurons express estrogen and progesterone re-ceptors, they are likely to relay the feedback effects of these steroids to GnRH neurons to regulate reproductive function.

PMID: 17823266 [PubMed - indexed for MEDLINE]

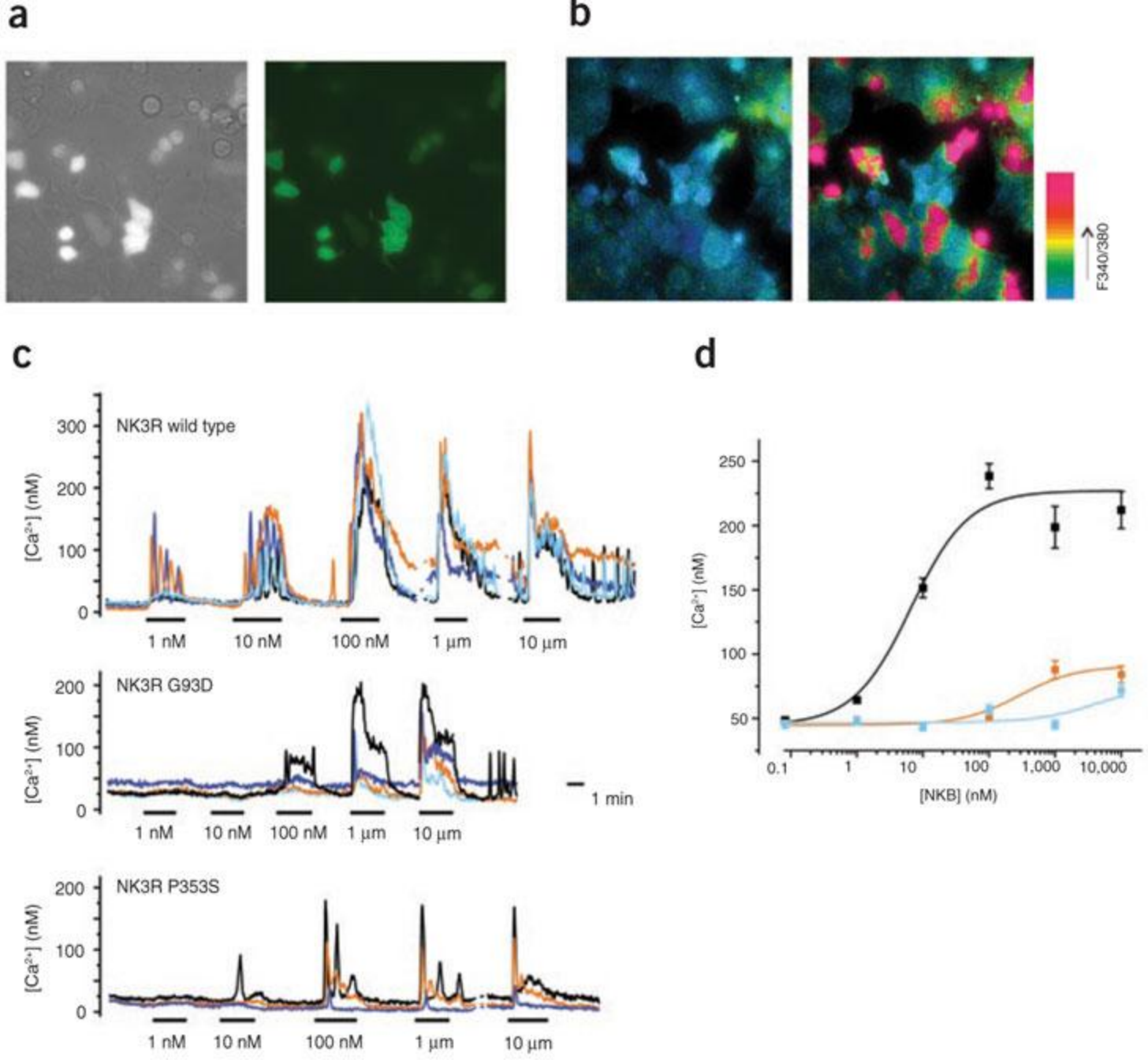
c

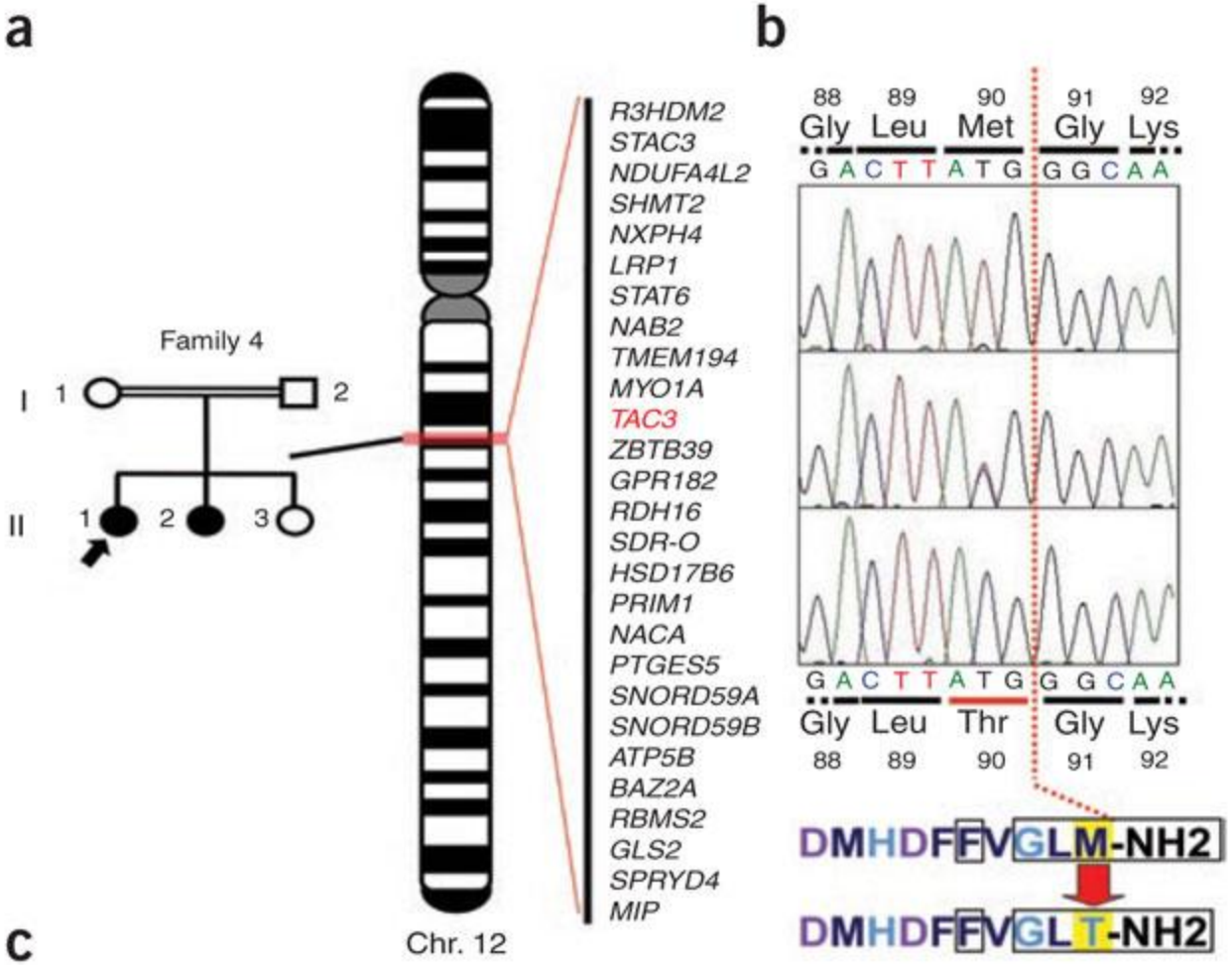


d

Human TACR3	346	MSSTMYNPIIYCCLN	360
Human TACR1	295	MSSTMYNPIIYCCLN	309
Human TACR2	297	MSSTMYNPIIYCCLN	311
Mouse TACR3	333	MSSTMYNPIIYCCLN	347
Zebrafish TACR3	286	MSSTMYNPIIYCCLN	300
Drosophila TACR3	371	MSNSMYNPIIYCWMN	385
Xenopus TACR3	172	MSSTMYNPIIYCCLN	186
Sea squirt TACR3	403	MSSSMYNPFIYCWNN	417

Human TACR3	83	ALWSLAYGVVVAVAV	98
Human TACR1	26	VLWAAAYTVIVVTSV	41
Human TACR2	27	ALWATAYLALVLVAV	42
Mosquito TACR3	65	VLWTLLFVCMVIVAT	80
Drosophila TACR3	99	VLWSILFGGMVIVAT	114
Mouse TACR3	66	ALWSLAYGLVVAVAV	81
Zebrafish TACR3	19	AVWSVAYSSVLAVAV	34
Sea squirt TACR3	129	FGWSVVYGLLVVVAL	150


a
b
F340/380
c
NK3R wild type
[Ca2+] (nM)
300
200
100
0
1 nM
10 nM
100 nM
1 µm
10 µm
NK3R G93D
200
100
0
1 min
NK3R P353S
d
250
200
150
100
50
0.1
1
10
100
1,000
10,000
[NKB] (nM)


a
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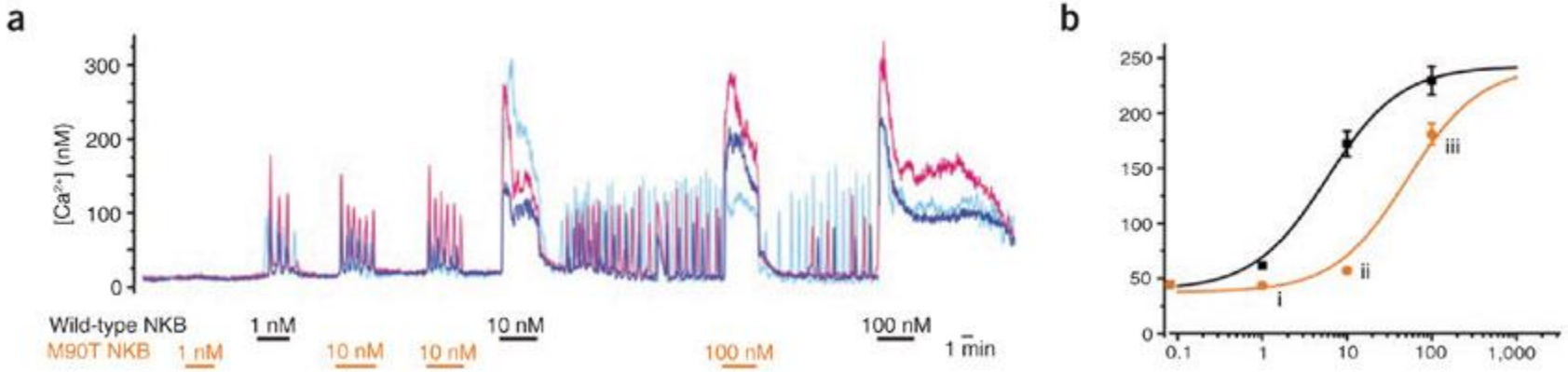
Chr. 12
R3HDM2
STAC3
NDUFA4L2
SHMT2
NXPH4
LRP1
STAT6
NAB2
TMEM194
MYO1A
TAC3
ZBTB39
GPR182
RDH16
SDR-O
HSD17B6
PRIM1
NACA
PTGES5
SNORD59A
SNORD59B
ATP5B
BAZ2A
RBMS2
GLS2
SPRYD4
MIP
b
88 89 90 91 92
Gly Leu Met Gly Lys
G A C T T A T G G G C A A
G A C T T A T G G G C A A
Gly Leu Thr Gly Lys
88 89 90 91 92
DMHDFFVGLM-NH2
DMHDFFVGLT-NH2
c
Neurokinin B orthologs
Human DMHDFFVGLM-NH2
Frog DMHDFFVGLM-NH2
Mouse DMHDFFVGLM-NH2
Cow DMHDFFVGLM-NH2
Rat DMHDFFVGLM-NH2
Neurokinin A orthologs
Human HKTDSFVGLM-NH2
Python HKTDSFVGLM-NH2
Mouse HKTDSFVGLM-NH2
Rat HKTDSFVGLM-NH2
Cow HKTDSFVGLM-NH2
Cod HKINSFVGLM-NH2
Lamprey HF-DEFVGLM-NH2
Chicken HKTDSFVGLM-NH2
Substance P orthologs
Human RPKPQQFFGLM-NH2
Tree shrew RPKPQQFFGLM-NH2
Guinea Pig RPKPQQSFGLM-NH2
Mouse RPKPQQFFGLM-NH2
Cow RPKPQQFFGLM-NH2
Alligator RPRPQQFFGLM-NH2
Goldfish KPRPHQFIGLM-NH2


a
300
200
100
0
[Ca2+] (nM)
Wild-type NKB
M90T NKB
1 nM
1 nM
10 nM
10 nM
10 nM
100 nM
100 nM
1 min
b
250
200
150
100
50
0
0.1
1
10
100
1,000
i
ii
iii

In summary

we have identified loss-of-function mutations in either neurokinin B or its receptor in four out of nine multiplex families affected by nIHH.

These findings establish that NKB action *via* the NK3R is necessary for the central neuroendocrine control of human reproduction.

These families represents the first examples of inherited defects of tachykinin signalling in any human disorder.

NKB signaling system may provide a novel avenue for the pharmacological manipulation of human fertility and the treatment of sex steroid-related diseases.

nature genetics

Letter abstract

Nature Genetics **41**, 354 - 358 (2008)
Published online: 11 December 2008 | doi:10.1038/ng.306

TAC3 and *TACR3* mutations in familial hypogonadotropic hypogonadism reveal a key role for Neurokinin B in the central control of reproduction

A Kemal Topaloglu[1,7], Frank Reimann[2,7], Metin Guclu[3], Ayse Serap Yalin[4], L Damla Kotan[5], Keith M Porter[6], Ayse Serin[5], Neslihan O Mungan[1], Joshua R Cook[6], Mehmet N Ozbek[1], Sazi Imamoglu[3], N Sema Akalin[4], Bilgin Yuksel[1], Stephen O'Rahilly[6] & Robert K Semple[6]

The timely secretion of gonadal sex steroids is essential for the initiation of puberty, the postpubertal maintenance of secondary sexual characteristics and the normal perinatal development of male external genitalia. Normal gonadal steroid production requires the

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Implications and the future

- NKB action is required for normal HPG function both *in utero* and peripubertally in humans
- Yet, Tacr3 knockout mice are fertile (Kung et al 2004) AND
- Central infusion of a potent NK3R agonist in rodents inhibits gonadotropin secretion(Sandoval-Guzman & Rance NE 2004).

Implications and the future

primates (but not rodents) exhibit true centrally-mediated suppression of GnRH secretion in the prepubertal period (Plant 2006).

divergence between rodents and humans is likely

testing NKB in a primate model may be very informative

Implications and the future

Although kisspeptin and now Neurokinin B pathways appear to be a prerequisite for human puberty, it is likely that there are many more actors yet to be discovered

It is extremely premature to assign a “master controller” for puberty

Implications and the future

As data accumulate (with a perceived large input from autozygosity mapping in multiplex nIHH families) the organization of the GNRH pulse generator will be characterized in a more detailed way including its functional hierarchy and the factor(s) that reactivate the system around the expected age of human pubertal onset.

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Uludag University: Metin Güçlü, Sazi İmamoğlu

Marmara University: Serap Yalın, Sema Akalın