

**Ring chromosome 17 not involving the Miller-Dieker region: a case with drug-resistant epilepsy**

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**Running title:** drug-resistant epilepsy in ring chromosome 17

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## **Abstract**

Chromosomal abnormalities are often identified in people with neurodevelopmental disorders including intellectual disability, autism and epilepsy. Ring chromosomes, which usually involve gene copy number loss, are formed by fusion of sub-telomeric or telomeric chromosomal regions. Some ring chromosomes, including ring 14, 17 and 20, are strongly associated with seizure disorders. We report an individual with a ring chromosome 17 (r(17)(p13.3q25.3)) with a terminal 17q25.3 deletion and no short arm copy number loss, with a phenotype characterized by intellectual disability and drug-resistant epilepsy including a propensity for non-convulsive status epilepticus.

Established facts: Ring 17 syndrome is a rare condition with different clinical features depending on whether the Miller-Dieker Critical Region (MDCR), is involved. Ring 17 syndrome not including the MDCR is considered milder but only 12 cases have been previously described.. The genetic and clinical evaluation of this condition might be challenging.

Novel Insights: The reported phenotype includes growth delay, intellectual disability, seizures, *café au lait* skin lesions, minor dysmorphism and a flecked retina. Only two previous cases provide description of the epileptic phenotype. We report in detail the epilepsy-related features of an individual with ring 17 syndrome not involving the MDCR helping to delineate a possible common phenotype of this

condition. The present case has also been assessed through Exome Sequencing to exclude the possibility that known possible genetic factors might have influenced the epileptic phenotype. A comparison with the linear 17q deletion is also discussed.

**Key words:** copy number variation, non-convulsive status epilepticus, epilepsy comorbidity

## **Introduction**

Chromosomal abnormalities identified by newer cytogenetic technologies are emerging as an important genetic association of some epilepsies (Xiang et al., 2010). In recent years, the increasing number of reports of people with overlapping genetic changes and phenotypes has allowed the delineation of new syndromes (<http://decipher.sanger.ac.uk/>) (Firth et al., 2009).

Ring chromosomes have been described with all human autosomes. Associated phenotypes vary greatly depending on the chromosomes involved, the size of deleted segments and the presence or absence of mosaicism (Wyandt, 1988). Some ring chromosomes, such as ring 20 and ring 14, are associated with a specific seizure phenotype, including status epilepticus. Ring 17 syndrome is a rare condition with different clinical features depending on whether the Miller-Dieker Critical Region (MDCR), which maps to the short arm of chromosome 17 at 17p13.3, is involved (Shashi et al., 2003). When this region is deleted (with or without ring 17 chromosome formation), the phenotype, includes lissencephaly, severe intellectual

disability and typical facial dysmorphism (Shashi et al., 2003). Epilepsy has also been reported but a definite phenotype has not been described (Shimajima et al., 2010). If the MDCR region is not involved in ring 17 formation, the phenotype is milder and may include growth delay, intellectual disability, seizures, *café au lait* skin lesions, minor dysmorphism and a flecked retina (Charles et al., 1991; Shashi et al., 2003; Surace et al., 2009).

Only 12 cases of ring chromosome 17 without involvement of the MDCR have been described (de Palma et al., 2015; Endo et al., 1999; Gass & Taney, 1994; Kumari et al., 2009; Qazi et al., 1979; Ricard-Mousnier et al., 2007; Shashi et al., 2003; Surace et al., 2009). Epilepsy has been described in detail in only two such cases, with focal seizures from sleep and prolonged non-convulsive status epilepticus in wakefulness (de Palma et al., 2015; Ricard-Mousnier et al., 2007). We describe the clinical and EEG features of a further case with ring chromosome 17.

### **Clinical report**

This 31 year old woman is the second child of healthy unrelated parents. There is no family history of epilepsy or any other neurological condition. She was born at term through natural delivery weighing 2.9kg (10<sup>th</sup> percentile). She remained consistently below the 25<sup>th</sup> percentile for height afterwards. She initially attained normal developmental milestones, being able to speak two languages.

Seizure onset was at age four years, when she developed clonic seizures in sleep, followed by different types of seizures in wakefulness. With seizure onset, she lost her ability to speak, which was initially regained when the seizures were well controlled. When seizures recurred and increased in frequency, she lost her language ability permanently.

She has had multiple seizure types including generalized tonic-clonic seizures, clonic seizures, tonic seizures from sleep and, complex partial seizures which are the commonest type. She has had treatments with many drugs including lamotrigine, phenobarbital, zonisamide, valproate, phenytoin, carbamazepine, vigabatrin, topiramate, levetiracetam, clobazam, clonazepam, gabapentin and felbamate. She derived some benefit only from lamotrigine and phenobarbital, which reduced seizure frequency by about 50%. The ketogenic was abandoned as she could not tolerate it.

At age ten, EEG showed marked epileptiform abnormality bi-laterally but with some left sided predominance. A brain MRI scan at age of 11 was normal. A subsequent interictal SPECT suggested asymmetry of the temporal lobes with the right showing reduced perfusion in the anterior portion, extending posteriorly.

She was referred at age of 24. She had moderate intellectual disability. She could walk with assistance, did not speak and did not have anal sphincter control.

Examination disclosed multiple café au lait patches on the back and abdomen and multiple white patches on the chest; no obvious dysmorphism was noted. She had

daily complex partial seizures, a median of three monthly generalized tonic-clonic seizures and 1-2 monthly tonic seizures from sleep.. Complex partial seizures were characterized by sudden arrest of activity, staring gaze, hand wringing and lip smacking usually brief lasting 1-2 minutes but associated with significant post-ictal tiredness. On referral, her treatment was lamotrigine 375 mg/day, zonisamide 300 mg/day and phenobarbital 30 mg/day. Phenobarbital and zonisamide were discontinued and lamotrigine increased to 550 mg/day. Her social interaction and alertness improved without worsening of seizure frequency.

At age 28, she was admitted due to an increased seizure frequency and cognitive decline. EEG showed almost continuous bilateral bursts of spike and wave complexes over a background of theta/delta activity. No convulsive seizures had been documented, but she was much less alert, drowsy and slower than usual (Fig.1). A diagnosis of non-convulsive status epilepticus was made and treated with benzodiazepines which lead to an improvement.

### **Cytogenetics**

Array-CGH analysis using a NimbleGen 135K whole genome v3.0 array chip showed a near-terminal interstitial 0.45 Mb microdeletion within 17q25.3 (chr17: 80,572,212,-80,983,971; the genomic region of the corresponding benign CNV is chr17: 44,353,924-44,766,072; HG19; Fig. 2a). No copy number gain or loss was

noted on the chromosome 17 short arm. Examination of 30 G-banded metaphases and FISH analysis using Cytocell Aquarius 17pter and RP11-567016 probes identified a single ring chromosome r(17)(p13.3q25.3), in all metaphases examined (Fig. 2b). The deletion involved eight OMIM protein-coding genes (*FOXK2*, *WDR45B*, *RAB40B*, *FN3KRP*, *FN3K*, *TBCD*, *ZNF750*, *B3GNTL1*) (Figure 2c). Parental FISH analysis demonstrated that the ring chromosome had arisen *de novo*.

We performed whole exome sequencing (WES) [mean target coverage of 102.27; 82.6% of bases read at least 30X] and interrogated exonic variants occurring in epilepsy-related genes (see Supplementary table). The exonic library was amplified by Nextera Exome Kit (Illumina), and sequenced on a HiSeq 2500 platform (Illumina). After read alignment, candidate genes were analyzed by vcf tools (Danecek et al., 2011) and annotated using Annovar. (Wang et al., 2010). None of the variants identified were considered pathogenic using the criteria applied (see supplementary methods for more details).

## **Discussion**

Ring chromosome 17 without MDCR involvement is rare (Charles et al., 1991; Chudley et al., 1982; de Palma et al., 2015; Endo et al., 1999; Gass & Taney, 1994; Kumari et al., 2009; Qazi et al., 1979; Ricard-Mousnier et al., 2007; Shashi et al., 2003; Surace et al., 2009). Epilepsy, although reported in all cases, is well described in only two cases, both presenting with nocturnal focal seizures and prolonged

diurnal non-convulsive status epilepticus (de Palma et al., 2015; Ricard-Mousnier et al., 2007) Isolated linear 17q deletions are also rare: thirteen cases are reported in the Decipher database (<https://decipher.sanger.ac.uk/search?q=znf750#consented-patients/results>). These present with various dysmorphic features and eye abnormalities other than a flecked retina. Epilepsy is not described in linear 17q deletion. The clinical features of the cases with ring (17) chromosome and linear 17q deletion are summarized in Tables 1 and 2.

Our case had all the features reported for the other ring (17) cases, although as she had not had an ophthalmological review we are not certain if she has a flecked retina. The main clinical symptom was drug-resistant epilepsy which amounted to an epileptic encephalopathy with multiple seizures type, especially featuring nocturnal tonic seizures and non-convulsive status epilepticus. Language was most severely affected, with normal acquisition and subsequent loss.

Clinical features of Landau-Kleffner Syndrome (LKS) and Lennox-Gastaut Syndrome (LGS) are present in our case and also in a previously reported case (Ricard-Mousnier et al., 2007), but the electroclinical features are not consistent with either of these conditions. Non-convulsive status epilepticus was a shared epilepsy phenotype (de Palma et al., 2015). Detailed data are available from only these three cases (our own and ref. 8, 13), but it is possible that polymorphic seizures and non-convulsive status epilepticus represent the typical presentation of epilepsy in this condition. In our view, a ring (17) abnormality should be considered if a child is



affected by drug-resistant epilepsy, featuring non-convulsive status epilepticus associated with other clinical conditions such as growth delay, intellectual disability, *café au lait* skin lesions, minor dysmorphism or a flecked retina.

MDCR involvement in ring chromosome 17 helps predict a homogeneous and severe phenotype but less information is available when this region is not involved. Thus, to date there is no candidate gene for the epilepsy phenotype in the ring chromosome 17 syndrome without MDCR involvement. There are no reports of simple deletions of the long arm of chromosome 17: del(17)(q25.3) to allow comparison.

The chromosomal abnormality in our case is quite small (only 0.45 Mb) and only includes eight protein-coding genes, five of which are expressed in the brain (*FN3KRP*, *FN3K*, *TBCD*, *ZNF750*, *BGNTL1*). Biallelic mutations affecting *TBCD* have been found in a rare condition called Sanjad-Sakati syndrome. Epilepsy has been reported in 46% of these individuals but never characterized except in one child with generalized tonic-clonic convulsive status (Ahmed-Farag-Elhassanien et al 2013) (Rajniti Prasad et al 2013) The exact functions of the other genes are not known. Whole exome sequencing did not detect variants predicted to be damaging that could possibly explain the phenotype of this individual beyond the ring chromosome.

Ring chromosomes are chromosomal abnormalities which can arise through the fusion of the telomeric regions with or without loss of genetic material (Wyandt, 1988). Rings are invariably unstable, resulting in cumulative somatic mutations which may be tissue-specific as a result of secondary imbalances involving the ring chromosome. In a recent series of rings such secondary imbalances were observed in 4/29 cases (Guilherme et al., 2013). We were unable to demonstrate secondary instability in peripheral blood in our case, but this does not preclude the possibility in other tissues. However the multiple café au lait and white patches is indicative of an underlying mosaicism indicating instability of the ring chromosome in this tissue (even if not seen in the cultured blood).

A previous review of all ring chromosome 17 cases, without MDCR involvement, showed shared common phenotypic features with no consistency in copy number losses (Charles et al., 1991; Surace et al., 2009). This observation is supported by our case, which in comparison to the only two other cases with a detailed description of epilepsy, showed a discrepant copy number loss (de Palma et al., 2015; Ricard-Mousnier et al., 2007). It was suggested that alterations in gene expression (other than simply haploinsufficiency) could explain the common phenotypic features found in mild ring 17 syndrome (Surace et al., 2009). Variation in gene expression within or between tissues could arise either from ring chromosome instability in vivo (Wyandt, 1988) (Chudley et al., 1982) and/or as a result of telomere position effects resulting from juxtaposition of actively

transcribed sequences in proximity to telomere ends (as a result of ring formation), which may have an impact over long distances (Robin et al., 2014). Additional cases, with more functional data, may lead to the identification of novel candidate epilepsy genes in ring chromosome 17 syndromes.

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Figure1: a) array plot showing two CNVs on chromosome 17: a terminal deletion (red spots) which is pathogenic and a benign duplication (green spots); b) FISH preparation showing metaphase chromosomes. Bluegnome BAC probe RP11-567016 (orange) which maps to 17q25.3 and Cytocell Aquarius subtelomeric probe for 17p (green); signals are consistent with deletion of RP11-567016 on a ring chromosome identified as a ring chromosome 17 by the presence of a green (17p) signal. The ring chromosome 17 replaces a normal chromosome 17.

c) UCSC snapshot of chromosome 17 showing the CNV of our case (red box). The black bar indicates a magnification of the CNV and below are reported the included genes. Red and blue bars indicate respectively deletions and duplications reported in Decipher and occurring in the same genomic area.

**Figure 2 (a,b,c,d):** EEGs recorded at the age of 28 during an episode of non-convulsive status epilepticus, represented by sub-continuous generalized spike and waves discharges.

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