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Subcortical band heterotopia (SBH) in males: clinical, imaging and genetic findings in comparison with females

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Summary

Subcortical band heterotopia (SBH) or double cortex syndrome is a neuronal migration disorder, which occurs very rarely in males: to date, at least 110 females but only 11 in males have been reported. The syndrome is usually associated with mutations in the *doublecortin* (*DCX*) (Xq22.3-q23) gene, and much less frequently in the *LIS1* (17p13.3) gene. To determine whether the phenotypic spectrum, the genetic basis and genotype–phenotype correlations of SBH in males are similar to those in females, we compared the clinical, imaging and molecular features in 30 personally evaluated males and 60 previously reported females with SBH. Based on the MRI findings, we defined the following band subtypes: partial, involving one or two cerebral lobes; intermediate, involving two lobes and a portion of a third; diffuse, with substantial involvement of three or more lobes; and pachygyria-SBH, in which posterior SBH merges with anterior pachygyria. Karyotyping and mutation analysis of *DCX* and/or *LIS1* were performed in 23 and 24 patients, respectively. The range of clinical phenotypes in males with SBH greatly overlapped that in females. MRI studies revealed that some anatomical subtypes of SBH, such as partial and intermediate posterior, pachygyria-SBH and diffuse bands with posterior predominance, were more frequently or exclusively present in males. Conversely, classical diffuse SBH and diffuse bands with anterior predominance were more frequent in females. Males had either mild or the most severe band subtypes, and these correlated with the over-representation of normal/borderline intelligence and severe mental retardation, respectively. Conversely, females who had predomin-

antly diffuse bands exhibited mostly mild or moderate mental retardation. Seven patients (29%) had missense mutations in *DCX*; in four, these were germline mutations, whereas in three there was evidence for somatic mosaicism. A germline missense mutation of *LIS1* and a partial trisomy of chromosome 9p were identified in one patient (4%) each. One male each had a possible pathogenic intronic base change in both *DCX* and *LIS1* genes. Our study shows that SBH in males is a clinically heterogeneous syndrome, mostly occurring sporadically. The clinical spectrum is similar to that of females with SBH. However, the greater cognitive and neuroradiological heterogeneity and the small number of mutations identified to date in the coding sequences of the *DCX* and *LIS1* genes in males differ from the findings in females. This suggests other genetic mechanisms such as mutations in the non-coding regions of the *DCX* or *LIS1* genes, gonadal or somatic mosaicism, and finally mutations of other genes.

Keywords: *DCX*; double cortex; *LIS1*; male; subcortical band heterotopia

Abbreviations: AED = antiepileptic drugs; a–p = anterior–posterior; *DCX* = *doublecortin* gene; FISH = fluorescence *in situ* hybridization; FSIQ = full-scale intelligence quotient; IQ = intelligence quotient; *LIS1* = *lissencephaly* gene 1; SBH = subcortical band heterotopia

Introduction

Subcortical band heterotopia (SBH), also known as subcortical laminar heterotopia or double cortex syndrome, is a cortical malformation characterized by the presence of symmetrical and bilateral bands of heterotopic grey matter located between the ventricular wall and the cortical mantle, and clearly separated from both (Dobyns *et al*., 1996; Harding, 1996).

Affected individuals typically present with epilepsy and variable degrees of mental retardation. Seizures often start in the first decade and vary from partial to generalized attacks. They may progress to multiple seizure types and are usually refractory to medication. Neurological examination may be normal, but dysarthria, hypotonia, poor fine motor control or, rarely, a pyramidal syndrome may be present (Palmini *et al*., 1991; Barkovich *et al*., 1994).

Diagnosis is based on MRI, which shows the characteristic isointensity of the heterotopic band with the cortex in all imaging sequences (Barkovich *et al*., 1989). The thickness and extent of the band can vary (Barkovich *et al*., 1994; Gleeson *et al*., 2000*a*), while the appearance of the overlying cortical mantle on MRI may be normal, show a simplified gyral pattern or, rarely, true pachygyria (Barkovich *et al*., 1994; Dobyns *et al*., 1996; Guerrini and Carrozzo, 2001).

Most patients with SBH are females (Andermann and Andermann, 1996; Dobyns *et al*., 1996): to date, at least 110 females with SBH (Matell, 1893; Jacob, 1936, 1938; Wiest and Hallervorden, 1958; Barkovich *et al*., 1989; Palmini *et al*., 1991; Gallucci *et al*., 1991; Ricci *et al*., 1992; Soucek *et al*., 1992; Hashimoto *et al*., 1993; Iannetti *et al*., 1993; Landy *et al*., 1993; Miura *et al*., 1993; Tohyama *et al*., 1993; Barkovich *et al*., 1994; De Volder *et al*., 1994; Parmeggiani *et al*., 1994; Scheffer *et al*., 1994; Harding, 1996; Berg *et al*., 1998; Vossler *et al*., 1999; Gleeson *et al*., 2000*a*), but only 13 males have been reported (Barkovich *et al*., 1994; Ketonen *et al*., 1994; Franzoni *et al*., 1995; Gigli *et al*. 1996; Ono *et al*., 1997; Federico *et al*., 1999; Pilz *et al*., 1999; Vossler *et al*., 1999; Pinard *et al*., 2000; Kato *et al*., 2001; Poolos *et al*., 2002).

Although most patients with SBH are sporadic, a syndrome of familial SBH with X-linked inheritance, in which the vast majority of carrier females have SBH and affected males usually have classical lissencephaly, has been described (Pinard *et al*., 1994; Scheffer *et al*., 1994; Andermann and Andermann, 1996; Dobyns *et al*., 1996). However, in at least one family, a mother and son were both found to have SBH (Pilz *et al*., 1999).

Two genes have been demonstrated to be involved in the aetiology of SBH: (i) *DCX* (also known as *doublecortin* or *XLIS*), located on chromosome Xq22.3-q23 (des Portes *et al*., 1997, 1998*a*, *b*; Ross *et al*., 1997; Gleeson *et al*., 1998, 1999; Sossey-Alaoui *et al*., 1998; Horesh *et al*., 1999) and (ii) *LIS1* (also called *PAFAH1B1* because it codes for the beta 1 subunit of brain platelet activating factor acetylhydrolase) on chromosome 17p13.3 (Ledbetter *et al*., 1992; Reiner *et al*., 1993; Hattori *et al*., 1994; Chong *et al*., 1997; Lo Nigro *et al*., 1997; Sapir *et al*., 1997; Pilz *et al*., 1999).

DCX mutations have been found in ~80% of sporadic females with SBH and in all multiplex families with SBH, both in SBH females and in males with lissencephaly (des Portes *et al*., 1998*a*, *b*; Gleeson *et al*., 1998, 1999, 2000*a*; Matsumoto *et al*., 2001). In sporadic males, mutations of this gene are usually associated with lissencephaly (agyria/pachygyria) (Pilz *et al*., 1998, 2002). These data suggest that SBH is a mild form of lissencephaly that usually results from the effects of random inactivation in heterozygous females (des Portes *et al*., 1998*a*, *b*; Gleeson *et al*., 1998, 1999). Palmini *et al*. (1993) previously postulated the developmental continuum among SBH, pachygyria and lissencephaly.

Only one sporadic male with SBH has been demonstrated to carry a missense mutation of the *LIS1* gene located on chromosome 17p13.3 (Pilz *et al*., 1999). *LIS1* mutations are

responsible for 65% of classical lissencephaly (Pilz *et al*., 1998; Cardoso *et al*., 2002).

Several recent reports have demonstrated that *LIS1* mutations are associated with more severe lissencephaly or SBH over the parietal and occipital regions, whereas *DCX* mutations are associated with more severe abnormalities over anterior brain regions (Pilz *et al*., 1998, 1999; Dobyns *et al*., 1999; Gleeson *et al*., 2000*a*).

Despite the progress in understanding the molecular basis of SBH in females, mutations in males have largely remained unidentified to date. It is also unclear whether the phenotypic spectrum of the rare males with SBH is the same as that in affected females, and whether similar genotype–phenotype correlations can be demonstrated in males with SBH. To clarify these issues, we present the clinical and imaging phenotypes and molecular genetic data for 30 males with SBH, and compare these with the corresponding features in 60 SBH females.

Patients and methods

Patients

Five male patients (1, 4, 10, 20 and 24) were studied at the Montreal Neurological Hospital. The remaining 25 patients were from 21 centres on five continents. Eight patients have been reported previously: patients 2 and 25 (Pilz *et al*., 1999); 7 (Franzoni *et al*., 1995); 15 (Ketonen *et al*., 1994; Gleeson *et al*., 2000*b*); 18 (Gigli *et al*. 1996; Federico *et al*., 1999); 19 (Barkovich *et al*., 1994; Pilz *et al*., 1999); 21 (Ono *et al*., 1997); and 27 (Vossler *et al*., 1999). All of these patients were known personally to at least one of the authors. For all males, detailed information regarding family history, abnormal pre- and perinatal events, age at seizure onset, psychomotor development, cognitive function, neurological examination, EEG and neuroimaging findings was available (Tables 1 and 2).

For the meta-analysis, the 30 males were compared with 60 females with SBH whose detailed clinical information has been published (Palmini *et al*., 1991; Gallucci *et al*., 1991; Ricci *et al*., 1992; Soucek *et al*., 1992; Hashimoto *et al*., 1993; Iannetti *et al*., 1993; Landy *et al*., 1993; Miura *et al*., 1993; Tohyama *et al*., 1993; Barkovich *et al*., 1994; De Volder *et al*., 1994; Parmeggiani *et al*., 1994; Scheffer *et al*., 1994; Harding, 1996; Berg *et al*., 1998; Vossler *et al*., 1999) (Table 3). The same electroclinical and neuroradiological classification criteria were used for males and females (Table 3).

Methods

The International Classifications of Seizures [Commission on Classification and Terminology of the International League Against Epilepsy (CCTILAE, 1981)] and of Epilepsies and Epileptic Syndromes (CCTILAE, 1989) were utilized for classification of seizures and epileptic syndromes, respectively. Both routine scalp EEG and prolonged video-EEG recordings were performed in all patients (Table 1). Seven patients in our series underwent surgery (Table 1). In patient 20, a frontal lobe biopsy was performed in association with callosotomy.

To stratify the patients according to cognitive function (Table 2), we defined six cohorts based on available clinical information and IQ: (i) normal, IQ $\geqslant$80; (ii) borderline, IQ 70–80; (iii) mild delay, IQ 55–69; (iv) moderate delay, IQ 40–54; (v) severe delay, IQ 25–39; (vi) profound delay, untestable. Eight patients had a formal psychometric evaluation: four children were evaluated using the Wechsler Intelligence Scale—Revised (WISC-R), and in three patients the Wechsler Adult Intelligence Scale was used. Patient 20 underwent testing with the Vineland Adaptive Scale instead of the WISC-R. Eight patients were too young and four too severely retarded for formal psychometric evaluation. In the meta-analysis, the six categories of cognitive functioning were reduced to three: normal–borderline intelligence, mild–moderate mental retardation and severe–profound mental retardation (Table 3).

Cranial MRI studies were performed in all patients (Table 2). We termed SBH partial when it was highly localized involving one to two cerebral lobes, intermediate when it involved two lobes and a small portion of a third lobe, and diffuse when there was substantial involvement of at least three lobes. In practice, partial bands involved either the frontal lobe only or both parietal and occipital lobes. Intermediate bands involved part of the posterior frontal lobe plus the parietal and occipital lobes. Diffuse bands involved most of the frontal, parietal and occipital lobes, with anterior extension at least to the mid-frontal lobe and usually into the anterior frontal lobe. Extension into the temporal lobes was quite variable in all types. The term 'simplified gyral pattern' was used to denote an abnormal gyral pattern consisting of gyri of normal width ($\leqslant$1 cm) and cortical thickness ($\leqslant$4 mm) that were separated by wide and shallow sulci. We used the term pachygyria to denote gyri with a width of $\geqslant$1.5 cm and abnormally thick cortex.

Routine or high-resolution karyotyping was performed in 23 patients, and fluorescence *in situ* hybridization (FISH) studies on 11 patients (3, 4, 8, 9, 11, 17, 19, 20, 22, 24 and 25) using cosmid probes (D17S370, D17S379, L132, 37E9 or 120A7) corresponding to the lissencephaly syndrome critical region of 17p13.3. In patient 18, FISH analysis was performed with a chromosome 9 'painting' probe (ONCOR).

Clinical data and blood samples were obtained with informed consent from 24 patients, and DNA was extracted using a standard protocol. Mutation analysis was performed for both *DCX* and *LIS1* in 16 patients, for *DCX* only in seven patients, and for *LIS1* only in one patient. We did not analyse the second gene when a convincing mutation was detected in the first gene tested. Mutation detection was performed by direct sequencing of genomic DNA as described previously (Lo Nigro *et al*., 1997; Pilz *et al*., 1999; Cardoso *et al*., 2000). In most patients, the mutation was confirmed to be *de novo* by direct sequencing of both parents. The investigators were

Table 1 *Clinical and EEG findings in 30 males with SBH*

Patient	Seizure onset	Seizure type	Main EEG findings	Neurological and physical examination	Response to AEDs/surgery
1	5 years	CPS, DA	Multifocal spikes, SBS	Slow finger movements > Lt hand	Anterior callosotomy
2	21 months	CPS	Syn bil spikes and sharp waves, Diffuse slw	Minimal hypotonia and motor incoordination	Good
3	3 years	CPS	Spikes over Rt post T	Anisocoria Lt >Rt	Good
4	5 years	CPS	Bil independent F-C, Gen irregular spw	Normal	Good
5	5 months	Blank spells, SMA	Rt C-T-P spikes	Normal	Rt post-central gyrus removal
6	7 years	S, M, DA	Bil independent C-P-O spikes and sz onset	Dysarthria, hypotonia	Post callosotomy, Rt P-O corticectomy
7[a]	7 years	CPS	Rt T-O spikes	Normal	Good
8	4.5 years	Gen myocl, DA, CPS, GTC	Normal	Normal	Poor
9	6 years	CPS	Multifocal spikes > P-O	Normal	Good
10	3 years	CPS, DA, Foc and Gen myocl	Syn bil slw and spw > P-O	Anisocoria Rt > Lt, dysarthria, gross and fine motor incoordination	Poor
11	3 months	CPS, infantile spasms, GTC	Multifocal spikes	Diffuse hypotonia, dysmorphic features	Poor
12	16 years	CPS	Bil independent T spikes and sz onset	Normal	Rt temporal lobectomy
13	–	–	Mild slowing of BA	Normal	–
14	4 years	Foc myocl	Bil independent F spikes	Normal	Rt F-C-P subpial transection
15[b]	6 years	CPS	Mild disorganization of BA	Mild mirror movements on finger opposition	Good
16	3 years	Foc and Gen myocl, GTC, DA	Bil independent F-C-T spikes, Gen slw, SBS	Rt hemiparesis	Two-stage complete callosotomy
17	7 months	Infantile spasms, GT, GTC, AA, Gen myocl	Hypsarrhythmia, Gen polysp, Multifocal spikes	Mild generalized spasticity	Intractable
18	–	–	Multifocal spw > P-O	Dysmorphic features, dysarthria	–
19	7.5 years	CPS, AA, DA, GTC, SS, M	Gen slow spw, Multifocal spikes	Gross and fine motor incoordination	Intractable
20	3.5 years	DA, AA, Gen myocl, GTC, S	Gen slow spw, Multifocal spikes > F-C	Anisocoria, Lt > Rt, gross and fine motor incoordination, growth retardation	Two-stage complete callosotomy
21	5 days	Subtle Sz, Gen myocl, GTC	Multifocal spikes	Tetraparesis, dysmorphic features	Poor
22	1.5 years	GTC status, GTC, DA, AA	Normal	Incoordination, dysmorphic features	Poor
23	2 months	M, CPS, Gen myocl, GTC, DA	Rt O-T, Lt F sharp waves, Bil spikes, Gen polyspw	Tetraparesis	Intractable
24	7 months	DA, Gen myocl, GT, AA	Gen polyspikes, Gen slow spw, Multifocal spikes	Severe hypotonia, dysmorphic features, growth retardation	Intractable
25	15 days	M	N/A	Dysmorphic features	Poor
26	2 years	GT, rare GTC	Gen rhythmic spikes, Multifocal spikes > P-O	Spastic tetraparesis	Intractable
27[c]	2 years	Gen and Foc myocl, GT, GTC	Gen polyspikes, Gen slow spw, Multifocal spikes	Paraparesis	Good
28	13 years	CPS	Lt P-O spikes	N/A	Intractable
29	16 years	CPS, DA	Multifocal spikes	Normal	Poor
30	7 months	GTC, CPS	Multifocal and Gen spikes, Polyspikes and slw	Normal	Poor

[a]From Franzoni *et al*., 1995; [b]from Ketonen *et al*., 1994; [c]from Vossler *et al*., 1999. AA = atypical absences; BA = background activity; Bil = bilateral; C = central; CPS = complex partial seizures; DA = drop attacks; F = frontal; Foc = focal; Gen = generalized; GT = generalized tonic seizures; GTC = generalized tonic clonic seizures; Irreg = irregular; Lt = left; M = simple motor seizures; Myocl = myoclonic; N/A= not available; O = occipital; P = parietal; Post = posterior; Rt = right; S = simple sensory seizures; SBS = secondary bilateral synchrony; SMA = supplementary motor area seizures; Spw = spike and waves; SS = special sensory seizures; Sz = seizure; Slw = slow waves; Syn = synchronous; T = temporal.

unaware of mutation data at the time of initial neuroimaging review. DNA samples from six patients were unavailable.

Differences in age at seizure onset between males and females were analysed using the Student's *t*-test. Differences in seizure types at onset, clinical syndromes, cognitive function, neurological examination, dysmorphic features, imaging characteristics of the SBH and presence of additional brain abnormalities were analysed using Fisher's exact test.

Results

Prenatal and perinatal events

Prenatal events occurring within the first 4 months of pregnancy included: application of local heat to the lower abdomen, but with no skin burns (mother of patient 6, up to second month of pregnancy); moderate flu-like symptoms (mothers of patients 8 and 22, at 3 and 4 months of pregnancy, respectively, the former having been treated with paracetamol); exposure to bonding glue (mother of patient 8, at 4 months of pregnancy); and intake of doxylamine and an antihistaminic (H1-receptor)-decongestant preparation (mother of patient 19, at 2–3 months of pregnancy). Perinatal complications included cord around the neck (patient 8), foetal distress and marked meconium staining of the amniotic fluid (patient 19), and cephalo-pelvic disproportion (patient 22). The last two led to emergency Caesarian sections.

Clinical findings

The age range at the time of study was 1 month to 34 years. Patients 25 and 30 were the youngest, aged 2 and 9 months, respectively, at the time of the clinical evaluation.

Motor development and status were mildly delayed in eight patients (walking between 18 and 24 months: patients 2, 6, 10, 14, 16, 19, 20 and 22), moderately delayed in one (walking between 24 and 30 months: patient 18) and severely delayed in four (started walking only after 30 months: patients 17, 21, 23 and 26). Patient 11 did not walk at 16 months, and patient 24 was never able to walk, even with proper support. Patient 30 had a 3-month delay in gross motor development at the age of 9 months. Early motor development and function were normal in 14 males.

Language development and use were abnormal in 13 patients: three had mild delay (first words and sentences between 18 and 30 months of age: patients 10, 16 and 22), four had moderate delay (2, 7, 19 and 20: first words and sentences between 30 and 42 months), and three had severe delay (a few words, no sentences after 3.5 years in patients 21, 23 and 26). Patients 17, 24 and 27 were non-verbal at the ages of 5, 34 and 42 years, respectively. The remaining males had normal language development.

Cognitive function (Table 2) was normal in six patients and borderline in six. The rest had variable mental retardation, assessed to be mild in five, mild to moderate in one, moderate in one, severe in seven and profound in two. Given their very young age, no definitive statement could be made about the cognitive function of patients 25 and 30.

Abnormalities on examination

Eighteen patients had abnormal neurological findings (Tables 1 and 4). Of the associated malformations and dysmorphic features, microcephaly was the most frequently observed.

Epilepsy

Seizure histories (Table 1)

Two patients (13 and 18) never had seizures. In one patient (18), the investigations that led to the diagnosis were initiated because of dysmorphic features and mental retardation. Ten of 28 patients (36%) had a single seizure type, mainly complex partial seizures. Eighteen patients (64%) had more than one seizure type. Age at onset of epilepsy ranged from 5 days to 16 years (mean 50.7 months, median 36 months) (Table 1). Eleven patients had tonic, atonic or myoclonic drop attacks. In one of them, this consisted of axial myoclonus precipitated by unexpected noise or when suddenly touched. Twelve patients had generalized tonic clonic seizures, and nine had generalized myoclonic jerks that often occurred in clusters after awakening. Atypical absences were present in five patients. Four had generalized tonic attacks; in one (26), these occurred as tonic spasms elicited by seeing food or eating. In patient 22, epilepsy began at the age of 18 months with generalized convulsive status epilepticus.

Sixteen patients had complex partial seizures. Simple motor and sensory attacks were noted in four and two patients, respectively; one patient had supplementary motor attacks occurring up to 30 times a night and several times during the day. One patient had daily minor attacks with subtle blinking or blurred vision, and occasionally appeared frightened or covered his eyes during these 'blind spells'. Four patients had focal myoclonic attacks; in one (14), these were often followed by a drop attack.

Four patients had Lennox–Gastaut syndrome (19, 20, 24 and 27). Two had infantile spasms with developmental regression after their onset (11 and 17). The clinical picture in patients 16, 22 and 23 was also suggestive of Lennox–Gastaut syndrome, but the EEG findings were inconsistent with this diagnosis.

EEG results

Four patients had only focal spikes. In 14, bilateral independent or multifocal epileptic abnormalities were found, with side predominance noted in four (1, 6, 12 and 14) and secondary bilateral synchrony in two (1 and 16). Two patients had mostly generalized bilateral and synchronous epileptic activity; in one (2), this occurred during sleep. Generalized slow spike and waves in association with multifocal spikes or

Table 2 *Neuroimaging and molecular findings in 30 males with SBH*

Patient	Research no.	FSIQ or retardation	Band heterotopia			Other brain anomalies	Chromosome analysis	Mutation analysis	
			Type	Thickness	Distribution			*DCX*	*LIS1*
1	LP99-197	Mild	1, a > p	Thin	Frontal	Simplified gyral pattern	46, XY	Missense, 532C→T, R178C	Not done
2	LP98-060	Borderline	1, a > p	Thin	Frontal	Dysmorphic right caudate head and frontal horn	46, XY	Missense, 265C→G, R89G[a]	Not done
3	LP95-081	Normal	2, p > a	Thin	Parietal	–	46, XY	No mutations	No mutations
4	LP97-008	98	2, p > a	Thin	Parietal-occipital	–	46, XY	No mutations	No mutations
5	N/A	Normal	2, p > a	Thin	Parietal-occipital	–	N/A	N/A	N/A
6	N/A	Borderline	2, p > a	Thin	Parietal	–	N/A	N/A	N/A
7[b]	LR02-050	Normal	3, p > a	Medium–thick	Posterior temporal to occipital	–	N/A	No mutations	No mutations
8	LP95-083	76	3, p > a	Medium	Posterior frontal to occipital	Simplified gyral pattern	46, XY	No mutations	No mutations
9	LP95-075	61	3, p > a	Medium	Posterior frontal to occipital	Simplified gyral pattern	46, XY	No mutations	No mutations
10	LR01-284	59	3, p > a	Thick	Posterior frontal to occipital	Posterior true pachygyria, enlarged ventricles	46, XY	No mutations	No mutations
11	LP98-012	Moderate	3, p > a	Thick	Posterior frontal to occipital	Posterior simplified gyral pattern	46, XY	No mutations	No mutations
12	LP99-135	89	4, a = p	Thin	Anterior-frontal to occipital	–	46, XY	Missense, 556C→T, R186C[c]	Not done
13	LP97-026	83	5, a = p	Medium–thick	Anterior-frontal to occipital	Simplified gyral pattern, enlarged ventricles	46, XY	Missense, 200G→A, G67E	Not done
14	N/A	Borderline	5, a > p	Medium	Anterior-frontal to occipital	–	N/A	N/A	N/A
15[d]	N/A	69	5, a = p	Thick	Anterior-frontal to occipital	–	46, XY	Missense, 628G→T, V210F[c,e]	Not done
16	N/A	Mild to moderate	5, a = p	Medium	Anterior-frontal to occipital	–	N/A	N/A	N/A
17	LR01-208	Severe	5, p > a	Medium–thick	Anterior-frontal to occipital	–	46, XY	No mutations	1160–37G→A, intron 10
18[f]	LR00-065	Severe	5, a = p	Medium	Anterior-frontal to occipital	Enlarged ventricles	47,XY,+der(9)(q11-pter) de novo[f]	No mutations	No mutations
19	LP94-051	Severe	5, p > a	Medium–thick	Mid-frontal to occipital	Simplified gyral pattern	46, XY	Not done	Missense, 499T→C, S169P[a]
20	LP96-036	Severe	5, p > a	Thick	Mid-frontal to occipital	Simplified gyral pattern, enlarged ventricles	46, XY	No mutations	No mutations
21	LR02-028	Severe	5, a = p	Thick	Anterior-frontal to occipital	Simplified gyral pattern, enlarged ventricles	46, XY	No mutations	No mutations
22	LP96-093	Mild	6, a > p	Pachygyria-SBH	Parietal-occipital	Anterior true pachygyria, delayed myelination	46, XY	No mutations	No mutations
23	N/A	Severe	6, a > p	Pachygyria-SBH	Posterior-frontal to occipital	Anterior true pachygyria	N/A	N/A	N/A
24	LP96-091	Profound	6, a > p	Pachygyria-SBH	Posterior-frontal to occipital	Anterior true pachygyria, enlarged ventricles, thin CC, cerebellar atrophy	46, XY	No mutations	No mutations
25	LP98-046	N/A	6, a > p	Pachygyria-SBH	Posterior-frontal to occipital	Anterior true pachygyria, enlarged ventricles, thin CC	46, XY	Missense, 233G→A, R78H[a]	Not done

Table 2 *Continued*

Patient	Research no.	FSIQ or retardation	Band heterotopia			Other brain anomalies	Chromosome analysis	Mutation analysis	
			Type	Thickness	Distribution			*DCX*	*LIS1*
26	N/A	Severe	6, a > p	Pachygyria-SBH	Posterior-frontal to occipital	Anterior true pachygyria, enlarged ventricles	N/A	N/A	N/A
27[g]	LR01-322	Profound	3, p > a	Medium	Mid-posterior-frontal to occipital	N/A	46, XY	705+48 A→G, intron 5	No mutations
28	LP99-004	70	3, p > a	Medium	Mid-posterior-frontal to occipital	N/A	46, XY	No mutations	No mutations
29	LR01-358	Borderline	4, a = p	Thin	Anterior-frontal to occipital	Bifrontal simplified gyral pattern	46, XY	No mutations	No mutations
30	LR02-036	N/A	5, a = p	Thick	Anterior-frontal to occipital	Simplified gyral pattern	46, XY	Missense, 683T→C, L228P[c]	Not done

[a]From Pilz *et al*., 1999; [b]from Franzoni *et al*., 1995; [c]somatic mosaicism; [d]from Ketonen *et al*., 1994; [e]from Gleeson *et al*., 2000*b*; [f]from Federico *et al*., 1999; [g]from Vossler *et al*., 1999. CC = corpus callosum; FSIQ = full-scale intelligence quotient; N/A = not available.

generalized polyspikes or polyspike and wave complexes were found in four patients. Hypsarrhythmia was the main finding in one patient (17). Background activity anomalies only and normal EEGs were found in two patients each. We had no information on the EEGs of patient 25.

Response to antiepileptic drugs

Seven patients (2, 3, 4, 7, 9, 15 and 27; 25%) responded satisfactorily to antiepileptic drugs (AEDs). Except for patient 27, these patients had normal or borderline intelligence or mild mental retardation and only one seizure type. Patient 9 had no more than two seizures per month with carbamazepine and vigabatrin, and patient 27 had a dramatic and sustained improvement upon addition of lamotrigine to valproic acid and phenytoin. The remaining five individuals received monotherapy: either valproic acid (2, 3 and 4) or carbamazepine (7 and 15). Patients 2 and 3 had no further seizures after starting treatment and the other three patients had two to five minor attacks per month, with no detectable effects on behaviour or cognition. Eight patients (8, 10, 11, 21, 22, 25, 29 and 30; 28%) responded poorly to AEDs, with slight reduction in seizure frequency, control of only some types of seizures, or persistence of multiple seizures at night. Thirteen patients (46%) were refractory to medical therapy, with up to 20–30 seizures daily despite appropriate treatment and trials of multiple drug regimens. Most developed severe behavioural problems, mainly aggression and decline in learning abilities.

Surgical therapy

The surgical procedures carried out in our patients are detailed in Table 1. Despite some early improvement, there was no sustained reduction in the frequency and severity of seizures in four out of seven patients who were surgically treated. Patient 14 had infrequent focal myoclonic jerks of the left hand 1 year after subpial transection, but no further progression to drop attacks. The marked reduction of drop attacks in patients 1 and 20 continued at 1 year after callosotomy.

MRI abnormalities (Table 2 and Fig. 1)

The subcortical band varied substantially in extent and thickness. Six major groups were seen: (1) thin partial frontal SBH with no involvement of the posterior regions (two patients); (2) thin partial posterior SBH with no involvement of the frontal regions (four patients); (3) medium or thick intermediate SBH that was always more prominent posteriorly (seven patients); (4) diffuse thin SBH (two patients); (5) diffuse medium or thick SBH (10 patients); and (6) anterior pachygyria that merged into posterior SBH (five patients). In intermediate posterior SBH, the band extended from the occipital pole to just reach the posterior frontal or temporal regions. In the two patients (12 and 29) with diffuse thin SBH (group 4), the band was asymmetric with a discontinuous appearance on the right side. Further heterogeneity was also found among patients with diffuse medium or thick SBH (group 5), three of whom had clear posterior predominance. Among the latter, one appeared thickest in the centro-parietal region (patient 17) and two had sparing of the anterior frontal regions (patients 19 and 20). The other patients in group 5 appeared thickest frontally (patient 14) or had no obvious differences in thickness between the front and the back. In this study, some of the scans did not permit precise localization of the central sulcus. These groups are different from and should not be confused with the lissencephaly-SBH grading system used in several other papers (Pilz *et al*., 1998; Dobyns *et al*., 1999). In this paper, group 6 with mixed pachygyria-SBH corresponds to lissencephaly-SBH grade 5, while the other groups are variants of lissencephaly-SBH grade 6.

When we considered the distribution of the malformations regardless of severity or thickness of the band, we were able to identify three main groups. In the first, SBH and the overlying cortical malformation were only present or more severe in anterior brain regions representing an anterior-to-posterior (a > p) gradient (eight patients). This cluster included partial frontal bands (group 1), diffuse bands that were thicker in the anterior head regions (patient 14 from group 5), and the anterior pachygyria-posterior SBH (group 6). In the next group, the posterior aspects of the cerebral hemispheres were the more severely involved comprising a posterior-to-anterior (p > a) gradient (14 patients). This category encompassed thin partial posterior (group 2) or intermediate posterior (group 3) bands and diffuse bands with clear posterior predominance (patients 17, 19 and 20 from group 5). Finally, we reviewed several patients with diffuse bands in whom the MRI showed no differences between anterior and posterior regions, or an a = p gradient (eight patients, comprising those from group 4 and patients 13, 15, 16, 18, 21 and 30 from group 5). Most bands were symmetric.

A simplified gyral pattern with short gyri and shallow sulci was observed in 10 patients. Five patients had true pachygyria. Eight had enlarged ventricles, two had a thin corpus callosum and one had cerebellar hypoplasia.

Neuropathology

In patient 20, a right frontal biopsy revealed a decreased number of neurones in the cortex, with some attempt at columnar alignment from white matter to the surface. The first two layers appeared well formed, but in the underlying layers, disorganization and neuronal clumps were seen. In all sections, the white matter contained an excessive number of neurones resembling those normally present in the lower layers of the cortex. In patient 12, examination of the resected tissue showed heterotopic grey matter arranged in a linear band and normal cortical thickness.

Table 3 *Comparison of clinical features between males and females with SBH*

	Males[a]	Females[a]	*P* value
Total	30	60	
Patients who presented with seizures[b]	20/30 (67%)	35/53 (66%)	NS
Patients who developed epilepsy	28/30 (93%)	56/60 (93%)	NS
Mean age at seizure onset (months)	50.7 (±53)	69.2 (±54)	NS
Clinical phenotype			
Seizure type at onset			
Focal seizures	13/28 (46%)	19/49 (39%)	NS
Generalized seizures	8/28 (29%)	17/49 (35%)	NS
Infantile spasms	1/28 (4%)	4/49 (8%)	NS
Multiple seizure types	3/28 (11%)	7/49 (14%)	NS
Undetermined if focal or generalized	3/28 (11%)	3/49 (6%)	NS
Habitual seizures			
Focal seizures[c]	21/27 (78%)	38/55 (69%)	NS
Generalized tonic-clonic seizures	12/27 (44%)	22/55 (40%)	NS
Drop attacks	11/27 (41%)	20/55 (36%)	NS
Atypical absences	5/27 (19%)	16/55 (29%)	NS
Myoclonic seizures[d]	7/27 (26%)	9/55 (16%)	NS
Other generalized type of seizures[e]	4/27 (15%)	13/55 (24%)	NS
Multiple seizure types in combination	18/27 (67%)	33/55 (60%)	NS
Lennox–Gastaut syndrome	4/27 (15%)	11/55 (20%)	NS
Infantile spasms	2/27 (7%)	5/55 (9%)	NS
Intractable epilepsy	21/27 (78%)	19/29 (65%)	NS
Surgical treatment	7/27 (26%)	5/19 (26%)	NS
Cognitive function			
Normal–borderline intelligence	12/28 (43%)	14/59 (24%)	0.08
Mild–moderate mental retardation	7/28 (25%)	31/59 (52%)	0.02
Severe–profound mental retardation	9/28 (32%)	14/59 (24%)	NS
Abnormal neurological examination	18/29 (62%)	26/53 (49%)	NS
Dysmorphic features	6/29 (21%)	3/53 (6%)	NS
MRI			
Subcortical band heterotopia			
Partial/intermediate SBH	13/30 (43%)	6/60 (10%)	0.0006
Anterior band heterotopia	2/13	5/6	0.01
Posterior band heterotopia	11/13	1/6	0.01
Diffuse band heterotopia	12/30 (40%)	54/60 (90%)	0.000001
Diffuse SBH with posterior predominance	3/12	3/21	NS
Diffuse SBH with anterior predominance	1/12	12/21	0.01
Anterior pachygyria-posterior SBH	5/30 (17%)	0/60	0.003
Cortical anomalies[f]	16/28 (57%)	31/60 (52%)	NS

[a]The number in the denominator indicates the number of patients on whom specific information was available. [b]The other patients presented with developmental delay, sleep disorders, dysmorphic features, behavioural problems, learning difficulties, alone or in association. [c]Includes simple partial seizures, complex partial seizures, focal tonic seizures, partial sensory seizures and focal myoclonic seizures, with or without secondary generalization. [d]Includes all clearly myoclonic seizures and those where it could not be distinguished whether they were focal or generalized. Clearly focal myoclonic seizures are classified in the focal seizures group. [e]Includes absence seizures, generalized tonic seizures, generalized clonic seizures, spasms. [f]Includes simplified gyral pattern and true pachygyria.
NS = not significant.

Family history

The mother of patient 1 has epilepsy and mental retardation (FSIQ 59), and lives in a foster home. She has two sisters and a mentally retarded brother with epilepsy. Her MRI as well as that of one of her affected sisters gave no evidence for SBH. Ten other siblings are healthy. The mother of patient 2 has had epilepsy since the age of 18 years with myoclonic seizures leading to secondary generalization and episodes of speech arrest. Her MRI showed bilateral asymmetric frontal SBH. Her family history was unremarkable. Patient 18 was the youngest child of probably consanguineous parents. A sister died on the 25th day of life with intractable seizures,

Table 4 *Dysmorphic features*

	Patient No.
Microcephaly	11, 18[a], 21[b], 24, 25
Flat occiput	11
Mild trigonocephaly	22
Plagiocephaly	25
Bitemporal hollowing	22
Sloping forehead	25
High forehead	24
Synophrys	24
Low hairline	18[a]
Upslanting eyes	25
Deep set and down slanting eyes	18[a]
Small epicanthal folds	22
Small cup shaped ears	18[a]
Under folding of the posterior helices	22
Low set ears	24
Wide nasal bridge	18[a], 22
Deep philtral crease	25
Anteverted nares	25
High arched palate	21[b], 24, 25
Small jaw	25
Protruding tongue	24
Dysodonthiasis	18[a]
Small hands with chondropathic articulations	18[a]
Broad, square hands	24
Sydney line	24
Tapering fingers	24
Short fingers	18[a]
Short feet	24
Short toes	18[a], 24
Dysonichia	18[a]
Micropenis	18[a]
Bilateral cryptorchidism	18[a]
Hypospadias	22
Short stature	18[a], 24
Hirsutism	24
Depigmented spots on the trunk	24
Large pilonidal dimple	24

[a]Gigli *et al*., 1996; [b]Ono *et al*., 1997.

and three brothers died at birth of unknown causes (Gigli *et al*. 1996; Federico *et al*., 1999). The remaining histories were unremarkable with respect to family history of seizures or mental retardation.

Genetic findings (Table 2)

A normal male karyotype was found in 22 of the 23 patients tested (96%). In patient 18, chromosome analysis revealed a *de novo* partial trisomy of the entire short arm of chromosome 9 in all cells analysed: 47,XY,+der(9)(q11-pter). In this male, mutation analysis of the coding sequences of both *DCX* and *LIS1* was negative. FISH analysis for chromosome 17p13.3 was normal in all 11 males tested.

We detected mutations of either *DCX* or *LIS1* in 10 of the 24 patients who underwent mutation analysis, which included the coding sequences as well as some flanking intronic sequences. Thus, the overall rate of mutation detection in males with SBH (42%) is much lower than in females with SBH (85%; Matsumoto *et al*., 2001). The mutations included seven missense and one intron mutations in *DCX*, and one missense and one intron mutation of *LIS1*. Genotype–phenotype comparison showed consistent differences between the groups defined by MRI appearance, as *DCX* mutations were detected in patients with a = p and a > p phenotypes, while *LIS1* mutations were found in patients with p > a phenotypes. Specifically, *DCX* mutations were found in seven of 12 (58%) patients with a = p or a > p gradients, and *LIS1* mutations were found in two of 12 (17%) patients with a p > a gradient.

The two patients with partial frontal bands (1 and 2) were found to have novel familial missense mutations of *DCX* inherited from their mothers. In the family of patient 1, a missense mutation of exon 5 of *DCX* was also detected in a maternal aunt with epilepsy and no evidence of SBH.

Discussion

Our series demonstrates that the clinical spectrum of SBH in males overlaps with that in females in terms of representation of seizure types, epilepsy syndromes and response to antiepileptic therapy. However, there is increased heterogeneity with respect to cognitive function, neuroimaging and molecular genetic data in males compared with females (Tables 1–3).

Onset of symptoms and clinical course

In ~65% of both males and females, the brain malformation was revealed by onset of seizures. In the remaining patients, the investigations that led to the diagnosis were prompted by the presence of developmental delay, sleep disorders, dysmorphic features, behavioural or learning problems alone or in association. Epilepsy was eventually diagnosed in ~95% of patients of both sexes. Age at seizure onset was earlier in males than in females, but the difference was not statistically significant (Table 3).

No significant differences were found in the seizure types at onset or in types of habitual seizures (Table 3). Focal seizures predominated both at the onset of epilepsy and as habitual seizures, but in the latter they were usually combined with multiple seizure types. The Lennox–Gastaut syndrome and infantile spasms were equally represented in male and female patients. A similar percentage of patients from both groups had intractable epilepsy and underwent surgery (Table 3).

Neuropsychological investigations showed that the two extremes of the cognitive function levels: normal–borderline intelligence and severe–profound mental retardation were over-represented within the male group, whereas females exhibited mostly mild–moderate mental retardation (Table 3).

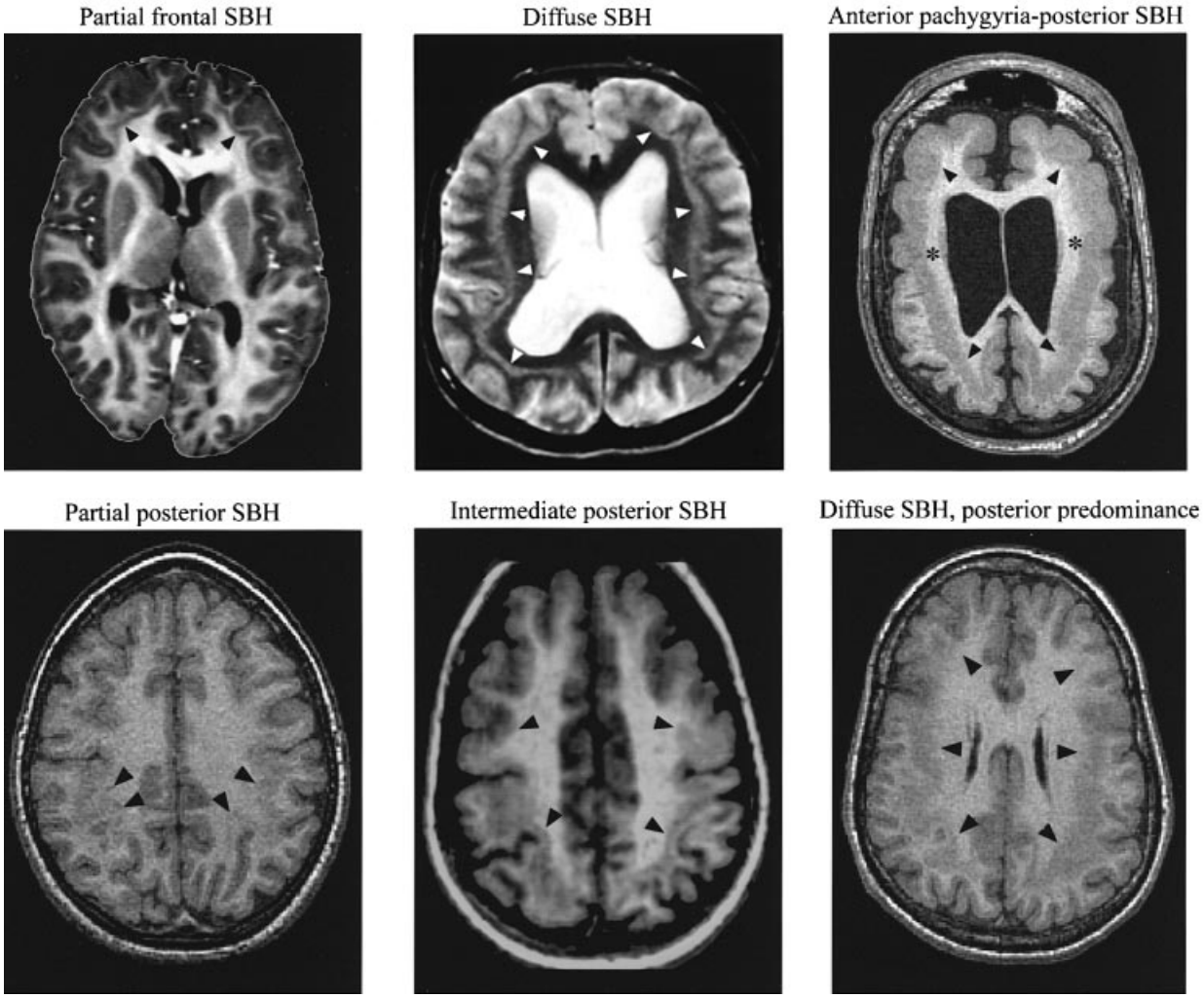

Fig. 1 Brain MRI axial cuts from six patients included in this study. Pictures are representative of SBH subtypes. The arrows indicate the location and extent of the SBH and pachygyria. In the anterior pachygyria-posterior SBH pattern, asterisks indicate the point where posterior SBH merges with anterior pachygyria.

Analysis of SBH subtypes

The most striking differences among patients were revealed by the MRI findings. First, partial and intermediate SBH were significantly more frequent in males than in females (43% versus 10%, $P = 0.0006$) (Table 3). In addition, the SBH subtypes seen among males were more frequently confined to the posterior aspects of the cerebral hemispheres and highly localized when compared with females (patients 3–11, 27 and 28; Tables 2 and 3). Only two males (1 and 2) had partial frontal bands, while five out of six (83%) females with partial or intermediate SBH had frontal bands (Table 3). However, three other sporadic females with posterior bands out of a series of 30 females with SBH have been reported (Gleeson *et al*., 2000*a*). Thus, all the atypical band subtypes seen in males have been observed in females, although they are rare (Gleeson *et al*., 2000*a*; W.B.D., unpublished observations). Both male and female patients with partial frontal SBH were familial, whereas those with posterior SBH were sporadic.

The majority of the males with posterior partial or intermediate SBH had normal or borderline intelligence or mild mental retardation, normal early development and focal epilepsy. Only one male (11) with an intermediate posterior band had a moderate degree of mental retardation; his SBH was thick (1 cm) and he had infantile spasms. Patient 27 (Vossler *et al*., 1999) may also have had a posterior intermediate band. He had Lennox–Gastaut syndrome and had profound mental delay. Three of the four females with partial or intermediate posterior SBH (including those described by Gleeson *et al*., 2000*a*) had moderate or profound mental retardation: one had a thick intermediate posterior SBH and the remaining two had Lennox–Gastaut syndrome.

Secondly, diffuse bands were significantly over-represented among females (90% versus 40%, $P = 0.000001$) (Table 3). Within the diffuse SBH group, the malformation tended to predominate over the posterior brain regions in males and over the anterior brain regions in females. Similar to females, males with this type of SBH had mental

development ranging from normal to severe delay depending on the thickness of the band, the degree of cortical pattern derangement, associated brain abnormalities and epileptic syndrome. For example, patient 12 with a thin, almost discontinuous band and only complex partial seizures had normal intelligence, whereas patient 17, whose SBH was thick and predominated over the posterior aspects, had had infantile spasms and showed severe mental retardation (Tables 1 and 2). Seizure type at presentation was mostly complex partial or myoclonic. Subsequently, myoclonic seizures and drop attacks in association predominated (Table 1). Attacks were intractable in all but one male with diffuse SBH, and four underwent surgery.

Finally, the pachygyria-SBH pattern was only observed in males, while no females have been reported (Table 3); however, we are aware of two such females (W.B.D., unpublished data). The males with this SBH subtype (22, 23, 24, 25 and 26) exhibited the most severe clinical phenotype (Tables 1–3). Early development and cognitive functioning ranged from normal to profound mental retardation. Patient 22 with mild delay and patient 25 with normal early development were the youngest, aged 3 years and 2 months, respectively, at the time of evaluation. The most severe degree of malformation in this group of patients is in the frontal cortex, an area that is relatively silent until 7 years of age, when its full maturation is achieved (Luria, 1973). In the other three patients, now aged 8, 20 and 34 years, interaction with the environment was virtually absent. In this group, generalized tonic clonic status epilepticus, atypical absences or myoclonic drop attacks marked the onset of habitual seizures, and epilepsy was intractable. Patient 24 is the most severely affected individual with SBH ever reported.

Mean age of onset of epilepsy was earlier in the pachygyria-SBH group (10.3 months) as compared with the partial/intermediate (4.4 years) and the diffuse (5.7 years) SBH groups. This finding contributes to the difference in age of onset of epilepsy between males and females (Table 3), as well as to the differences in developmental and cognitive functioning. The increased number of partial posterior bands as well as of pachygyria-SBH among males may explain, respectively, the significant over-representation of normal/borderline intelligence and severe/profound mental retardation within this group. Conversely, females who had predominantly diffuse bands, a paucity of partial bands and no pachygyria-SBH exhibited mostly mild/moderate mental retardation.

The genetic basis of SBH in males

Mutations of *DCX* are the major cause of SBH. In all but one or two SBH patients in whom a mutation has been demonstrated, this involves the *DCX* gene (Pilz *et al*., 1999; Gleeson *et al*., 2000*b*). In females, *DCX* mutations have been associated either with diffuse bands with no apparent gradient (a = p) or with bands compatible with an anterior-to-posterior gradient (a > p) of the malformation (Pilz *et al*., 1999; Gleeson *et al*., 2000*a*). However, because *DCX* is located on the X chromosome, males who are hemizygous for a *DCX* mutation usually have lissencephaly (Dobyns *et al*., 1996; Pilz *et al*., 1999). The molecular findings in patients 1, 2, 13 and 25 illustrate that some missense mutations in the *DCX* gene have sufficient residual function to result in SBH rather than lissencephaly (Pilz *et al*., 1999). Patients 1, 2 and 25 have band types that, although different in their severity, fit the anterior-to-posterior (a > p) gradient, which is mostly associated with *DCX* mutations (Pilz *et al*., 1999; Gleeson *et al*., 2000*a*), whereas patient 13 has an a = p gradient. Conversely, the specific amino acid alteration and its position in the protein may explain the differences in the band types.

An additional mechanism by which DCX mutations may be responsible for SBH in males is that of somatic mosaicism, which simulates the situation found in females due to random X-inactivation. In our series, three (12, 15 and 30) of the seven patients with missense mutations were found to have somatic mosaicism, and all three had an a = p gradient.

Another patient (27), with a p > a gradient, had a mutation in intron 5 of *DCX*, which may or may not be pathogenic. Based on the unexpected gradient for *DCX*, which was the only exception to the usual rule, we hypothesize that this mutation is not pathogenic.

Only seven out of 24 (29%) of the males tested had a mutation in the coding region of the *DCX* gene. This confirms the rare occurrence of DCX mutations in males. The over-representation of atypical bands (partial, intermediate and pachygyria-SBH), the paucity of classical diffuse and anteriorly predominating SBH, and the predominance of sporadic cases in males suggests that mutations of other genes responsible for corticogenesis (D'Arcangelo *et al*., 1995; Ogawa *et al*., 1995; Rakic and Caviness, 1995; Anton *et al*., 1997, 1999; Gonzales *et al*., 1997; Rio *et al*., 1997; Yoneshima *et al*., 1997; Fox *et al*., 1998; Rice *et al*., 1998) may operate to cause SBH in males.

An ideal candidate gene is *LIS1*. *LIS1* mutations are usually associated with isolated lissencephaly sequence (ILS) or with Miller Dieker syndrome (MDS), diseases with agyria/pachygyria (Dobyns *et al*., 1991, 1993; Lo Nigro *et al*., 1997; Pilz *et al*., 1998). The malformations caused by mutations in *LIS1* have a posterior predominance unless the deletion size is very large and involves a second neuronal migration gene located more distally in chromosome 17p. The children all have MDS and diffuse lissencephaly (W.B.D., unpublished results). The *LIS1* point mutation detected in patient 19 confirms the role played by *LIS1* mutations in some patients with SBH who exhibit a posterior-to-anterior gradient. This also suggests that a single amino acid change in a critical domain of the LIS1 protein, rather than a deletion, may explain a milder phenotype such as SBH (Pilz *et al*., 1999). The novel mutation in intron 10 of *LIS1* found in patient 17 may or may not be pathogenic. We hypothesize that this mutation is pathogenic, as the MRI of this patient closely resembles the MRI of the patient who has a missense mutation of *LIS1*. If indeed it is pathogenic, this

would represent only the second mutation of *LIS1* associated with SBH. In general, the functional consequences of intron mutations are uncertain. While they may be pathogenic, functional studies are required to prove this conclusively.

These results recapitulate previous observations in patients with lissencephaly, in whom a = p and a > p gradients were seen in males with *DCX* mutations, and p > a gradients were seen in patients with *LIS1* mutations (Pilz *et al*., 1998; Dobyns *et al*., 1999). A few a = p gradients were also seen among patients with *LIS1* mutations, all of whom have deletions extending from *LIS1* toward the telomere that deletes a second gene known to be involved in neuronal migration (W.B.D., unpublished data). No mutation was detected in 14 of the 24 patients studied. While sequencing of the coding region may miss some mutations of a gene, usually ~10%, failure to detect mutations in more than half of the patients tested suggests additional loci.

One of these loci is likely to be located on chromosome 9p (Federico *et al*., 1999), based on observation of trisomy 9p in patient 18. Mutation analysis of *DCX* and *LIS1* in this patient with a diffuse band was negative. Several other reported patients with this same duplication have had epilepsy, mental retardation and brain abnormalities, possibly due to a neuronal migration disorder (Stern, 1996; Saneto *et al*., 1998). The preponderance of females [14 out of 20 (70%) in the review by Wilson *et al*., 1985] with this syndrome would suggest that males are more severely affected with a higher likelihood of prenatal and perinatal death (Wilson *et al*., 1985).

It should be noted that *DCX* mutations can be observed in mothers and maternal relatives of patients with SBH who may or may not be symptomatic, and may or may not have detectable MRI evidence of SBH.

The small number of mutations identified to date in males with SBH and the preponderance of sporadic cases might suggest that additional mechanisms may be responsible for SBH in males. These include mutations in the non-coding regions of the *LIS1* or *DCX* genes, as suggested by the findings in patients 17 and 27, alternative splicing or somatic mosaicism (patients 12, 15 and 30), gonadal mosaicism (Matsumoto *et al*., 2001) and finally mutations of other genes.

Conclusion

In conclusion, our report demonstrates that SBH in males is a clinically heterogeneous syndrome, mostly occurring sporadically. The clinical spectrum is similar to that of females with SBH, but there is increased heterogeneity with respect to the neuroradiological, cognitive and molecular genetic aspects. Males had a higher frequency of partial and intermediate SBH, diffuse SBH with posterior predominance and pachygyria-SBH, as well as a significantly lower frequency of classical diffuse band heterotopia compared with females. Seven patients had missense mutations in *DCX*, three of whom had somatic mosaicism. Four additional male patients with somatic mosaicism have recently been identified (Kato *et al*., 2001; Poolos *et al*., 2002), confirming the importance of this mechanism in the aetiology of SBH in males.

A missense mutation of *LIS1* and partial trisomy of chromosome 9p have been identified in one patient each. The absence of mutations in the coding sequences of these genes in the remaining patients differs from the findings in females and suggests other genetic mechanisms.

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References

Andermann E, Andermann F. Genetic aspects of neuronal migration disorders. X-linked inheritance in subcortical band and periventricular nodular heterotopia; familial occurrence of bilateral perisylvian polymicrogyria. In: Guerrini R, Andermann F, Canapicchi R, Roger J, Zifkin BG, Pfanner P, editors. Dysplasias of cerebral cortex and epilepsy. Philadelphia: Lippincott-Raven; 1996. p. 11–5.

Anton ES, Marchionni MA, Lee KF, Rakic P. Role of GGF/neuregulin signaling in interactions between migrating neurons and radial glia in the developing cerebral cortex. Development 1997; 124: 3501–10.

Anton ES, Kreidberg JA, Rakic P. Distinct functions of α_3 and α_V integrin receptors in neuronal migration and laminar organization of the cerebral cortex. Neuron 1999; 22: 277–89.

Barkovich AJ, Jackson DE Jr, Boyer RS. Band heterotopias: a newly recognized neuronal migration anomaly. Radiology 1989; 171: 455–8.

Barkovich AJ, Guerrini R, Battaglia G, Kalifa G, N'Guyen T, Parmeggiani A, et al. Band heterotopia: correlation of outcome with magnetic resonance imaging parameters. Ann Neurol 1994; 36: 609–17.

Berg MJ, Schiffitto G, Powers JM, Martinez-Capolino C, Fong CT, Myers GJ, et al. X-linked female band heterotopia-male lissencephaly syndrome. Neurology 1998; 50: 1143–6.

Cardoso C, Leventer RJ, Matsumoto N, Kuc JA, Ramocki MB, Mewborn SK, et al. The location and type of mutation predict malformation severity in isolated lissencephaly caused by abnormalities within the LIS1 gene. Hum Mol Genet 2000; 9: 3019–28.

Cardoso C, Leventer RJ, Dowling JJ, Ward HL, Chung J, Petras KS, et al. Clinical and molecular basis of classical lissencephaly:

mutations in the LIS1 gene (PAFAHIBI). [Review]. Hum Mutat 2002; 19: 4–15.

Chong SS, Pack SD, Roschke AV, Tanigami A, Carrozzo R, Smith AC, et al. A revision of the lissencephaly and Miller–Dieker syndrome critical regions in chromosome 17p13.3. Hum Mol Genet 1997; 6: 147–55.

CCTILAE. Proposal for revised clinical and electroencephalographic classification of epileptic seizures. Epilepsia 1981; 22: 489–501.

CCTILAE. Proposal for revised classification of epilepsies and epileptic syndromes. Epilepsia 1989; 30: 389–99.

D'Arcangelo G, Miao GG, Chen S-C, Soares HD, Morgan JI, Curran T. A protein related to extracellular matrix proteins deleted in the mouse mutant reeler. Nature 1995; 374: 719–23.

De Volder AG, Gadisseux J-F, Michel CJ, Maloteaux J-M, Bol AC, Grandin CB, et al. Brain glucose utilization in band heterotopia: synaptic activity of 'double cortex'. Pediatr Neurol 1994; 11: 290–4.

des Portes V, Pinard JM, Smadja D, Motte J, Boespflug-Tanguy O, Moutard ML, et al. Dominant X-linked subcortical laminar heterotopia and lissencephaly syndrome (X-SCLH/LIS): evidence for the occurrence of mutation in males and mapping of a potential locus in Xq22. J Med Genet 1997; 34: 177–83.

des Portes V, Pinard JM, Billuart P, Vinet MC, Koulakoff A, Carrié A, et al. A novel CNS gene required for neuronal migration and involved in X-linked subcortical laminar heterotopia and lissencephaly syndrome. Cell 1998a; 92: 51–61.

des Portes V, Francis F, Pinard JM, Desguerre I, Moutard ML, Snoeck I, et al. Doublecortin is the major gene causing X-linked subcortical laminar heterotopia (SCLH). Hum Mol Genet 1998b; 7: 1063–70.

Dobyns WB, Curry CJ, Hoyme HE, Turlington L, Ledbetter DH. Clinical and molecular diagnosis of Miller–Dieker syndrome. Am J Hum Genet 1991; 48: 584–94.

Dobyns WB, Reiner O, Carrozzo R, Ledbetter DH. Lissencephaly: a human brain malformation associated with deletion of the LIS1 gene located at chromosome 17p13. [Review]. JAMA 1993; 270: 2838–42.

Dobyns WB, Andermann E, Andermann F, Czapansky-Beilman D, Dubeau F, Dulac O, et al. X-linked malformations of neuronal migration. [Review]. Neurology 1996; 47: 331–9.

Dobyns WB, Truwit CL, Ross ME, Matsumoto N, Pilz DT, Ledbetter DH, et al. Differences in the gyral pattern distinguish chromosome 17-linked and X-linked lissencephaly. Neurology 1999; 53: 270–7.

Federico A, Tomasetti P, Zollino M, Diomedi M, Dotti MT, De Stefano N, et al. Association of trisomy 9p and band heterotopia. Neurology 1999; 53: 430–2.

Fox JW, Lamperti ED, Eksioglu YZ, Hong SE, Feng Y, Graham DA, et al. Mutations in filamin 1 prevent migration of cerebral cortical neurons in human periventricular heterotopia. Neuron 1998; 21: 1315–25.

Franzoni E, Bernardi B, Marchiani V, Crisanti AF, Marchi R, Fonda C. Band brain heterotopia. Case report and literature review. [Review]. Neuropediatrics 1995; 26: 37–40.

Gallucci M, Bozzao A, Curatolo P, Splendiani A, Cifani A, Passariello R. MR imaging of incomplete band heterotopia. AJNR Am J Neuroradiol 1991; 12: 701–2.

Gigli GL, Tomassetti P, Diomedi M, Chierichetti F, Blasi C, Dotti MT, et al. Doppia corteccia, epilessia e ritardo mentale grave in un soggetto maschio con cariotipo 47XY+der(9). Boll Lega Ital Epil 1996; 95/96: 399–401.

Gleeson JG, Allen KM, Fox JF, Lamperti ED, Berkovic S, Scheffer I, et al. Doublecortin, a brain-specific gene mutated in human X-linked lissencephaly and double cortex syndrome, encodes a putative signaling protein. Cell 1998; 92: 63–72.

Gleeson JG, Minnerath SR, Fox JF, Allen KM, Luo RF, Hong SE, et al. Characterization of mutations in the gene doublecortin in patients with the double cortex syndrome. Ann Neurol 1999; 45: 146–53.

Gleeson JG, Luo RF, Grant PE, Guerrini R, Huttenlocher PR, Berg MJ, et al. Genetic and neuroradiological heterogeneity of double cortex syndrome. Ann Neurol 2000a; 47: 265–9.

Gleeson JG, Minnerath S, Kuzniecky RI, Dobyns WB, Young ID, Ross ME, et al. Somatic and germline mosaic mutations in the doublecortin gene are associated with variable phenotypes. Am J Hum Genet 2000b; 67: 574–81.

Gonzales JL, Russo CJ, Goldowitz D, Sweet HO, Davisson MT, Walsh CA. Birthdate and cell marker analysis of scrambler: a novel mutation affecting cortical development with a reeler-like phenotype. J Neurosci 1997; 17: 9204–11.

Guerrini R, Carrozzo R. Epilepsy and genetic malformations of the cerebral cortex. [Review]. Am J Med Genet 2001; 106: 160–73.

Harding B. Gray matter heterotopia. In: Guerrini R, Andermann F, Canapicchi R, Roger J, Zifkin BG, Pfanner P, editors. Dysplasias of cerebral cortex and epilepsy. Philadelphia: Lippincott-Raven; 1996. p. 81–8.

Hashimoto R, Seki T, Takuma Y, Suzuki N. The 'double cortex' syndrome on MRI. Brain Dev 1993; 15: 57–9.

Hattori M, Adachi H, Tsujimoto M, Arai N, Inoue K. Miller–Dieker lissencephaly gene encodes a subunit of brain platelet-activating factor acetylhydrolase [corrected]. Nature 1994; 370: 216–8.

Horesh D, Shapir T, Francis F, Wolf SG, Caspi M, Elbaum M, et al. Doublecortin, a stabilizer of microtubules. Hum Mol Genet 1999; 8: 1599–610.

Iannetti P, Raucci U, Basile LA, Spalice A, Di Biasi C, Trasimeni G, et al. Neuronal migrational disorders: diffuse cortical dysplasia or the 'double cortex' syndrome. Acta Paediatr 1993; 82: 501–3.

Jacob H. Faktoren bei der Entstehung der normalen und der entwicklungsgestörten Hirnrinde. Z Ges Neurol Psychiat 1936; 155: 1–39.

Jacob H. Genetisch verschiedene Gruppen entwicklungsgestörter Gehirne. Z Ges Neurol Psychiat 1938; 160: 615–48.

Kato M, Kanai M, Soma O, Takusa Y, Kimura T, Numakura C, et al. Mutation of the doublecortin gene in male patients with double

cortex syndrome: somatic mosaicism detected by hair root analysis. Ann Neurol 2001; 50: 547–51.

Ketonen L, Roddy S, Lannan M. Band heterotopia. J Child Neurol 1994; 9: 384–5.

Landy HJ, Curless RG, Ramsay RE, Slater J, Ajmone-Marsan C, Quencer RM. Corpus callosotomy for seizures associated with band heterotopia. Epilepsia 1993; 34: 79–83.

Ledbetter SA, Kuwano A, Dobyns WB, Ledbetter DH. Microdeletions of chromosome 17p13 as a cause of isolated lissencephaly. Am J Hum Genet 1992; 50: 182–9.

Lo Nigro C, Chong CS, Smith AC, Dobyns WB, Carrozzo R, Ledbetter DH. Point mutations and an intragenic deletion in LIS1, the lissencephaly causative gene in isolated lissencephaly sequence and Miller–Dieker syndrome. Hum Mol Genet 1997; 6: 157–64.

Luria AR. The working brain: an introduction to neuropsychology. New York: Basic Books; 1973.

Matell M. Ein Fall von Heterotopie der grauen Substanz in den beiden Hemisphären des Grosshirns. Arch Psychiat Nervkrankh 1893; 25: 124–36.

Matsumoto N, Leventer RJ, Kuc JA, Mewborn SK, Dudlicek LL, Ramocki MB, et al. Mutation analysis of the DCX gene and genotype/phenotype correlation in subcortical band heterotopia. Eur J Hum Genet 2001; 9: 5–12.

Miura K, Watanabe K, Maeda N, Matsumoto A, Kumagai T, Ito K, et al. Magnetic resonance imaging and positron emission tomography of band heterotopia. Brain Dev 1993; 15: 288–90.

Ogawa M, Miyata T, Nakajima K Yagyu K, Seike M, Ikenaka K, et al. The reeler gene-associated antigen on Cajal-Retzius neurons is a crucial molecule for laminar organization of cortical neurons. Neuron 1995; 14: 899–912.

Ono J, Mano T, Andermann E, Harada K, Sakurai K, Ikeda T, et al. Band heterotopia or double cortex in a male: bridging structures suggest abnormality of the radial glial guide system. Neurology 1997; 48: 1701–3.

Palmini A, Andermann F, Aicardi J, Dulac O, Chaves F, Ponsot G, et al. Diffuse cortical dysplasia, or the 'double cortex' syndrome: the clinical and epileptic spectrum in 10 patients. Neurology 1991; 41: 1656–62.

Palmini A, Andermann F, de Grissac H, Tampieri D, Robitaille Y, Langevin P, et al. Stages and patterns of centrifugal arrest of diffuse neuronal migration disorders. Dev Med Child Neurol 1993; 35: 331–9.

Parmeggiani A, Santucci M, Ambrosetto P, Amadi A, Baioni E, Rossi PG. Interictal EEG findings in two cases with 'double cortex' syndrome. Brain Dev 1994; 16: 320–4.

Pilz DT, Matsumoto N, Minnerath S, Mills P, Gleeson JG, Allen KM, et al. LIS1 and XLIS (DCX) mutations cause most classical lissencephaly, but different patterns of malformation. Hum Mol Genet 1998; 7: 2029–37.

Pilz DT, Kuc J, Matsumoto N, Bodurtha J, Bernadi B, Tassinari CA, et al. Subcortical band heterotopia in rare affected males can be caused by missense mutations in DCX (XLIS) or LIS1. Hum Mol Genet 1999; 8: 1757–60.

Pilz D, Stoodley N, Golden JA. Neuronal migration, cerebral cortical development and cerebral cortical anomalies. [Review]. J Neuropathol Exp Neurol 2002; 61: 1–11.

Pinard JM, Motte J, Chiron C, Brian R, Andermann E, Dulac O. Subcortical laminar heterotopia and lissencephaly in two families: a single X linked dominant gene. J Neurol Neurosurg Psychiatry 1994; 57: 914–20.

Pinard JM, Feydy A, Carlier R, Perez N, Pierot L, Burnod Y. Functional MRI in double cortex: functionality of heterotopia. Neurology 2000; 54: 1531–3.

Poolos NP, Das S, Clark GD, Lardizabal D, Noebels JL, Wyllie E, et al. Males with epilepsy, complete subcortical band heterotopia, and somatic mosaicism for DCX. Neurology 2002; 58: 1559–62.

Rakic P, Caviness VS Jr. Cortical development: view from neurological mutants two decades later. [Review]. Neuron 1995; 14: 1101–4.

Reiner O, Carrozzo R, Shen Y, Wehnert M, Faustinella F, Dobyns WB, et al. Isolation of a Miller–Dieker lissencephaly gene containing G protein β-subunit-like repeats. Nature 1993; 364: 717–21.

Ricci S, Cusmai R, Fariello G, Fusco L, Vigevano F. Double cortex. A neuronal migration anomaly as possible cause of Lennox–Gastaut syndrome. Arch Neurol 1992; 49: 61–4.

Rice DS, Sheldon M, D'Arcangelo G Nakajima K, Goldowitz D, Curran T. Disabled-1 acts downstream of reelin in a signaling pathway that controls laminar organization in the mammalian brain. Development 1998; 125: 3719–29.

Rio C, Rieff HI, Qi P, Khurana TS, Corfas G. Neuregulin and erbB receptors play a critical role in neuronal migration. Neuron 1997; 19: 39–50.

Ross ME, Allen KM, Srivastava AK, Featherstone T, Gleeson JG, Hirsch B, et al. Linkage and physical mapping of X-linked lissencephaly/SBH (XLIS): a gene causing neuronal migration defects in human brain. Hum Mol Genet 1997; 6: 555–62.

Saneto RP, Applegate KE, Frankel DG. Atypical manifestations of two cases of trisomy 9 syndrome: rethinking development delay. Am J Med Genet 1998; 80: 42–5.

Sapir T, Elbaum M, Reiner O. Reduction in microtubule catastrophe events by LIS1, platelet-activating factor acetylhydrolase subunit. EMBO J 1997; 16: 6977–84.

Scheffer IE, Mitchell LA, Howell RA, Fitt G, Syngeniotis A, Saling M, et al. Familial band heterotopias: an X linked dominant disorder with variable severity [abstract]. Ann Neurol 1994; 36: 511.

Sossey-Alaoui K, Hartung AJ, Guerrini R, Manchester DK, Posar A, Puche-Mira A, et al. Human doublecortin (DCX) and the homologous gene in mouse encode a putative Ca^{2+}-dependent signaling protein which is mutated in human X-linked neuronal migration defects. Hum Mol Genet 1998; 7: 1372–32.

Soucek D, Birbamer G, Luef G, Felber S, Kristmann E, Bauer G. Laminar heterotopic grey matter (double cortex) in a patient with late onset Lennox–Gastaut syndrome. Wien Klin Wochenschr 1992; 104: 607–8.

Stern JM. The epilepsy of trisomy 9p. Neurology 1996; 47: 821–4.

Tohyama J, Kato M, Koeda T, Inagaki M, Ohno K. The double cortex syndrome. Brain Dev 1993; 15: 83–4.

Vossler GD, Lee JK, Ko TS. Treatment of seizures in subcortical laminar heterotopia with corpus callosotomy and lamotrigine. J Child Neurol 1999; 14: 282–8.

Wiest WD, Hallervorden J. Migrationshemmung in Groß- und Kleinhirn. Dt Z Nervheilk 1958; 178: 224–38.

Wilson GN, Raj A, Baker D. The phenotypic and cytogenetic spectrum of partial trisomy 9. Am J Med Genet 1985; 20: 277–82.

Yoneshima H, Nagata E, Matsumoto M, Yamada M, Nakajima K, Miyata T, et al. A novel neurological mutation of mouse, yotari, which exhibits a reeler-like phenotype but expresses CR-50 antigen/reelin. Neurosci Res 1997; 29: 217–23.

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