Numerical chromosome changes in epileptogenic focal cortical dysplasia

To the Editor,

Type II focal cortical dysplasia (FCD) corresponds to a specific malformation of cortical development associated with intractable focal epilepsy of early onset, frequent neurological deficits, and cognitive impairment (Chassoux et al., 2012). Unlike FCD type IIb, which is consistently associated with low-grade brain tumours and frequent genomic imbalances, the aetiology of FCD types I and II is unclear.

Here, we describe a unique cytogenetic finding of a case of FCD in order to illustrate new findings in this field. An 11-year-old female presented with left focal seizures since 2 years of age. Seizures were very frequent (up to 100 episodes per day) and refractory to antiepileptic drugs despite attempts to optimise medication. Video-electroencephalography monitoring depicted right rolandic epilepsy. The patient was also shown to have mild neurocognitive deficiency. She was referred for a right parietal lesionectomy. Pathological examination revealed typical Taylor FCD type IIb. For cytogenetic studies, a fresh sample of FCD tissue (adjacent to areas verified by frozen section) was collected aseptically and minced with scissors in a Petri dish. The minced pieces were divided into T30 flasks and enzymatically disaggregated for two hours on 0.5% collagenase type IV (Sigma Chemical Co., St. Louis, MO, USA) in DMEM-F10 medium (Gibco BRL, Life Technologies, Carlsbad, CA, USA), supplemented with 15% foetal bovine serum (FBS). Following disaggregation, the cells were centrifuged and the collagenase solution removed and replaced with medium. Disaggregated cells were cultured in HAM F10 (T24) and RPMI 1640 (5637; GibcoBRL, Life Technologies®, Carlsbad, CA, USA), supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml) at 37°C in a humidified 5% CO2 incubator. Cultures were harvested after one week with overnight colcemid (Gibco) treatment at a final concentration of 0.25 µg/mL. Cells were collected by trypsinization and centrifuged at 1,000 rpm. Trypsin activity was inhibited by resuspension in medium containing 10% FBS. The cells were pelleted by centrifugation, washed with phosphate-buffered saline, and resuspended in hypotonic medium (0.075 M KCl) for 20 minutes at 37°C. Following hypotonic treatment, preparations were fixed three times with methanol and acetic acid (3:1). Cytogenetic analysis of cultured cells by GTG-banding showed a composite karyotype, designated as 44-46, XX, +9[4], [cp7] (figure 1). The patient has remained seizure-free since surgery.

Compelling evidence indicates that common epilepsies are polygenic, although enrichment of copy number variants (CNVs) in cohorts of individuals affected with epilepsy advocates that certain structural changes in the genome may confer a significant risk for epilepsy (Williams and Battaglia, 2011). Recently, Kariminejad et al. (2011) investigated the CNVs in a large cohort of patients with structural brain malformations by array-comparative genomic hybridization (CGH). Cerebral malformation included lissencephaly, polymicrogyria, corpus callosum agenesis, and FCD. The authors found both inherited and de novo duplications in chromosome 9 in their cohort, although none of the patients with this genetic finding had FCD. Possible candidate genes which may influence structural brain malformations in this chromosome include: SMARCA2 and VLDLR (at 9p24.2), TEK and MOBKL2B (at 9p21.2), and FCMD, SLC31A2, SLC31A1 and AMBP (at 9q31.1-33.1). Moreover, somatic cases of mosaicism for trisomy 9 have been sporadically described. Reports usually portray severe cerebral malformations, including holoprosencephaly, corpus callosum dysgenesis, bilateral subependymal cysts, ventriculomegaly, and complex cerebral malformation including Dandy-Walker syndrome (Chen et al., 2003; Gérard-Blanluet et al., 2002; Murru et al., 2002). Unfortunately, in this case, information regarding somatic karyotyping was lacking due to the refusal of additional genetic testing by the parents. Nevertheless, a recognisable trisomy 9 phenotype (either complete or mosaic), as reviewed by Arnold et al. (1995), was absent in this patient. A meticulous review of the clinical data depicted a phenotypically normal female, with normal mental development and no major or minor dimorphisms. Since surgical treatment of severe epilepsy is becoming more widespread, cytogenetic studies of biological material from these epileptogenic regions are now more affordable. Although the application of conventional cytogenetic techniques appears to be limited, a combination with molecular karyotyping techniques may depict both structural chromosomal changes and CNVs in FCD and other epileptogenic malformations. This may be an interesting model to address the true biological origin of this lesion and hopefully provide new insight into epilepsies in this setting.
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Figure 1. Cytogenetic analysis of FCD cells by GTG-banding showing 46, X, -X, +9.

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References


