A Comparative Phenotypic Study of Kallmann Syndrome Patients Carrying Monoallelic and Biallelic Mutations in the Prokineticin 2 or Prokineticin Receptor 2 Genes

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Context: Both biallelic and monoallelic mutations in *PROK2* or *PROKR2* have been found in Kallmann syndrome (KS).

Objective: The objective of the study was to compare the phenotypes of KS patients harboring monoallelic and biallelic mutations in these genes.

Design and Patients: We studied clinical and endocrine features that reflect the functioning of the pituitary-gonadal axis, and the nonreproductive phenotype, in 55 adult KS patients (42 men and 13 women), of whom 41 had monoallelic mutations and 14 biallelic mutations in *PROK2* or *PROKR2*.

Results: Biallelic mutations were associated with more frequent cryptorchidism (70% *vs.* 34%, $P < 0.05$) and microphallus (90% *vs.* 28%, $P < 0.001$) and lower mean testicular volume (1.2 ± 0.4 *vs.* 4.5 ± 6.0 ml; $P < 0.01$) in male patients. Likewise, the testosterone level as well as the basal FSH level and peak LH level under GnRH-stimulation were lower in males with biallelic mutations (0.2 ± 0.1 *vs.* 0.7 ± 0.8 ng/ml; $P = 0.05$, 0.3 ± 0.1 *vs.* 1.8 ± 3.0 IU/liter; $P < 0.05$, and 0.8 ± 0.8 *vs.* 5.2 ± 5.5 IU/liter; $P < 0.05$, respectively). Nonreproductive, nonolfactory anomalies were rare in both sexes and were never found in patients with biallelic mutations. The mean body mass index of the patients (23.9 ± 4.2 kg/m^2 in males and 26.3 ± 6.6 kg/m^2 in females) did not differ significantly from that of gender-, age-, and treatment-matched KS individuals who did not carry a mutation in *PROK2* or *PROKR2*. Finally, circadian cortisol levels evaluated in five patients, including one with biallelic *PROKR2* mutations, were normal in all cases.

Conclusion: Male patients carrying biallelic mutations in *PROK2* or *PROKR2* have a less variable and on average a more severe reproductive phenotype than patients carrying monoallelic mutations in these genes. Nonreproductive, nonolfactory clinical anomalies associated with KS seem to be restricted to patients with monoallelic mutations. **(*J Clin Endocrinol Metab* 95: 659–669, 2010)**

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Abbreviations: BMI, Body mass index; CV, coefficient of variation; FGF, fibroblast growth factor; FGFR1, FGF receptor 1; GNRHR, GnRH receptor; HH, hypogonadotropic hypogonadism; KISS1R, kisspeptin receptor; KS, Kallmann syndrome; MRI, magnetic resonance imaging.

Kallmann syndrome (KS) is a developmental disorder combining congenital hypogonadotropic hypogonadism (HH) with anosmia or hyposmia (1). The deficiency of the sense of smell is related to olfactory bulb hypoplasia or aplasia. HH is due to GnRH deficiency (2), which likely results from the failed embryonic migration of neuroendocrine GnRH-synthesizing cells (3). KS is genetically heterogeneous. In 1991 *KAL1*, encoding an extracellular matrix glycoprotein, was implicated in the X chromosome-linked form of KS (4–6). In 2003 we showed that the fibroblast growth factor receptor 1 (*FGFR1*) gene was involved in an autosomal dominant form of KS (7). More recently Falardeau *et al.* showed that the gene encoding fibroblast growth factor (FGF)-8, a ligand of FGFR1, was also involved in a few KS cases (8). Mutations in the chromodomain helicase DNA-binding protein 7 gene (*CHD7*), which underlie coloboma, heart defect, choanal atresia, retardation, genital hypoplasia, ear anomalies (CHARGE) syndrome, have also been reported in a few patients initially diagnosed with KS (9, 10).

In 2006 we identified, in several KS patients, mutations in *PROK2* (National Center for Biotechnology Information gene ID 60675; Bethesda, MD) or *PROKR2* (National Center for Biotechnology Information gene ID 128674) that encode prokineticin-2 and prokineticin receptor-2 (a G protein-coupled receptor), respectively (11). Since then, we and others reported on the presence of *PROKR2* or *PROK2* mutations (mainly missense but also nonsense and frameshift mutations) in additional patients (12–16). Most patients carry monoallelic mutations, but some patients carry mutations on both alleles of either gene. Moreover, most of the mutations identified in homozygous (or compound heterozygous) patients have also been found in heterozygous state in other patients (11, 12, 15 and this study). Finally, many of the mutations reported so far, whose deleterious effects on the receptor signaling activity are predicted or have been confirmed by *in vitro* studies (12, 14, 17), are also present in some clinically unaffected individuals [(11, 12), and C. Dodé unpublished results)]. This, together with the observation that only homozygous null mice for *Prok2* or *Prokr2* reproduce the KS phenotype (12, 18) strongly suggests that heterozygous patients carry additional mutations in other KS genes. Accordingly, we did not find a dominant-negative effect of any of the *PROKR2* missense mutations on the wild-type receptor *in vitro* (17). Therefore, *PROK2* and *PROKR2* are likely to be involved both in recessive monogenic and digenic/oligogenic forms of KS (1). Digenic inheritance has indeed been shown in a few patients who bear mutations both in *PROKR2* and *PROK2*, *KAL1*, or *FGFR1* (11, 14 and this study). Notably, a few additional patients have been found to carry mutations in both *PROKR2* and the GnRH1, GnRH receptor (GNRHR), or kisspeptin receptor (KISS1R) genes that have been implicated in normosmic congenital HH (19 and this study). Most patients heterozygous for *PROKR2* or *PROK2* mutations, however, are expected to carry additional mutations in as-yet-undiscovered KS genes. Indeed, mutations in the KS genes identified so far have been found in less than 30% of all KS patients, indicating that other disease genes remain to be discovered (1). Because these genes, when mutated, may cause variable degrees of reproductive and olfactory dysfunction, as well as diverse nonreproductive, nonolfactory anomalies [as previously shown for *KAL1*, *FGFR1*, and *FGF8* (1)], some clinical heterogeneity can be anticipated among KS patients harboring monoallelic *PROKR2* or *PROK2* mutations, depending on the other gene(s) implicated in each patient. In contrast, patients carrying biallelic mutations in *PROKR2* or in *PROK2*, which may be sufficient to cause the KS phenotype as suggested by *Prokr2* and *Prok2* mouse mutants, could have less variable phenotypes. To test this hypothesis, we conducted a comparative phenotypic study

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in 55 KS patients who carry either monoallelic or biallelic mutations in *PROK2* or *PROKR2*.

Patients and Methods

Patients

From a cohort of approximately 600 KS patients who have been screened for the presence of *PROK2* or *PROKR2* mutations, the 55 patients (42 men and 13 women) carrying either monoallelic (41 patients) or biallelic (14 patients) mutations in *PROK2* or *PROKR2* were selected for this study. Twenty-seven of these patients have been previously reported (11, 15), whereas the remaining 28 patients have not. In all KS patients carrying *PROK2* or *PROKR2* monoallelic mutations, additional mutations in *KAL1*, *FGFR1*, *FGF8*, *GNRHR*, *GNRH1*, *KISS1R*, *KISS1*, TAC3R, or *TAC3* were searched for as described elsewhere (7, 8, 20–24). In addition, we sought mutations in *CHD7* in the KS patients who had a heart defect or hearing impairment (patients 23 and 39), *i.e.* two clinical anomalies that can be found in the CHARGE syndrome. Table 1 provides a list of all the mutations identified in the patients.

Gonadotropin deficiency in the patients was characterized by: 1) absent or incomplete puberty at age 18 yr; 2) low plasma testosterone levels in men and low estradiol levels in women plus low or normal serum gonadotropin levels; 3) otherwise normal pituitary function; 4) normal serum ferritin concentrations; and 5) normal magnetic resonance imaging (MRI) of the hypothalamic-pituitary region.

Spontaneous sexual maturation was assessed through a detailed history taking (virilization in males, breast development and menses in females and growth spurt in both gender), and physical examination. In males, stretched penile length was measured at diagnosis before any androgen or gonadotropin therapy; microphallus was defined as a length less than 2.5 cm (25). Testicular volume was evaluated before any treatment in 10 patients with biallelic mutations and 22 patients with monoallelic mutations by using both a Prader orchidometer and ultrasonography. None of the patients had received gonadotropin replacement therapy before this study. Patients who were receiving androgen replacement therapy were taken off testosterone enanthate for at least 2 months before hormonal investigations. On the day of admission, a blood sample was drawn between 0800 and 1000 h and stored until baseline serum FSH, LH, and plasma testosterone assays in males and estradiol assay in females. Analysis of gonadotropin secretion was carried out using the GnRH challenge test (100 μg iv) as reported (22, 26).

Olfactory acuity was assessed by interview in all patients and analyzed by olfactometry (27) in 15 patients. In addition, 30 patients underwent MRI of the olfactory bulb region. In all patients, bimanual synkinesia and hypodontia were searched for by clinical examination. In addition, four patients had dental panoramic x-ray. Renal ultrasound examination was performed in 29 patients. One patient underwent continuous sleep monitoring during 48 h as described (28). Finally, in five KS patients, plasma cortisol level was measured in serial blood samples taken at 0800, 1200, 1600, 2000, 2400 and 0400 h, and values were compared with values in 12 healthy volunteers.

TABLE 1. List of the mutations found in the patients

Gene	Exon	Nucleotide change	Amino acid change
PROKR2	1	c.58delC	p.20fsX24
	1	c.253C>T[a]	p.R85C
	1	c.253C>G[a]	p.R85G
	1	c.254G>A	p.R85H
	1	c.254G>T[a]	p.R85L
	2	c.491G>A	p.R164Q
	2	c.518T>G	p.L173R
	2	c.533G>C	p.W178S
	2	c.629A>G	p.Q210R
	2	c.752G>T[a]	p.W251L
	2	c.802C>T	p.R268C
	2	c.868C>T	p.P290S
	2	c.969G>A	p.M323I
	2	c.989delC[a]	p.T330fsX5
	2	c.991G>A	p.V331M
PROK2	1	c.94G>C	p.G32R
	2	c.161G>A[a]	p.S54N
	2	c.163delA	p.I55fsX1
	2	c.217C>T	p.R73C
	4	c.297-299insT	p.G100fsX22
	4	c.310C>T[a]	p.H104Y
KAL1[b]	8	c.1187C>T	p.S396L
	9	c.1267C>T	p.R423X
FGFR1[b]	13	c.1810G>A[a]	p.A604T
GNRHR[b]	2	c.719G>A[a]	p.R240Q
KISS1R[b]	4	c.565G>A	p.A189T

[a] New sequence variants.

[b] These five mutations were found in five patients who also carry monoallelic mutations in *PROKR2*. The pathogenic effects of the *KAL1* mutations have been previously discussed (11, 20). The missense mutation in *FGFR1* is located in the tyrosine kinase domain of the receptor and presumably interferes with its enzymatic activity. The missense mutation in *GNRHR* is located in the third intracellular loop of this G protein-coupled receptor and has both a deleterious effect on the intracellular release of Ca^{2+} *in vitro* and a lower amount of the receptor at the cell surface (<50% of that of wild-type receptor, see supplemental Fig. S1). Finally, the missense mutation in *KISS1R* has already been reported both in a patient with normosmic congenital HH and clinically unaffected individuals (33). It is located in the second extracellular loop of this G protein-coupled receptor and may thus interfere with ligand binding.

The study was approved by the local ethics committee, and the patients and controls included gave their written informed consent to participate.

Hormone measurements

All the hormone measurements were performed in a single run. Plasma LH, FSH, and inhibin B levels were measured with immunoradiometric assay or ELISAs, as reported elsewhere (21, 26, 29). The intra- and interassay coefficients of variation (CVs) were, respectively, 1.5 and 5.2% for LH, 2.7 and 5.5% for FSH, and 15% for inhibin B. The detection limits were 0.15 IU/liter, 0.2 IU/liter, and 10 pg/ml for LH, FSH, and inhibin B, respectively. Plasma testosterone was measured with a commercial RIA method with a detection limit of 0.05 ng/ml (0.18 nmol/liter) and intra- and interassay CVs of 5.8 and 8.0%, respectively. Estradiol was measured by RIA as previously described (21), with a detection limit of 3.3 pg/ml (11.1 pmol/liter) and intra- and interassay CVs of 4.8 and 7.0%. Plasma cortisol was measured by RIA as previously reported (30).

TABLE 2. KS patients with a monoallelic mutation in *PROKR2* or *PROK2*

Cases	Gender	Familial cases	BMI (kg/m^2)	Sense of smell	Olfactory bulb MRI	Cryptorchidism/ microphallus	Spontaneous puberty	Mean testis volume (ml)	LH (IU/liter) basal-peak	FSH (IU/liter) basal-peak	E2 (pg/ml)	T (ng/ml)	Inhibin B (pg/ml)	Associated phenotypes	Genotype	Reference
															PROKR2	
1	M	No	22.8	Hyposmia (H)	Aplasia	No/yes	No	3.0	0.1–3.6	5.3–6.6		0.08	87	Hypodontia	R85C/+	
2	M	No	21.8	Anosmia (H)	Aplasia	No/no	No	0.6	0.1–0.1	0.2–1.2		1	22		R85C/+	
3	F	No	NA	Normosmia? (H)	NA		No		1.0–3.2	5.7–11.2	<		NA		R85C/+	
4	M	No	20.9	Anosmia (H)	Aplasia	Yes/no	No	2.0	0.4–2.6	0.4–1.7		0.8	<		R85G/+	
5[#]	M	Yes	17.3	Normosmia? (H)	NA	Yes/no	No	1.5	0.9–15.1	1.2–7.6		0.05	NA	Sleep disorder Bimanual synkinesia	R85H/+	
6[#]	M	Yes	NA	Normosmia? (H)	NA	No/no	Yes (delayed)	NA	NA–NA	NA–NA		NA	NA	Sleep disorder	R85H/+	
7*	F	Yes	24.2	Hyposmia (O)	Aplasia		No		0.3–NA	0.9–NA	5.2		NA		R85H/+	(11)
8*	F	Yes	27.1	Hyposmia (O)	Aplasia		Yes		NA–NA	NA–NA	NA		NA	High-arched palate	R85H/+	(11)
9	M	No	NA	Anosmia (O)	NA	Yes/no	No	NA	2.4–15	4.8–12		0.7	NA		R85L/+ *FGFR1*: A604T/+	
10	M	No	24.0	Hyposmia (H)	Aplasia	Yes/yes	No	1.3	0.2–0.9	1.5–1.5		0.4	<	Facial dysmorphy	R164Q/+	(11)
11	M	No	34.0	Anosmia (H)	NA	No/yes	No	2.2	0.5–1.0	1.2–2.6		0.3	NA		L173R/+	(11)
12	F	?	NA	Anosmia (O)	NA		No		0.8–NA	2.6–NA	14		NA		L173R/+	
13	M	Yes	23.8	Hyposmia (H)	Hypoplasia	No/no	No	3.5	2.0–19.8	3.5–6.1		1.1	59		L173R/+	
14	M	No	26.3	Anosmia (H)	Aplasia	No/no	No	2.5	0.1–4.2	0.9–2.2		0.4	NA		L173R/+	
15	F	Yes	NA	Anosmia (O)	NA		No		NA–NA	NA–NA	NA		NA	Ptosis High-arched palate	L173R/+	
16	M	No	NA	Anosmia (H)	NA	No/no	No	NA	0.1–NA	0.2–NA		0.4	NA	Severe depression	L173R/+	(11)
17	M	Yes	NA	Hyposmia (H)	Hypoplasia	Yes/no	No	NA	NA–NA	NA–NA		NA	NA	Brachydactyly (hands) + Polydactyly (right foot)	L173R/+	
18	M	Yes	24.6	Normosmia? (H)	Left hypoplasia	No/no	No	5.5	2.1–9.3	1.7–2.9		0.6	126		L173R/+	
19	M	No	16.6	Anosmia (H)	Normal	No/yes	No	1.2	0.1–2.0	0.3–4.0		0.2	NA		L173R/+	

(*Continued*)

TABLE 2. Continued

Cases	Gender	Familial cases	BMI (kg/m^2)	Sense of smell	Olfactory bulb MRI	Cryptorchidism/ microphallus	Spontaneous puberty	Mean testis volume (ml)	LH (IU/liter) basal-peak	FSH (IU/liter) basal-peak	E2 (pg/ml)	T (ng/ml)	Inhibin B (pg/ml)	Associated phenotypes	Genotype	Reference
20	M	Yes	16.5	Anosmia (H)	Aplasia	No/no	No	2.6	0.1–0.1	0.2–4.5		0.2	NA	High-arched palate Psychomotor troubles	L173R/+	(11)
21	M	No	27.0	Hyposmia (O)	Aplasia	No/no	Yes	20	1.1–6.8	NA–NA		1.2	NA		L173R/+ *KAL1*: S396L	(11)
22	M	Yes	23.3	Hyposmia (H)	NA	No/Yes	No	NA	0.4–6.0	1.4–5.8		0.5	NA		L173R/+ *KAL1*: R423X	
23	F	No	22.8	Hyposmia (H)	NA		No		0.5–0.8	0.5–1.9	NA		15	Interventricular septum heart defect	W178S/+	(11)
24^	M	Yes	NA	Anosmia (H)	Aplasia	No/no	Yes	NA	NA–NA	NA–NA		NA	NA		Q210R/+	(11)
25^	M	Yes	NA	Anosmia (H)	NA	No/no	Yes	NA	3.5–NA	14.1–NA		3.9	NA		Q210R/+	(11)
26	M	No	NA	Anosmia (O)	Asymmetric hypoplasia	Yes/no	No	NA	0.1–0.6	0.3–2.1		0.3	NA		W251L/+	
27	M	No	28.4	Anosmia (H)	Aplasia	No/no	No	3.1	0.1–2.1	0.2–2.5		0.3	18		R268C/+	(11)
28	M	No	23.6	Anosmia (H)	Hypoplasia	No/no	Yes	25	3.0–NA	7.0–NA		2.2	NA	Nonsecretory pituitary macroadenoma	R268C/+ *KISS1R*: A189T/+	
29	M	No	22.5	Anosmia (O)	NA	No/no	No	2.5	0.1–0.1	0.2–1.2		NA	<		P290S/ +	
30	M	No	28.0	Hyposmia (H)	NA	No/no	No	1.3	0.7–10.5	0.7–2.4		0.4	NA		P290S/+	
31	M	No	28.3	Anosmia (O)	Aplasia	Yes/no	No	1.2	0.2–0.5	0.5–2.0		0.06	75	Alleged sleep disorder	P290S/+	(11)
32	M	No	20.3	Anosmia (H)	Normal	Yes/yes	No	5.0	0.3–1.3	0.2–3.4		0.3	NA		P290S/+	(11)
33	F	Yes	NA	Hyposmia (H)	NA		Partial (breast development)		4.0–NA	4.0–NA	28		NA		P290S/+	
34	M	No	26.9	Anosmia (H)	Aplasia	Yes/yes	No	6.0	0.1–10	2.5–8.0		1.8	15	Diabetes mellitus Ptosis	V331 M/+ *GNRHR*: R240Q/+ *PROK2*	(11)
35	M	Yes	26.6	Normosmia? (H)	NA	No/no	No	NA	1.0–10	1.0–3.5		0.5	NA		G32R/+	(11)
36	M	No	28.0	Anosmia (O)	Hypoplasia	No/no	No	2.0	1.5–3.0	0.8–2.0		0.2	NA		S54N/+	
37	M	No	29.6	Anosmia (H)	Hypoplasia	Yes/yes	No	2.2	0.2–1.4	0.3–1.3		0.3	NA	Horizontal nystagmus	R73C/+	
38	F	Yes	43.0	Anosmia (H)	NA		Partial (breast development)		0.3–4.9	0.9–2.9	73		NA	Sleep disorder	R73C/+	(11)

(*Continued*)

TABLE 2. Continued

Cases	Gender	Familial cases	BMI (kg/m^2)	Sense of smell	Olfactory bulb MRI	Cryptorchidism/ microphallus	Spontaneous puberty	Mean testis volume (ml)	LH (IU/liter) basal-peak	FSH (IU/liter) basal-peak	E2 (pg/ml)	T (ng/ml)	Inhibin B (pg/ml)	Associated phenotypes	Genotype	Reference
39	M	Yes	NA	Anosmia (O)	NA	Yes/yes	No	NA	0.1–0.5	0.2–0.5		0.1	NA	Pectus excavatum High–arched palate Bilateral perceptive deafness	H104Y/+	
40″	F	Yes	21.9	Anosmia (O)	Aplasia		No		0.1–3.0	0.2–3.0	5		NA		G100fsX22/+	(11)
41″	M	Yes	18.9	Normosmia (O)	NA	No/no	No	5.2	0.1–9.0	0.2–3.0		1.3	NA		G100fsX22/+	(11)

Superscript symbols denote related patients. M, Male; F, female; NA, not available; H, by history; O, olfactometry; E2, estradiol; T, testosterone; <, below detection limit (see *Patients and Methods*).

Statistical analyses

Quantitative results are reported as individual values in the figures and tables, and as means ± SD in the text. Hormonal parameters, testicular volume and body mass index (BMI) were compared using the Mann-Whitney, Wilcoxon, or Kolmogorov-Smirnov nonparametric tests. Qualitative variables were compared using the χ^2 test. $P \leq 0.05$ were considered to denote significant differences.

Results

The phenotypic features of the patients carrying monoallelic and biallelic mutations in *PROKR2* or *PROK2* are presented in Tables 2 and 3, respectively. Pedigrees of the new familial cases are shown in Fig. 1.

Reproductive phenotype in men

Five of 32 male patients with monoallelic mutations (16%) had undergone spontaneous puberty *vs.* none in the 10 patients with biallelic mutations. The difference between the two groups was, however, not statistically significant ($P = 0.18$).

Prevalence of microphallus and cryptorchidism

Nine of the 10 male patients with biallelic mutations (90%) and nine of the 32 male patients with monoallelic mutations (28%) had microphallus at diagnosis ($P < 0.001$). Cryptorchidism was also more frequent in patients with biallelic mutations than patients with monoallelic mutations (70 *vs.* 34%; $P < 0.05$). Notably, all the male patients with biallelic mutations had either microphallus or cryptorchidism (four patients) or both (six patients).

Testicular volume and testosterone secretion

Mean testicular volume was lower in the patients with biallelic mutations (1.2 ± 0.4 ml) than the patients with monoallelic mutations (4.5 ± 6.0 ml; $P < 0.01$) (Fig. 2A). Circulating testosterone levels were always below normal and were on average lower in patients with biallelic mutations (0.2 ± 0.1 *vs.* 0.7 ± 0.8 ng/ml; $P = 0.05$) (Fig. 2B).

Basal and stimulated gonadotropin levels

The mean basal FSH level was lower in patients with biallelic mutations than patients with monoallelic mutations (0.3 ± 0.1 *vs.* 1.8 ± 3.0 IU/liter; $P < 0.05$) and was below normal in both groups of patients (Fig. 2C). The mean poststimulation LH peak level was also lower in patients with biallelic mutations (0.8 ± 0.8 *vs.* 5.2 ± 5.5 IU/liter; $P < 0.05$) (Fig. 2D). In contrast, the mean basal LH levels (0.3 ± 0.4 *vs.* 0.7 ± 1.0 IU/liter; $P = 0.57$) and poststimulation FSH levels (3.3 ± 1.6 *vs.* 3.6 ± 2.7 IU/liter; $P = 0.60$) were not significantly different between these two groups.

TABLE 3. KS patients with biallelic mutations in *PROKR2* or *PROK2*

Cases	Gender	Familial cases	BMI (kg/m²)	Sense of smell	Olfactory bulb MRI	Cryptorchidism/ microphallus	Spontaneous puberty	Testis volume (ml)	LH (IU/liter) basal-peak	FSH (IU/liter) basal-peak	E2 (pg/ml)	T (ng/ml)	Inhibin B (pg/ml)	Associated phenotypes	Genotype	Reference
															PROKR2	
42	M	No	19.6	Hyposmia (H)	Hypoplasia	Yes/yes	No	0.9	0.3–2.4	0.5–4.1		0.08	<		R85C/R85C	
43	F	No	28.0	Anosmia (H)	NA		No		NA–NA	NA–NA	NA		NA		R85H/R85H	(11)
44	F	No	24.2	Anosmia (H)	NA		No		0.5–2.7	0.3–1.9	<		NA		R164Q/T330fsX5	
45	M	No	24.5	Anosmia (H)	Aplasia	Yes/yes	No	0.6	0.6–1.2	0.3–0.5		0.1	11		L173R/L173R	(11)
46	M	No	30.3	Hyposmia (H)	Aplasia	Yes/yes	No	0.7	0.2–1.0	0.4–5.0		0.4	NA		L173R/L173R	
47*	M	Yes	21.7	Anosmia (H)	NA	No/yes	No	1.5	0.1–0.1	0.2–2.6		0.3	NA		Q210R/L173R	(11)
48*	M	Yes	26.8	Anosmia (H)	NA	No/yes	No	1.2	0.1–0.1	0.2–3.5		0.2	NA		Q210R/L173R	(11)
49*	M	Yes	26.2	Anosmia (H)	NA	Yes/yes	No	1.5	0.1–0.1	0.2–2.9		0.3	NA		Q210R/L173R	(11)
50	F	No	24.3	Anosmia (O)	Hypoplasia		No		0.1–0.5	0.2–1.5	NA		NA		P290S/P290S	
51	F	No	21.2	Anosmia (O)	Aplasia		No		0.1–2.2	0.6–4.0	<		NA		P290S/P290S	
52α	M	Yes	21.7	Hyposmia (H)	Aplasia	Yes/yes	No	2.0	0.5–0.5	0.3–5.0		0.4	NA		M323I/H20fsX24	(11)
53α	M	Yes	21.7	Anosmia (H)	NA	Yes/yes	No	1.5	NA–NA	NA–NA		0.3	NA		M323I/H20fsX24	(11)
															PROK2	
54	M	No	NA	Anosmia (H)	Aplasia	Yes/no	No	1	1.2–NA	0.3–NA		0.3	NA		I55fsX1/I55fsX1	(15)
55	M	No	18.1	Anosmia (H)	Aplasia	No/yes	No	1.2	0.1–NA	0.2–NA		0.1	NA		R73C/R73C	(15)

Superscript symbols denote related patients. Note that patients 24 and 25 (Table 2) and 47, 48, and 49 (Table 3) are from the same family. M, Male; F, female; NA, not available; H, by history; O, olfactometry; E2, estradiol; T, testosterone; <, below detection limit (see *Patients and Methods*).

Reproductive phenotype in women

The four women with biallelic *PROKR2* mutations all had primary amenorrhea and no spontaneous breast development. Their gonadotropin and estradiol levels were low. Eight of nine women with monoallelic mutations in *PROKR2* or *PROK2* had primary amenorrhea. Six had no breast development at diagnosis before any estrogen replacement therapy, whereas two had near-normal spontaneous breast development (Tanner stage IV). Their plasma estradiol levels were low or reached values observed in normal women in follicular phase. Finally, one woman had anosmia without hypogonadism.

Olfactory phenotype

All 14 patients with biallelic mutations in *PROKR2* or *PROK2* had an abnormal sense of smell, either anosmia (11 patients) or hyposmia (three patients), whereas six of 41 patients with monoallelic mutations in either gene (15%) had a seemingly normal sense of smell, as assessed by history and confirmed by olfactometry in one patient. The difference between the two groups was, however, statistically nonsignificant ($P = 0.13$).

Other clinical anomalies

Nonreproductive, nonolfactory clinical anomalies were rare. Twenty-nine patients (21 with monoallelic and eight with biallelic mutations) underwent kidney ultrasonography, and all had normal findings. Four patients had high-arched palate, one patient had hypodontia (one missing molar), and two patients had a ptosis. Four patients reported sleep disorders, one of whom (patient 31) complained of recurrent sleep onset insomnia. During nighttime and daytime sleep monitoring, however, his sleep quality and duration were normal (supplemental table, published as supplemental data on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org). One patient had bimanual synkinesia, one had audiometrically documented hearing loss, one had horizontal nystagmus, one had facial dysmorphism (hypertelorism, epicanthus, flat nose), and one had brachydactyly of the hands and postaxial type B polydactyly of the right foot. Finally, an interventricular septum heart defect, severe depression, psychomotor difficulties, and diabetes mellitus were each found in a patient with a monoallelic *PROKR2* mutation. None of the patients had cleft lip/palate.

BMI

Because obesity has been reported in some KS patients with *PROK2* or *PROKR2* mutations (11, 13), we compared BMI values in nine male KS patients with biallelic mutations, 24 male patients with monoallelic mutations,

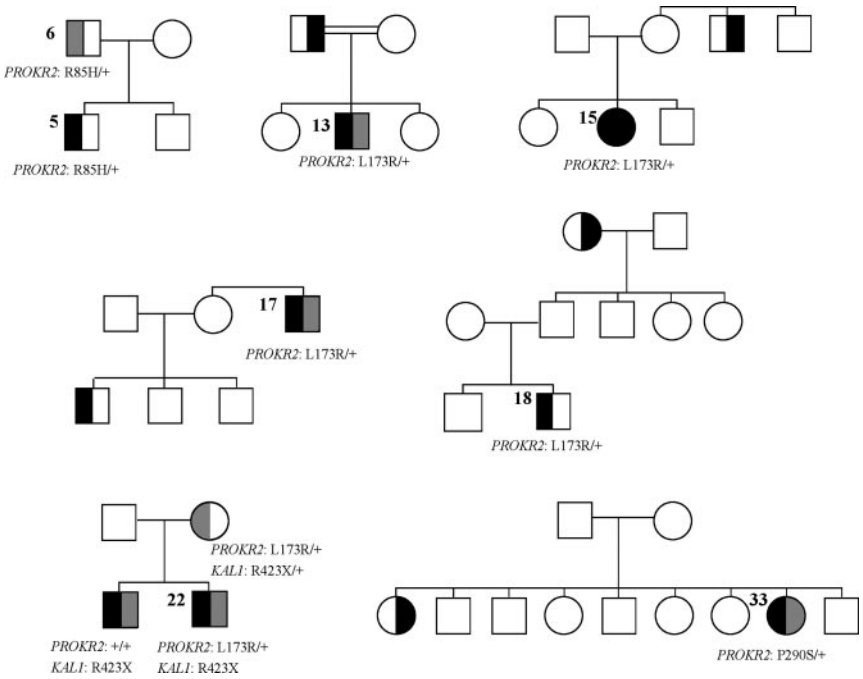

FIG. 1. Pedigrees of the new familial cases. *Filled symbols* denote individuals affected by KS (both hypogonadism and anosmia or hyposmia). *Right half-filled symbols* denote individuals with hyposmia (*gray*) or anosmia (*black*). *Left half-filled symbols* denote individuals with delayed puberty (*gray*) or hypogonadism (*black*). The patients' numbers are indicated. Notably, patient 22 (*PROKR2*: L173R/+; *KAL1*: R423X) has the same clinical status as his affected brother who does not harbor the *PROKR2* mutation. Therefore, the nonsense mutation in the X chromosome-linked *KAL1* gene is probably sufficient to account for the KS in these male patients. This mutation is, however, unlikely to account for the mother's delayed puberty, which may rather result from synergistic effects of the *KAL1* and *PROKR2* monoallelic mutations.

and 39 age- and treatment-matched male KS patients without detected mutations in *PROK2* or *PROKR2*. These controls include 14 patients with mutations in *KAL1* (eight patients) or *FGFR1* (six patients). We also compared BMI values between the nine female patients with monoallelic or biallelic mutations in *PROK2* or *PROKR2* and nine age- and treatment-matched female KS patients without detected mutations in these genes, including four patients with *FGFR1* mutations. Three patients, comprising a man with a biallelic *PROKR2* mutation, a man with a monoallelic *PROKR2* mutation and a woman with a monoallelic *PROK2* mutation were obese (BMI > 30 kg/m^2), but on the whole the mean BMI values in the male and female patients carrying *PROK2* or *PROKR2* mutations were not different from those in the control KS patients of the same gender (23.9 ± 4.2 *vs.* 23.8 ± 5.2 kg/m^2, $P = 0.43$, and 26.3 ± 6.6 *vs.* 25.4 ± 3.8 kg/m^2, $P = 0.50$, for males and females, respectively). Moreover, no significant differences were found between the male patients carrying biallelic mutations (23.4 ± 3.8 kg/m^2) and those carrying monoallelic mutations (24.1 ± 4.4 kg/m^2) or those who did not carry mutations in *PROK2* or *PROKR2* ($P = 0.50$ and $P = 0.55$, respectively) (Fig. 3).

Circadian cortisol pattern

Because reduced rhythmicity of circulating glucocorticoid levels has been reported in Prok2-null mutant mice (31), we examined circadian plasma cortisol levels in five patients with mutations in *PROKR2* or *PROK2*, including one patient with biallelic mutations in *PROKR2*. As shown in Fig. 4, the circadian pattern was normal in all five patients, including a normal nadir at midnight.

Discussion

One aim of this study was to compare, in a significant number of KS patients, the reproductive and olfactory phenotypes of subjects harboring biallelic *vs.* monoallelic mutations in *PROK2* or *PROKR2*. We found that male patients harboring biallelic mutations have a more severe reproductive phenotype, with higher prevalence of both microphallus and cryptorchism, and a lower mean testicular volume, associated with lower basal FSH and stimulated LH levels. In females, no meaningful statistical comparisons could be made, owing to the small number of female patients with biallelic mutations, but the more severe reproductive phenotype (primary amenorrhea and no breast development) was found in these four women. Therefore, in our cohort, both male and female patients carrying biallelic mutations in *PROKR2* or *PROK2* had uniformly severe reproductive phenotypes before hormone replacement therapy, an observation reinforced by the seven additional patients homozygous for *PROKR2* or *PROK2* mutations (six males and one female) who have been reported so far (12, 13, 16). By contrast, the reproductive phenotype of patients harboring monoallelic mutations in PROK2 or PROKR2 was more variable, in agreement with our prediction. These results suggest that a presumably deep impairment in prokineticin signaling through prokineticin receptor 2 due to biallelic mutations either in *PROKR2* or *PROK2* often results in a more severe reproductive phenotype than a mild dysfunction in this pathway due to *PROKR2* or *PROK2* monoallelic mutations, even though this dysfunction is likely to be combined with an impairment in another putative pathway not yet discovered, within the framework of a digenic/oligogenic mode of inheritance of the disease.

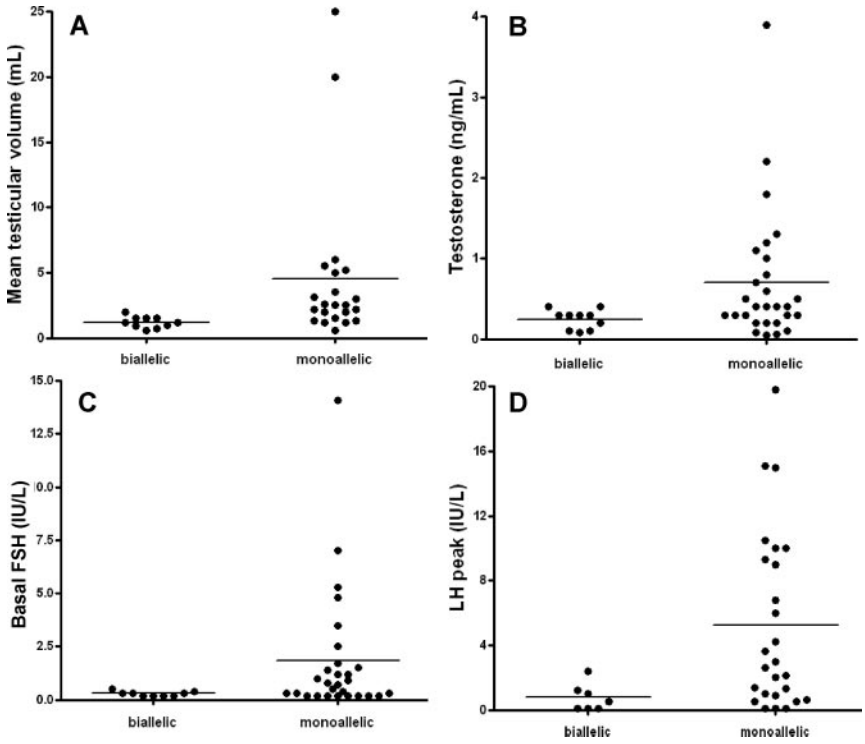

FIG. 2. Individual values of mean testicular volume (A), plasma total testosterone (B), basal serum FSH (C), and the LH response (peak) to GnRH (D) in male patients with biallelic and monoallelic mutations in *PROKR2* or *PROK2*. The normal ranges in men are 15–30 ml for testicular volume, 2.9–8.3 ng/ml for testosterone, 3.0–7.0 IU/liter for basal FSH, and 6.0–23 IU/liter for stimulated LH. A significant difference between patients with biallelic and monoallelic mutations is observed in testicular volume ($P < 0.01$), testosterone ($P = 0.05$), basal FSH ($P < 0.05$), and LH peak ($P < 0.05$).

In keeping with other descriptions of KS patients harboring mutations in *PROKR2* or *PROK2*, anosmia or hyposmia was observed in all the patients with biallelic mutations studied here, whereas this cardinal KS sign was apparently absent (by history) in 15% of the patients with monoallelic mutations. Only one woman with biallelic mutations in *PROK2* has been reported to have isolated congenital HH (12), but the existence of hyposmia in this patient cannot be ruled out in the absence of olfactometry.

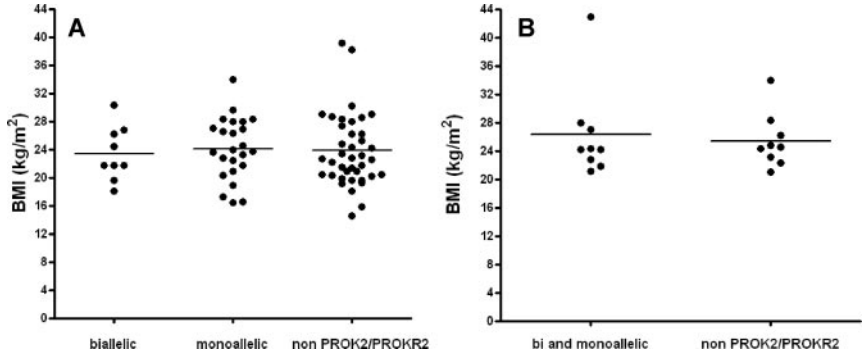

FIG. 3. Individual BMI values in male and female patients. A, From left to right, male KS patients with biallelic and monoallelic *PROKR2* or *PROK2* mutations and without mutations in these genes. No significant differences between the groups are observed. B, From left to right, female KS patients with mutations in *PROKR2* or *PROK2* (monoallelic or biallelic) and without mutations in these genes. No significant difference between the two groups is observed.

Another aim of this study was to identify associated nonreproductive, nonolfactory manifestations in these KS patients harboring mutations in *PROKR2* or *PROK2*. In keeping with previous reports (11–16), additional clinical anomalies frequently reported together with *KAL1* and *FGFR1* mutations were rare in our series of patients. Indeed, no cases of renal agenesis or cleft lip/palate were seen, and there was only one case each of bimanual synkinesia, hearing loss, and hypodontia (1). Moreover, some additional disorders that were observed in only one patient each may represent fortuitous associations, including diabetes mellitus, psychomotor troubles, and depression. These data are reassuring for candidates for medically assisted reproduction because they show the rarity of additional disorders potentially affecting their offspring's health (*e.g.* renal agenesis) or quality of life (cleft lip or palate, hearing loss, bimanual synkinesia).

Obesity has been tentatively linked to *PROK2* and *PROKR2* mutations on the basis of a few reported human cases (11, 13) and the known involvement of this signaling pathway in rodent eating behavior (31). We therefore compared BMI values in patients carrying monoallelic and biallelic mutations in *PROK2* or *PROKR2* and in KS patients without mutations in these genes. The mean BMI values were not significantly different, thus challenging the hypothetical link between obesity and *PROK2*/*PROKR2* mutations.

Prok2-null mice display significantly reduced rhythmicity in a variety of physiological parameters, including the sleep-wake cycle and circulating glucocorticoid levels (31, 32). Four of the 55 patients complained of sleep disorders. However, sleep was analyzed in one of these individuals and had a normal structure. Therefore, a causal relationship between *PROK2* or *PROKR2* mutations and sleep disorders remains to be established in humans. We also measured plasma cortisol levels at 4-h intervals for 24 h in five of the *PROK2*/*PROKR2*-mutated patients (including a patient with biallelic mutations in

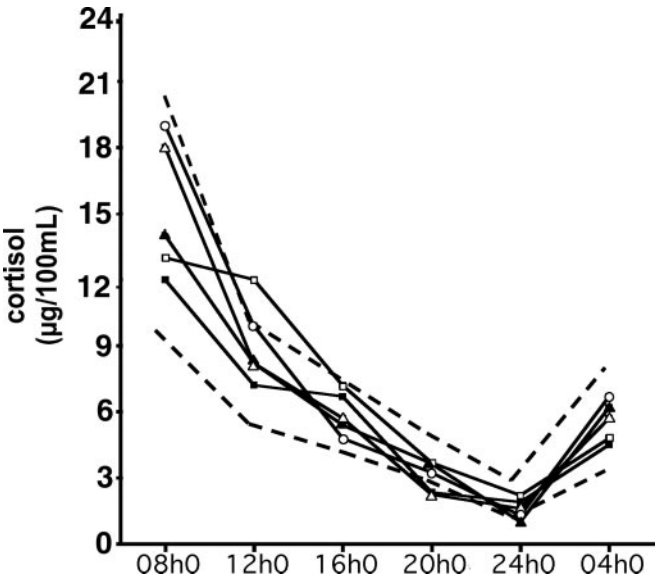

FIG. 4. Individual circadian plasma cortisol levels in five patients. Patients 5 (*white triangles*), 30 (*white squares*), 31 (*white circles*), 38 (*black squares*), and 45 (*black triangles*) were studied. The normal range in 12 adults is indicated by the *dotted lines*.

PROKR2 and three patients claiming to have sleep disorders) and observed a normal circadian rhythm in all patients. These results, which do not exclude more subtle defects not discernible by 4-h cortisol measurements, argue against a major contribution of the PROKR2 receptor signaling to physiological circadian cortisol variations in humans.

In conclusion, KS patients harboring biallelic mutations in *PROK2* or *PROKR2* have a more severe reproductive phenotype than patients with monoallelic mutations in these genes. Associated disorders are infrequent and were not observed in patients with biallelic mutations. Finally, we conclude from our series of patients that mutations in these genes do not appear to be associated with obesity or with a clear abnormal circadian glucocorticoid pattern.

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