4. Genetics of FCD: an emerging scenario

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Key points
- Post-zygotic somatic variants arising during brain development have emerged as a major cause of FCD.
- Somatic variants in the PI3K-AKT-mTOR pathway genes for FCD II: single-hit somatic gain-of-function variants in mTOR activators and two-hit germline/somatic loss-of-function variants in mTOR repressors.
- Galactose transporter SLC35A2 loss-of-function variants account for mild MCD with oligodendrogial hyperplasia and epilepsy.
- Somatic variants are enriched in abnormal FCD II cell types, i.e. dysmorphic neurons and balloon cells.
- The size of the dysplastic lesion depends on when the mutation occurs, with mutations occurring in early progenitors causing larger lesions than late mutations.
- Genetic testing in cerebrospinal fluid is a promising tool for early genetic diagnosis.
- Advances in genetics will pinpoint novel therapeutic targets.

Introduction

FCD are malformations of brain cortical development affecting a localized brain region and manifesting with drug-resistant focal epilepsy and developmental delay (Guerrini and Dobyns, 2014). Resective neurosurgery is often required to control seizures, allowing access to dysplastic tissue for diagnostic and research purposes. In 2011, the Diagnostic Methods Commission of the International League Against Epilepsy (ILAE) released a first international consensus classification of FCD into 3 subtypes (FCD I, II, III) based on histopathological findings, when the genetic etiology was still unknown (Blumcke et al., 2011). The classification was revised in 2018 (Najm et al., 2018) and recently updated (Najm et al., 2022, in press).

Most FCD cases are isolated (sporadic), but few familial cases have also been reported, suggesting a genetic origin of the malformations. FCD cases can be identified in families with recurrence of focal epilepsy, or co-occurrence of FCD, HME and brain tumors (ganglioglioma, dysembryoplastic neuroepithelial tumor) (Leventer et al., 2014, Baulac et al., 2015). In the past years, an increasingly recognized role in neurodevelopmental and neuropsychiatric diseases have been attributed to somatic variants (which occur post-zygotically in soma cells) (McConnell et al., 2017). It is now well recognized that during normal human development, each dividing cell propagates mutations to its daughter cells and these mutations are considered clonal. The human brain is therefore a mosaic of cells with their own unique genome (Lodato et al., 2015, Bizzotto et al., 2021) (Figure 1). Based on the timing of the mutational event during development, a somatic variant can be present in multiple tissues (early event) or restricted to a single tissue or cell population (late event). Variants present only in neuronal cells can be identified only by accessing the mutated brain tissue.

The advent of high-coverage next-generation sequencing approaches and dedicated bioinformatic tools facilitated the identification of somatic variants in resected brain tissues from focal cortical malformations (D’Gama and Walsh, 2018, Koh and Lee, 2018, Marsan and Baulac, 2018). Recent findings have emphasized that FCD subgroups are genetically distinct: a subset of FCD I (which also includes mMCD and MOGHE) is caused by glycosylation defects as a consequence of loss-of-function SCL35A2 somatic variants while FCD II are caused by somatic variants in the PI3K-AKT-mTOR pathway (Mirzaa et al., 2016, D’Gama et al., 2017, Baldassari et al., 2019b, Sim et al., 2019). The genetic etiology of FCD III remains unknown.

In this chapter, we review the most recent genetic findings of FCD, focusing especially on FCD II, and explore future perspectives for early genetic diagnosis and targeted therapies.
Genetics of FCD II

FCD II is histologically characterized by localized cortical disorganization and the presence of dysmorphic neurons (present in FCD IIa and FCD IIb) and balloon cells (present in FCD IIb only) (chapter 3).

The first indications pointing to mechanistic target of rapamycin complex 1 (mTORC1) pathway hyperactivation in FCD II emerged from histological comparative studies with cortical tubers found in patients with TSC. TSC is a multisystem disorder caused by germline loss-of-function variants in genes encoding components of the TSC complex (TSC1 and TSC2), a repressor of the mTOR complex 1 (mTORC1). Cortical tubers, are characterized by the presence of giant cells and cytomegalic neurons, which resemble the balloon cells and dysmorphic neurons observed in FCD II. In the context of these histopathological similarities, two research groups in 2004 showed that abnormal cell types from both tubers in TSC and FCD II specimens displayed mTOR hyperactivation, as shown by increased phosphorylated-S6 (pS6) protein immunoreactivity (a standard readout of mTOR activation) (Baybis et al., 2004, Miyata et al., 2004) (Figure 2). These studies indicated that mTOR pathway hyperactivation is a hallmark common to both TSC and FCD II and may be the cause of a spectrum of developmental brain malformations with cytomegalic cells. Nonetheless, the genetic components leading to FCD II and HME remained elusive until recently (see chronological narrative review (Lee et al., 2022)).

The mammalian target of rapamycin (mTOR) is an evolutionarily conserved serine/threonine kinase present in every cell of the body within two protein complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). The mTOR signaling cascade controls several fundamental processes, such as protein synthesis, cell growth, metabolism and autophagy (Saxton and Sabatini, 2017, Kim and Guan, 2019). mTORC1 and mTORC2 are finely regulated by several upstream factors (e.g., nutrients such as glucose and amino acids, growth factors, ATP) that signal on the energetic/metabolic state of the cell. In the brain, the mTOR signaling pathway contributes to proper neural development, synaptic transmission and plasticity and neural network activity (reviewed in (Lipton and Sahin, 2014, Lasarge and Danzer, 2014, Crino, 2016)). Given the pathway's central role in directing cell growth, it is not surprising that mTOR pathway hyperactivation leads to morphologically abnormal cells with enlarged soma size as observed in TSC and FCD II/ HME specimens. A myriad of proteins, upstream effectors and substrates participate in the different branches of the mTOR pathway, to activate (e.g., in conditions of high nutrients availability), or to repress (e.g., the above cited TSC complex components, in conditions of low nutrient cellular levels) the pathway. Downstream substrates of mTORC1 signalling that modulate these pivotal cellular processes include ribosomal protein S6 kinase (S6K) and eukaryotic initiation factor 4E-binding protein, which are commonly used to monitor the activity of the pathway.
The focal nature of FCD II, combined with the mosaic pattern of mTOR hyperactivation in the resected tissues (presence of cytomegalic mTOR-hyperactivated cells intermingled with normal cells), is evocative of a possible signature of postzygotic somatic variants. This hypothesis was initially confirmed in large lesions such as megalencephaly and hemispheric dysplasia (HME), with the identification of brain somatic activating variants in the mTOR pathway components PIK3CA, AKT3 and MTOR genes and somatic duplication of chromosome 1q (that includes AKT3 gene) (Lee et al., 2012, Riviere et al., 2012, Poduri et al., 2012). Pathogenic variants were identified at high mosaic rates (or variant allele frequency (VAF), ≥ 8%). Few years later, it became clear that smaller malformations such as FCD II are also due to mutations in the PI3K-AKT-mTOR pathway genes, but are restricted to a smaller group of cells (therefore at smaller VAFs), thus requiring more sensitive sequencing techniques for their detection (e.g., high-coverage targeted gene panel or high-depth exome sequencing). Brain somatic variants in PIK3CA, AKT3, MTOR genes were later on discovered also in smaller FCD II malformations at lower mosaic rates (usually less than 5%) compared to those found in HME (Jansen et al., 2015, Mirzaa et al., 2016, D’Gama et al., 2015, Baek et al., 2015).

It is now well established that somatic mTOR pathway hyperactivating variants in a small set of genes account for most FCD II and HME cases (reviewed in (Blumcke et al., 2021, Lee et al., 2022). Two molecular mechanisms, both leading to mTOR hyperactivation are involved: 1/ single-hit gain-of-function somatic variants in an mTOR pathway activator encoding gene, and 2/ two-hit loss-of-function variants in an mTOR pathway inhibitor encoding gene. One study reported a possible cumulative effect of two mosaic variants (MTOR p.S2215F and RPS6 p.R232H) in mTOR pathway activators in a patient with HME (Pelorosso et al., 2019).

- **Somatic single-hit mutations in mTOR pathway activators**

Recent studies on large cohorts of FCD II and HME have demonstrated that somatic gain-of-function variants in activators of mTOR signaling pathway (PIK3CA, AKT3, RHEB, MTOR) are the most frequently encountered (Figure 3) (Jansen et al., 2015, Baldassari et al., 2019b, Sim et al., 2019, D’Gama et al., 2017, Lai et al., 2021). These variants, usually missense variants, are brain-specific (e.g., not detected in DNA derived from blood) and are sufficient to cause the dysplastic lesion. The diagnostic rate is variable (from ~30 to ~60%) across different studies, and may depend on the sequencing approach applied (e.g., targeted panel or whole
exome sequencing), the DNA quality (whether it is extracted from formalin-fixed paraffin embedded (FFPE) or frozen tissue) or the degree of pathology of the sequenced tissue (i.e., the density of dysmorphic neurons and balloon cells in FCD II). Hotspot variants (also observed in cancer tissues) are commonly detected in PIK3CA (e.g. p.E545K, p.H1047R), AKT3 (p.E17K) and MTOR (e.g. p.L1460P, p.T1977R, p.S2215F, p.S2215Y) gene itself, which is mutated in more than half of the FCD II cases (Baldassari et al., 2019b, Sim et al., 2019). A minority of FCD II/HME cases present with somatic activating variants in RHEB gene (Salinas et al., 2018, ...