**Searching for somatic mutations in focal cortical dysplasia by using next generations sequencing**

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**Introduction:** Epilepsies are one of the most frequent neurological diseases, affecting 1.5-2% of the worldwide population [1]. Malformations of cortical development (MCD), including Focal Cortical Dysplasia (FCD), can cause epilepsy and are often associated with the occurrence of refractory seizures [2]. FCD is characterized by alterations in cytoarchitecture also observed in other MDCs, such as in Tuberous Sclerosis (ET) and Hemimegaencephaly (HME) [3,4]. Recently, an association among mosaic mutations, ET, HME and FCD has been reported [5]. The identification of mosaic mutations in FCD can contribute to the understanding of complex diseases.

**Materials and Methods:** Deep-Whole-Exome sequencing was performed on genomic DNA extracted from brain tissue resected by surgery (BTRS) and blood of four patients with FCD. We performed capturing and enrichment with Nextera® Expanded Kit (Illumina®). Samples were sequenced following a 200bp paired-end protocol in a Hiseq2500 (Illumina®) to achieve at least 200x of average coverage.

We aligned sequences using BWA MEM and performed realignment around SNPs and indels, quality recalibration and variant calling using the Genome Analysis Toolkit (GATK). We evaluated mosaicisms using SomaticSniper and finally we added annotation to the variants found using Variant Effect Predictor. Variants were classified as mosaic mutations when less than 50% of reads are not aligned to human genome reference and are present only in BTRS. Variants were filtered prioritizing frameshift, missense, nonsense and splicing site mutations that were localized in coding regions or exon-intron boundaries. In addition, we also focused in not described variants or variants whose minor allele frequency (MAF) is < 0.1.

**Results:** We identified a total of eight mosaic mutations in BTRS, including four variants localized in genes belonging to mTOR pathway (*EIF4EBP1, IRS1, ULK1* and *STRADA*), three mutations localized in genes belonging to Tau pathway (*GAPDH, ADAM10* and *ERN1*) and one mutation in the *CLIC6* gene.

**Discussion:** Somatic mutations were identified in genes with roles and expression in central nervous system (CNS). Since mosaic mutations in genes associated with Tau and mTor pathways have been reported in patients with FCD, we believe that the variants identified in our cohort of patients are strong candidates to be involved in FCD etiology.

**Conclusion:** Further experiments will be necessary to validate our results**.** In addition**,** more patients should be sequenced in order to search for additional somatic mutations. We hope that this project will contribute to a better understanding of the genetic etiology of FCD as well as to identify molecular mechanisms involved in the developing of the cerebral cortex.

**References:** [1] Angevine JB Jr, Sidman RL. Nature 192:766-8, 1961. [2] Kuzniecky RI. Epilepsia. 35 Suppl 6:S44-56, 1994. [3] Fauser S, Huppertz HJ, Bast T, et al., Brain 129:1907-16, 2006. [4] Mühlebner A, Coras R, Kobow K, et al., Acta Neuropathol 123:259-72, 2012. [5] Becker AJ, Urbach H, Scheffler B, et al., Ann Neurol 52:29-37, 2002.

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