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ARTICLE

14q12 microdeletions excluding *FOXG1* give rise to a congenital variant Rett syndrome-like phenotype

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Rett syndrome is a clinically defined neurodevelopmental disorder almost exclusively affecting females. Usually sporadic, Rett syndrome is caused by mutations in the X-linked *MECP2* gene in ~90–95% of classic cases and 40–60% of individuals with atypical Rett syndrome. Mutations in the *CDKL5* gene have been associated with the early-onset seizure variant of Rett syndrome and mutations in *FOXG1* have been associated with the congenital Rett syndrome variant. We report the clinical features and array CGH findings of three atypical Rett syndrome patients who had severe intellectual impairment, early-onset developmental delay, postnatal microcephaly and hypotonia. In addition, the females had a seizure disorder, agenesis of the corpus callosum and subtle dysmorphism. All three were found to have an interstitial deletion of 14q12. The deleted region in common included the *PRKD1* gene but not the *FOXG1* gene. Gene expression analysis suggested a decrease in *FOXG1* levels in two of the patients. Screening of 32 atypical Rett syndrome patients did not identify any pathogenic mutations in the *PRKD1* gene, although a previously reported frameshift mutation affecting *FOXG1* (c.256dupC, p.Gln86ProfsX35) was identified in a patient with the congenital Rett syndrome variant. There is phenotypic overlap between congenital Rett syndrome variants with *FOXG1* mutations and the clinical presentation of our three patients with this 14q12 microdeletion, not encompassing the *FOXG1* gene. We propose that the primary defect in these patients is misregulation of the *FOXG1* gene rather than a primary abnormality of *PRKD1*.
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INTRODUCTION

Rett syndrome is a severe neurodevelopmental disorder caused by mutations in the X-linked gene encoding the methyl-CpG-binding protein, *MECP2*. In the classic form of Rett syndrome (MIM 312750), females have psychomotor regression, profound intellectual impairment, seizures and stereotypic hand movements.[1] Usually sporadic, Rett syndrome is caused by mutations in the X-linked *MECP2* gene in ~90–95% of classic cases and 40–60% of individuals with atypical Rett syndrome.[2] A number of Rett syndrome variants have been described including the congenital, the early-onset seizure and the preserved speech variants. Mutations in the *CDKL5* gene have been associated with the early-onset seizure variant of Rett syndrome, although the predominant phenotype is an epileptic encephalopathy.[3–5] The congenital variant is characterised by infantile hypotonia and developmental delay evident earlier than in classic Rett syndrome but causative mutations have previously rarely been identified in these patients.[6]

Recent reports of microdeletions in the 14q12 region found in Rett syndrome patients have found a third gene, forkhead box protein G1 (*FOXG1*), to be highly associated with the congenital variant.[7,8] The FoxG1 protein is a winged-helix transcription factor and its expression is highest in fetal and infant brain. It is involved in telencephalic development,[9] promotes neurogenesis and antagonises neuronal differentiation.[10,11] At least eight instances of 14q12 microdeletions have been reported.[7,8,12–14] Mutation analyses have identified *de novo* loss-of-function point mutations in *FOXG1*, particularly in patients with the congenital form of Rett syndrome.[15–17] Duplications of *FOXG1* are also found in patients with epilepsy and intellectual impairment,[18–21] highlighting the importance of gene dosage at this locus, although more recently the pathogenicity of *FOXG1* duplications has been questioned.[22]

We report the clinical features and array CGH findings of three atypical Rett syndrome patients. The three patients, two female and one male, all had intellectual impairment, early-onset developmental delay without regression, microcephaly and hypotonia. In addition, the females had a seizure disorder and agenesis of the corpus callosum. All three were found to have an interstitial deletion of 14q12. The commonly deleted region included the *PRKD1* gene but not the *FOXG1* gene.

MATERIALS AND METHODS

Case report 1

We first reviewed this girl at age 7 years and 8 months. She was referred for an opinion regarding the possible underlying diagnosis of Rett syndrome as a cause of developmental delay, microcephaly, hypotonia and seizure disorder.

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The girl was the second child to non-consanguineous parents. She was born by normal vaginal delivery at 42 weeks of gestation, after a normal pregnancy. Her birth weight was 3.46 kg (25–50th percentile), length was 53 cm (50–75th percentile) and head circumference was 33.5 cm (8th percentile). Developmental milestones were delayed. She rolled at 4 months, but was not able to sit without support until ~1 year of age and never learnt to walk. She was never able to hold a bottle, feed herself and did not develop a pincer grip. Her parents reported that she constantly had her hands in her mouth. Her hearing was normal. At 1 year of age she developed infantile spasms. A gastrostomy was placed for poor weight gain and feeding difficulties. She suffered with constipation, had bruxism and a disturbed sleep pattern.

Repeated examinations over time demonstrated subtle dysmorphic features, including an oval-shaped face, depressed nasal bridge, anteverted nares, a full and everted lower lip and limited facial expression with a tendency to hold the mouth open. At 7 years and 8 months of age, her weight was 14 kg (<3rd percentile) and head circumference was 43.5 cm (<<1st percentile). She demonstrated mid-line hand grasping and hand wringing movements, tongue thrusting and hand mouthing. She had a left esotropia, small, cold hands and feet with relatively long fingers and toes, left second toe clinodactyly and shortening of the 4th and 5th metatarsals. There was one café-au-lait lesion over the right buttock and she had a relatively hirsute back. She had truncal hypotonia and was unable to sit without support. She had mild limb hypertonia and brisk deep tendon reflexes, which over time developed into spastic quadriparesis associated with fixed flexion deformities. Her seizures continued, becoming tonic–clonic, and were associated with choreoathetoid movements. She subsequently developed a thoracolumbar scoliosis and a disturbed breathing pattern with episodes of hyperventilation. She was noted to have reduced bone mineral density and was treated with pamidronate infusions.

A cerebral MRI scan revealed agenesis of the corpus callosum and relatively small brain size. Karyotype, methylation studies for Angelman syndrome, subtelomere FISH studies, *MECP2* mutation testing, including MLPA studies and *CDKL5* analysis, were normal. In addition, urinary amino and organic acid analyses, plasma lactate, pyruvate, ammonia, transferrin isoforms, very long chain fatty acids and TORCH serology were normal. Array CGH studies detected a 1.17-Mb deletion of 14q12. Parental studies were normal. See 'Chromosomal microarray analysis methods' for procedural details.

The patient died at age 16 years from an acute respiratory illness complicating a slow deterioration in her general health.

Case report 2

This 11-year-old girl was referred for assessment of profound global developmental delay, microcephaly, truncal hypotonia, seizures and hand stereotypies. She was born after a normal pregnancy and vaginal delivery at 38 weeks of gestation. Her birth weight was 2.6 kg (9th percentile), length was 46 cm (6th percentile) and head circumference was 31 cm (1st percentile). She was hypotonic from birth, resulting in some initial feeding difficulties. At 3 months, she was noted to have a convergent strabismus and tongue thrusting movements. All developmental milestones were significantly delayed – she smiled at 2 months, rolled at 14 months and sat unsupported in her second year. She never learnt to walk. She reaches for an object but her hand function was restricted to swiping at objects and she has not developed a pincer grip. She had limited babbled only. A formal assessment at age 13 months indicated profound developmental delay in all areas (Griffith Mental Developmental Scale). Her general health and appetite were good but she was not able to feed herself and suffered with constipation. She had a disturbed sleep pattern, episodes of deep breathing, tongue thrusting and hand mouthing movements. At 11 years of age she developed a seizure disorder. The EEG demonstrated occasional centro-parietal epileptiform activity on a slow background and hence she was commenced on carbamazepine.

On examination at 11 years and 9 months, her weight was 39 kg (40th percentile), length was 142 cm (20th percentile) and head circumference was 50.5 cm (<1st percentile). She had truncal hypotonia, limb hypertonia and brisk deep tendon reflexes. There was no scoliosis. She demonstrated midline hand grasping, hand wringing and hand-to-mouth movements. She had an alternating esotropia corrected with spectacles for hypermetropia. She had relatively long tapered fingers and toes (75th centile). Her facial features were subtly different to her parents with microcephaly, long palpebral fissures, slightly upturned palpebral fissures, short upturned nose, cupped ears with simple thickened helices and mouth held open. She had a laughing 'happy' disposition and startled easily to noise, movement or light stimulation.

A cerebral MRI scan revealed slight prominence of the body of the lateral ventricles, especially posteriorly and agenesis of the corpus callosum. *MECP2* gene sequencing and deletion screening (MLPA studies) were normal. Normal results were obtained for other investigations including a karyotype, transferrin isoforms, methylation studies for Angelman syndrome (*SNRPN1* methylation) and urinary glycosaminoglycans, amino and organic acids. Chromosome microarray detected a 1.15-Mb deletion in chromosome 14q12. Parental studies indicated this was *de novo*.

Case report 3

We first saw this boy at age 6 months, when he was referred for an opinion regarding developmental delay and microcephaly. He was the second child to non-consanguineous parents. A left-sided pelvi-ureteric junction obstruction was detected antenatally. The mother carried a known balanced translocation (46, XX, t(12;21)(p11.2;p11.2) and the boy's karyotype, performed by amniocentesis, was reported as normal. He was born at term by caesarean section. His birth weight was 4.4 kg (97th percentile), length was 52.5 cm (50–75th percentile) and head circumference was 34 cm (14th percentile). Postnatally, he required surgery for the pelvi-ureteric junction obstruction. By 6 months of age, he had developed microcephaly with a head circumference of 39.7 cm (<3rd percentile) and positional plagiocephaly. He was nondysmorphic and his other growth parameters were normal; weight was 7.7 kg (50th percentile) and height was 68 cm (50th percentile). His fine and gross motor developmental milestones were delayed, although he had good social development. He had poor use of his hands. He had increased muscle tone in all limbs with brisk deep tendon reflexes in his lower limbs. Hearing and vision were normal. Following several apnoeic episodes at 10 months of age, he underwent a formal sleep study (polysomnography), which showed mixed central and obstructive sleep disordered breathing. There was progressive deceleration of head growth; at 1 year of age, his head circumference was 41.5 cm (>2 cm below the 2nd percentile). By 4 years of age, he had severe developmental delay. He had learnt to crawl and stand with support. His hand function was restricted to picking up objects and finger feeding. He had minimal speech, limited to a few single words and had learnt some sign language. He had difficulty feeding with tongue thrusting movements and gagging. He also had bruxism and cold feet. He suffered with constipation, had a disturbed sleep pattern with night laughter but had never had a seizure. He had midline hand grasping, hand tapping and hand-to-mouth movements. He had a happy disposition and startled easily.

Postnatally, a karyotype and TORCH screen were normal. Subsequently, at 2 years of age, array CGH detected a 1.80-Mb deletion of 14q12. Parental studies were normal.

The clinical features of all three patients are summarised in Table 1.

Sample collection

Patient samples were received for clinical diagnostic testing and parental samples were subsequently obtained. Informed consent was obtained from the parents of the patients.

Chromosomal microarray analysis methods

For all three patients, CGH microarray analysis was performed at the Cytogenetics Department at The Children's Hospital at Westmead, using *Agilent Sureprint G3Hmn 400K* arrays (Agilent Technologies, Santa Clara, CA, USA), with data analysed at an effective median resolution of 0.06 Mb. Arrays were handled and loaded as per the manufacturer's specifications [http://www.genomics.agilent.com/]. Array scans were performed at 3 μm resolution, using an *Agilent* DNA Microarray Scanner, model 'SYS-SN-ARRAY'. Data analysis was performed using the ADM-2 algorithm, as supplied within '*Genomic Workbench Standard Edition 5.0*', with routine settings as follows: Aberration Algorithm: ADM-2, Threshold: 6.7, Centralisation: ON, Bin Size: 10, Centralisation Threshold 6.0, Fuzzy Zero: ON, Combine Replicates

Table 1 Clinical summary of patients from this study and previous reports of 14q12 microdeletions and translocations, and *FOXG1* mutations

	Patient 1	*Patient 2*	*Patient 3*	*Patients (n = 4) with 14q12 microdeletions*[a]	*Patients (n = 2) with translocations at 14q12*[b]	*Patients with FOXG1 point mutations*[c]	*Patient with FOXG1 mutation*
Age	Died at 16 years	12 years	4 years				7 years
Sex	Female	Female	Male	3 Female, 1 Male	2 Female	14 Female, 2 Male	Female
Gestation	42 weeks	36 weeks	40 weeks				40 weeks
Birth weight	3.46 kg	2.6 kg	4.4 kg	2.73–3.94 kg	3.36 kg, 3.35 kg	2.57–4.0 kg	2.78 kg
Birth length	53 cm	46 cm	52.5 cm	48.5–53 cm	49.5 cm, 51 cm	48–54 cm	49 cm
Birth head circumference	33.5 cm (8th percentile)	31 cm (1st percentile)	34 cm (14th percentile)	32–33 cm	36 cm, 32.5 cm	30–34 cm	32.5 cm (3rd percentile)
Postnatal microcephaly	Yes	Yes	Yes	4/4, Yes	2/2, Yes	11/12, Yes	Yes
Early development	Delayed	Delayed	Delayed	4/4, Delayed	2/2, Delayed	8/9, Delayed	Delayed
Psychomotor retardation	Profound	Profound	Severe	4/4, Yes, degree uncertain	2/2, Severe	16/16, Yes, degree uncertain	Profound
Hypotonia	Yes	Yes	Yes	4/4, Yes	1/1, Yes	6/7, Yes	Yes
Ability to walk	No	No	No	3/3, No	1/1, No	3/16, Yes (with aid)	No
Speech	No	No	Single words, no longer used	3/3, No	1 single words, lost at 2 years	15/16, No words, 1 single words	No
Hand function	Reach for object	Reach for object	Finger feed, pick up and hold object	1 with no hand movement, 2 with some movement	N/A	9/11, no hand function, 2 with grasping	Reach for object
Facial dysmorphism	Yes	Yes	Epicanthic folds	4/4, Yes	N/A	No	No
Scoliosis	Yes	No	No	2/3, Yes	N/A	6/14, Yes	No
Feeding difficulties	Yes	Yes	Yes	3/3, Yes	N/A	7/9, Yes	Yes
Bruxism	Yes	Yes	Yes	2/3, Yes	N/A	12/15, Yes	Yes
Cold extremities	Yes	Yes	Yes	1/3, Yes	N/A	5/9, Yes	Yes
Stereotypic movements	Hand grasping, wringing, mouthing	Hand wringing, mouthing	Hand grasping, tapping, mouthing	3/3 Yes	N/A	16/16, Yes	Midline hand clasping and hand wringing
Protruding tongue	Yes	Yes	Yes	2/3, Yes	N/A	5/8, Yes	No
Disturbed sleep pattern	Yes	Yes	Yes	1/1, Yes	N/A	6/10, Yes	Yes
Constipation	Yes	Yes	Yes	2/2, Yes	1/1, Yes	4/5, Yes	Yes
Awake breathing pattern	Hyperventilation	Episodes of deep breathing	Normal	N/A	N/A	1 report of exaggerated breathing	Normal
Seizures	Yes, onset 1 year	Yes, onset 11 years	No	3/4, Yes	2/2, Yes	8/16, Yes, onset between 4 months to 14 years	Yes, onset 1 year
Brain MRI	Agenesis of corpus callosum	Agenesis of corpus callosum Prominent ventricles	Normal	$\frac{3}{4}$, Normal, 1 with agenesis of corpus callosum	2/2, Agenesis of corpus callosum	2 normal, 13 with abnormalities, include hypoplasia of corpus callosum (5) and delayed myelination (5), also frontal gyral simplification (3) and enlargement of ventricles (1)	Delayed myelination, enlargement of ventricles
CGH array band, breakpoints and size of the deletion [Genome build = HG18]	14q12: 28 765 009–29 942 125 [1.17 Mb]	14q12: 28 407 180–29 557 749 [1.15 Mb]	14q12: 28 499 292–29 386 470 [0.89 Mb]	microdeletions of 14q12 ranging from 2.6 to 3.65 Mb	translocations with breakpoints 5 and 265 kb downstream of the primary transcript of *FOXG1*		
Parental studies	Normal	Normal	Normal	All deletions were *de novo*	*De novo* translocations		Normal

N/A, data not available.
[a]Data collected from Jacob *et al*,[7] Papa *et al*,[8] Bisgaard *et al*[12] and Mencarelli *et al*;[14] data from Kortüm *et al*[13] were excluded as microdeletion patients and point mutations were grouped together; information was not available (N/A) for all of the fields from all patients.
[b]Data collected from Shoichet *et al*[31] and Kortüm *et al*.[13]
[c]Data collected from Ariani *et al*,[17] Philippe *et al*,[25] Le Guen *et al*,[24,35] Mencarelli *et al*,[15] Bahi-Buisson *et al*,[16] Takahashi *et al*[26] and Van der Aa *et al*.[36]

(Intra Array): OFF, Genome: hg18, Aberration Filters: minProbes = 5 AND minAvgAbsLogRatio = 0.25 AND maxAberrations = 30 AND percent Penetrance = 0, Expand Non Unique Probes: OFF. For parental follow-up testing, the *Agilent SurePrint G3 Human CGH Microarray 8 × 60K* nontargeted array design was used, with effective median resolution of 0.25 Mb. All other test parameters were as above.

PRKD1 and *FOXG1* mutation screening

PRKD1 and *FOXG1* screening was carried out on 32 atypical Rett syndrome patients, previously excluded for mutations in *MECP2* and *CDKL5*. The coding region of the primary *FOXG1* transcript (NM_005249.4), and the 18 exons, including exon–intron boundaries, of *PRKD1* (NM_002742.2) were amplified from genomic DNA with primers designed with Primer3 software,[23] using GenBank reference sequence NC_000014.8 as the genomic reference. The A in the initiation codon is designated as +1. Primer sequences are given in Supplementary Table 1, with some *FOXG1* primers identical to those from other studies.[24,25] Bi-directional sequencing was carried out by Macrogen (Seoul, Korea) using an ABI3730XL sequencing apparatus (Life Technologies, Carlsbad, CA, USA). Sequence traces were analysed using Mutation Surveyor (SoftGenetics, State College, PA, USA).

Expression of *FOXG1* and *PRKD1* in patients with 14q12 microdeletions

Expression levels of *FOXG1* and *PRKD1* were measured in a lymphoblastoid cell line derived from white blood cells from case 1 and in whole blood collected in PAXgene Blood RNA tubes (Qiagen, Doncaster VIC, Australia) from cases 2 and 3. Two normal controls for each sample type were available. RNA extractions were carried out using RNeasy Mini Kit (Qiagen) for lymphoblastoid cell lines and PAXgene Blood RNA kit (Qiagen) for blood samples, followed by triplicate cDNA synthesis. Messenger levels for *FOXG1*, *PRKD1* and *GAPDH* were measured in duplicate using reverse-transcription quantitative PCR using a Corbett Rotorgene 6000 (Qiagen) and associated software. Statistical analysis (nonparametric tests for independent samples) was carried out using IBM SPSS Statistics package, Version 19 (Armonk, NY, USA).

RESULTS

Chromosomal microarray analysis

For all three patients in this study, the finding of a chromosome 14q microdeletion was initially determined by clinical comparative genomic hybridisation analysis. Parental samples were also tested, and no parents were found to carry any 14q12 deletion. The three deletions were all within chromosome band 14q12. Further details are outlined in Figure 1. Chromosome coordinates refer to chromosome build HG18.

The deletion in case 1 was of estimated minimum extent 1.17 Mb, extending from 28.765 to 29.942 Mb. Maximum extent was estimated at 1.21 Mb, from 28.747 to 29.962 Mb. The gene *PRKD1* lies within the deleted region. In case 2, the deletion was of minimum size 1.15 Mb, extending from 28.407 to 29.558 Mb (maximum extent was 1.18 Mb, from 28.396 to 29.574). The deletion includes the entire *PRKD1* gene, whereas *FOXG1* maps 0.1 Mb outside its proximal boundary. In case 3, the minimum deletion size was 0.89 Mb, extending from position 28.499 to 29.386 Mb (maximum size was 0.91 Mb, from 28.487 to 29.393). The deletion includes part of *PRKD1*, whereas *FOXG1* maps 0.18 Mb outside its proximal boundary.

PRKD1 and *FOXG1* mutation screening

A single-point mutation of *FOXG1* was identified in the cohort of 32 atypical Rett syndrome patients. A cytosine duplication at nucleotide 256 (c.256dupC) was identified in patient 4 with the clinical diagnosis of congenital Rett syndrome variant. The duplication is predicted to lead to a shift in the open-reading frame of the protein (p.Gln86Pro*34). Screening of the parents indicated that this mutation was *de novo*. The mutation has been previously identified in both a female and a male with congenital variant of Rett syndrome.[24,26]

Screening of the same 32 patients did not reveal any pathogenic mutations in *PRKD1*, a protein kinase involved in extracellular receptor-mediated signal transduction pathways. A number of known nonpathogenic polymorphisms were identified and shown in Table 2. A 5′UTR variation (c.-9C>A) was also identified in one patient. The variation was not reported in the NCBI dbSNP, and parental DNA was not available for screening. The promoter region of *PRKD1* was analysed using two software programs (BDGP Neural Network promoter prediction software, http://www.fruitfly.org/seq_tools/promoter.html[27] and Promo 3.0, http://alggen.lsi.upc.es/cgi-bin/promo_v3/promo/promoinit.cgi?dirDB=TF_8.3).[28] There were no differences between the predictions from the wild-type sequence and

Table 2 Known *PRKD1* polymorphisms identified in Rett syndrome patients

Variation[a]	*Heterozygosity*[b]	*Allele frequency in cohort*	*dbSNP ID*
c.91_96dupTCCGGG (p.G34_P35dup)	4.5%	8.6%	rs45471692
c.1673-50A>G	45.8%	64.1%	rs1191601
c.2167-12T>A	43.3%	41.2%	rs3783299
c.2202T>C (p.I734I)	50.4%	43.9%	rs2230505
c.2435-14G>A	45.0%	87.5%	rs2273815

[a]Variation naming based on GenBank accession file NC_000014.8, with the A in the initiation codon designated as +1.
[b]Heterozygosity level from dbSNP using the HapMap European population data.

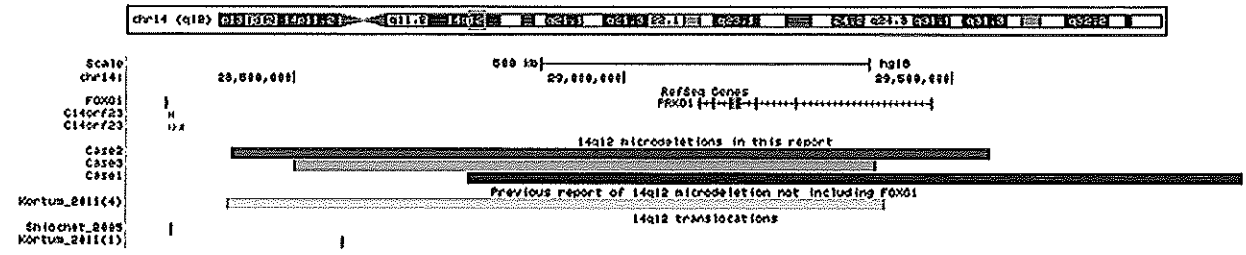

Figure 1 14q12 microdeletions excluding coding region of *FOXG1*. Microdeletions at 14q12 were detected in three patients in this report (cases 1–3) by array CGH. The *PRKD1* gene was disrupted but *FOXG1* remained intact in all three patients. A similar cytogenetic finding has been reported in patient 4 in Kortüm *et al*.[13] There were also two patients of similar phenotypes reported by Shoichet *et al*[31] and Kortüm *et al*,[13] with translocations affecting 14q12 and breakpoints downstream of *FOXG1*.

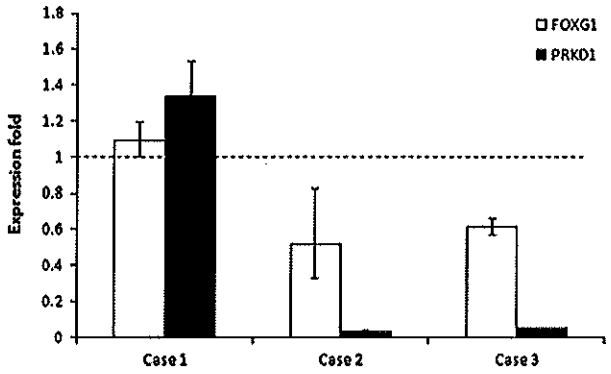

Figure 2 Expression of *PRKD1* and *FOXG1* in 14q12 microdeletion patients. Folds of expression, relative to two normal controls, of *FOXG1* (white bars) and *PRKD1* (black bars) in the three patients were measured in a lymphoblast cell line for case 1 and in whole blood from cases 2 and 3. Expressions of both genes and a housekeeping gene (*glyceraldehyde 3-phosphate dehydrogenase*) were measured using triplicate cDNA per sample (means of triplicate cDNA shown, with error bars showing SD).

that containing c.-9C>A. It is unlikely that this sequence variation has any effect on the initiation of transcription of *PRKD1*. Similarly, prediction for translation initiation using NetStart1.0[29] (http://www.cbs.dtu.dk/services/NetStart/) gave near-identical results between the wild-type and mutated sequences, suggesting that c.-9C>A also has no effect on translation of *PRKD1* protein.

Expression of *FOXG1* and *PRKD1* in patients with 14q microdeletions

Both *FOXG1* and *PRKD1* mRNA expression levels appeared to be decreased in whole-blood samples from cases 2 and 3 (Figure 2). Statistical analysis carried out by considering triplicate cDNA as distinct samples, giving $n=6$, indicated that the expression differences observed in both genes were statistically significantly reduced (nonparametric Mann–Whitney *U*-test, $P<0.005$). Using lymphoblast cell lines from case 1 and controls, there was no difference in either *FOXG1* or *PRKD1* expression between the patient and the controls. However, these triplicates did not represent true biological replicates and inclusion of similar samples in the future would be required to establish the significance of these findings.

DISCUSSION

The 14q12 microdeletion is associated with a clinically recognisable phenotype, characterised by severe mental retardation with a normal perinatal period and early delay in achievement of developmental milestones.[14] The clinical course resembles Rett syndrome with postnatal microcephaly and growth retardation, hypotonia, epilepsy, stereotypic hand movements and feeding problems. However, unlike classical Rett syndrome, there is early developmental delay with little or no regression and more significant microcephaly. In addition, some patients have been reported to have mild dysmorphism, protruding tongue movements and agenesis of the corpus callosum. The clinical characteristics of patients with mutations of the *FOXG1* gene reported to date are similar,[13,30] although there has been one case report of a child with classical Rett syndrome having a *FOXG1* mutation.[25] There is phenotypic overlap between congenital Rett syndrome variants with *FOXG1* mutations and the clinical presentation of our three patients with this 14q12 microdeletion, not encompassing the *FOXG1* gene. The commonly deleted region is ~260 kb and includes the *PRKD1* gene but not the *FOXG1* gene.

The clinical features of our three cases are consistent with the diagnosis of atypical Rett syndrome of the congenital onset variant.[1] Cases 1 and 2 both had abnormal initial development with subsequent severe psychomotor delay, loss of some hand function, midline stereotypic hand movements, postnatal microcephaly, peripheral vasomotor disturbance, breathing abnormalities and tongue stereotypies. The girls did not learn to walk. In addition, they had a seizure disorder. Case 1 had infantile spasms at 1 year of age, whereas case 2 did not have seizures until 11 years of age. Both had agenesis of the corpus callosum on neuroimaging. Case 3 had abnormal initial development with subsequent severe psychomotor delay and some loss of acquired speech, midline stereotypic hand movements, bruxism, cold extremities, tongue stereotypies, impaired sleep pattern and postnatal microcephaly. We believe that there is a subtle pattern of dysmorphology associated with deletions of the 14q12 region. Cases 1 and 2 demonstrate some similarities of facial appearance, including an oval-shaped face, strabismus, full and everted lower lip with a tendency to hold the mouth open. In addition, the females have long fingers and toes, clinodactyly of the second toe (case 1) and shortening of the 4th and 5th metatarsals bilaterally. Case 3 had epicanthic folds. All three cases were described as having a 'happy disposition' and tongue thrusting movements.

Other reports of chromosomal abnormalities involving 14q12 have described patients with similar phenotypes to the three cases in this study (summarised in Table 1).[7,8,12,14] Of these, there has only been one previous microdeletion of 14q12 not involving *FOXG1*.[13] In addition, there were two translocations affecting chromosome 14 identified in patients with similar phenotypes, although the breakpoints on chromosome 14 mapped 5 and 265 kb downstream of the primary transcript of *FOXG1*.[13,31] The collective data suggest that *cis*-regulatory elements exist in the region between *FOXG1* and *PRKD1*, and we propose that they play an important role in the regulation of *FOXG1* expression. *In silico* analysis of the shared-deleted region identified two conserved regions containing potential regulatory elements, located 620 and 684 kb downstream from *FOXG1* (see Supplementary Table 2). The former region has also been proposed by Kortüm *et al*[13] to play a regulatory role.[13]

In support of this hypothesis, a decrease in *FOXG1* expression level was indicated in cases 2 and 3, despite its coding region remaining intact in both patients. *PRKD1* expression was also affected in both patients, although this might be partially attributed to the disruption of the *PRKD1* coding region caused by the microdeletions. Additional information confirming the specificity of the quantitation process can be found in Supplementary Figures S1–S4. The decreases were not observed in case 1, but this may be because of the change in sample type, as a clonal lymphoblastoid cell line was used instead of whole blood for case 1. Gene enhancer assays carried out in transgenic mice by another group indicated that some conserved sequence elements in the deleted region in common may have regulatory roles.[13]

In most of the reported 14q12 microdeletions thus far, the *PRKD1* gene has also been disrupted.[8,12–14] Including this report, only four cases of 14q12 microdeletions have found *FOXG1* gene to be intact, the other being reported by Kortüm *et al*.[13] In earlier reports and mutation analyses, *FOXG1* has been considered the primary pathogenic cause in the patients because of its role in the formation of the developing brain.[9] *FOXG1* is a transcriptional repressor with expression restricted to fetal and adult brain and testis.[32] In addition, overlapping expression of *FOXG1* and *MECP2* has been demonstrated in differentiating forebrain cortex.[17] FoxG1 is hypothesised to protect

postmitotic neurons from neurotoxic effects of MeCP2.[33] Although their exact interaction is yet to be defined, this may suggest a common pathway or interaction at crucial points of brain development.

We propose that the primary defect in our patients is misregulation of the *FOXG1* gene rather than a primary abnormality of *PRKD1* because of the similarities between the phenotype of those patients with 14q12 microdeletions and those with *FOXG1* point mutations, as well as the reductions in *FOXG1* expression observed in cases 2 and 3. However, a possible pathogenic role of loss of *PRKD1* in all the patients with 14q12 microdeletions cannot be disregarded. *PRKD1* encodes a serine–threonine kinase that plays a role in cell proliferation and differentiation, apoptosis, cardiac contraction and cancer.[34] There are currently no known mutations or disorders linked to mutations in *PRKD1*, nor is much known about the mechanism of its regulation of gene expression. We suggest that array CGH is a mandatory investigation in patients with a congenital Rett syndrome phenotype without a pathogenic mutation in *MECP2*, *CDKL5* or *FOXG1*.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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