Chapter 9
Large De Novo Microdeletion in Epilepsy with Intellectual and Developmental Disabilities, with a Systems Biology Analysis

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9.1 Introduction

Epilepsy is a disease which arises from largely unknown cellular and genetic mechanisms. It is a common neurological disease that reflects neuronal hyperexcitability induced by many different factors such as trauma, neurotoxicity and genetic variation (Lee and Heo 2014). ID/DD is one of the most common pediatric neurological diseases and is also one of the most important unsolved problems in health care. Studies have shown that the prevalence rate of ID/DD is 1–3% (Chelly et al. 2006). It is estimated that approximately 30% of patients with ID/DD have seizures (Tuchman et al. 2009). These associations indicate that epilepsy shares a similar pathogenic mechanism with those diseases in some situations (Williams et al. 2009; Cooper et al. 2011; Grayton et al. 2012).

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Copy number variation (CNV) has been reported to be associated with a group of neuropsychiatric disorders, including epilepsy and ID/DD. Recent studies of CNV in patients with epilepsy have revealed a series of CNV hotspots, such as 1q21.1 (de Kovel et al. 2010; Mefford et al. 2010), 15q11.2 (Zhang et al. 2009; de Kovel et al. 2010; Mefford et al. 2010; Vanlerberghe et al. 2015), 15q13.3 (Mulley et al. 2011; Sisodiya and Mefford 2011; Kogan et al. 2015), 16p11.2 (Mefford et al. 2011; Bassuk et al. 2013; Tiwari et al. 2013), 16p13.11 (Sisodiya and Mefford 2011) and 22q11.2 (Helbig et al. 2013; Kim et al. 2016). At the same time, a group of ID/DD-related CNV hotspots was been found, as 1q21.1 (Harvard et al. 2011), 2q13 (Yu et al. 2012; Riley et al. 2015), 15q11.2 (Derks et al. 2013; Caciotti et al. 2015), 16p11.2 (Bassuk et al. 2013; Derks et al. 2013), 22q11.2 (Mertz et al. 2013; Olszewski et al. 2014). The overlap of those CNV hotpots between epilepsy and ID/DD, indicate these two disease share a similar pathogenic genetic mechanism.

To elucidate whether CNV is a causal factor in epilepsy with ID/DD in Chinese children, we utilized a custom high-density oligonucleotide-based comparative genomic hybridization (CGH) microarrays to detect the CNVs in 96 epilepsy patients with ID/DD.

9.2 Large De Novo Rare Microdeletion Is an Important Pathological Cause of Epilepsy with ID/DD

9.2.1 Ethics and Patients

The study protocol was approved by Medical Ethics Committee of Peking University First Hospital. Informed consent was obtained from the parents. All data of this study were analyzed anonymously. DNA samples were collected from 96 epileptic patients with ID/DD and from their parents. All of the patients were recruited from the Department of Pediatrics, Peking University First Hospital from 2006 to 2014. These samples were prepared from a collection of whole blood samples by DNeasy Blood & Tissue Kit (QIAGEN).

Patient with both epilepsy and ID/DD who fulfilled the following inclusion criteria were assumed to be cryptogenic: (1). no perinatal brain injury (2). no hypoxia, ischemia, trauma or infection of the central nervous system (CNS); (3). no evidence of typical inherited metabolic disorder or specific neurodegenerative disorders, as found by physical examination, cranial neuroimaging and blood/urinary metabolic diseases screening; (4). negative from a gene screen by 300 epilepsy gene panel (Zhang et al. 2015). Finally, according to the inclusion criteria, 96 participating Han ethnicity patients were recruited from Peking University First Hospital.

Table 9.1 Large CNVs identified in 8 of 96 individuals affected by epilepsy and ID/DD

Sample	CNV locus	Start	Stop	Size (bp)	Gene number	de novo or inherited
3940	2q24.1	157,183,677	159,479,627	2,295,951	10	de novo
2332	2q33.1-q34	199,750,753	210,748,712	10,997,960	79	de novo
1549	5q13.2	68,830,699	70,600,323	1,769,625	15	de novo
	Xp22.31	6,705,268	7,942,835	1,237,568	4	de novo
5332	5q13.2	68,828,322	69,732,251	903,930	12	de novo
5319	5q33.1-q34	151,040,072	160,070,141	9,030,070	51	de novo
1277	17p13.3	2,165,369	3,058,821	893,453	17	de novo
1583	22q11.21-q11.22	19,058,829	21,360,978	2,302,150	48	de novo
3568	22q11.21-q11.22	19,058,829	21,360,978	2,302,150	48	de novo

9.2.2 CNV Detection by Array CGH

To detect the changes of CNVs in the genomic DNA, we applied high-density oligonucleotide-based CGH microarrays, a custom-designed Agilent SurePrint G3 Microarray (4 × 180K) was used to verify CNVs. The high-density areas covered the known epilepsy associated genes or related chromosome loci (including genes and CNVs in epilepsy including early infantile epileptic encephalopathy and idiopathic generalized epilepsy, listed in Supplementary Table 9.1). DNA digestion, Cy5-dUTP or Cy3-dUTP labeling, purification, array hybridization, washing, scanning, and data analysis were conducted by following the Agilent oligonucleotide aCGH protocol (version 6.3).

We performed whole-genome array CGH in a series of 96 patients. All of them had a presenting diagnosis of epilepsy with ID/DD. Our goal was to discover novel CNVs associated with epilepsy and ID/DD. In this study, we gathered data from whole-genome analysis and extended our analysis to other idiopathic epilepsy syndromes, such as infantile spasms and early onset epileptic encephalopathy (EOEE). In total 96 patients, we identified 8 individuals (8.3%) with 9 long rare microdeletions. If the CNV is larger than 500 kb, it will be identified as a long/large one.

9.2.3 The Loci of Microdeletions

In this study, we identified 8 of 96 (8.3%) patients with 1 or 2 large microdeletions (more than 500 kb). The biggest deletion was about 11 Mb, and the smallest was 893 kb. The mean CNV size was 3.5 Mb and the median size was 2.9 Mb. The number of deleted genes in each patient was from 12 to 79 (Table 9.1). Figure 9.1 shows the loci of the CNVs in the genome. There were two identical microdeletions at 22q11.21-q11.22 (Fig. 9.2) and two similar microdeletions at 5q13.2 (Fig. 9.3). The other CNVs were located on 2q33.1-q34, 2q24.1, 5q33.1-q34, 17p13.2, and Xp22.31 (Fig. 9.4). All the CNVs were de novo and heterozygous. Microdeletion of 5q13.2

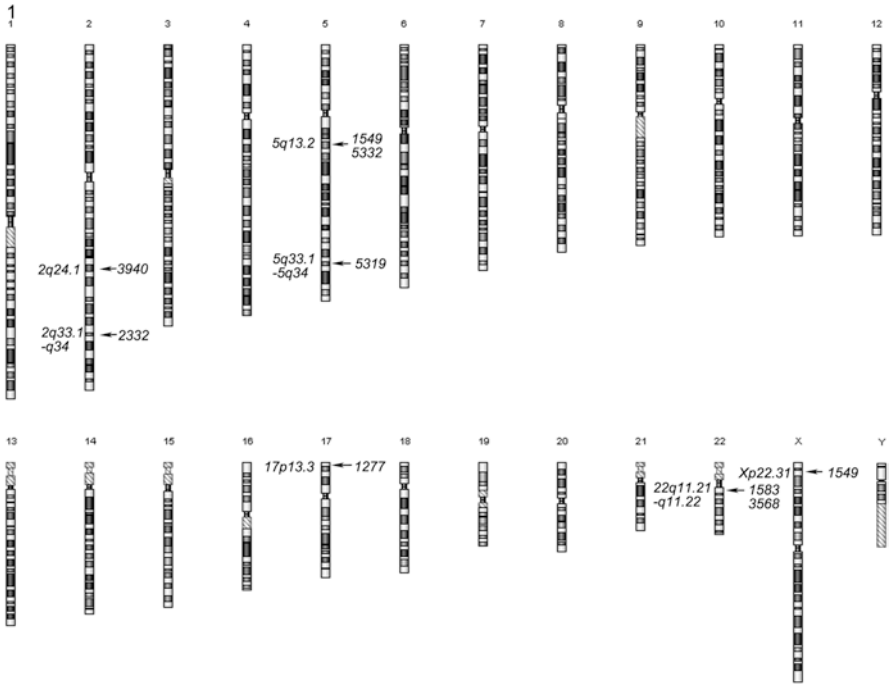

Fig. 9.1 The loci of the 9 CNVs of our cohort in the genome. 2 CNVs (2q24.1 of patient 3940 and 2q33.1-q34 of patient 2332) in chromosome 2, 3 CNVs (5q13.2 of patient 1549 and 5332, 5q33.1-q34 of patient 5319), 1 CNV (17p13.3 of 1277) in chromosome 17, 2 CNVs (22q11.21-q11.22 of patient 1583 and 3568) in chromosome 22, 1 CNV (Xp22.31 of patient 1539)

and 17p13.2 was not found in patients of epilepsy with ID/DD before. The clinical features of the patients with large microdeletion were summarized in Table 9.2. Of the 8 patients with large microdeletions, 5 were male and 3 were female. Besides epilepsy and ID/DD, the phenotypes of the patients were diverse. Six out of 7 patients who had a MRI scan had encephalodysplasia. Of 5 patients who had a psychiatric test, 2 patients suffered from autism. For craniofacial characteristics, 3 patients had facial dysmorphism, 1 patient had cleft lip/palate, and 2 patients had strabismus (Tables 9.2 and 9.3).

From these results, most of patients have only one large microdeletions. Those CNVs should course epilepsy and ID/DD by two different situations, one is the CNVs have both epilepsy-related genes and ID/DD related genes, the other is the CNVs have one or more genes associating both epilepsy and ID/DD. We also found that most of the long rare CNVs in this study were not in the most well-known idiopathic epilepsy CNV hotspots, such as 1q21.1, 15q11.2, 15q13.3, 16p11.2, 16p13.11, 22q11.2 (Table 9.4). The reason for this difference might be that the known CNV hotpots come from studies of idiopathic epilepsy, while our patients suffered from both epilepsy and ID/DD. Most of them are epileptic encephalopathy. The reported CNV regions of epileptic encephalopathy, such as 1q36, 2q32.3,

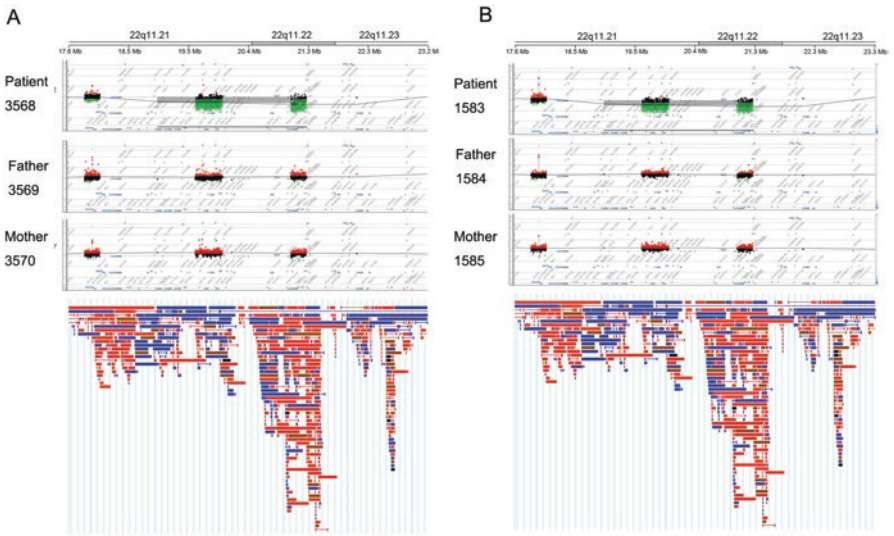

Fig. 9.2 The 2 same CNV in 22q11.21-q11.22. (**a**) The microdeletion in 22q11.21-q11.22 of patient 3568. Compared with the genome DNA of his father and mother, the CNVs is de novo. From the data of SV database in this area, the CNV is rare. (**b**) The microdeletion in 22q11.21-q11.22 of patient 1583. Compared with the genome DNA of his father and mother, the CNVs is de novo. From the data of SV database in this area, the CNV is rare

2q24.3, 3q11, 4q3.1-q3.2, 7q11.23, 14q12, 15q11-13, 16p11.2, Xp22 (Table 9.5) are not in accordance with idiopathic epilepsy.

9.3 Pathogenic Mechanism Analysis

We analyzed the genes in the CNVs and discovered some genes which were candidate pathogenic genes. We screened for candidates by determining whether genes were epilepsy/seizure related, ID/DD related, synapse related, ion channel/receptor related, transmitter related, and neurodevelopment related, or having high expression in the CNS (Table 9.3). We found that 4 out of the 9 CNVs included epilepsy related genes, while 6 out of the 9 CNVs included reported ID/DD related genes. Besides the known epilepsy- or ID/DD-related genes, some novel candidate genes might be involved in epilepsy with ID/DD: *NR4A*, *KCTD18*, *TRAK2*, *UNC80*, *CASP8*, *NRP2*, *KLF7*, *OCLN*, *SMN1*, *SMN2*, *NAIP*, *ADRA1B*, *HAND1*, *SRR*, *PAFAH1B1*, *SEPT5*, *RTN4R*, *TBX1*, *ARVCF*, *RTN4R* and *CRKL* (Table 9.6).

Patient 3940 with a 2q24.1 deletion was suffering from autism with epilepsy and ID/DD. 2q24.1 was reported to be involved in juvenile myoclonic epilepsy (Layouni et al. 2010), ID/DD (Daoud et al. 2009) and schizophrenia (Yamada et al. 2012). Therefore, 2q24.1 should be a potential CNV locus for neuropsychiatric disorders. The pathogenic genes in this patient could be *GPD2* and *NR4A2*. *GPD2*, which is

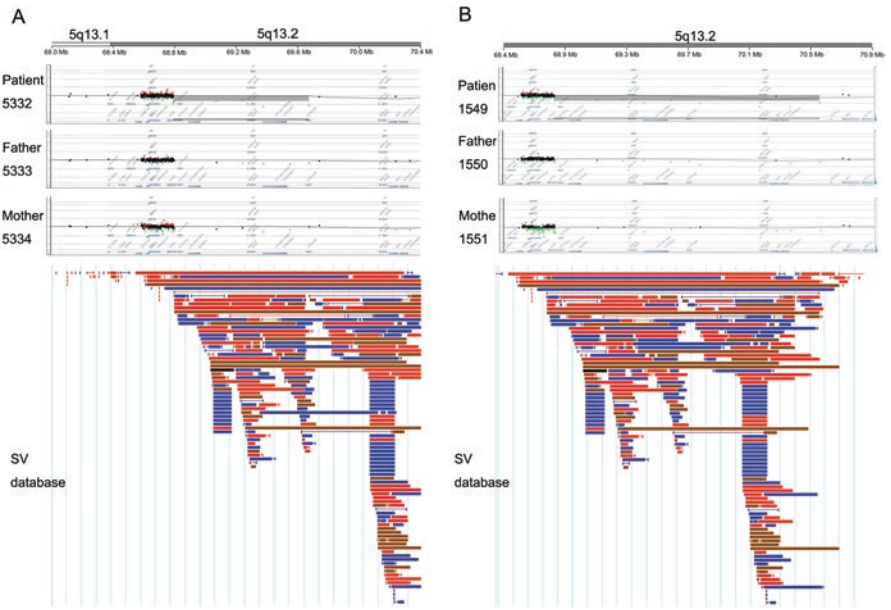

Fig. 9.3 The 2 similar CNV in 5q13.2. (**a**) The microdeletion in 5q13.2 of patient 5332. Compared with the genome DNA of his father and mother, the CNVs is de novo. From the data of SV database in this area, the CNV is not rare. (**b**) The microdeletion in 5q13.2 of patient 1549. Compared with the genome DNA of his father and mother, the CNVs is de novo. From the data of SV database in this area, the CNV is not rare

highly expressed in brain, was reported to be a candidate gene for ID/DD in a female with nonsyndromic ID/DD (Daoud et al. 2009; Barge-Schaapveld et al. 2013). *NR4A2* is the gene for Nuclear Receptor Subfamily 4, Group A, Member 2, which is crucial for expression of a set of genes such as *SLC6A3*, *SLC18A2*, *TH* and *DRD2* for the development of neurons (Messmer et al. 2007).

The clinical features of patient 2332 with a 2q33.1-q34 deletion were similar to 2q32-q33 deletion syndrome. 2q32-q33 deletion syndrome (OMIM # 612313), first reported at 1989, (Glass et al. 1989) is characterized by severe ID/DD, microcephaly and craniofacial dysmorphism. A *STAB2* gene deletion might be the most likely pathogenic gene in the 2q33.1-q34 region. The *SATB2* is a candidate brain developmental gene which should be responsible for the 2q32-q33 deletion syndrome (Van Buggenhout et al. 2005; Rosenfeld et al. 2009; Usui et al. 2013). The *STAB2* gene encodes a transmembrane receptor which has always been a marker of the upper layer of the normal fetal neocortex (Arai et al. 2012). In the 2q33.1-q34 CNV of our patient, there are both epilepsy-related genes and ID/DD related genes. The reported epilepsy-related genes are *ADAM23* (Owuor et al. 2009; Fukata et al. 2010), *MAP2* (Chulanova et al. 2001; Jalava et al. 2007). *SATB2* (Leoyklang et al. 2007) and *CREB1* (Barco et al. 2003) were reported to be ID/DD related gene. Besides those genes, *KCTD18* (Pichler et al. 2013), *TRAK2* (Grishin et al. 2006), and *UNC80*

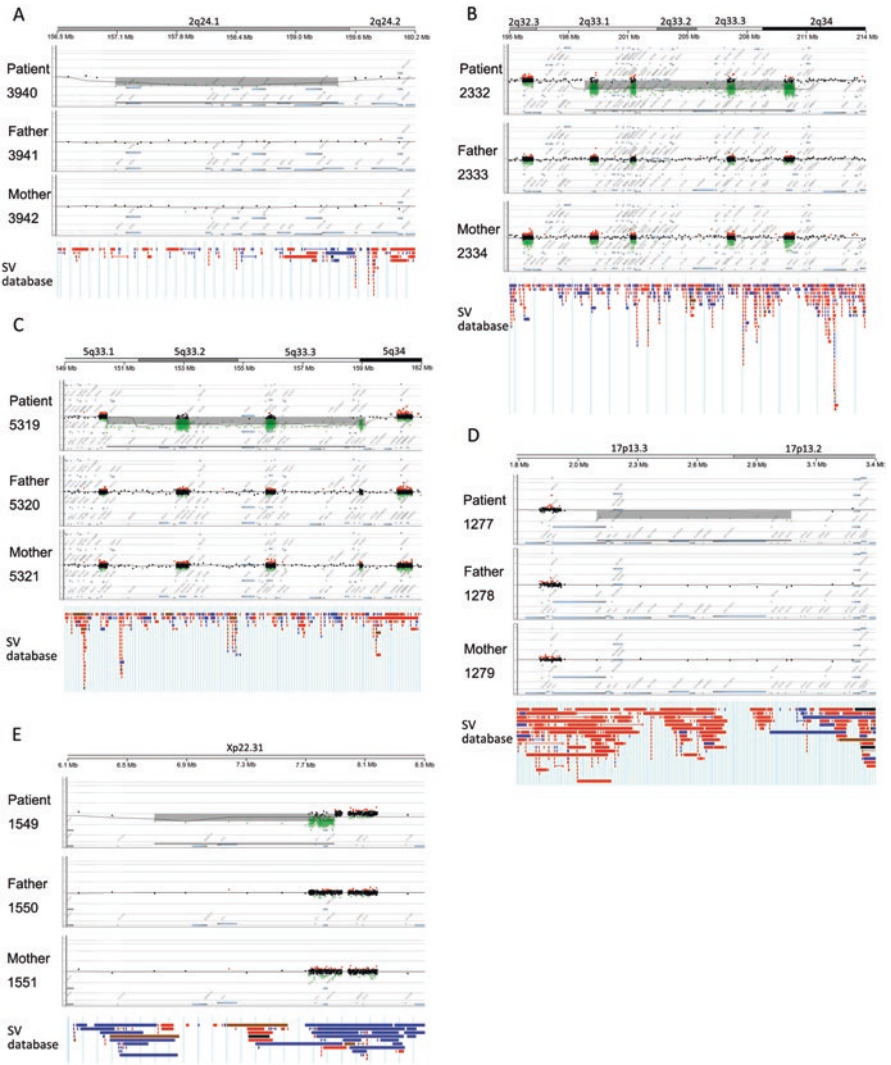

Fig. 9.4 The other CNVs in our cohort. (**a**) The microdeletion in 2q24.1 of patient 3940. Compared with the genome DNA of his father and mother, the CNVs is de novo. From the data of SV database in this area, the CNV is rare. (**b**) The microdeletion in 2q33.1-q34 of patient 2332. Compared with the genome DNA of his father and mother, the CNVs is de novo. From the data of SV database in this area, the CNV is rare. (**c**) The microdeletion in 5q33.1-q34 of patient 5319. Compared with the genome DNA of his father and mother, the CNVs is de novo. From the data of SV database in this area, the CNV is rare. (**d**) The microdeletion in 17p13.2 of patient 1277. Compared with the genome DNA of his father and mother, the CNVs is de novo. From the data of SV database in this area, the CNV is rare. (**e**) The microdeletion in Xp22.31 of patient 1549. Compared with the genome DNA of his father and mother, the CNVs is de novo. From the data of SV database in this area, the CNV is rare

Table 9.2 The clinical features of patients

Patient (F/M)	CNV loci	Diagnosis	Seizure	ID/DD	EEG	MRI	Other clinical features
3940 (F)	2q24.1	Epilepsy	Onset 4 years, tonic seizure (always in sleep)	Moderate	Epileptiform wave	/	Hyperactivity, flat foot
2332 (M)	2q33.2-q34	EOEE	Onset 3 months, generalized seizure, 5–6 times per year	Severe	Normal	Dysplasia of corpus callosum, strabismus	Autism, atrial septal defect, microcephaly, ocular hypertelorism, micrognathia, big ear, blepharoptosis
1549 (F)	5q13.2, Xp22.31	Infantile spasm	Onset 4 years, infantile spasm, generalized seizure, 9 times per year	Severe	Spike slow wave	Normal	None
5332 (M)	5q13.2	Epilepsy	Onset 1 year 1 month, tonic seizure, 6–30 times per year	Severe	Generalized and multifocal spike wave and spike slow wave	Myelin dysplasia, dysplasia of corpus callosum	Hypotonia
5319 (M)	5q33.1-q34	Epilepsy	Onset 1.5 years, generalized seizure with fever, twice per year	Moderate	Normal	Dysplasia of cerebral white matter	Small ear, strabismus, high palatine arch, autism
1277 (M)	17p13.2	Infantile spasm	Onset 5 months, infantile spasm last 3.5 years then cured by hormonotherapy	Severe	High-amplitude sharp wave	Pachygyria, agyria, schizencephaly, hydrocephalus	None
1583 (M)	22q11	Epilepsy	Onset 5 months, tonic seizure usually with fever, 1.5 times per months	Severe	Low to medium amplitude spike wave and spike slow wave with interictal EEGs in sleep	Cerebral dysplasia, increased lateral ventricle especially on the left side, wide arachnoid at temporal lobe and frontal lobe	Anomalous face with short philtrum, small auricle, high palatal arch, and up-warped upper lip; high muscular tension of both lower limbs
3568 (F)	22q11	Epilepsy	Onset 2.5 years, complex focal seizure, 2 times per months	Moderate	/	Hippocampal sclerosis, abnormal signal at temporal lobe and parietal lobe (especially right side)	None

(Gogliotti et al. 2011) are related to ion channels or receptors, and *CASP8* (Ma et al. 2007), *NRP2* (Maden et al. 2012) and *KLF7* (Caiazzo et al. 2011) takes part in neurodevelopment.

There were two patients (1583 and 3568) sharing the identical 48 gene-deleted CNV in 22q11.21-q11.22. Besides epilepsy and ID/DD, they also suffered from encephalodysplasia. Patient 1583 also had an anomalous face. Patient 1583 was a boy born after uneventful term delivery. He was the product of healthy parents without family history of epilepsy and ID/DD. However, this boy had tonic seizures since 5 months. The seizures are intermittent with a frequency of 1.5 times per month. His intellectual and motive developments were delayed. The patient also had an anomalous face with short philtrum, small auricle, high palatal arch, and up-warped upper lip. A brain MRI showed cerebral dysplasia, increased lateral ventricle especially on his left side, wide arachnoid at temporal lobe and frontal lobe. Interictal EEGs found some low to medium amplitude spike wave and spike slow wave in sleep. Patient 3568 was a girl born after uneventful term delivery. There was no history of epilepsy and ID/DD in her family. The girl had complex focal seizures since 2.5 years. The seizures occurred intermittently with a frequency of 2 time per month. Her intellectual development was delayed. The MRI showed that two-side hippocampal sclerosis and abnormal signal at parietal lobe and temporal lobe (especially at the right sides). Besides epilepsy and ID/DD, they also suffered from encephalodysplasia. The reason may be this CNV have some genes of neurodevelopment, such as *TBX1*, *ARVCF*, *RTN4R*, *and CRKL* (Tables 9.2 and 9.4).

Based on the locus of the deletion and the clinic features, these patients would almost certainly suffer from 22q11.2 deletion syndrome. This syndrome involves a series of syndromes such as DiGeorge syndrome (DGS) (Kelley et al. 1982), velocardiofacial syndrome (VCFS) (Scambler et al. 1991; Driscoll et al. 1992), conotruncal anomaly face syndrome (CTAF) (Matsuoka et al. 1994), some cases of autosomal dominant Opitz G/BBB syndrome (McDonald-McGinn et al. 1995; Fryburg et al. 1996; Lacassie and Arriaza 1996), and Cayler cardiofacial syndrome (asymmetric crying facies) (Giannotti et al. 1994). Among candidate pathogenic genes, *COMT* is related to both epilepsy (Doyle and Sellinger 1980) and ID/DD (Zhang et al. 2007; Li et al. 2009). *SNAP29* (Elfving et al. 2008) and *TBX1* (Sedghi et al. 2012) have been proven to related to epilepsy.

In 2006, 5q34 was reported to be a susceptibility locus for idiopathic generalized epilepsy (Hempelmann et al. 2006). A 6.45 Mb deletion in 5q33-q34 and a 713 Kb deletion in 5q33.2 were reported by Mefford in 2010 and 2011 (Mefford et al. 2010, 2011) to be related to epilepsy and ID/DD. Our study found a patient was with a deletion in 5q33.1-q34. Among candidate pathogenic genes, *CYFIP2* is highly expressed in the brain and contributes to both epilepsy (Hideyama et al. 2010) and ID/DD (Hoeffer et al. 2012). The other ID/DD gene is *GLRA1* (Al-Futaisi et al. 2012), which encodes a subunit of glycine receptor.

In a research in 2013, Speriz reported that 17p13.2 may be an epilepsy and ID/DD related genetic region as a duplication (Spreiz et al. 2014). A deletion of 17p13.2 was also reported to be associated with Miller-Dieker lissencephaly syndrome (Chen et al. 2010). This report, together with our findings, indicates that 17p13.2

Table 9.3 Phenotype description of patients with long deletion

Phenotype	Frequency	Patients
Neurologic		
ID/DD	100% (8/8)	All
Epilepsy	100% (8/8)	All
Microcephaly	12.5% (1/8)	2332
Encephalodysplasia	85.7% (6/7)	1227, 1583, 2332, 3568, 3519, 5332
Psychiatric		
Autism	4% (2/5)	2332, 5319
Craniofacial		
Facial dysmorphism	37.5% (3/8)	1583, 2332, 5319
Cleft lip/palate	12.5% (1/8)	2332
Strabismus	37.5% (2/8)	5319, 2332
Dyskinesia	50% (4/8)	5332, 1583, 1549, 1277
Syndrome		
EOEE	37.5% (3/8)	2332,1277, 1549

Table 9.4 The CNV hotpots of idiopathic epilepsy

CNV locus	Candidate gene	Type of CNVs	Subtype	Reference
1q21.1	GJA5, GJA8, HYDIN2	Deletion	JAE, JME	de Kovel et al. (2010), Mefford et al. (2010)
15q11.2	CYFIP1, NIPA2	Deletion	JAE, JME, CAE, EGTCS only	Zhang et al. (2009), de Kovel et al. (2010), Mefford et al. (2010)
15q13.3	CHRNA7	Deletion	JAE, JME, CAE, EGTCS only	Dibbens et al. (2009), Helbig et al. (2009), de Kovel et al. (2010)
16p11.2	KCTD13, SEZ6L2	Deletion	JME	Mefford et al. (2010)
16p13.11	NDE1	Deletion	JME, CAE, EGTCS only	de Kovel et al. (2010), Mefford et al. (2010)
22q11.2	SLC25A18	Deletion	EGTCS only	de Kovel et al. (2010)

may be an important genetic region for gyrus development. Among pathogenic gene candidates, *SRR*, *PAFAH1B1* and *MRPL40* should be considered. *AFAH1B1* was reported to be associated with lissencephaly (Cardoso et al. 2000; Kerjan and Gleeson 2007). *R* encodes serine racemase, which catalyzes L-serine to D-serine. D-serine is an important transmitter in brain and may be related to epilepsy (Ryu et al. 2010) and ID/DD (Klatte et al. 2013).

There were two patients (1549 and 5332) sharing the similar gene-deleted CNV in 5q13.2. Patient 1549 was a girl born after uneventful term delivery. She was the product of healthy parents without family history of epilepsy and ID/DD. However, this girl had generalized seizure since 4 years. The seizures are with a most frequency of 9 times per day. Her intellectual and motive developments were delayed.

Table 9.5 The CNV hotpots of epileptic encephalopathy

CNV locus	Candidate gene	Type of CNVs	Subtype	Reference
1p36	KLHL17	Deletion	IS	Paciorkowski et al. (2011)
2q32.3		Deletion	IS	Tiwari et al. (2013)
2q24.3	SCN1A	Deletion	Dravet syndrome	Wang et al. (2012)
3q11	EPHA6, GABRR3	Duplication	IS	Mefford et al. (2011)
4q3.1-q3.2	EPHA5	Duplication	Dravet syndrome	Lin et al. (2013)
7q11.23	STX1A	Deletion	IS	Paciorkowski et al. (2011)
14q12	FOXG1	Duplication	IS	Paciorkowski et al. (2011)
15q11-13	GABRA5, GABRB3,GABRG3	Duplication	IS	Paciorkowski et al. (2011), Tiwari et al. (2013)
16p11.2		Duplication	IS	Mefford et al. (2011), Tiwari et al. (2013)
Xp22	CDKL5	Deletion	IS	Mefford et al. (2011), Tiwari et al. (2013)

Her brain MRI was normal. Interictal EEGs found some low to medium amplitude spike wave and spike slow wave in sleep. Patient 5332 was a boy born after uneventful term delivery. There was no history of epilepsy and ID/DD in her family. The boy got complex focal seizures since 1 year 4 month. The seizures occurred intermittently with a frequency of 6–30 time per day. His intellectual development was delayed. EEGs found Generalized and multifocal spike wave and spike slow wave. The MRI showed that brain dysplasia with defect of myelination of white matter.

Patient 1549 also has a long deletion in Xp22.31. The patient 5332 also suffered from aphasia and muscle hypotonia. The level of galactose in urine was a little higher than the normal standard. 5q13.2 was not reported to be related to epilepsy or ID/DD. In this CNV, no gene in this CNV have been reported to involve in epilepsy and ID/DD. The candidate pathogenic genes in this deletion were *SMN1*, *SMN2*, *OCLN*, and *NAIP*. These genes are involved in neurodevelopment. *SMN1* and *SMN2* are important factor for motor neuron development, and associate with spinal muscular atrophy (Prior 2007). Knocking out *SMN2* would increase seizure susceptibility (Gogliotti et al. 2011). *OCLN* encodes tight junction protein occludin, which is involved in the early stage of neurodevelopment (Virgintino et al. 2004). Occludin was reported to be overexpressed in Alzheimer's disease and vascular dementia (Romanitan et al. 2007), so it may be related to ID/DD. Neurodevelopment related gene *NAIP*, which encodes Neuronal Apoptosis Inhibitory Protein (Mercer et al. 2000), was reported to decrease in brains of patients suffering with Down syndrome or Alzheimer's disease (Seidl et al. 1999). It is indicated that *NAIP* may be related to ID/DD.

In an infantile spasms related deletion in Xp22 reported by Mefford, *CDKL5* was reported as the candidate pathogenic gene (Mefford et al. 2011). In our study, a deletion of Xp22.31 in patient 1594 (who also had a 5q13.2 deletion) contained 4

Table 9.6 Candidate pathogenic genes in the CNVs

CNV locus	All	Epilepsy (seizure)	ID/DD	Synapse	Ion channel/ receptor	Transmitter	Neurodevelopment	High expression in CNS
2q24.1	*NR4A2, GPD2*		*GPD2*				*NR4A2*	*GPD2*
2q33.1-q34	*SATB2, KCTD18, CASP8, TRAK2, NRP2, ADAM23, KLF7, CREB1, MAP2, UNC80*	*ADAM23 MAP2*	*SATB2, CREB1*		*ADAM23, KCTD18, TRAK2, UNC80*		*SATB2, CASP8, NRP2, ADAM23, KLF7, CREB1, MAP2*	
5q13.2	*OCLN, SMN1, SMN2, NAIP*						*OCLN, SMN1, SMN2, NAIP*	
5q33.1-q34	*GLRA1, HAND1, CYFIP2, ADRA1B*	*CYFIP2*	*GLRA1, CYFIP2*		*GLRA1, ADRA1B*		*HAND1*	*CYFIP2*
17p13.2	*SRR, PAFAH1B1, MRPL40*		*MRPL40*			*SRR*	*PAFAH1B1*	
22q11.21-q11.22	*SEPT5, TBX1, COMT, ARVCF, RTN4R, SNAP29, CRKL*	*COMT, SNAP29 TBX1*	*COMT*	*SEPT5, COMT, SNAP29*	*RTN4R*	*COMT*	*TBX1, ARVCF, RTN4R, CRKL*	
Xp22.31	*STS, VCX, PNPLA4*	*PNPLA4*	*STS, VCX*					

genes, *HDHD1A*, *STS*, *VCX*, and *PNPLA4*. *PNPLA4* may be involved to epilepsy and ID/DD (Carrascosa-Romero et al. 2012). *STS* and *VCX* was proven to take part in X-linked ID/DD (Ben Khelifa et al. 2013). As this patient is a girl, this heterozygous microdeletion in X chromosome may play a less role in the pathogenic mechanism.

9.4 Systems Biological Analysis

Recently, system biology has provided a series of powerful tools for biomedicine studies. As an important analysis method, network reconstruction was used in biomarker detection (Mitra et al. 2013), drug discovery (Zou et al. 2013), and for studying the synaptic plasticity (He et al. 2014) mechanism of learning and memory (Kandel et al. 2014). Network reconstruction is very suitable for studying the pathogenic mechanism of complex disease in CNS, such as autism (Corominas et al. 2014), schizophrenia (Sun et al. 2010), and tumor induced epilepsy (Mittal et al. 2013).

In this study, we also tried to use the analysis tools of systems biology to predict the common pathogenic mechanism for epilepsy and ID/DD as complex diseases. By the Cystoscope 3.1.0 (Shannon et al. 2003), the network of the known epilepsy genes (in Supplementary Table 9.1) and CNV genes was constructed based on genetic interaction, pathway, and physical interaction in GENEMANIA database (Montojo et al. 2010). Form the constructed network, we found 70.5% of the CNV genes (158/224) to be involved in a network, while only 3.5% CNV genes are known epilepsy-related genes (Fig. 9.5a). This result indicated that most of these genes are potential epilepsy related genes. All of the patients in our cohort have suffered from epilepsy and ID/DD, so we believe that there is some common pathogenic mechanism.

Interestingly, we found the BGNADP motif which was constructed by *BTD*, *GALNT10*, *NMUR2*, *AUTS2*, *DLG2* and *PTPRD* (Fig. 9.5b). This motif was connected with each of the CNVs in our patients. The BGNADP motif is a small epilepsy and ID/DD related gene network. *BTD* is the gene of biotinidase. Mutations in *BTD* caused a disease called biotinidase deficiency, which is characterized by seizures, hypotonia, skin rash, ataxia hearing loss and optic atrophy (Hymes et al. 2001). *AUTS2* (autism susceptibility candidate 2) is associated with a series of neurologic disorders, such as autism, attention deficit hyperactivity disorder, dyslexia, ID/DD and epilepsy (Poot et al. 2011; Jolley et al. 2013; Nagamani et al. 2013; Oksenberg et al. 2013). *DLG2* encodes a membrane-associated guanylate kinase called PSD-93, which interacts at postsynaptic sites of neurons and forms a scaffold for the clustering receptors and ion channel. *DLG2* expression was reported to increase in epilepsy, indicating the role of *DLG2* in epilepsy (Liu et al. 2007). *PTPRD* is a member of the protein tyrosine phosphatase gene family. Deficiency of *PTPRD* results in ID/DD (Choucair et al. 2015). *PTPRD* is also an epilepsy candidate gene according to a genome-wide association study (Speed et al. 2014). *GALNT10* and *NMUR2* are members of the CNVs of our cohort. They have not yet

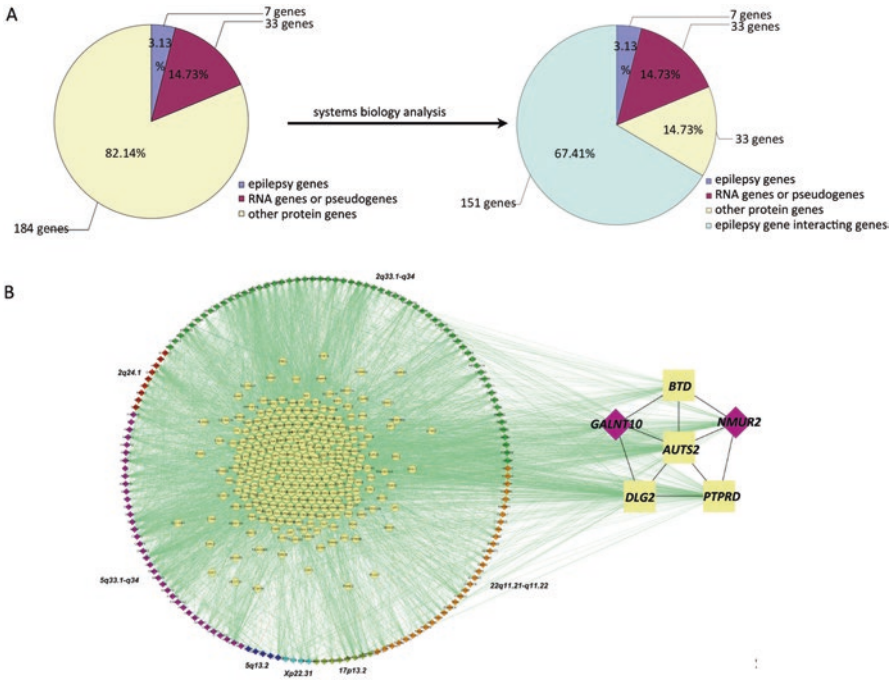

Fig. 9.5 The network of epilepsy genes and CNV genes. (**a**) Systems biology analysis showed 151 CNV genes (151/224, 67.41%) were interacting with the known epilepsy genes. (**b**) The network of epilepsy genes with CNV genes. The genes in different CNVs was labeled by different colors (2q33.1-q34 in green, 5q33.1-q34 in purple, 22q11.21-q11.22 in orange, 17p13.2 in deep green, 5q13.2 in blue, 2q24.1 in red and Xp22.31 in cyan)

been subjected to serious study, and should be targeted as candidate epilepsy and/or ID/DD genes in a further study. Our study has indicated that this BGNADP motif could be an important component in the common pathogenic mechanism. Further study should be needed to delineate the role of BGNADP in epilepsy and ID/DD.

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