Searching for somatic mutations in focal cortical dysplasia using next generation sequencing

V.S. de Almeida[1], S.H. Avansini[1], M. Borges[1], F.R. Torres[1], F. Rogerio[2], B.S. Carvalho[3], F. Cendes[4], I. Lopes-Cendes[1]

[1]Department of Medical Genetics, School of Medical Sciences, [2]Department of Anatomical Pathology, School of Medical Sciences, [3]Department of Statistics, Institute of Mathematics, Statistics and Scientific Computing, IMECC, [4] Department of Neurology, School of Medical Sciences; University of Campinas, UNICAMP, Campinas, SP, BRAZIL and the Brazilian Institute of Neuroscience and Neurotechnology (BRAINN), Campinas, SP, BRAZIL.

Introduction: Malformations of cortical development (MCD), including focal cortical dysplasia (FCD), can cause epilepsy and are often associated with the occurrence of refractory seizures [1]. FCD is characterized by alterations in cytoarchitecture also observed in other MDCs, such as Tuberous Sclerosis (TS) and Hemimegaencephaly (HME) [2,3]. Recently, mosaic mutations were detected in TS, HME and FCD [4]; however, it is still unclear whether somatic mosaicism is indeed frequent in FCD [4].

Materials and Methods: Deep sequencing of the mTOR pathway genes was performed on genomic DNA extracted from brain tissue resected by surgery (BTRS) and blood samples of five patients with FCD type II. We performed capturing and enrichment with SeqCap EZ Choice Library (NimbleGen, Roche). Samples were sequenced following a 150bp paired-end protocol in a Miseq (Illumina), to achieve at least 600x 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 mosaicism using Mutect2. Variants were classified as mosaic mutations when less than 10% 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 variants not described previously or variants whose minor allele frequency (MAF) is $\leq$ 0.01. Effect of variants was evaluated using Variant Effect Predictor (VEP).

Results: We identified somatic mutations in 2/5 patients (40%). Patient P20 presents a *MTOR* somatic mutation c.4379T>C/p.Leu1460Pro (rs1057519779, NM_004958.3) with allele frequency of 9/503 reads (1.8%) in BTRS and 0/438 (0%) in blood. Patient G118 presents a *TSC2* somatic mutation c.3781G>A/p.Ala1261Thr (not reported previously; NM_000548.3) with allele frequency of 7/409 reads (1.7%) in BTRS and 0/373 reads (0%) in blood and an *AKT1* somatic mutation c.349_351del/ p.Glu117del (rs768025881, NM_005163.2) with allele frequency of 10/454 (2.2%) in BTRS and 1/350 reads (0.29%) in blood. These mutations were not found in the Exome Aggregation Consortium (ExAC) and in a Brazilian database of genomic variants (www.BIPMed.org). VEP classified all variants as probably damaging. In addition, is already reported in a patient with FCD.

Discussion/Conclusion: Somatic mutations identified are potentially deleterious since they were found in genes previously associated with FCD. Furthermore, somatic mutations in mTOR genes seems to be common in patients with FCD. Additional deep sequencing experiments, including more patients with FCD, will be carried out in order to confirm our preliminary findings.

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

Supported by: CEPID-FAPESP