Original article

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Phenotypes of children with 20q13.3 microdeletion affecting *KCNQ2* and *CHRNA4*

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ABSTRACT – In order to clarify the phenotypes of 20q13.33 microdeletion, clinical manifestations and genetic findings from four patients are discussed in relation to chromosomal microdeletions at 20q13.33. All patients had epileptic seizures mostly beginning within the neonatal period and disappearing by 4 months of age, similar to epilepsy phenotypes of benign familial neonatal seizures. We performed array comparative genomic hybridization analysis in order to investigate the chromosomal aberration. Developmental outcome was good in two patients with deletion restricted to three genes (*CHRNA4*, *KCNQ2*, and *COL20A1)*, whereas delay in developmental milestones was observed in the other two with a wider range of deletion. Information obtained from array comparative genomic hybridization may be useful to predict seizure and developmental outcome, however, there is no distinctive pattern of abnormalities that would arouse clinical suspicion of a 20q13.33 microdeletion. Deletion of *KCNQ2* and *CHRNA4* does not appear to affect seizure phenotype. Molecular cytogenetic techniques, such as array comparative genomic hybridization, will be necessary to clarify the relationship between phenotypes and individual genes within this region.

Key words: 20q microdeletion, *KCNQ2*, *CHRNA4*, epilepsy, developmental delay

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KCNQ2 was first discovered as a cause of benign familial neonatal epilepsy (BFNE) (Singh *et al.*, 1998). Recent studies have identified mutations in *KCNQ2* among children with neonatal or early infantile epileptic encephalopathy (Weckhuysen *et al.*, 2012). Located alongside *KCNQ2,* in tandem, is the *CHRNA4* gene, located on chromosome 20q13.33. *CHRNA4* is known as one of the causative genes of autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) (Steinlein *et al.*, 1995).

We previously reported children with 20q13.33 microdeletion, involving both *KCNQ2* and *CHRNA4,* showing a phenotype compatible with BFNE (Kurahashi *et al.*, 2009). Several researchers have reported variable phenotypes of 20q13.33 microdeletion (Béri-Deixheimer *et al.*, 2007; Traylor *et al.*, 2010; Mefford *et al.*, 2012; Pascual *et al.*, 2013). At present, phenotypes of 20q13.33 microdeletion have not been fully described. Here, we report clinical manifestations and genetic findings of two novel patients, as well as two previously reported cases, with 20q13.33 microdeletion, affecting both *KCNQ2* and *CHRNA4*. The epilepsy phenotypes associated with chromosomal microdeletions are discussed, and further compared with previous reports in the literature *(table 1)*.

Case studies

Patient 1 was an 18-month-old girl. She was the first child born to non-consanguineous healthy parents. She was born at 39 weeks of gestation by uneventful spontaneous delivery, with a birthweight of 2,946 g. She was noted to have seizures since 10 days of age. Her seizures were characterized by apnoea and motion arrest, followed by tonic convulsion of the upper extremities, lasting for 5 to 30 minutes and occurring several times a day. Although phenobarbital (PB) was first administered, her seizures persisted. Thereafter, carbamazepine (CBZ) was used and her seizures were completely controlled after 4 months of age. EEG at 4 and 12 months of age showed no abnormal findings. Head MRI at 12 months of age was also unremarkable. CBZ was discontinued at 15 months of age and a recurrence of seizures was not observed. No dysmorphic features were recognized and neurological examination showed no abnormalities. Her psychomotor developmental was mildly delayed. She walked alone at 18 months of age. At the last follow-up visit, at 25 months of age, she could speak several words and was capable of verbal communication, whereas two-word sentences were not recognized.

Patient 2 was a boy, the second child of non-consanguineous healthy parents. The patient was born at 37 weeks of gestation by spontaneous delivery, with a birthweight of 2,688 g. This patient had poor oral feeding, necessitating nasogastric feeding. At 3 months of age, the patient had a generalized convulsion, lasting for 90 seconds. Five days later, the patient had clustered unprovoked seizures, characterized by upper eye deviation, followed by blinking, right hemifacial twitching, and clonic convulsion of the right arm lasting for one minute. PB was administered and his seizures were completely controlled at a dose of 4 mg/kg/day. He had no dysmorphic features. EEG at 8 months of age revealed sporadic spikes in the left frontal area. MRI at 8 months of age showed mild atrophy with no myelination delay. His developmental milestones were mildly delayed. Sitting without support was recognized at 9 months of age, but he could not stand with support at 11 months of age.

Patients 3 and 4 have formerly been described in our previous report as IV-5 of Family 11 and III-1 of Family 17, respectively (Kurahashi *et al.*, 2009). Patient 3 had seizures at two days old. Her seizures were transiently controlled by PB, whereas seizures recurred at 3 months of age. Her seizures ceased after addition of zonisamide. She had no dysmorphic features. Her EEG and head MRI were unremarkable. Her development was unremarkable at the last follow-up visit at 13 years of age. Regarding family history, neonatal seizures were also observed in five paternal ancestors, four of whom had the same copy number variant at chromosome 20q. Patient 4 had seizures at one day old and seizures disappeared spontaneously at three days old. Her psychomotor development was normal at the last follow-up visit at 8 years of age. Her father also had neonatal seizures, as well as the same copy number variant at chromosome 20q.

Genetic studies

We performed array comparative genomic hybridization (aCGH) analysis, after obtaining written informed consent from the parents, using genomic DNA extracted from the patients' peripheral blood. For Patient 1, aCGH showed a 1.09-Mb deletion at 20q13.33, affecting 31 genes (*figure 1*). Her parents declined further genetic analysis. For Patient 2, a *de novo* 765-kb deletion was identified at 20q13.33, affecting 16 genes (*figure 1*). As shown in our previous report (Kurahashi *et al.*, 2009), Patient 3 had a paternally inherited 136.4-kb deletion, affecting *KCNQ2* (partial), *CHRN4* (complete), and *COL20A1* (partial). Patient 4 had a paternally inherited 171.8-kb deletion, affecting *KCNQ2* (complete), *CHRN4*

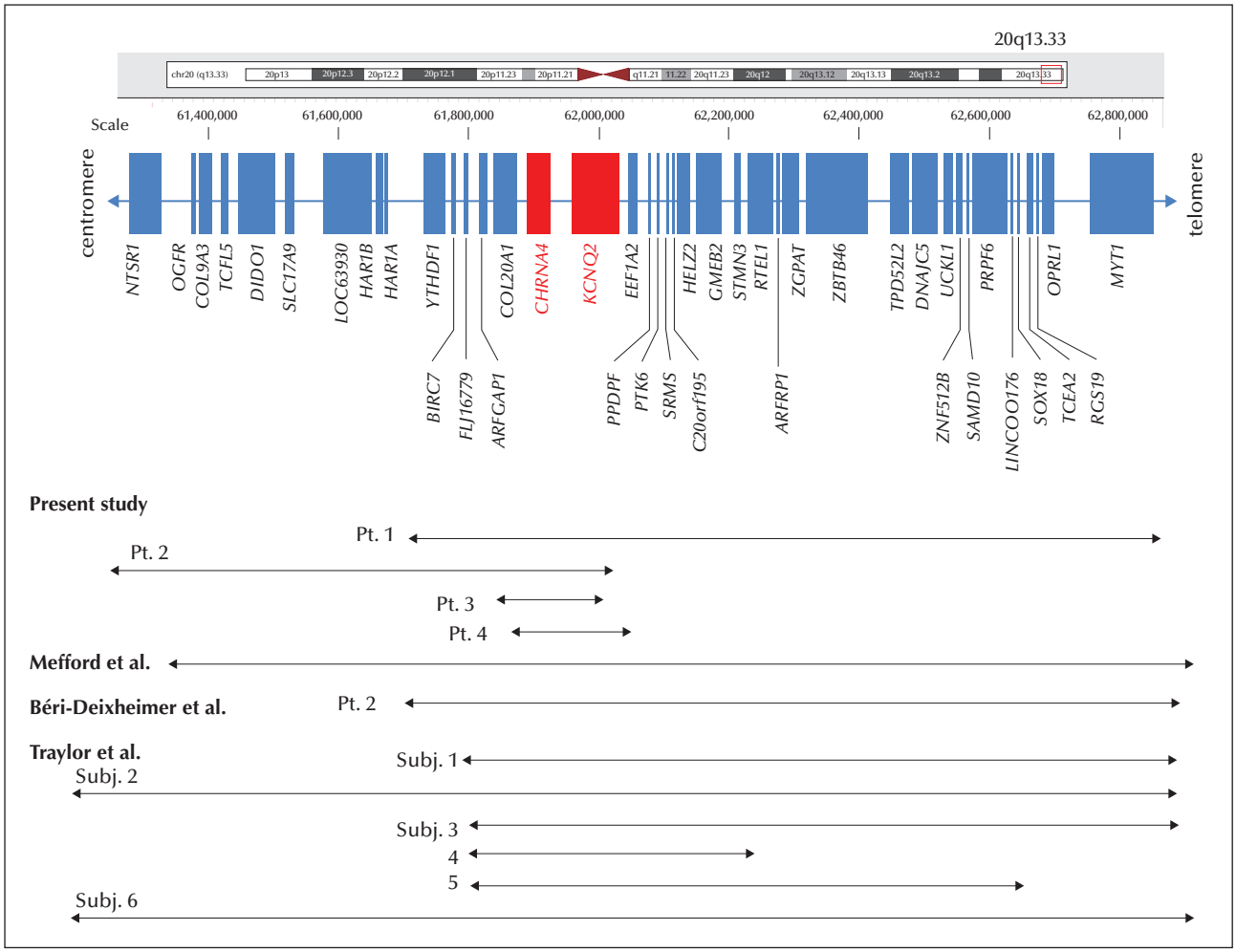

Figure 1. Microarray-based characterization of microdeletions at 20q13.33. Schematic representation of deletions in Patients 1-4 and those previously reported (Béri-Deixheimer *et al.*, 2007; Traylor *et al.*, 2010; Mefford *et al.*, 2012). The patients reported by Pascual *et al.* (2013) were not included because the range of deletion was not mentioned in their report. *KCNQ2* and *CHRNA4* are shown in red. Genes which are known to be related to central nervous system disorders are shown in orange.

(complete), and *COL20A1* (partial). No other pathological copy number variants were detected in our patients.

Discussion

In our series, patients with 20q13.33 microdeletion, involving both *KCNQ2* and *CHRNA4*, invariably showed a favourable epilepsy phenotype, similar to BFNE. The onset of epilepsy was within the first two weeks of life in all but one, and seizures disappeared by 4 months of age. Psychomotor development was good in two patients with a restricted deletion around *KCNQ2* and *CHRNA4*, whereas a delay in psychomotor development was observed in the other two with a larger deletion. These results imply that information based on aCGH may be useful to predict seizure and developmental outcome of infants with 20q13.33 microdeletion, with onset during the neonatal or early-infantile period.

There are several reports published on children with 20q13.33 microdeletion (Béri-Deixheimer *et al.*, 2007; Kurahashi *et al.*, 2009; Traylor *et al.*, 2010; Mefford *et al.*, 2012; Pascual *et al.*, 2013). Among them, at least 11 patients had deletion of both *KCNQ2* and *CHRNA4* genes. The clinical features of these 11 patients, along with our patients, are summarized in *table 1*. A comparison between all the patients reveals a phenotypic overlap, which includes developmental delay and epilepsy. However, it is difficult to identify a clinically recognizable constellation of dysmorphic features among patients with 20q13.33 microdeletion. Molecular cytogenetic techniques, such as aCGH, will be necessary for precise diagnosis.

Table 1.

□	Sex	Inheritance	Deletion size	*CHRNA4* deletion	*KCNQ2* deletion	Age at epilepsy onset	Age at epilepsy offset	Seizure semiology	Febrile seizure	Interictal EEG	MRI	Treatment	Psychomotor development	Dysmorphic features
Patient 1	F	unknown	2.47 mb	C	C	10 d	4 m	asymmetric tonic posturing	no	normal	normal	PB ineffective CBZ effective	mildly delayed	none
Patient 2	M	*de novo*	1.09 mb	C	C	3 m	4 m	right hemiconvulsion	no	focal spikes	atrophic	PB effective	mildly delayed	none
Patient 3	F	paternal	765 kb	C	C	2 d	3 m	blank eyes, reduced responsiveness	yes	normal	normal	PB ineffective ZNS effective	normal	none
Patient 4	F	paternal	136.4 kb	C	P	1 d	3 d	hemiconvulsion	no	normal	normal	none	normal	none
Traylor *et al.*, *2010*														
Patient 1	M	*de novo*	171.8 kb	C	C	6 m	single seizure	generalized	no	normal	n/a	none	global IQ 40	none
Patient 2	F	*de novo*	1.1 mb	C	C	2 w	intractable	complex partial	no	n/a	delayed myelination	n/a	severely delayed	present
Patient 3	M	unknown	1.61 mb	C	C	none			no	n/a	n/a	n/a	severely delayed	present
Patient 4	M	paternal	1.08 mb	C	C	yes	n/a	n/a	n/a	n/a	n/a	n/a	delayed	present
Patient 5	M	*de novo*	560 kb	C	C	yes	n/a	n/a	n/a	n/a	n/a	n/a	delayed	n/a
Patient 6	F	unknown	1.0 mb	C	C	n/a	n/a	n/a	n/a	n/a	n/a	n/a	expired due to NEC	present

Table 1. (*Continued*).

□	Sex	Inheritance	Deletion size	*CHRNA4* deletion	*KCNQ2* deletion	Age at epilepsy onset	Age at epilepsy offset	Seizure semiology	Febrile seizure	Interictal EEG	MRI	Treatment	Psychomotor development	Dysmorphic features
Béri-Deixheimer *et al.*, *2007*														
Patient 2	F	unknown	6.8 mb	C	C	2 m	single seizure	n/a	no	abnormal	thin cc	none	severely delayed	present
Mefford et al., 2012	M	unknown	1.6 mb	C	C	2 w	8 w	red complexion, tonic stiffening	no	hypsarrhythmia	delayed myelination	PB and pyridoxine effective	severely delayed	none
Pascual *et al.*, *2013*														
Patient 1	M	maternal*	1.5 mb	C	C	7 d	1 m	left head turns, secondary generalization	yes	transiently abnormal	normal	LEV ineffective OXC effective	mildly delayed	none
Patient 2	F	maternal*	521 kb	C	C	2 d	6 m	n/a	n/a	transiently abnormal	normal	LEV, ZNS, TPM	global delay	none
Patient 3	F	*de novo*	520.7 kb	C	C	2 d	n/a	focal motor	n/a	multifocal spikes	normal	PB effective	global delay	none

n/a: not available, CC: corpus callosum, IQ: intelligence quotient, NEC: necrotizing enterocolitis, PB: phenobarbital, CBZ: carbamazepine, ZNS: zonisamide, LEV: levetiracetam, OXC: oxcarbazepine: TPM: topiramate; d: days; w: weeks; m: months; C: complete; P: partial.
*In these families, genetic analyses were not performed in family members other than the proband.

Epilepsy was observed in all but one patient (*table 1*), and detailed information of epilepsy was obtained in 11 patients (our four patients and seven in the literature). The age at the onset of epilepsy ranged from two days to 6 months of age. It is remarkable that seizure outcome was mostly favourable in such patients. Two patients had only one seizure, and seizures disappeared by 6 months of age in eight. These features are similar to BFNE, related to *KCNQ2* mutation (Bellini *et al*., 2013), and are different from those of ADNFLE, related to *CHRNA4* mutation (Steinlein *et al*., 1995). The known mutations in *CHRNA4* associated with ADNFLE are missense or insertion mutations without frameshift, and are located in the pore region. Knockout mice with defective *chrna4* were reported not to have epileptic seizures (Marubio *et al*., 1999). These findings suggest that complete deletion of *CHRNA4* does not result in the development of epilepsy. However, long-term follow-up will be necessary to determine epilepsy phenotype more precisely.

Developmental outcome is presumed to be related to the size and location of deletion. Two of our patients (Patient 3 and 4), in whom only three genes were affected (*KCNQ2*, *CHRN4*, and *COL20A1)*, achieved normal psychomotor development, whereas the other patients with a larger deletion had delay in psychomotor development. This suggests that deletion of *KCNQ2* and *CHRN4* does not affect psychomotor development. We consider that 20q13.33 deletion is likely to manifest with benign neonatal seizures and favourable developmental outcome when the deletion is restricted to within *KCNQ2*, *CHRNA4*, and *COL20A1*.

There are a number of other genes within the deleted regions in our patients and those in the previous reports (*figure 1*). Among them, the following genes are known to be related to central nervous system disorders: *NTSR1*, *ARFGAP1*, *EEF1A2*, *RTEL1*, *ZBTB46*, *DNAJC5*, *ZNF512B*, and *MYT1*. Kroepfl *et al*. (2008) reported a patient with intellectual disability associated with a complete deletion of *MYT1*. An experimental study showed that *MYT1* may regulate oligodendrocytes, and may affect developmental outcome. *DNAJC5* mutations have been proven to cause adult-onset neuronal ceroid lipofuscinosis (Nosková *et al*., 2011). The dominant negative effect due to *DNAJC5* mutation is thought to contribute to brain disorders. It is unlikely that deletion of *DNAJC5* affected the phenotypes of our patients. A non-synonymous mutation of *EEF1A2* was reported in one patient with epileptic encephalopathy (Veeramah *et al*., 2013). Patients 1 and 4 reported here, as well as those in the literature, had *EEF1A2* deletion, however, their epilepsy phenotypes were mostly benign. *RTEL1* mutation causes Hoyeraal-Hreidarsson syndrome in an autosomal recessive manner (Le Guen *et al*., 2013). Deletion of *RTEL1* did not affect the phenotypes of our patients. The contribution of *NTSR1*, *ARFGAP1*, *ZBTB46*, and *ZNF512B* is difficult to determine at present.

In conclusion, 20q13.33 microdeletion is associated with a favourable outcome of epileptic seizures and a varying degree of developmental delay. There is no characteristic pattern of abnormalities that would arouse clinical suspicion of a 20q13.33 microdeletion. Deletion of *KCNQ2* or *CHRNA4* does not appear to affect seizure phenotype. The severity of developmental delay is related to the size and location of deletion.

Supplementary Data.

Summary didactic slides are available on the www.epilepticdisorders.com website.

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None of the authors have any conflict of interest to disclose.

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TEST YOURSELF

(1) Which gene in the 20q13.3 region is related to neonatal or early infantile epilepsy, with favourable outcome?

(2) Does deletion of *CHRNA4* influence the phenotype of epilepsy in children with microdeletion affecting *CHRNA4* and *KCNQ2*?

(3) What is the effect on psychomotor development in children with microdeletion affecting CHRNA4 and KCNQ2?

Note: Reading the manuscript provides an answer to all questions. Correct answers may be accessed on the website, www.epilepticdisorders.com, under the section "The EpiCentre".