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Idiopathic gonadotrophin deficiency: genetic questions addressed through phenotypic characterization*

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Summary

OBJECTIVE The association of idiopathic hypogonadotrophic hypogonadism (IHH) with congenital olfactory deficit defines Kallmann's syndrome (KS). Although a small proportion of IHH patients have been found to harbour defined genetic lesions, the genetic basis of most IHH cases remains to be elucidated. Genes currently recognized to be involved comprise KAL (associated with X-linked-KS), the GnRH receptor (associated with resistance to GnRH therapy), DAX 1 (associated with adrenohypoplasia congenita) and three loci also associated with obesity, leptin (OB), leptin receptor (DB) and prohormone convertase (PC1). Because of the rarity of the condition and the observation that patients are almost universally infertile without assistance, familial transmission of IHH is encountered infrequently and pedigrees tend to be small. This has constrained the ability of conventional linkage studies to identify other candidate loci for genetic IHH. We hypothesized that a systematic clinical evaluation of a large patient sample might provide new insights into the genetics of this rare disorder. Specifically, we wished to examine the following propositions. First, whether normosmic (nIHH) and anosmic (KS) forms of IHH were likely to be genetically discrete entities, on the basis of quantitative olfactory testing, analysis of autosomal pedigrees and the prevalence of developmental defects such as cryptorchidism and cleft palate. Second, whether mirror movements and/or unilateral renal agenesis were specific phenotypic markers for X-linked-KS.

DESIGN AND PATIENTS We conducted a clinical study of 170 male and 45 female IHH patients attending the endocrinology departments of three London University teaching hospitals. Approximately 80% of data were obtained from case records and 20% collected prospectively. Parameters assessed included olfaction, testicular volume, family history of hypogonadism, anosmia or pubertal delay, and history or presence of testicular maldescent, neurological, renal or craniofacial anomalies. Where possible, the clinical information was correlated with published data on genetic analysis of the KAL locus.

RESULTS Olfactory acuity was bimodally distributed with no evidence for a spectrum of olfactory deficit. Testicular volume, a marker of integrated gonadotrophin secretion, did not differ significantly between anosmic and normosmic patients, at 2·0 ml and 2·2 ml, respectively. Nevertheless, the prevalence of cryptorchidism was nearly three times greater in anosmic (70·3%, of which 75·0% bilateral) than in normosmic (23·2%, of which 43·8% bilateral) patients. Individuals with nIHH, eugonadal isolated anosmia and/or KS were observed to coexist within 6/13 autosomal IHH pedigrees. On three occasions, fertility treatment given to an IHH patient had resulted in the condition being transmitted to the resulting offspring. Mirror movements and unilateral renal agenesis were observed in 24/98 and 9/87 IHH patients, respectively, all of whom were identifiable as X-KS males on the basis of pedigree analysis and/or defective KAL coding sequence. Abnormalities of eye movement and unilateral sensorineural deafness were observed in 10/21 and 6/111 KS patients, respectively, but not in nIHH patients.

*For commentary see page 159.

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DISCUSSION **Patients with IHH are almost invariably either anosmic (KS) or normosmic (nIHH), rather than exhibiting intermediate degrees of olfactory deficit. Moreover, the prevalence of cryptorchidism is nearly three times greater in KS than in nIHH despite comparable testicular volumes, suggesting a primary defect of testicular descent in KS independent of gonadotrophin deficiency. Disorders of eye movement and hearing appear only to occur in association with KS. Taken together, these findings indicate a clear *phenotypic* separation between KS and nIHH. However, pedigree studies suggest that autosomal KS is an heterogeneous condition, with incomplete phenotypic penetrance within pedigrees, and that some cases of autosomal KS, nIHH and even isolated anosmia are likely to have a common *genetic* basis. The prevalences of anosmia, mirror movements and unilateral renal agenesis among X-KS men are estimated to be 100, 85 and 31%, respectively. In sporadic IHH, mirror movements and unilateral renal agenesis are 100% specific phenotypic markers of *de novo* X-KS. By comparison, only 7/10 X-KS families harboured KAL coding defects. Clinical ascertainment, using mirror movements, renal agenesis and ichthyosis as X-KS-specific phenotypic markers, suggested that *de novo* X-KS was unlikely to comprise more than 11% of sporadic cases. The majority of sporadic KS cases are therefore presumed to have an autosomal basis and, hence, the preponderance of affected KS males over females remains unexplained, though reduced penetrance in women would be a possibility.**

Idiopathic hypogonadotrophic hypogonadism (IHH) is classically a congenital disorder, diagnosed through low plasma levels of gonadal steroids with inappropriately low/normal gonadotrophins. IHH is irreversible, distinguishing it from the functional gonadotrophin deficiency observed in nutritional or exercise-related amenorrhoea, sleep deprivation and systemic disease. Kallmann's syndrome (KS) is defined by the association of IHH with olfactory deficit, though it is unclear whether the diagnosis should be reserved for anosmic individuals, or whether hyposmia is also compatible.

Although mutations of the gonadotrophin releasing hormone (GnRH) receptor gene (*GnRH-R*) are identifiable in about 2% of normosmic (nIHH) patients, resulting in varying degrees of GnRH resistance (De Roux *et al*., 1997; Layman *et al*., 1998; Seminara et al. 2000), IHH usually results from impaired secretion of GnRH (Naftolin *et al*., 1971; Mortimer *et al*., 1974; Yoshimoto *et al*., 1975). IHH occurs in the obesity syndromes associated with mutations of leptin (*OB*), leptin receptor (*DB*) and prohormone convertase (*PC1*) genes (Ströbel *et al*., 1998; Jackson *et al*., 1997; Clément *et al*., 1998). *DAX1* mutations also result in IHH, but the initial presentation is invariably with neonatal adrenal insufficiency (Zanaria *et al*., 1994; Muscatelli *et al*., 1994; Achermann *et al*., 1999).

IHH patients are almost invariably infertile and most cases therefore occur sporadically, presumably as a result of *de novo* mutation, though vertical disease transmission may increase as a result of the therapeutic use of gonadotrophins and pulsatile GnRH for induction of ovulation or spermatogenesis. Nevertheless, both X-linked (X-KS) and autosomal KS pedigrees are well described (McKusick, 1998), even though autosomal KS (and nIHH) loci have yet to be identified. *KAL*, the gene implicated in X-KS, lies adjacent to the steroid sulphatase (*STS*) locus at Xp22.3 (Franco *et al*., 1991; Legouis *et al*., 1991). Patients with large Xp22.3 deletions may therefore exhibit a contiguous gene syndrome comprising X-KS and ichthyosis. A number of different *KAL* point mutations have also been described. Hardelin *et al*. (1993b) and Quinton *et al*. (1996a) identified up to 10 potentially X-KS pedigrees (male-limited disease, no consanguinity) with no demonstrable defect of the *KAL* coding region. In two of Hardelin's pedigrees, segregation analysis using a polymorphic dinucleotide repeat just proximal to the *KAL* locus tended to confirm X-linked inheritance (Bouloux *et al*., 1991). More recently, Maya-Nuñez *et al*. (1999) described a family exhibiting male-limited KS and ichthyosis, in which *KAL* coding sequence was normal despite deletion of the adjacent *STS* gene. Taken together, these observations predict the existence of a regulatory locus proximal to the *KAL* coding region, defects of which result in an identical X-KS phenotype.

In X-KS, there is a proven developmental link between the aetiology of hypothalamic GnRH deficiency and the dysgenesis of olfactory-related structures (Schwanzel-Fukuda *et al*., 1989). It can be diagnosed by demonstrating abnormalities of *KAL* coding sequence, and the relationship between the results of olfactory and genetic studies is thus subject to scrutiny. Significantly, *KAL* mutations or deletions have hitherto not been found in nIHH patients (Bick *et al*., 1992; Quinton *et al*., 1996a; Georgopoulos *et al*., 1997; Oliveira *et al*. 2000). With no autosomal IHH gene(s) identified, such genotype–phenotype correlations cannot be performed in non-X-linked (autosomal and sporadic) patients, in whom the diagnosis of KS or nIHH is purely clinical. However, two other lines of enquiry have the potential to provide indirect evidence. First, quantitative olfactory assessment of IHH patients might reveal: (a) a continuous spectrum of olfactory deficit, consistent with phenotypic variation within a single disease process (IHH) as

Table 1 Characteristics of the 24 KS men exhibiting renal agenesis and/or mirror movements

	X-linked pedigree ($n = 20$)		Sporadic presentation ($n = 4$)	
	no *KAL* defect	*KAL* defect	Ichthyosis	*KAL* defect
Mirror movements ($n = 24$)	$n = 5$	$n = 19$	$n = 2$	$n = 4$
Renal agenesis ($n = 9$)	$n = 7$	$n = 2$	–	–

proposed by Hudson *et al*. (1994); or (b) a bimodal distribution of olfactory acuity, compatible with two aetiologically discrete diseases (KS and nIHH). Second, pedigree analysis might indicate (a) the occurrence of both anosmic and normosmic patients within the same autosomal IHH families, or (b) that familial nIHH and familial KS are entirely distinct entities.

A variety of phenotypic anomalies are described in IHH, but it is not clear which of these represent significant aetiological associations. These include mirror movements (Kallmann *et al*., 1944; Conrad *et al*., 1978; Schwankhaus *et al*., 1989; Quinton *et al*., 1996a), defective visuospatial attention (Kertzman *et al*., 1990), disorders of eye movement and gaze (Rubinstein *et al*., 1975; Schwankhaus *et al*., 1989; Cordonnier *et al*., 1990), sensorineural deafness (Wegenke *et al*., 1975; Lieblich *et al*., 1982; Schwankhaus *et al*., 1989; Hill *et al*., 1992; Cortez *et al*., 1993; Levy & Knudtzon, 1993; Quinton *et al*., 1996a; Tompach & Zeitler, 1996; Waldstreicher *et al*., 1996), cerebellar dysfunction (Schwankhaus *et al*., 1989; Quinton *et al*., 1999b), agenesis of the corpus callosum (De Morsier, 1954; Parr, 1988), coloboma (Lieblich *et al*., 1982; Jaffe *et al.*, 1987; Quinton *et al*., 1996a), spastic paraparesis (Tuck *et al.*, 1983), mental retardation (see below), renal agenesis (see below), craniofacial/palatal (Lieblich *et al*., 1982; van Dop *et al.*, 1987; Honig *et al.*, 1992; Tompach & Zeitler, 1996; Molsted *et al*., 1997) and cardiovascular defects (Moorman *et al*., 1984; Cortez *et al*., 1993; Fromantin *et al*., 1972).

The rarity of the condition means that significant numbers of IHH patients will only accumulate at tertiary referral centres. The patients presented in this study represent the largest cohort of well-phenotyped IHH subjects to have been investigated in such detail. The intention was to identify those abnormalities which represented real phenotypic associations and hence to shed further light on the pathophysiology and genetics of IHH. For instance, mirror movements can be demonstrated by asking an affected individual to perform unilateral, rapid, repetitive actions, such as finger-tapping, forearm pronation–supination, wrist and elbow flexion–extension and 'piano-playing-in-the-air'. Voluntary movements of one limb are associated with involuntary, nonsuppressible and homologous 'mirror' movements of the contralateral limb. Preliminary work led us to hypothesize that both mirror movements and renal agenesis might be specific clinical markers for X-KS and, in a sporadic KS patient, might therefore predict a *de novo KAL* coding defect (Kirk *et al*., 1994; Quinton *et al*., 1996a, 1996b).

Patients and methods

Patient population

The study population comprised all IHH patients registered at St Bartholomew's Hospital (up to 1992) and the Royal Free and UCL-Middlesex Hospitals (up to 1998), London, UK. The patients had accumulated over a period of approximately 25 years. The group consisted of 170 males and 45 females with biochemical and physical evidence of congenital gonadotrophin deficiency, but otherwise normal biochemical tests of anterior pituitary function and no evidence of a hypothalamo–pituitary mass lesion: LH $\leq 4{\cdot}0$ mIU/l, FSH $\leq 5{\cdot}0$ mIU/l, testosterone $\leq 5{\cdot}0$ nmol/l (males), oestradiol ≤ 100 pmol/l (females). In eight children of prepubertal age, the diagnosis of IHH could only be presumptive, on the basis of micropenis and bilateral cryptorchidism ($n = 7$), anosmia with a

Table 2 Phenotypic abnormalities in IHH (n = number of subjects screened for a particular defect)

	Total KS	nIHH	Total IHH	X-KS
Cryptorchidism	70% ($n = 91$)	23% ($n = 70$)	50% ($n = 161$)	83% ($n = 29$)
Mirror movements	31% ($n = 77$)	0% ($n = 46$)	20% ($n = 123$)	82% ($n = 29$)
Renal agenesis	15% ($n = 61$)	0% ($n = 26$)	10% ($n = 87$)	32% ($n = 28$)
Eye movement disorders	27% ($n = 44$)	4·5% ($n = 22$)	20% ($n = 66$)	33% ($n = 9$)
Sensorineural deafness	8% ($n = 77$)	0% ($n = 46$)	5% ($n = 123$)	3% ($n = 29$)
Craniofacial/palatal defect	4% ($n = 112$)	5% ($n = 103$)	5% ($n = 215$)	0% ($n = 29$)

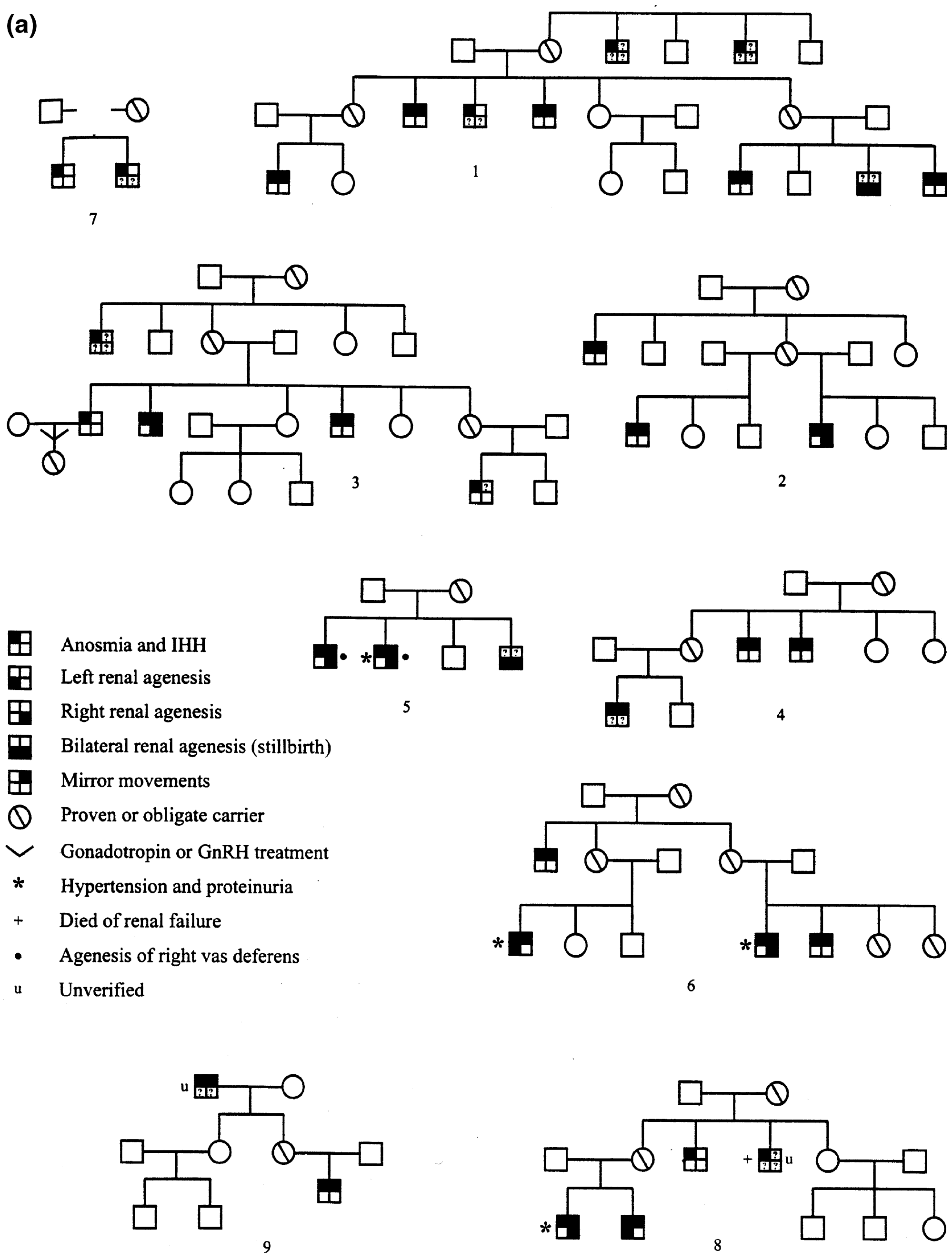

Fig. 1 (a) Families with X–linked Kallmann's syndrome.

family history of IHH ($n = 2$) and/or demonstration of a *KAL* coding sequence mutation ($n = 2$). Individuals in whom the gonadotrophin deficiency appeared to have been acquired (Nachtigall *et al.*, 1997), reversible (Quinton *et al.*, 1999a) or associated with adrenal insufficiency were excluded from the study.

Data relating to the following parameters were collected by a combination of direct patient evaluation and examination of case notes: olfaction; history or presence of cryptorchidism; family history of hypogonadism, pubertal delay or olfactory deficit; *Prader* orchidometric estimation of baseline (pre-GnRH or gonadotrophin therapy) mean testicular volume; neurological

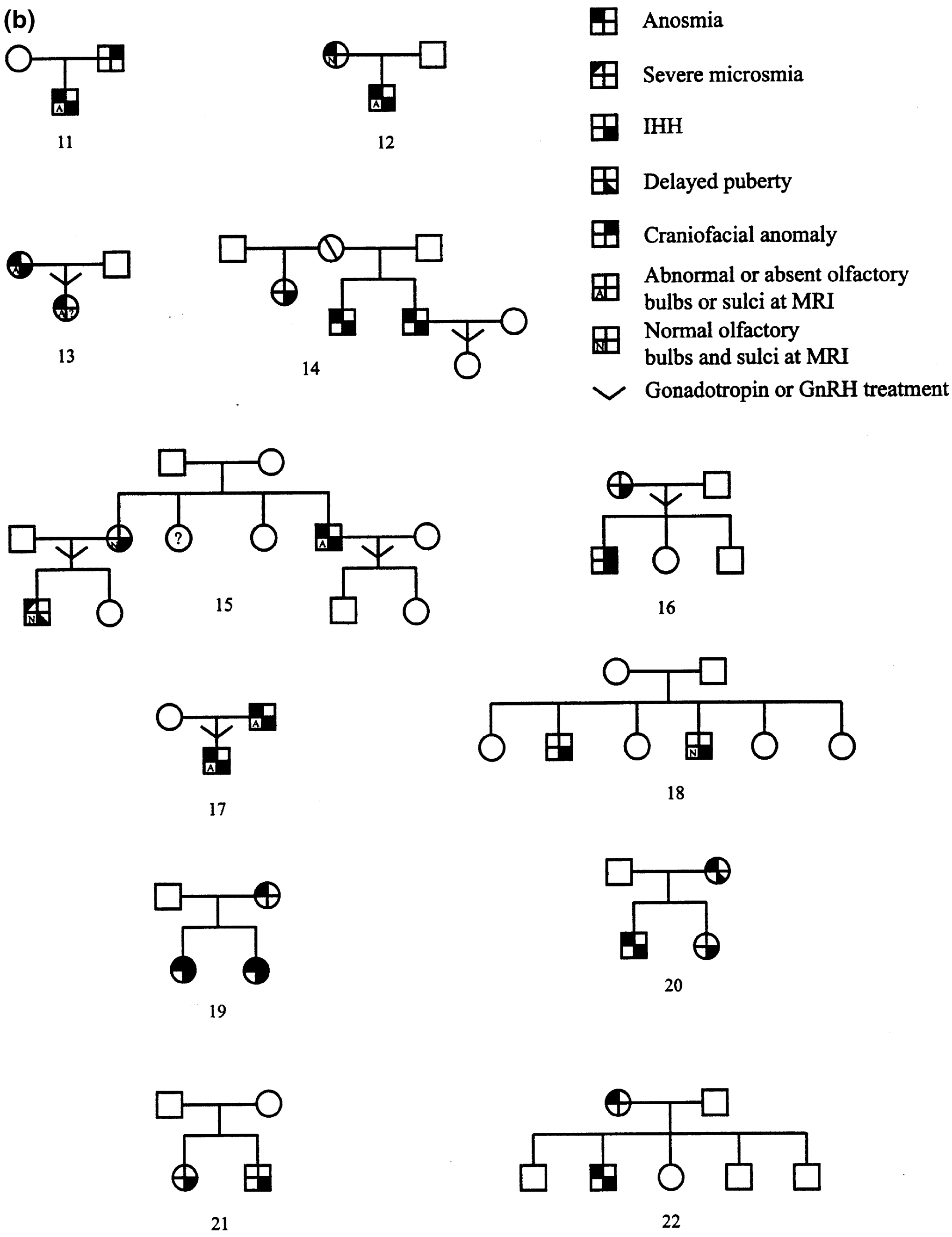

Fig. 1 (b) Families with autosomally inherited hypogonadotrophic hypogonadism. Pedigree 1: exon 5, G861A (LON 77A10; Hardelin *et al.*, 1992); pedigree 2: exon 9, C147T (LON 80D5; Hardelin *et al.*, 1992); pedigree 3: exon 6, G924A (LON 36K4; Hardelin *et al.*, 1992); pedigree 4: normal *KAL* coding sequence (LON S14; Hardelin *et al.*, 1993b) – analysis of polymorphism at *KAL* locus was not suggestive of autosomal transmission (P-MG Bouloux, personal observation); pedigree 5: Xp22.3 deletion spanning entire *KAL* locus (LON G; Hardelin *et al.*, 1993a); pedigree 6: Xpter deletion spanning *STS* and *KAL* loci (Bouloux *et al.*, 1993); pedigree 7: exon 11 deleted (xK12; Quinton *et al.*, 1996a); pedigree 8: normal *KAL* coding sequence xK9/10; Quinton *et al.*, 1996a); pedigree 9: exon 12, C1847Del (xK8; Quinton *et al.*, 1996a); pedigree 10: normal *KAL* coding sequence (pedigree not shown because of verification problem: affected brothers, uncle and male cousins reported by proband all live abroad); pedigree 18: all 14 *KAL* coding exons present (H5; Quinton *et al.*, 1996a) – normal *GnRH-R* coding sequence (N de Roux, personal communication); pedigree 22: not shown because of insufficient verification of family members (paternal cousin was diagnosed with KS at Massachusetts General Hospital).

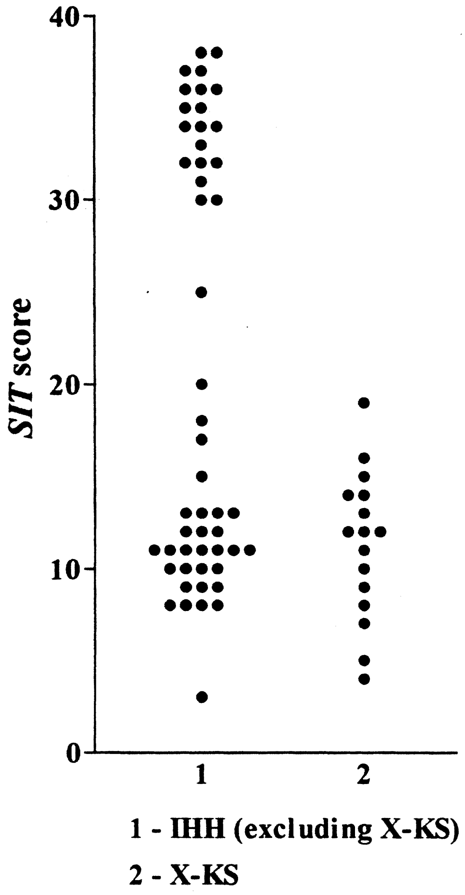

Fig. 2 Assessment of olfactory function using the Smell Identification Test®.

examination, specifically screening for distal upper-limb mirror movements, and ultrasound screening for unilateral renal agenesis. As this study was predominantly retrospective, a complete data set was not available for every single patient although, wherever possible, patients were recalled to clinic so as to fill gaps in the data. When the history indicated the possibility of other affected family members, the proband was asked to encourage them to make contact with our units, with a view to being screened. Details of some of the patients have been presented in previous publications (Hardelin *et al.*, 1992, 1993a, 1993b; Kirk *et al.*, 1994; Quinton *et al.*, 1996a, 1996b, 1997, 1999b; Krams *et al.*, 1997, 1999; Mayston *et al.*, 1997; Duke *et al.*, 1998).

Assessment of olfaction

Conventional smell testing kits consisting of volatile oils or essences (e.g. lemon, cloves, peppermint, wintergreen) or even readily available odourants (e.g. coffee granules, orange peel) are quite adequate for identifying complete absence of olfaction (anosmia). However, because such test results cannot be quantified, intermediate degrees of olfactory deficit may not be reliably distinguished. Smell tests based on olfactory threshold, using varying molar concentrations of odourants, are theoretically highly sensitive. However, their usefulness is limited by lack of reproducibility and the 100-fold variation in olfactory thresholds observed in normal human populations. We therefore used the Smell Identification Test® (SIT) wherever possible (Doty, 1995; http://www.smelltest.com). This consists of 40 scratch-and-sniff cards for self-completion, each with a choice of four possible answers (forced multiple choice). A subject who is completely anosmic will thus score approximately 10/40. Age- and sex-matched tables enable subjects to be assigned centile scores and thus be categorized as normosmic, anosmic or hyposmic (mild, moderate or severe). Although SIT is well validated in US anglophones, some of the odourants (e.g. pumpkin pie, fruit punch, root beer, etc.) would be unfamiliar to most UK nationals and our experience with SIT has led us to interpret test scores indicating very borderline hyposmia as being of cultural rather than pathophysiological significance.

Genetic analysis

It was deemed impractical to sequence all 14 *KAL* exons individually in 170 male IHH patients. We therefore sought to develop a logical, yet resource-efficient paradigm to test our original hypothesis. For the hypothesis to stand, the following propositions would need to be verified: (a) mirror movements and/or renal agenesis would only be exhibited by IHH patients who were both male and anosmic, (b) the majority of patients exhibiting these anomalies would belong to male-limited KS pedigrees, and (c) most sporadic KS patients found to exhibit mirror movements and/or renal agenesis would be found to harbour *KAL* coding defects. The data necessary to verify these propositions would therefore largely be obtainable through the clinical history and examination. Genetic screening for *KAL* coding defects could therefore be targeted at anosmic male patients observed to exhibit mirror movements and/or renal agenesis and/or ichthyosis and/or a family history compatible with X-linked inheritance. For all but one of these patients, results have already been published (Hardelin *et al.*, 1992, 1993a, 1993b; Quinton *et al.*, 1996a, 1997; see also legend to Fig. 1a).

In addition, restriction fragment length polymorphism (RFLP) analysis was used to identify female Xp22.3 heterozygotes whose carrier status was not already evident from pedigree analysis. For each of the four X-KS families known to harbour a point mutation within a particular exon (Fig. 1a: pedigrees 1, 2, 3 and 9), a detailed restriction map was constructed. In pedigrees 1, 3 and 9, the mutation was predicted to disrupt an enzyme restriction site, such that PCR products of the exon would no longer be enzymatically cleaved (*Bsc*I, *Rsa*I and *Age*I, respectively). Exons were amplified and PCR products purified as described in Quinton *et al*. (1996a) and RFLP analysis was performed as described by Grau & Griffais (1994).

Results

Olfactory assessment

Qualitative olfactory testing using simple odourants demonstrated normosmia in 73/170 male and 28/45 female IHH subjects, and suggested hyposmia in 8/170 males. The reliability of simple olfactory testing was validated when 66 of the patients underwent more detailed olfactory evaluation using SIT (Fig. 2). SIT confirmed the original qualitative findings of normosmia in 17/17 patients and anosmia in 43/43 patients. Six of the eight men originally felt to be qualitatively hyposmic were retested using SIT and only two of these proved to be truly hyposmic (two were, in fact, anosmic and two normosmic). Thirteen men harbouring *KAL* coding defects who were tested with SIT were all found to be anosmic.

The bimodal distribution of SIT scores illustrated in Fig. 2 is striking and appears to indicate two discrete IHH patient subgroups, KS and nIHH, rather than a continuous spectrum of olfactory deficit. Two boys were too young to have their sense of smell reliably assessed but, as they each belonged to a known X-KS family and harboured the particular *KAL* mutation characteristic of that family (Fig. 1a: pedigrees 1 and 3), they were assigned to the KS group for the purpose of phenotyping. Thus, of 215 IHH patients in this series, 48% could be said to have nIHH and 52% KS.

Inheritance and genetics

Although there was evidence of familial transmission in 52/215 patients (24%), the majority of cases were sporadic, giving no family history of hypogonadism, pubertal delay or olfactory dysfunction. Overall, 10 putative X-KS (Fig. 1a) and 13 autosomal IHH pedigrees (Fig. 1b) were identified. Published *KAL* coding sequence data from KS men belonging to pedigrees 1–9 are referenced in the legend to Fig. 1(a). The proband in pedigree 10 did not supply a detailed family tree, but confirmed that one brother and at least two maternal cousins were anosmic, hypogonadal and, like himself, exhibited mirror movements. No *KAL* coding defect was, however, identified. A precise family tree was likewise unavailable for the KS proband in pedigree 23, but his paternal cousin was a known KS patient, having attended Massachusetts General Hospital.

Of 37 sporadic KS men who had been fully evaluated for mirror movements, ichthyosis and unilateral renal agenesis, four (11%) were found to exhibit one or more these three phenotypic anomalies (Table 1). All four patients harboured a *KAL* mutation or deletion. These comprised: C1847Del (exon 12), deletion of exon 1 (both reported by Quinton *et al*., 1996a); pericentric inversion at Xp22.3 with deletion of *STS* and exons 10–14 of *KAL* (Quinton *et al*., 1997), and deletion of *STS* and *KAL* exons 1–10 (not previously reported). We can therefore estimate that *de novo* X-KS, accounts for about 11% of sporadic KS cases.

RFLP analysis demonstrated the following: Pedigree 1: the teenage sister of an X-KS male was found not to carry the G861A mutation. Pedigree 2: the 28-year-old sister of two X-KS males was found to be heterozygous for G924A. Following counselling, she nevertheless proceeded to have two male children, one of whom harboured the mutation. Pedigree 9: the proband presented as a sporadic case exhibiting mirror movements, but it emerged that the maternal grandfather had been anosmic with marked mirror movements and absent facial hair. The proband's mother was found to be heterozygous for C1847Del, suggesting that the maternal grandfather may have been an X-KS 'fertile eunuch'.

Of significance to fertility units were three IHH patients who had received induction of ovulation or spermatogenesis and whose offspring had inherited the condition (Fig. 1b: pedigrees 12, 15 and 16). Figure 1(b) illustrates the considerable phenotypic heterogeneity exhibited by autosomal families, with related individuals having KS, nIHH, pubertal delay and even isolated congenital anosmia. Inheritance of 'pure' nIHH was only seen in three families and this reflects the scarcity of such reports in the literature. By contrast, the X-KS families shown in Fig. 1(a) reveal a relatively homogeneous phenotype, with affected individuals differing only in the expression of mirror movements and renal agenesis.

Neurological examination

One hundred and five male (including 71 KS) and 18 female (including six KS) IHH patients were screened neurologically. Twenty-four patients were observed to exhibit mirror movements, which were usually limited to fine finger movements but, in some cases, with evident involuntary cocontraction of *biceps*, *triceps* and *supinator* muscles. All mirroring subjects were found to be male and anosmic, i.e. KS. Twenty-one belonged to male-limited KS families but three were sporadic cases. However, these three were shown to harbour *KAL* coding defects, indicating *de novo* X-linked disease (C1847Del in exon 12; deletion of exon 1; pericentric inversion at Xp22.3 with deletion of *STS* and *KAL* exons 10–14). Overall, 24/29 X-KS, but no autosomal KS or nIHH subjects, exhibited mirror movements. Thus, in IHH patients, the presence of mirror movements as a marker for the X-linked form of Kallmann's syndrome is 100% specific with an estimated diagnostic sensitivity of 82% (Tables 1 and 2).

Associations have been proposed between KS and both mental retardation (Kallmann *et al*., 1944; Obaydi *et al*., 1992) and schizophrenia (Cowen & Green, 1993). Although specific psychometric testing was not performed in this series, only four IHH subjects ($n = 3$ KS) had empirical evidence of cognitive impairment, requiring special schooling or being unable to live and work independently in the community, and the single schizophrenic patient was normosmic. No nIHH patient was found to be hearing impaired, but unilateral sensorineural deafness was noted in six sporadic KS males, one of whom one also exhibited mirror movements and was found to harbour a C1847Del mutation (*KAL* exon 12). Indeed, incomplete phenotyping, particularly with respect to more subtle defects of VIIIth nerve function, might have resulted in hearing impairment being relatively under-reported in this study. Gaze-evoked horizontal nystagmus, jerky ocular pursuit and saccadic dysmetria represent a triad of minor eye movement disorders recognized to occur in association with IHH (Schwankhaus *et al*., 1989). Apart from a sporadic nIHH male with spinocerebellar ataxia (Gordon Holmes syndrome), disordered eye movements were only observed in KS subjects (Table 2). In two cases, these were associated with chorioretinal coloboma and, in two others, with neonatal neurosurgery (for hydrocephalus with optic nerve atrophy and Dandy–Walker variant with vertex encephalocele, respectively). Two sporadic KS patients ($n = 1$ female) had a clear history of ocular motor dyspraxia during childhood, a hitherto undescribed clinical association. Sensorineural deafness and disorders of eye movement appear therefore to represent real associations with KS, not specific to X-linked disease (Table 2).

Renal agenesis

Eighty-one male (of whom 59 KS) and six female patients were screened with transabdominal ultrasound for unilateral renal agenesis. In each of the cases where one kidney could not be visualized, isotope renography was used to confirm its absence. Unilateral renal agenesis was demonstrated in only nine subjects, all of whom belonged to known X-KS families (Fig. 1a). In 15 affected males from X-KS pedigrees and four sporadic male KS cases found to harbour *KAL* mutations or deletions, both kidneys were identifiable, i.e. 9/29 X-KS males exhibited unilateral renal agenesis. In subjects with IHH, renal agenesis is therefore a marker for X-KS with a diagnostic specificity of 100% and an estimated sensitivity of 31% (Tables 1 and 2).

Gonadal volume

In 52 men who had been treated with GnRH or gonadotrophins, inspection of medical records failed to identify pretreatment measurements of testicular size. These men were therefore excluded from our analysis. However, reliable data were available for 120 men who were either untreated at the time of examination or had only received therapy with testosterone. Testicular volume ranged from 1·0 to 6·0 ml with a mean of 2·1 ml (95% CI: 1·9–2·3 ml). Similar volumes were seen in KS (mean 2·0 ml; $n = 65$, 95% CI: 1·7–2·3) and nIHH (mean 2·2 ml; $n = 55$, 95% CI: 1·8–2·5), implying a similar underlying degree of gonadotrophin deficiency. Although testes appeared to be smaller in proven X-KS subjects (1·7 ml; $n = 23$, 95% CI: 1·3–2·0), this difference did not reach statistical significance using the Mann–Whitney *U*-test. In all 35 females who were imaged ultrasonographically, uterine dimensions appeared prepubertal and ovaries, where visualized, were small and inactive.

Cryptorchidism and other anomalies

The overall prevalence of cryptorchidism in 161 males with congenital IHH was 50% and this was bilateral in 69% of cases (Table 2). By comparison, the prevalence of cryptorchidism in the general population is around 3–5% (Rozanski & Bloom, 1995). Testicular maldescent was more frequent in KS as a whole (70%, of which 75·0% bilateral) than in nIHH (23%, of which 44% bilateral). The prevalence was particularly high in X-KS (83%, of which 83% bilateral). Due to the largely retrospective nature of this study, precise prevalences of the following anomalies (none associated with X-KS) were difficult to establish: bilaterally short clavicles ($n = 1$ KS), 47XXY karyotype ($n = 1$ KS), club foot ($n = 1$ nIHH), cleft lip and/or palate ($n = 5$ nIHH; $n = 3$ KS), hemifacial microsomia ($n = 2$ KS sisters). However, given that the incidence of clefting in the general population is estimated to be 0·1% (Tompach & Zeitler, 1996), this study does not suggest an excess in KS (Table 2).

Discussion

X-linked KS was phenotypically well-defined and characterized by upper-limb mirror movements and unilateral renal agenesis. Our best estimates of the prevalence of these anomalies (which were not observed in autosomal KS or nIHH subjects) among X-KS males were 85% and 31%, respectively. Mirror movements were first described in association with anosmia and hypogonadism by Kallmann *et al*. (1944) and assessed electromyographically by Conrad *et al*. (1978). The underlying pathology appears to be an abnormal fast-conducting ipsilateral corticospinal tract projection (Krams *et al*., 1997, 1999; Mayston *et al*., 1997), suggesting a role for *KAL* in the embryonic development of the descending motor pathways. Although we did not test visuospatial ability, a previous study has shown that, among IHH patients, abnormalities in spatial perception are limited to anosmic subjects exhibiting mirror movements (Kertzman *et al*., 1990), i.e. X-KS. The association of unilateral renal agenesis with male-specific familial hypogonadism was first noted by Sainton (1902) and was subsequently described in relation to KS (Nowakowski & Lenz, 1961; Wegenke *et al*., 1975; Kirk *et al*., 1994). Expression of *KAL* mRNA in the embryonic human urogenital system, mesonephros and spinal cord suggests the gene has a wider remit than olfactory system development (Duke *et al*., 1995; Hardelin *et al*., 1999). A number of the X-KS men with single kidneys exhibit overt nephropathy (Duke *et al*., 1998; see also Fig. 1a: pedigrees 5, 6 and 8). However, it remains unclear whether this merely represents hyperfiltration damage to the single kidney, or whether *KAL* mutations or deletion do result in subtle defects of renal ultrastructure or function.

In this series of 215 IHH patients, 76% of cases were sporadic, 79% were male (of which 44% normosmic), 21% were female (of which 62% normosmic) and 52% were KS. Quantitative olfactory testing of patients with IHH clearly discriminated between normosmic (nIHH) and anosmic (KS) patients, and identified very few cases with partial olfactory deficit. Extended autosomal pedigrees with first cousin marriages were not seen in this study. Consequently, we found no cases of autosomal recessive transmission, but did elicit considerable evidence for autosomal dominant inheritance with incomplete penetrance (Fig. 1b). In some families, individuals with KS, nIHH, pubertal delay and even isolated anosmia were observed to coexist (Fig. 1b: pedigrees 11, 13, 14, 18, 19 and 21). Although other researchers have identified eugonadal individuals with isolated anosmia or severe pubertal delay in KS families (Waldstreicher *et al*., 1996), the occurrence of nIHH subjects in KS pedigrees is poorly recognized.

The medial olfactory placode is the site of origin both of the accessory olfactory nerves (terminal and vomeronasal) and of GnRH neurones. These migrate intracranially along ingrowing nerve fascicles, in relation to tissue expression of anosmin-1, the *KAL*-encoded protein (Hardelin *et al*., 1999). The olfactory nerve, however, originates from the lateral olfactory placode and X-KS results from developmental failure of all these olfactory placode-derived processes (Schwanzel-Fukuda *et al*., 1989). Incomplete penetrance in autosomal KS could thus be expressed pathophysiologically through selective failure of either the lateral or the medial placode, resulting, respectively, in isolated anosmia or nIHH.

Georgopoulos *et al*. (1997) and Oliveira *et al*. (2000) screened sporadic KS patients, finding *KAL* coding defects in 1/13 (8%) and 4/36 (11%), respectively, but genetic analysis would necessarily fail to identify patients with X-KS resulting from noncoding defects of *KAL*. However, clinical ascertainment of IHH patients for X-KS-specific phenotypic markers, demonstrated that *de novo* X-KS does indeed represent about 11% of sporadic KS patients. IHH patients treated with gonadotrophin or GnRH may pass on the condition to their offspring in an autosomal dominant manner. There has previously only been a single case report of this (Merriam et al., 1997). Given that the genetic basis of the majority of IHH individuals remains unknown, it is difficult to provide accurate pretreatment counselling on the risk of the condition being inherited by the a patient's offspring. Nevertheless, the existence of a defined risk should be specifically discussed with all patients prior to fertility treatment. In X-KS families where there is a known *KAL* exon mutation, RFLP analysis can provide simple, inexpensive and rapid identification both of female heterozygotes and of affected male neonates.

There is a 50% prevalence of cryptorchidism (largely bilateral) among IHH males, with testicular maldescent being nearly three times as common in KS than nIHH. *KAL* expression has been demonstrated in the developing human urogenital system and this gene and its autosomal homologue(s) may therefore play a specific role in testicular descent (Duke *et al*., 1995). Although 34% of IHH males in this series had undergone surgery for bilateral undescended testes during childhood, only 34% of these were referred at the time for a paediatric endocrine opinion. The significance of pubertal delay in the context of anosmia or a history of bilateral cryptorchidism appears to be poorly appreciated by nonspecialists, resulting in delayed diagnosis and treatment. IHH patients and their physicians are strongly recommended to visit http://www.hypohh.net.

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