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Mirror movements in X-linked Kallmann's syndrome
I. A neurophysiological study

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Summary
Possible mechanisms underlying the pathological mirror movements that are seen in the majority of patients with X-linked Kallmann's syndrome have been investigated using neurophysiological techniques. An EMG was recorded from the first dorsal interosseous muscle (1DI) during voluntary self-paced abduction of one index finger; EMG activity could also be recorded simultaneously from the contralateral 1DI. There was no significant difference between the time of onset of the bursts of voluntary and involuntary mirroring EMG. Focal magnetic stimulation of the hand area of the motor cortex revealed the presence of fast conducting bilateral corticospinal projections from each motor cortex in all subjects. However, both inter- and intra-subject differences exist when considering the ratio of ipsilaterally to contralaterally projecting axons. Cross-correlation analysis of multi-unit EMGs recorded during simultaneous voluntary sustained activation of homologous left and right pairs of distal upper limb muscles was performed. A short duration central peak was seen in the cross-correlograms indicating the presence of a common drive to left and right homologous motor neuron pools. This common drive may result from the synchronous activation of intermingled ipsilaterally and contralaterally projecting corticospinal neurons in the motor cortex. Cutaneomuscular reflexes were recorded from the 1DI following stimulation of the digital nerves of the index finger. Typically each reflex comprises spinal and longer latency trans-cortical components. In these subjects, the long latency components of the reflex response could, in addition, be recorded from the 1DI of the non-stimulated side. We conclude that these subjects have a novel ipsilateral corticospinal tract and that activity in this tract is responsible, at least in part, for the pathological mirroring.

Keywords: Kallmann's syndrome; cross-correlation analysis; corticospinal tract; mirror movements

Abbreviations: 1DI = first dorsal interosseous muscle; MEP = motor evoked potential; XKS = X-linked Kallmann's syndrome

Introduction
The major characteristics of Kallmann's syndrome are hypogonadotrophic hypogonadism and anosmia; additional features, only observed in patients with the X-linked form of this syndrome, are unilateral renal agenesis (Kirk *et al*., 1994) and mirror movements (Kallmann *et al*., 1944; Conrad *et al*., 1978; Schwankhaus, 1989) which are seen in 85% of these patients (Quinton *et al*., 1996*a*, *b*). Mirror movements are involuntary movements of one side of the body that accompany and mirror intentional movements of the other side; they are seen most often when using the distal muscles of the upper limb. Mirror movements are frequently present in young children but the prevalence and degree of mirroring decreases with increasing age (Connolly and Straton, 1968). The origin of these mirror movements seen during childhood is unknown, but it has been suggested that mirroring results from activity in the ipsilateral corticospinal tract (Nass, 1985). Nass (1985) suggested that, during a unilateral voluntary task, this ipsilateral tract is normally inhibited by activity in fibres of the corpus callosum originating from the motor cortex ipsilateral to the voluntary movement. Myelination of

callosal fibres is not complete until 10–13 years of age (Yakovlev and Lecours, 1967) and therefore the callosal pathway may not be fully functional until this age. If mirror movements are marked and persist into adulthood they are considered to be pathological; such is the case for those patients with X-linked Kallmann's syndrome (XKS). It is thought that the Kallmann gene product is involved in axonal guidance within the olfactory system (Franco *et al*., 1991; Legouis *et al*., 1991); it seems likely that growth of other axonal pathways could also be affected and hence mirror movements in these patients could result from a defect in axonal guidance within the motor system. Bearing in mind the above hypothesis to explain mirror movements in children, the defect in guidance could be a failure of callosal fibres to cross the midline. Alternatively, some corticospinal fibres could fail to decussate at the medulla, resulting in a considerable novel ipsilateral projection in addition to the normally occurring ipsilateral projection. In the present study we have used neurophysiological techniques to examine these hypotheses.

A preliminary account of some of this work has been presented to the Physiological Society (Mayston *et al*., 1995*a*).

Methods

Subjects

Recordings were made with ethical approval from the Joint University College and University College Hospital Committee on the Ethics of Human Research, and with informed consent, from control subjects and from 14 male patients (aged 16–60 years), derived from six pedigrees with XKS as evidenced by the family history or by demonstration of a mutation of *KAL* (the gene which is mutated in XKS); *see* the Appendix. Thirteen of these 14 patients have mirror movements. Details of control subjects are given in the relevant sections below.

Mirror movements

The degree of mirroring was assessed by observing subjects while they made the following movements. (i) They sequentially opposed the tip of each finger to the tip of the thumb, from index to little finger and back again. This was repeated several times as quickly and neatly as possible using the right hand and then the same routine was repeated using the left hand. (ii) They held the hands horizontal with the fingers extended over the edge of a box and flexed each finger of the right hand in turn several times such that the finger moved down by 4 cm. The involuntary movement of the homologous finger of the other hand was noted. This was repeated using the left hand.

In each of these tasks, mirror movements were graded using a scale of 0 to 4 where 0 = no clearly imitable movement, 1 = barely discernible but repetititve movement, 2 = slight but unsustained movement, or stronger, but briefer, repetitive movement, 3 = strong and sustained repetitive movement, and 4 = movement equal to that of the intentional hand (Woods and Teuber, 1978). The average of the two scores was calculated for each hand.

Subjects were also asked to perform alternate supination and pronation using each forearm in turn.

Electromyographic recordings

Surface EMGs were recorded simultaneously at various times from the following homologous left and right muscle pairs using Teca electrodes (Teca bar/disk electrodes; Medelec, Woking, Surrey, UK) placed 20 mm apart, centre to centre: left and right first dorsal interosseous muscle (1DI), left and right forearm extensors with the electrodes placed over the index finger extensor muscle, left and right triceps with the electrodes placed over the belly of the medial head, and left and right deltoid muscles with electrodes placed over the belly of the middle portion. The EMGs were amplified and filtered (20 Hz–5 kHz) using a four-channel Medelec Sapphire EMG machine and stored on magnetic tape (Racal Store 4, Racal Ltd, Hythe, Southampton, UK) for future analyses.

EMG during phasic abduction of index finger

Subjects sat at a table with hands palm down, flat on the table. They were instructed to perform ~60 voluntary self-paced brief abductions of the left, then the right, index finger whilst the EMG was recorded simultaneously from the left and right 1DI. Using the beginning of the EMG burst of the 1DI of the voluntarily moved index finger as the trigger, the EMGs of the left and right 1DI were rectified and averaged for 50 sweeps using signal averaging software (Sigavg, Cambridge Electronic Design, Cambridge, UK). The ratio of the area of the burst of involuntary EMG to the voluntary EMG of the other side was calculated. Seven healthy control subjects (three females and four males, aged 20–46 years) were tested in the same way.

Magnetic stimulation

Investigation of the laterality of responses

Focal magnetic brain stimulation, using a 70 mm figure-of-eight coil with a Magstim 200 stimulator (The Magstim Company, Dyfed, UK), of the left and then the right motor cortex was used to study the laterality of corticospinal projections. EMGs were recorded simultaneously from left and right homologous muscle pairs.

The initial site of stimulation that was used depended upon the muscle pair being investigated (Table 1) and corresponded to the site from which a maximum response could be obtained as indicated in a previous study (Carr *et al*., 1994). Stimulation was initially given whilst the subject produced a weak contraction of the muscle pair under study. The threshold for

Table 1 *Sites of focal magnetic brain stimulation*

Muscle	Anterior to Cz (%)*	Lateral to Cz (%)†
First dorsal interosseous	7	13
Forearm extensor	2	13
Triceps	0	10

Position measured from the vertex (Cz) as a percentage of interpolar distance *between the nasion and inion, and †between the external auditory meati.

a response was defined as that output of the stimulator required to produce five sequential responses in the contralateral muscle observed at a gain of 200 μV per division. The output of the stimulator was then increased by 10% of the maximal output and the subject instructed to relax his muscles. When possible,10 responses were then recorded without background EMG. Responses were rectified and displayed using the signal averaging software. The area of each response was measured and the average calculated. The ratio of the area of the ipsilateral response to the area of the contralateral response was obtained.

In Subjects K2, K7, K8 and K9 and in four age-matched control subjects, the area of motor cortex over which a response in 1DI could be obtained was mapped whilst recording simultaneously from left and right 1DI. Subjects relaxed their hands unless otherwise instructed. Stimulation commenced as described above; stimulation was then given at 1 cm intervals in both the anterior and posterior directions and in the medial and lateral directions. Ten stimuli were given at each point and the mean area of the motor evoked potential (MEP) obtained.

Investigation of the corpus callosal pathway

Two double coned coils were used (each with 70-mm diameter coil); one was used to condition the response evoked by discharging the second (test) coil. One coil was positioned over the 1DI area of the left motor cortex and the other over the 1DI area of the right motor cortex. For each coil, the threshold for a response in 1DI was determined as described above. Threshold +5% of maximum output was the stimulus strength used for the test stimulus, and threshold +10% of maximum output was used for the conditioning stimulus. A BiStim Module (The Magstim Company) was used to enable the conditioning stimulus to be presented at various intervals before the test stimulus. At each interval 20 conditioned and 20 non-conditioned stimuli were presented in a random sequence and responses recorded from left and right 1DI while the hands were relaxed. Responses were rectified and the areas of the conditioned and non-conditioned responses measured; *t* tests for non-paired data were used to determine whether there was a significant difference.

Cross-correlation analysis

Multi-unit surface EMGs were recorded from homologous left and right muscle pairs during weak sustained voluntary isometric co-contraction as follows: index finger abduction against resistance for 1DI, wrist and finger extension for the forearm extensor, extension of the arm for the triceps and abduction of the arm for the deltoid muscle.

Medium- and large-amplitude spikes were selected for analysis using a level detector (Neurolog NL200, Neurolog, Hemel Hempstead, UK). Cross-correlograms were constructed using ~5000 spikes from each train with a bin width of 1 ms and a pre- and post-trigger period of 100 ms (Spike2 software, Cambridge Electronic Design, UK). Spikes from the left muscle were arbitrarily selected to be the trigger spikes. The size of any central peak was estimated in terms of E/M where E is the total number of spikes in the whole width of the peak, in excess of those expected by chance and M is the mean count in a 1-ms bin. Since this index is sensitive to the firing rates of the contributing units, the mean firing rate in each train was determined using spectral analysis and the index adjusted to that expected for a firing rate of 10 Hz (Harrison *et al*., 1991).

Cutaneomuscular reflexes

Cutaneomuscular reflexes were recorded simultaneously from the left and right 1DI during sustained abduction of the left and right index fingers. The subject was instructed to keep the EMG at 10–20% of that achieved during a maximal voluntary contraction, he was aided in this using visual feedback from a root-mean-square voltmeter. The digital nerves of the left, and then the right, index finger were stimulated using a pulse width of 100 μs and a frequency of 3 Hz, at a strength twice that required for perception. Such a stimulus is not painful and usually gives a readily identifiable response after 2–3 min. EMG was ampified, rectified and averaged (time locked to the stimulus) for 500 sweeps using the signal-averaging software.

Phasic stretch reflexes

EMGs were recorded simultaneously from the left and right 1DI muscles. The left 1DI muscle was stretched by pulling the index finger towards the middle finger while the right 1DI was abducted. The left 1DI muscle was tapped at its insertion using a tendon hammer to elicit a stretch reflex. Single sweeps of EMG were displayed and then up to 30 sweeps averaged, time locked to the stimulus. This procedure was repeated whilst the right 1DI was tapped. In addition, stretch reflexes were also recorded from left and right forearm flexors in four of the XKS patients with mirror movements, following a tap to the appropriate tendons.

Sensory evoked potentials

Sensory evoked potentials were recorded simultaneously from left and right sensory cortices. The scalp was prepared

for recording by mild abrasion of the appropriate sites using Omni Skin Prep (Weaver and Co; from Medelec, Woking, Surrey, UK). Using Biotach EEG paste (from Medelec), Ag/AgCl disk electrodes (Medelec) were placed 20% lateral and 2 cm posterior to the vertex with the reference electrode at Fz. The subject was seated in a reclining chair with the neck and head well supported, and was instructed to close their eyes and relax. The right median nerve was stimulated at the wrist at 3 Hz using a constant-current stimulator (Medelec Sapphire) at a stimulus strength sufficient to cause a small twitch of the thumb. Cortical potentials were averaged, time locked to the stimulus, for 500 sweeps. The experiment was repeated whilst stimulating the left median nerve. Twelve healthy control subjects (seven females and five males, aged 22–48 years) were studied in the same way.

Results

Mirror movements

Mirror movements, in those XKS patients who exhibited such movements, were most pronounced when the distal muscles of the upper limb were used. They were not seen in the lower limb. The degree of mirroring showed considerable between-subject variation ranging from slight (Grade 1) to marked (Grade 3); *see* Table 4. Patients with mirroring of Grade 2 or 3 also exhibited mirroring during supination or pronation of the forearm.

EMG during phasic index finger abduction

Figure 1A shows surface EMGs recorded during self-paced right index finger abduction whilst recording simultaneously from the left and right 1DI of a normal control subject. Each abduction of the right index finger is accompanied by a burst of EMG in the right 1DI; there was no movement of the left index finger and no EMG in the left 1DI. In contrast, Fig. 1B shows surface EMGs recorded from an XKS patient with mirror movements during similar self-paced right index finger abduction. For this subject, involuntary abduction movements of the left index finger accompanied the voluntary phasic abductions of the right index finger, and bursts of EMG are apparent on the left mirroring side in addition to those on the voluntarily activated right side. A single burst of voluntary EMG recorded from the right 1DI together with the mirroring activity recorded from the left 1DI can be seen in Fig 1C. Figure 1D shows the average of 50 of these bursts, the average being constructed by rectifying the EMG and averaging time locked to the start of the voluntary burst.

All patients with XKS and mirror movements produced involuntary movements of the contralateral index finger, although not necessarily for each phasic abduction. Variation in the amount of involuntary EMG is related to the strength of the voluntary index abduction which showed considerable variation as can be seen in Fig. 1B. In five subjects, although involuntary mirror movements could be seen some of the time, at other times involuntary EMG was present without observable movement and, in some instances, there was no involuntary EMG recorded from the contralateral 1DI. The latter situation occurred in patients who exhibited the weakest mirroring, i.e. their mirror movements were Grade 1–2. Taking the group as a whole, during voluntary abduction of the right index finger, the time of onset of the averaged rectified burst of EMG from the left 1DI on the mirroring side ranged from 38 ms before, to 34 ms after, the onset of EMG activity in the right 1DI; on average the involuntary EMG of the mirroring side commenced 2.3 ms later (SEM 5.1 ms, $n = 13$). During voluntary abduction of the left index finger, the time of onset of EMG activity in the right 1DI on the mirroring side ranged from 33.0 ms before, to 21.0 ms after, the onset of EMG activity in the left 1DI; on average the involuntary EMG activity in the mirroring side commenced 6.8 ms later (SEM 4.3 ms). Measurement of the times of onset of individual bursts of right voluntary and left involuntary EMG for each subject revealed that there was no significant difference (*t* test, $P > 0.05$) in one subject, that the involuntary EMG preceded the voluntary EMG in four subjects ($P < 0.05$) and that, for the remaining eight subjects, the involuntary EMG commenced significantly later than the voluntary EMG ($P < 0.05$). When the voluntary activity occurred on the left side, there was no significant difference in the time of onset of the voluntary and involuntary EMG in three subjects ($P > 0.05$) and, for the remaining 10 subjects, the involuntary EMG commenced significantly later than the voluntary EMG ($P < 0.05$). Because of the wide variation in these times of onset, for both voluntary and involuntary EMGs, there was no significant difference between them when all the subjects were grouped together ($P > 0.05$).

As stated above, Fig. 1D shows the rectified and averaged EMG recorded during 50 phasic abductions using the right index finger. For this subject, the ratio of the area of the mirroring, involuntary EMG to the area of the voluntary burst of EMG is 0.7. For all the subjects, the ratio of the area of the mirroring, involuntary EMG burst to the area of the EMG burst of the voluntary side (averaged for 50 voluntary phasic index finger abductions) ranged from 0.006 to 3.3 ($n = 13$) when the right index finger was activated and from 0.01 to 1.2 ($n = 13$) when the left index finger was voluntarily abducted (Table 4).

EMG was never seen contralateral to the voluntarily activated side in the patient who had XKS but no mirror movements (K13).

Magnetic brain stimulation

Bilateral MEPs

Sequential recordings were made from the following homologous muscle pairs.

Left and right 1DI. For all the XKS subjects with mirror movements, stimulation of the appropriate area of either the left or right motor cortex evoked responses both contralaterally and ipsilaterally when background EMG was present. There

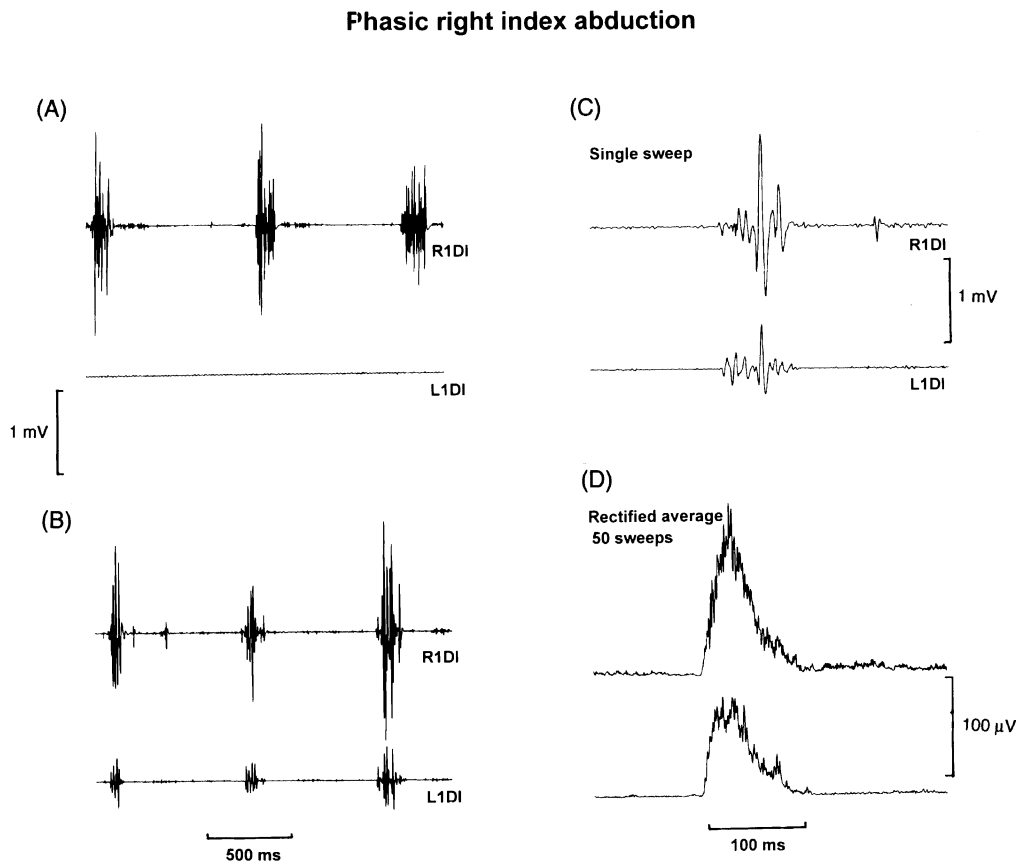

Fig. 1 Surface EMGs recorded simultaneously from the left and right first dorsal interossei muscles (L1DI and R1DI) during self-paced right index finger abduction. (**A**) Normal control subject: three bursts of EMG activity are present on the right side, each burst represents a single abduction of the right index finger, there is no EMG activity on the left side. (**B**) Patient K6 (with XKS and mirror movements): similarly, three bursts of voluntary EMG activity recorded from R1DI during right index abduction, but simultaneous involuntary bursts of EMG activity can also be seen in the L1DI. (**C**) Patient K6: EMG recorded from R1DI and L1DI during a single abduction of the right index finger. (**D**) Patient K6: the EMG has been rectified and 50 sweeps averaged, time locked to the beginning of the voluntary burst of EMG activity in R1DI.

was no significant difference in the latency of these contralateral and ipsilateral responses (paired *t* test $P > 0.05$) (Table 2). However, the relative sizes (averages of the areas of 10 responses) of the contralateral and ipsilateral responses showed considerable variation between subjects. In one out of 13 subjects, background EMG activity was required to enable contralateral responses to be seen when stimulating the left cortex; i.e. the threshold for a contralateral response was higher than that for an ipsilateral response. In contrast, in two subjects background EMG was required to see ipsilateral responses when stimulating the left cortex; in these subjects the threshold for a contralateral MEP was less than that for an ipsilateral MEP. In all other subjects, whether the left or the right cortex was stimulated, ipsilateral and contralateral MEPs had similar thresholds and were seen without preactivation of the 1DI (Fig. 2A and B). In seven out of 13 subjects, the ipsilateral response was larger than the contralateral response when the left motor cortex was stimulated. For this cortex, the ratio of the size of the ipsilateral to the contralateral response (13 subjects, average of 10 responses per subject) ranged from 34.0 to 0.04 (no preactivation except in the subjects mentioned above). With stimulation of the right cortex, in five out of 13 subjects the ipsilateral response was larger than the contralateral response; the ratio of the ipsilateral to the contralateral response ranged from to 23.7 to 0.05 (preactivation of contralateral side in one subject).

In normal subjects and in the XKS patient (K13) who did not have mirror movements, focal magnetic brain stimulation of either motor cortex evoked only a short latency contralateral response.

Left and right forearm extensors. In all XKS subjects with mirror movements, short latency bilateral responses were recorded from left and right forearm extensors when either the left or right cortex was stimulated; no pre-activation of the muscles was required. The ratio of the area of the ipsilateral to the contralateral response ranged from 16.7 to 0.02 when the left cortex was stimulated and from 2.0 to 0.3 when the right cortex was stimulated (Table 2).

Left and right triceps. Recordings from the left and right

Table 2 *Summary of data from focal magnetic brain stimulation of left and right motor cortices*

	Bilateral MEP	Ratio of MEP areas (ipsi-/contralateral range)	MEP latency					
			Contralateral			Ipsilateral		
			Range	Mean	SEM	Range	Mean	SEM
Left and right first dorsal interossei (n = 13 subjects)								
Stimulate left cortex	13/13	0.04–34.0	22.0–32.3	24.7	0.8	19.3–26.5	23.6*	0.6
Stimulate right cortex	13/13	0.05–23.7	21.0–26.8	22.9	0.5	20.7–25.0	22.9*	0.4
Left and right forearm extensors (n = 12 subjects)								
Stimulate left cortex	12/12	0.02–16.7	17.0–25.8	19.5	0.7	17.5–22.8	19.6*	0.5
Stimulate right cortex	12/12	0.03–2.0	16.6–20.8	18.7	0.4	17.0–22.5	19.7**	0.5
Left and right triceps (n = 7 subjects)								
Stimulate left cortex	3/7†	0.4–33.3	13.5–185‡	16.0	–	15.0–19.5	16.7*	–
Stimulate right cortex	4/7†	0.03–0.3	13.5–15.5‡	14.5	–	12.8–17.0	14.8*	–

For each subject and for each trial 10 stimuli were presented and the ratios of the areas of the rectified and averaged MEPs (ipsilateral/contralateral) calculated. There was no significant difference between the latencies of the contralateral and ipsilateral MEPs except when recording from left and right forearm extensors and stimulating the right motor cortex. *$P > 0.05$ and **$P < 0.05$: paired t test contralateral and ipsilateral MEP latencies. †Bilateral background EMG present in two subjects. ‡Only includes those with bilateral MEPs (motor evoked potentials).

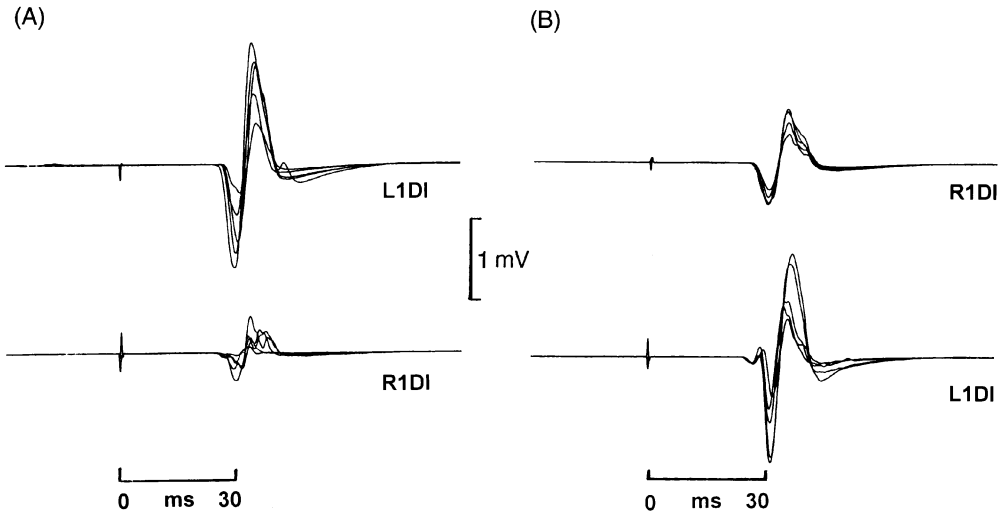

Fig. 2 Surface EMGs recorded simultaneously from the left and right first dorsal interossei muscles (L1DI and R1DI) during focal magnetic stimulation of hand area of the motor cortex of Patient K6, who had XKS and mirror movements: 5 superimposed responses. (**A**) Stimulation of the left motor cortex: for this patient, the ipsilateral response recorded from the L1DI is larger than the contralateral response recorded from the R1DI. (**B**) Stimulation of the right motor cortex of the same patient; the ipsilateral response recorded from R1DI is smaller than the contralateral response recorded from L1DI.

triceps were obtained from seven of the 13 subjects during stimulation of the motor cortex. Three of the patients with mirror movements had bilateral responses in triceps when the left cortex was stimulated and four had bilateral responses when the right cortex was stimulated. Pre-activation of the triceps bilaterally was required for two of these subjects. During stimulation of the left cortex, the ratio of the size of the ipsilateral response to the contralateral response ranged from 2.5 to 0.03 (n = 3) and during stimulation of the right cortex, this ratio ranged from 0.3 to 0.03 (n = 4) (Table 2).

Mapping of the motor cortex

In the normal subjects, unilateral stimulation of either motor cortex evoked contralateral responses only, in the left or right 1DI.

In contrast, in the four subjects with XKS who were studied (Patients K2, K7, K8 and K9), unilateral scalp stimulation evoked bilateral responses in relaxed left and right 1DI in three out of four of them, and bilateral responses in all of them with preactivation of left and right 1DI. The contralateral and ipsilateral MEPs were both maximal when

stimulating at the same point on the cortex, each then decreased in size as the coil was moved successively further away from this point, either in the lateral or medial direction or in the anterior or posterior direction. The latencies of these contralateral and ipsilateral responses were not significantly different (paired t test, $P > 0.05$). Figure 3 shows the amplitude of the MEPs recorded from relaxed left and right 1DIs, with points of stimulation lateral to the vertex, resulting from stimulation of the left and right motor cortex in Subjects K2, K8 and K9 without preactivation of the 1DIs. On the left hand side of the map the amplitudes of MEPs recorded contralateral and ipsilateral to the point of stimulation of the left cortex are shown and, on the right hand side, both contralateral and ipsilateral MEP amplitudes for stimulation of the right cortex. In one out of the four subjects with XKS (Patient K2) the site of stimulation at which the greatest amplitude MEP was recorded was not at the expected point of stimulation as described by Carr *et al*. (1994). In this subject, the ipsilateral response was always larger than the contralateral response, whichever site on the hand area of the cortex was stimulated. In all of the subjects with XKS it can be seen that the contralateral and ipsilateral responses, although of different amplitude, follow a similar pattern of modulation at points away from the site at which the greatest amplitude MEP was recorded. In the case of Patient K2, stimulation of the left motor cortex evoked only an ipsilateral response in the left 1DI, and preactivation was required in order to see the contralateral response recorded in right 1DI.

In both axes (i.e. medio–lateral and anterior–posterior) and for each cortex, there was no significant difference between the area from which responses could be elicited in the XKS patients and the normal controls (paired t test, $P > 0.05$), but the subjects with XKS and mirror movements demonstrated bilateral cortical hand representations.

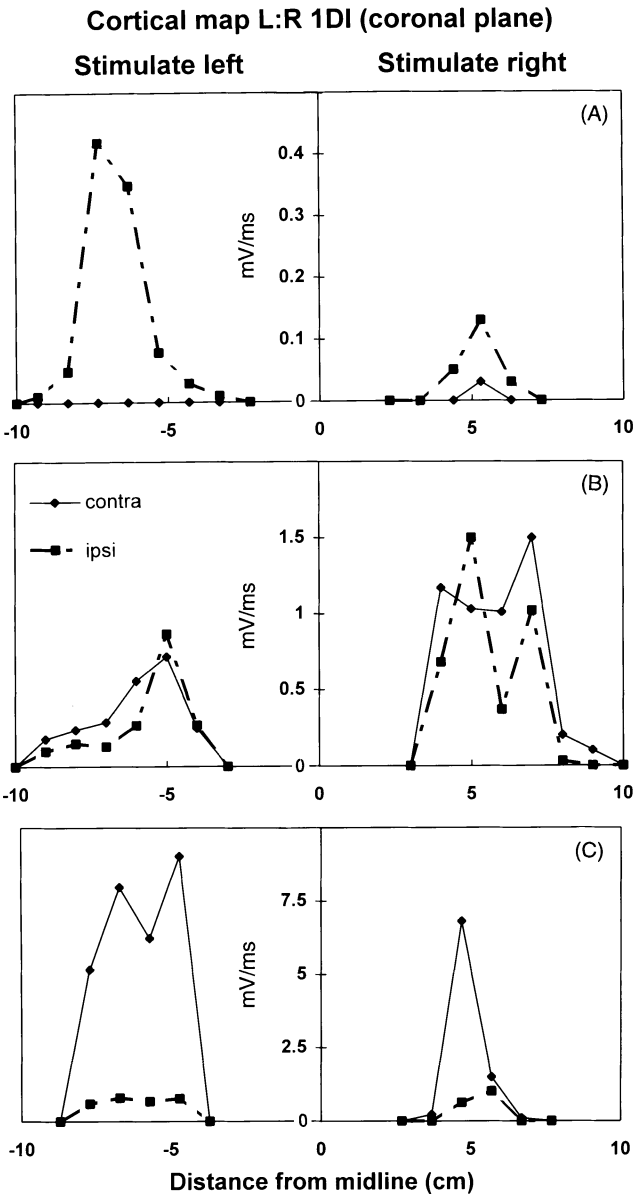

Fig. 3 Areas of contralateral and ipsilateral MEPs recorded from the left and right first dorsal interossei muscles (L1DI and R1DI) of Patients K2, K8 and K9. Stimulation given at 1 cm intervals medial and lateral to the site from which a maximal response could be obtained in relaxed muscle. In all cases the sizes of the contralateral and ipsilateral MEPs changed in a similar way as the position of the coil was moved. (**A**) Patient K2. Stimulation of the left motor cortex evoked ipsilateral responses only; stimulation of the right motor cortex evoked bilateral responses but the ipsilateral response was always larger. (**B**) Patient K8. Stimulation of either motor cortex evoked bilateral responses of similar size. (**C**) Patient K9. Stimulation of either motor cortex evoked bilateral responses but the contralateral response was always larger.

Interhemispherical conduction

To investigate callosal function, the response recorded in the right 1DI on stimulating the contralateral (left) motor cortex was conditioned by stimulating the ipsilateral (right) motor cortex in four normal right-handed control subjects and the response recorded in the left 1DI was conditioned by stimulating the left motor cortex in one normal left-handed control subject. The intervals tested between the conditioning and the test stimulus were 7 and 10 ms. For two out of five subjects at 7 ms and four out of five subjects at 10 ms, the conditioned response was significantly smaller than the non-conditioned response ($n = 5$, unpaired t test, $P < 0.05$).

This technique for investigating callosal function cannot be readily applied to the XKS patients with mirror movements, owing to the presence of a novel ipsilateral corticospinal projection. The existence of such a projection would result in the conditioning magnetic stimulus affecting the excitability of motor neurons contralateral to the test stimulus; this would have an effect on the size of the response to the test magnetic stimulus. Any decrease in the conditioned compared with the non-conditioned response could therefore be due the motor neurons being refractory following excitation by the conditioning stimulus. However, we were able to use this technique satisfactorily on one patient with XKS and mirror movements; this was Patient K10 whose corticospinal

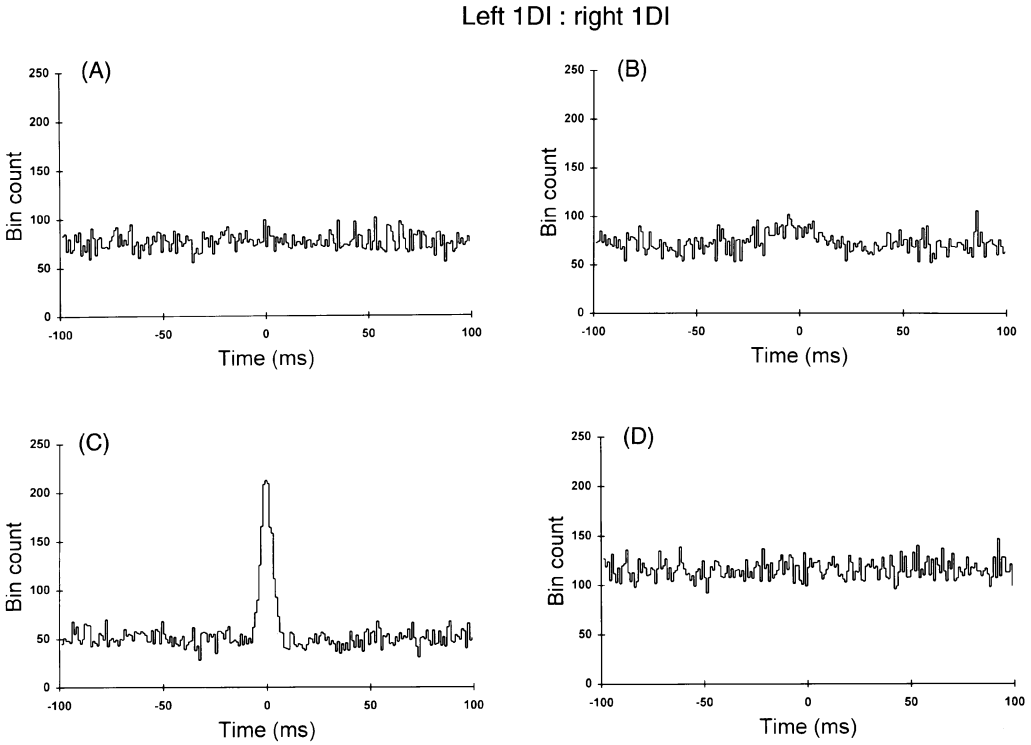

Fig. 4 Cross-correlograms constructed from multi-unit surface EMGs recorded during voluntary sustained left and right index finger abduction. Each correlogram was constructed from ~5000 trigger spikes from the left 1DI (left first dorsal interosseous muscle) and 5000 event spikes from the right 1DI, so that negative (and positive) time lags correspond to spikes in the right 1DI preceding (and following) spikes in the left 1DI, respectively. The bin width was 1 ms. (**A**) Patient K13 (with XKS but without mirror movements): the correlogram is flat. (**B**) Patient K1 (with XKS and mirror movements): there is a small short duration peak centred around time zero. (**C**) Patient K6 (with XKS and mirror movements): there is a large short duration central peak. (**D**) Patient K12 (with XKS and mirror movements): the correlogram is flat.

projection, as revealed using magnetic stimulation, is predominantly contralateral (Table 4). With both the 7- and 10-ms intervals between the conditioning and test stimuli, the conditioned response was significantly smaller than the non-conditioned response (unpaired t test, $P < 0.05$). For the normal control subjects, when a 7-ms time interval was used, the conditioned response varied from 20.2–95.5% of the non-conditioned response. At this time interval the conditioned response in Patient K10 was 6.0% of the non-conditioned. When a 10-ms delay was used, the conditioned response varied from 13.7–95.5% of the non-conditioned response in the control subjects and was 6.2% of the non-conditioned response in Patient K10.

Cross-correlation analysis

Cross-correlation analysis of multi-unit EMGs recorded from voluntarily co-activated left and right 1DI muscles was performed for the patient with XKS but no mirror movements and for the XKS patients with pathological mirror movements. The correlogram constructed from data recorded from the XKS patient without mirror movements was flat, i.e. there was no central peak (Fig. 4A). However, when the multi-unit EMG data obtained from voluntarily co-contracting left and right 1DI of the XKS patients with mirror movements was cross-correlated, a short duration central peak was seen for all but one of the patients. An example of a correlogram constructed from data obtained from this latter patient can be seen in Fig. 4D. The size of the central peak seen in the correlograms of the other patients exhibited considerable variation; two examples are given in (Fig. 4B and C). When the size of this central peak was estimated using the index E/M (where E = number of spikes in the peak in excess of those expected by chance, M = mean count in a 1-ms bin) it ranged from 3.1 to 17.9 (Tables 3 and 4). The duration ranged from 12.0 to 28.0 ms (mean 16.5 ms, SEM 1.3 ms, $n = 11$).

With recordings from voluntarily co-contracting left and right forearm extensors, central peaks were present in the cross-correlograms of 10 of the 12 subjects with mirror movements including the subject mentioned above who did not have a central peak in the correlogram constructed from data recorded from left and right 1DI. The duration of the central peak of left and right forearm extensor correlograms ranged from 10.0 to 19.0 ms, and the size, given as E/M, from 1.2 to 6.7. For the left and right triceps and left and

Table 3 *Summary of data from cross-correlation analyses obtained during voluntary sustained co-activation of left and right muscle-pairs*

	Subjects (*n*)	Peak present	Peak size (*E*/*M*)			Peak width		
			Range	Mean	SEM	Range	Mean	SEM
Left and right 1DI	13	12/13	3.1–17.9	5.6	1.4	12.0–28.0	16.8	1.4
Left and right forearm extensors	12	10/12	1.2–6.7	3.2	0.7	10.0–19.0	14.4	0.8
Left and right triceps	9	5/9	1.5–5.8	3.1	0.8	12.0–15.0	13.6	0.8
Left and right deltoid	9	2/9	2.2–2.4	2.3	–	13.0–13.0	13.0	–

The size of the cross-correlogram peak is expressed as E/M where E = the total number of spikes in excess of those expected by chance for the duration of the central peak and M = mean count in a 1-ms bin in an area away from the peak.

Table 4 *Summary from all XKS subjects of results from left and right 1DI EMGs*

Subject	Grade of mirror movements		Involuntary/ voluntary EMG		Ipsilateral/contralateral MEP after magnetic stimulation of		Cross-correlation peak		Cutaneomuscular reflexes	
	R→L	L→R	R active	L active	left cortex	right cortex	Size (*E/M*)	Width (ms)	Ipsilateral	Contralateral
K1	2	2	0.1*	0.1*	34.0	23.7†	3.9	28	E1	I1 E2
K2	1	2	0.5	0.01*	10.0†	3.6	3.3	25	E1	I1 E2
K3	2	2	0.1	0.2	8.2	2.5	4.6	15	E1	I1 E2
K4	2	3	0.1	0.6	3.8	1.2	3.2	17	E1 I1 E2	I1 E2
K4a	2	2	0.8	0.6	1.5	4.0	7.9	16	E1 I1 E2	I1 E2
K5	2	3	3.3	0.6	3.1	0.3	7	15	E1 I1 E2	I1 E2
K6	2	3	0.7	1.1	2.5	0.6	17.9	14	E1 I1 E2	I1 E2
K7	2	3	1.0	1.2	1.3	1.0	7.7	14	E1 I1 E2	I1 E2
K8	2	2	1.0	0.1	0.7	0.8	6.4	12	No reflex recorded	
K9	1	1	0.2	0.3	0.1	0.3	3.1	14	E1 I1 E2	I1 E2
K10	1	1	0.004*	0.2	0.01‡	0.2	6.1	13	E1 I1 E2	No response
K11	1	2	0.2	0.5	0.1	0.01	3.1	15	E1 I1 E2	I1 E2
K12	2	2	0.006*	0.04*	0.04‡	0.05	0.0		E1 I1 E2	§
K13	0	0	0.0	0.0	0.0	0.0	0.0		E1 I1 E2	No response

Mirror movements grades: 1 = slight; 2 = moderate; 3 = marked. R→L (L→R) = when using the left (right) hand. Involuntary/voluntary EMG: mean EMG recorded during 50 voluntary self-paced phasic index finger abduction movements, as a ratio of EMG areas during voluntary movement the right (R active) or left (L active) index finger. The cross-correlation was obtained from multi-unit EMGs during voluntary simultaneous abduction of left and right index fingers; for *E*/*M see* footnote to Table 3. Cutaneomuscular reflexes were recorded during stimulation of the digital nerves of the left or right index finger (same configuration of responses obtained). *Some frames without contralateral EMG. †With background EMG contralateral to the stimulated cortex. ‡With background EMG ipsilateral to stimulated cortex. §On stimulating the left digital nerves, a contralateral modulation of ongoing EMG activity was seen at the E1 latency, and following a tap to the left 1DI tendon, a contralateral modulation of ongoing EMG activity was seen at 39 ms (ipsilateral reflex latency was 33 ms).

right deltoid muscles, recordings were only obtained from nine subjects with mirror movements. Central peaks were found in five out of nine of these recordings for left and right triceps and in two out of nine for left and right deltoid muscles. For left and right triceps the duration of the central peak ranged from 12.0 to 15.0 ms and the size from 1.5 to 5.8 and for left and right deltoid the duration of the peak of the two correlograms was 13 ms while the size ranged from 2.2 to 2.4 (Table 3). Correlograms constructed from the muscle pairs in Patient K13 (with XKS but without mirror movements) were all flat.

The size of the central peak showed a distal to proximal gradient, being larger for distal muscle pairs (Table 3).

Reflex studies

Cutaneomuscular reflexes

A typical cutaneomuscular reflex recorded from the right 1DI can be seen in Fig. 5A; following stimulation of the digital nerves of a XKS patient with mirror movements, there was a triphasic modulation of the EMG ipsilateral to the stimulus. This modulation comprises a short latency increase in EMG (E1 component) followed by a decrease in EMG (I1 component) followed by a second larger increase in EMG (E2 component). However, in 10 out of 13 of the patients, reflex modulation of ongoing EMG was also seen contralateral to the side of stimulation, regardless of which side was being stimulated (Fig. 5B and Table 4). In three patients, only an

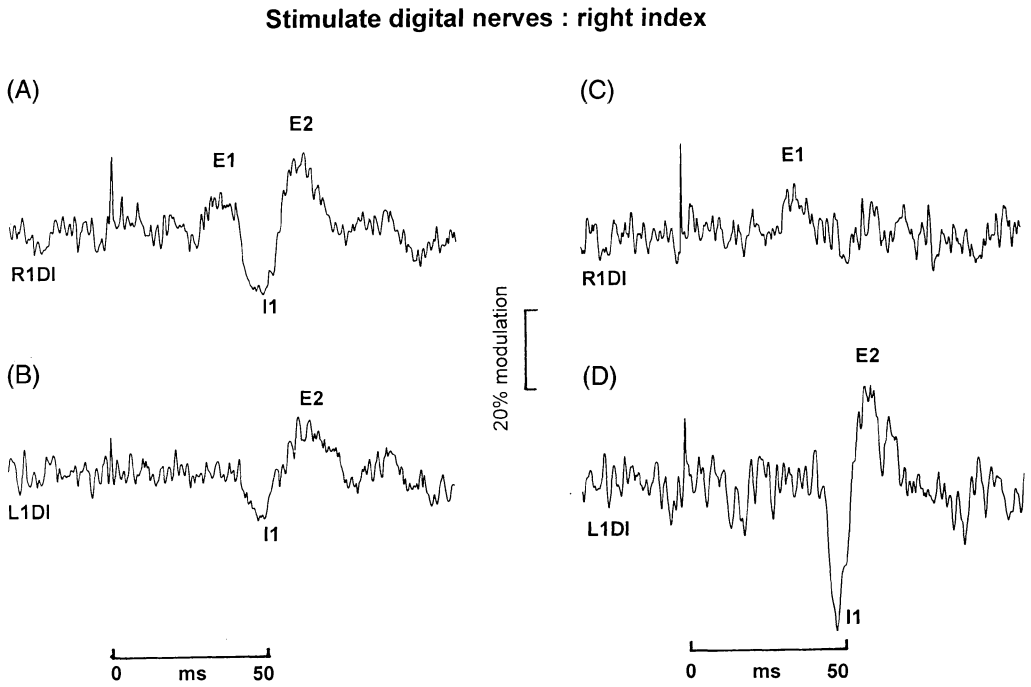

Fig. 5 Cutaneomuscular reflexes recorded from left and right first dorsal interossei muscles (L1DI and R1DI) following stimulation of the digital nerves of the right index finger at 3 Hz, at a stimulus strength twice threshold for perception during simulataneous sustained isometric voluntary abduction of left and right index fingers. Surface EMG activity was rectified and averaged for 500 sweeps, time locked to the time of stimulation. (**A**) and (**B**) show cutaneomuscular reflexes recorded from Patient K5, who had XKS and mirror movements. The response recorded from the R1DI (**A**) ipsilateral to the stimulus comprises E1, I1 and E2 components; a similar reflex configuration to that recorded from normal control subjects. In contrast to normal control subjects, a reflex response is also seen contralateral to the stimulated side (**B**). This contralateral reflex comprises I1 and E2 components; these components are believed to be of supraspinal origin. (**C**) and (**D**) show cutaneomuscular reflexes recorded from Patient K2 who had XKS and mirror movements. The response recorded from R1DI (**C**) ipsilateral to the stimulus only comprises an E1 component; this component is thought to be of spinal origin. The reflex response recorded from L1DI (**D**) contralateral to the stimulus comprises I1 and E2 components.

E1 component was recorded ipsilateral to the stimulus (Fig. 5C), whereas both the I1 and E2 components were recorded contralateral to the stimulus (Fig. 5D and Table 4).

No reflex was seen contralateral to the stimulus in records from the XKS patient with no mirror movements.

Phasic stretch reflexes

A short latency response could be seen in a single sweep of EMG from 1DI following a tap with a tendon hammer to the 1DI muscle; the average of 10 such reflex responses can be seen in Fig. 6A. There is no response contralateral to the side of stimulation, only ongoing voluntary EMG. A similar result was obtained for all but two of the subjects when a modulation of EMG contralateral to the stimulus was also seen at latencies which were 10.0 and 30.0 ms later than the ipsilateral spinal reflex responses. In four subjects, the tendons of the forearm flexors were also stretched. Short latency reflex responses were recorded ipsilateral to the tap in all these subjects but in one subject a reflex response was also seen contralateral to the stimulated side; the latency of the contralateral response was 19.3 ms longer than that of the ipsilateral response (Fig. 6B).

Sensory evoked potentials

Recordings of sensory evoked potentials following median nerve stimulation have been obtained from nine of the XKS patients with mirror movements and from 13 normal subjects. The size of the N20–P25 component was measured from the average of 500 sweeps. In records from normal subjects, the amplitude of the response ipsilateral to the stimulus ranged from 15.1% to 49.6% (mean 31.6%, SEM 3.4%, $n = 13$) of the contralateral response when the right median nerve was stimulated, and from 11.23% to 69.9% (mean 33.2%, SEM 5.0%, $n = 13$) of the contralateral response when the left median nerve was stimulated. Similar results were obtained from the patients with XKS and mirror movements. When the right median nerve was stimulated, the response recorded over the right sensory cortex ranged from 16.2% to 55.1% (mean 36.5%, SEM 5.3%, $n = 9$) of the response recorded over the left sensory cortex. When the left median nerve was

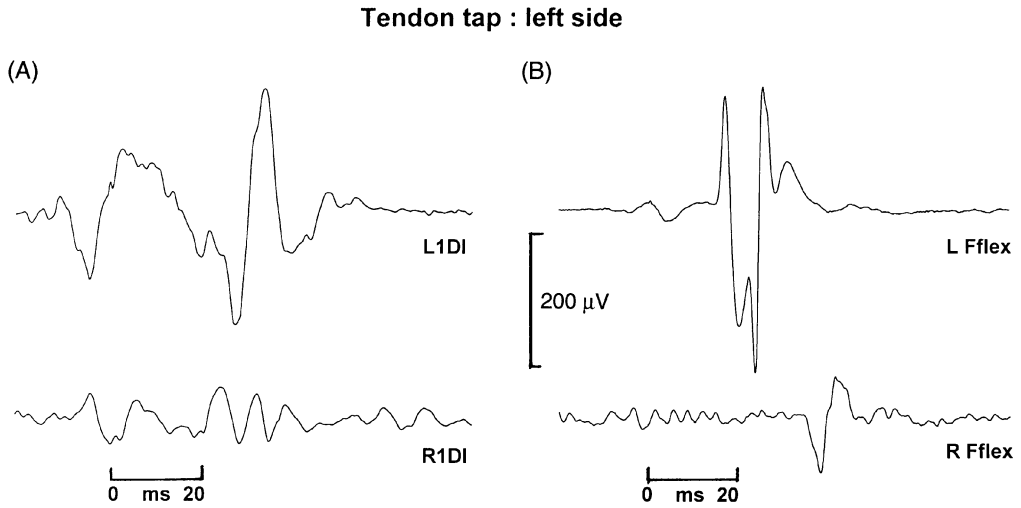

Fig. 6 Stretch reflexes recorded from patients with XKS and mirror movements. (**A**) Patient K7: surface EMGs recorded simultaneously from left and right first dorsal interossei muscles (L1DI and R1DI) following a tap to the L1DI at its insertion. EMGs averaged, time locked to the stimulus, 10 sweeps. A short latency reflex response is seen in L1DI; there is no reflex in R1DI contalateral to the stimulated side, only ongoing background EMG activity is present. (**B**) Patient K6: surface EMGs recorded simultaneously from left and right forearm flexors (L Fflex and R flex) following a tap to left tendon at wrist. EMGs averaged, time locked to the stimulus, 10 sweeps. A short latency (15.0 ms) response is seen in L Fflex; there is also a small reflex response of longer latency (34.3 ms) in R Fflex contralateral to the stimulus.

stimulated, the response recorded over the left sensory cortex ranged from 20.3% to 50.8% (mean 32.2%, SEM 4.4%, $n = 13$) of the response recorded over the right sensory cortex. There was no significant difference between these results from the XKS patients and those recorded from normal control subjects (unpaired t test, $P > 0.05$). In both normal subjects and those with XKS, the ratio of the ipsilateral response to the contralateral response was independent of whether the right or left nerve was being stimulated (paired t tests, $P > 0.05$).

Discussion

This study has used neurophysiological techniques to investigate mechanisms underlying mirror movements seen in patients with XKS. In all these patients, voluntary movements of the fingers of one side are accompanied by homologous involuntary mirroring movements of the contralateral fingers; however, the amplitude of the mirror movements varies between subjects. Taking the group as a whole, surface EMG recordings from left and right 1DI muscles during voluntary phasic unilateral index finger abduction have revealed that there is no significant difference in the time at which EMG activity commences on the voluntary and involuntary or mirroring side. The ability to perform independent finger movements, such as index finger abduction, is thought to be dependent upon the presence of monosynaptic cortico-motoneuronal connections which are associated with fast conducting corticospinal axons (Lawrence and Hopkins, 1976). This leads us to suppose that, in these mirroring patients, the simultaneous commands to the voluntarily moved and involuntarily moved index finger both travel via a fast conducting pathway.

Origin of involuntary EMG

Until recently, callosal fibres were not thought to be present in the hand area of the motor cortex but Rouiller *et al*. (1994), described the existence of a modest projection from the hand area in the macaque monkey. If some of the fibres of this pathway are excitatory then it might be argued that the motor command could spread via the corpus callosum. However, it is unlikely that the involuntary EMG response results from the spread of activity from the contralateral motor cortex to the ipsilateral motor cortex via such fibres, since this would be expected to take about 8–9 ms (Cracco *et al*., 1989) and the onset of the involuntary EMG should therefore be similarly delayed. Moreover, available evidence suggests that the callosal pathway between the two motor cortices is inhibitory since unilateral activation of one motor cortex results in a decrease in excitability of the contralateral motor cortex (Ferbert *et al*., 1992; Meyer *et al*., 1995*b*).

The involuntary EMG could result from activity in the normally occurring ipsilateral corticospinal tract. But from their experiments using split-brain monkeys, Brinkman and Kuypers (1973) concluded that ipsilateral pathways can control movements of the ipsilateral arm while distal muscles of the hand are under the control of the contralateral cortex. However, Colebatch and Gandevia (1989) found that adult patients with an acquired hemiplegia sometimes show a weakness on the ‘unaffected’ side; this weakness was most apparent during shoulder adduction and wrist extension but

some weakness was also apparent in the distal muscles of the hand. In addition, there are a number of reports based on imaging techniques that describe activation of the ipsilateral cortex during a unilateral task involving distal muscles (Kawashima *et al*., 1993; Kim *et al*., 1993) and recently Wassermann *et al*. (1994) reported that they were able to record ipsilateral EMG responses from distal muscles following magnetic stimulation. However, these ipsilateral responses were considerably smaller and of a longer latency than the contralateral responses. Finally, recordings from motor cortical cells in the awake monkey have revealed that there are neurons that are active during bilateral hand movement and some that are only active during an ipsilateral hand movement (Aizawa *et al*., 1990). Also, anatomical tracing experiments using the macaque monkey have revealed ipsilateral projections from the motor cortex which have a similar pattern of projection to contralateral projections but constitute only 8.1% of the corticospinal projection (Galea and Darian-Smith, 1994).

Thus, although there is an ipsilateral corticospinal projection in primates, available evidence points to it being a sparse, slowly conducting projection. Therefore, it is unlikely that the involuntary EMG recorded from the XKS patients originated from this normally occurring ipsilateral corticospinal tract.

The present study indicates the presence, in patients with XKS and mirror movements, of an abnormally developed or novel ipsilateral corticospinal tract which comprises fast conducting axons. Evidence for such a projection arises from three different experiments. These will now be discussed separately.

Focal magnetic brain stimulation

It is generally agreed that magnetic brain stimulation of the motor cortex excites fast conducting corticospinal neurons (Hess *et al*., 1987); this can occur both indirectly and directly (Edgley *et al*., 1990; Werhahn *et al*., 1994). Recently, Baker *et al*. (1994) succeeded in recording the pyramidal volley in the conscious macaque monkey following transcranial magnetic stimulation. Unilateral cortical stimulation of all patients with XKS and mirror movements evoked bilateral EMG responses in distal muscles. A similar result has been described by Danek *et al*. (1992) for patients with XKS and mirror movements. Patients with Klippel–Feil syndrome and marked mirror movements (Farmer *et al*., 1990) and patients with congenital hemiplegia who show marked mirroring also have bilateral responses (Carr *et al*., 1993). Finally, subjects with familial or idiopathic mirror movements have bilateral EMG responses (Cohen *et al*., 1991; Harrison *et al*., 1993). In our study of XKS patients with mirror movements, the ipsilateral response occurred at the same latency as the contralateral response, indicating that the conduction velocity of the ipsilaterally projecting axons is similar to that of the contralaterally projecting axons. The ratio of the size of the ipsilateral response to the contralateral reponse showed considerable variation both between subjects and within subjects when considering the two cortices separately (Tables 2 and 4). Table 4 has been organized according to the size of this ratio; the subject at the top has the largest ipsilateral projection while the subject at the bottom has the largest contralateral projection. In eight of the 13 subjects with mirror movements, the ipsilateral response was larger than the contralateral response when the left motor cortex was stimulated; the ipsilateral response was larger when the right motor cortex was stimulated in only five subjects. In those subjects in whom the motor cortex was mapped (K2, K7, K8 and K9), contralateral and ipsilateral MEPs were recorded simultaneously. Each MEP (contralateral and ipsilateral) decreased in size progressively as the coil was moved further away from the point where stimulation gave rise to the maximal responses. Thus, ipsilaterally projecting and contralaterally projecting axons are intermingled in all areas from which a response can be obtained using focal magnetic stimulation.

An ipsilateral projection could stem from defective axon guidance resulting in a lack of decussation of corticospinal fibres at the level of the pyramids. It is known that XKS results primarily from the failure of axons of the olfactory, vomeronasal and terminal nerves to project through the meninges overlying the cribriform plate and thus failure to synapse with second order neurons in the developing forebrain. Neurons synthesizing gonadotrophin releasing hormone originate in the olfactory epithelium but migrate along fascicles of these nerves to enter the forebrain eventually reaching the hypothalamus by gestational week 19 in humans (Schwanzel-Fukuda *et al*., 1989*a*). This migration is arrested in XKS (Schwanzel-Fukuda *et al*., 1989*b*). The gene which is affected in XKS, known as *KAL*, codes for a protein product whose greatest homology is with cell adhesion molecules (e.g. NCAM or nerve cell adhesion molecule). Such a protein would be well placed for a role in axon guidance and synaptogenesis (Franco *et al*., 1991; Legouis *et al*., 1991). It is conceivable that the same factor could affect the outgrowth of corticospinal axons as well as olfactory axons since the pyramidal decussation is first seen at postovulatory day 57 and the lateral olfactory tract is first seen at postovulatory day 52 in the human foetus (O'Rahilly and Muller, 1994). The variation seen with respect to the degree of (i.e. lack of) decussation (as inferred from the magnetic stimulation data) presumably results from inter-subject variability regarding the exact time of migration and/or variation in the protein gene product. A late decussation could result in the Kallmann protein being less important, since other genetic factors may promote decussation.

An ipsilateral corticospinal projection could also result from branching of some or all of the contralaterally projecting fibres and this was the conclusion of Carr *et al*. (1993) for children with hemiplegia and marked mirror movements. These authors argued that unilateral damage to the corticospinal tract had occurred during development and this led to branching of surviving corticospinal axons originating

from the cortex contralateral to the lesion; such branched axons then innervated homologous left and right motor neuron pools. This interpretation is supported by experiments using the hamster, in which branching of corticospinal axons was seen in the spinal cord following an early unilateral lesion (Kuang and Kalil, 1990). But in the present study, there appears to be no prima facie case for the occurrence of developmental damage. The recent work of Halloran and Kalil (1994) suggests it is unlikely that branching would occur at the level of the pyramidal decussation since these authors found that bifurcation of growth cones of growing axons, in their case callosal axons, was rare. Branching of axons is prompted by interaction with the target and, most recently, Dent *et al*. (1995) have demonstrated that membrane bound cues from spinal targets elicit branching. Furthermore, even if branching were present in the XKS patients of the present study, in some of these patients the ipsilateral projection, as revealed using magnetic stimulation, is greater than the contralateral projection. In these patients at least the ipsilateral projection must, in part, result from non-decussating, non-branching axons.

It therefore seems most likely that the novel ipsilateral projection seen in our XKS patients with mirror movements resulted from a lack of decussation of the corticospinal tract at the level of the pyramid.

Cross-correlation analysis

A short duration central peak was seen in all but one cross-correlogram constructed from multi-unit EMG recordings obtained from voluntarily co-contracting left and right 1DI muscles of XKS subjects with mirror movements. In some of these XKS subjects, the size of this peak was larger than that seen in correlograms for normal subjects constructed from recordings obtained from one limb of synergistic, co-contracting muscle pairs that share a common joint (Gibbs *et al*., 1995). Short-duration central peaks were also present in all but two cross-correlograms of records from voluntarily co-contracting left and right forearm extensors of the XKS patients with mirror movements, also in five out of nine subjects with records from left and right triceps and in two out of nine subjects with records from left and right deltoid. All correlograms were constructed using ~5000 spikes from each multi-unit EMG recording. It is possible that, if more spikes had contributed to the analysis, short duration central peaks might have become apparent in those flat correlograms mentioned above. No short duration central peak was seen when constructing cross-correlograms from data recorded from voluntarily co-contracted left and right homologous muscle pairs in the XKS patient without mirror movements, in normal adult subjects (Carr *et al*., 1994), or in normal children with marked mirror movements (Mayston *et al*., 1995*b*).

The presence of a short duration central peak in a cross-correlogram results from the near simultaneous arrival of excitatory postsynaptic potentials at the two motor neuron pools, indicating that there is a common drive to the motor neuron pools of the co-contracting muscles. A common drive may result from activity in branched last order neurons presynaptic to the motor neurons (Sears and Stagg, 1976, Kirkwood and Sears, 1978) and/or from presynaptic synchronization (Kirkwood *et al*., 1982; Kirkwood and Sears, 1991). Kirkwood and Sears (1991) emphasized that only the narrowest of peaks (half-width $\leqslant$2.1 ms) could confidently be interpreted as resulting from activity in last order branched fibres. The half-widths of the central peaks seen in the present study were considerably greater than this, thus indicating the presence of presynaptic synchronization. But our correlograms were constructed from multi-unit data which would lead to an increase in the width of the central peak (*see* Farmer *et al*., 1991 for discussion of central peak width). It therefore remains a possibility, that in the present study, activity in branched last order fibres did contribute to the cross-correlogram peaks.

The presence of a peak in the cross-correlogram constructed from motor unit firing either within a muscle or between synergistic muscles seems to be dependent upon the integrity of the corticospinal tract since synchrony is decreased following a stroke (Farmer *et al*., 1993) and is absent below the level of the lesion of paraplegic patients (Davey *et al*., 1990). Thus, in the present study, presynaptic synchronization of the firing of neurons within the motor cortex could underly the synchronization of motor neuron firing of homologous left and right muscles. In this group of XKS patients, neurons projecting contralaterally are close to neurons that project ipsilaterally, since the present study has shown that both contralateral and ipsilateral MEPs can be evoked from the same point of cortical stimulation. There is evidence for synchronization of cortical cells (Allum *et al*., 1982; Smith and Fetz, 1989; Fetz *et al*., 1991), although Smith and Fetz (1989), when studying post-spike facilitation, felt that any effect of cortical synchronization would contribute mainly to the tails of any peak and that a sharp rising central peak was indicative of a monosynaptic connection. Nevertheless, Baker and Lemon (1995), using a computer simulation, recently concluded that the contribution made by cortical synchronization to the post spike facilitation makes it difficult to estimate the strength of the monosynaptic connection between the corticospinal neuron and the motor neuron. In addition, intracellular recordings from pre- and postsynaptic pyramidal cells of a slice of rat somatomotor cortex have demonstrated the presence of excitatory connections; the most powerful connections appearing to be between cells in the same column or in neighbouring columns (Thomson and Deuchars, 1994). Perhaps in XKS patients with mirror movements the synchronization between adjacent motor cortical cells is greater than that normally found and therefore is sufficient to produce easily recognizable features in the cross-correlogram. Also, it is known that there are inhibitory interneurons present in the motor cortex and that these can have a strong effect on pyramidal neurons (Thomson and Deuchars, 1994); there may be a reduced level of inhibition

in XKS leading to a stronger synchronization of neighbouring corticospinal neurons. This could explain why the degree of synchronization seen in some of the XKS patients is greater than that seen in normal subjects when recording from two anatomically close synergistic muscles of one limb (Gibbs *et al*., 1995).

The above discussion suggests that the origin of the common drive to the left and right homologous motor neuron pools is likely to involve synchronization of contralaterally and ipsilaterally projecting pyramidal neurons within one motor cortex.

Reflex studies

Stretch reflexes

Phasic stretch reflexes were elicited to determine whether there had been any reorganization of the spinal monosynatic reflex pathway.

Stretching the 1DI or forearm flexor muscles produced a short latency, presumably monosynaptic, reflex response in the stretched muscle of XKS patients with mirror movements. In all but two subjects, there was no modulation of ongoing EMG of the contralateral homologous muscle. This indicates that, at least for this reflex pathway, the spinal circuitry is normal for most of these patients. For the two subjects with contralateral reflex responses, the latencies of these contralateral responses were longer than those of the ipsilateral responses but still compatible with a spinal origin. For one of these subjects, a contralateral cutaneomuscular reflex response was also seen at the E1 latency (Table 4, Subject K12). It would therefore appear that, in these two subjects at least, there had been some reorganization of spinal neuronal circuitry. The *KAL* gene is known to be expressed in the human spinal cord at day 45 following fertilization (Duke *et al*., 1995). At this time, corticospinal axons have not yet entered the cord; the corticospinal tract does not complete its caudal extension into the lumbar cord until week 29 (Humphrey, 1960). It is not known at what other times, if any, *KAL* may be expressed. Thus if the *KAL* gene or its protein product has any role in the guidance of corticospinal terminals, we might surmize that expression would be seen somewhat before and around this time.

Cutaneomuscular reflexes

Stimulation of the digital nerves modulates the ongoing EMG; an initial increase in EMG activity of spinal latency (E1 component) is followed by a decrease in the EMG (I1 component) which is followed by a further increase in EMG activity, of transcortical origin (E2 component). Recording cutaneomuscular reflexs therefore provides a mechanism for examining any reorganization of both spinal and supraspinal pathways.

In the present study, stimulation of the digital nerves of the index finger resulted in a readily identifiable reflex modulation of ongoing activity of the ipsilateral 1DI in 11 of the 13 XKS subjects with mirror movements. The lack of observable reflex responses in one subject probably indicates that the reflex pathway is less excitable in this individual.

In 10 of those subjects with an ipsilateral reflex response, reflex responses were also recorded contralaterally. Such responses have also been described for children with hemiplegia and marked mirroring (Carr *et al*., 1993). However, not all three components of the reflex were seen contralateral to the side of stimulation. Only once have we recorded a component contralateral to the stimulus at a latency suggestive of an E1 component (*see* above). This component, on the basis of its latency when recorded ipsilateral to the stimulus, is believed to result from spinal processing whereas the E2 component is dependent on the integrity of the dorsal columns, sensorimotor cortex and corticospinal tract (Jenner and Stephens, 1982). The crossing of the E2 component in XKS patients with mirror movements could result from activity in a novel ipsilateral projection (*see* earlier discussion). Given that magnetic brain stimulation revealed that neurons projecting ipsilaterally and those projecting contralaterally are intermingled; both sets could be simultaneously excited by the cutaneous input.

In some of the patients with cerebral palsy (Carr *et al*., 1993) and in all the XKS patients in whom crossed reflexes were seen in the present study, the I1 component was also seen contralateral to the stimulus. This component was believed to be of spinal origin (Jenner and Stephens, 1982) but as argued in Carr *et al*. (1993), the latency of the I1 component is sufficient for the reflex to be transcortical. It is difficult to explain how this component could be seen bilaterally in the XKS patients if it results from spinal processing, since, in three of the XKS patients (Patients K1–3, Table 4), the I1 and E2 components were only recorded contralateral to the stimulus, with just an E1 component being seen ipsilateral to the stimulus. If the I1 component does originate from spinal processing, then we might have expected to have seen both the E1 and I1 components ipsilateral to the stimulus. That the I1 component could only be recorded contralateral to the stimulated side argues in favour of a supraspinal, possibly cortical, origin. For these patients, the ipsilateral corticospinal projection from each motor cortex, as revealed using focal magnetic stimulation, was larger than the contralateral projection (Table 4). Thus, the cutaneous stimulus could have excited this ipsilateral projection resulting in contralateral reflex components. Any excitation of the small contralaterally projecting component was presumably insufficient to produce a visible modulation of ongoing EMG of the index finger being stimulated.

Theoretically, reflex EMG responses contralateral to the stimulated side could also result from an abnormal afferent pathway, i.e. if the ascending sensory volley projected to the ipsilateral sensory cortex instead of, or as well as, to the contralateral sensory cortex. To determine whether such an ipsilateral projection was present, we recorded sensory evoked potentials following median nerve stimulation. Potentials

were recorded from the ipsilateral in addition to the contralateral sensory cortex from both control and XKS subjects; the sizes of these ipsilateral responses showed a similar variability in both groups and were not significantly different beween the two groups. An ipsilateral response can be recorded due to volume conduction from the activated contralateral sensory cortex (Kakigi, 1986). Thus we could find no evidence to suggest the existence of any abnormally projecting ipsilateral afferents which could acount for the presence of the contralaterally reorded reflex components in our XKS patients.

Origin of the mirror movements

There are reports in the literature describing the presence of mirror movements in subjects with callosal agenesis (Schott and Wyke, 1981; Rothwell *et al*., 1991; Meyer *et al*., 1995*a*). But this is not a universal finding; recently Meyer *et al*. (1995*b*) described some patients with varying degrees of callosal agenesis who did not have mirror movements and the present authors have examined two subjects with complete callosal agenesis, revealed using MRI, who did not exhibit mirror movements. Nevertheless, at least in some instances, mirroring may be associated with callosal agenesis; thus it is possible that if the mutation of the *KAL* gene had affected the outgrowth of callosal axons in our XKS patients, this could have led to the presence of mirror movements by removing an inhibitory pathway. But 10 out 13 of these patients have undergone an MRI scan, and in all of these the corpus callosum is present. Given that the corpus callosum is present, it is possible that it is not functioning normally. Evidence suggests that in normal subjects, activity in the callosal pathway from one motor cortex inhibits the opposite motor cortex (Ferbert *et al*., 1992; Meyer *et al*., 1995*a*). Using the condition–test technique of Ferbert *et al*. (1992) inhibition of the test response recorded from 1DI, by a conditioning stimulus to the cortex ipsilateral to the 1DI, was seen in the present study when investigating the one XKS subject with mirror movements who could be studied using this technique (for explanation, *see* Interhemispheric conduction in the Results section). Thus in one XKS patient we have some evidence that the transcallosal pathway is functioning normally.

Danek *et al*. (1992) and Shibasaki and Nagae (1984) suggested that the mirror movements seen in patients with XKS result from bilateral activation of left and right motor cortices. The presence of short-duration central peaks in cross-correlograms constructed from the firing of motor units in homologous left and right motor neuron pools appears to be associated with the presence of mirror movements. Thus, if these movements do result from bilateral cortical activation, we would have to predict the presence of synchronization of left and right corticospinal neurons. Synchronization of neurons that are at a considerable distance from each other has been described but the time lag between cells is much greater (Amzica and Steriade, 1995) than that seen in the present study, where the mean width of the central peak is 16.5 ms, range 13.0–28.0 ms for left and right 1DI. This suggests that it is unlikely that the peaks in the cross-correlograms of the mirroring XKS patients result from synchronization of neurons situated in left and right motor cortices. Therefore, although the present study cannot rule out bilateral activation of the left and right motor cortices, bilaterally projecting synchronized activity originating from a single motor cortex must surely contribute to, if not fully account for, the mirroring seen in the XKS patients.

For most XKS subjects, there appears to be an association between the size of the cross-correlogram peak constructed from multi-unit EMG recorded from co-contracting left and right 1DI and intensity of the mirror movements which, across the subjects, varied from mild to marked. Those subjects with the most pronounced mirror movements, where the ratio of involuntary to voluntary EMG was close to unity, and in whom ipsilateral and contralateral responses in 1DI following focal magnetic brain stimulation were of similar size, have the largest central peaks in cross-correlograms. Conversely those with the smallest correlogram peaks have the smallest involuntary to voluntary EMG ratios.

However, although these observations provide strong evidence that mirroring in XKS is produced by a common synaptic drive from one cortex to homologous left and right muscles, this may not be the whole story. Two of our subjects with Grade 2 mirroring recorded while performing the complicated thumb to finger opposition task, appear at the two extremes in Table 4, i.e. the subject with the most predominant ipsilateral projection and the subject with the most predominant contralateral projection as judged by muscle responses to focal magnetic brain stimulation. This suggests that other factors may also contribute to the presence of mirror movements. As discussed earlier, there could be bilateral activation of the motor cortices, in addition to activity in the novel corticospinal tract. This hypothesis is further discussed in the following paper (Krams *et al*., 1997) which describes the use of PET to examine activation of the motor cortex.

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Appendix *Summary of clinical and genetic data from the XKS subjects in this study, grouped according to family*

Subject	Genotype	Other phenotypic anomalies
K1 = patient 4[1]	Terminal deletion of Xpter[3] involving entire STS and *KAL* loci, corresponds to LON BAR[4] and pedigree 6[5]	Mirror movements, hypertension, ichthyosis, micropenis
K8 = xK1[2] = patient 2[1]		Mirror movements, ichthyosis, left kidney absent, renal impairment, hypertension, proteinuria, hypothyroid, cryptorchid
K2 = xK2[2] = patient 3[1]		Mirror movements, ichthyosis, right kidney absent, hypertension, proteinuria, cryptorchid, micropenis
K11 = xK4[2] = LON G1[6]	Complete deletion of all *KAL* exons, corresponds to LON CRAI[4] and pedigree 5[5]	Mirror movements, cryptorchid, right kidney absent
K6 = xK5[2] = LON G2[6]		Mirror movements, cryptorchid, right kidney absent
K13 = xK6[2]	Point mutation exon 6, G→A substitution at base 924 creating premature stop codon, corresponds to pedigree 3[5]	No mirror movements
K10 = xK7[2] = LON 36K4[4,7,8]	As for K13	Mirror movements, cryptorchid, left kidney absent
K5 = xK11[2] = LON OBR[4]	Exon 1 deleted	Mirror movements, cryptorchid
K7 = xK8[2] = LON JB[4]	Deletion of C^{1847} in exon 12 resulting in frame shift and premature stop codon	Mirror movements, cryptorchid, right sensorineural deafness
K4 = xK9[2]	No coding sequence mutation	Mirror movements, cryptorchid, left kidney absent
K4a = xK10[2]	As for K4	Mirror movements, hypertension, proteinuria, right kidney absent
K9[9]	Chromosomal translocation (Xp22.3:Yq11.2), deletion of *STS* and exons 10–14 of *KAL*	Mirror movements, cryptorchid, ichthyosis, short stature
K12	Point mutation exon 5, G→A substitution at base 861	Mirror movements, cryptorchid creating premature stop codon
K3[4]	Corresponds to pedigree 1,[5] LON 77A10[4,7,8] is a maternal uncle	Mirror movements, cryptorchid

K2 and K8 are cousins; K1 is uncle to K8 and K2; K3 and K12 are cousins; K11 and K6 are brothers; K13 and K10 are brothers; K4 and K4a are brothers. STS = steroid sulphate gene.

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