Clinical and Genetic Analysis of Homozygous Epilepsy Cases in Consanguineous Pashtoon Families

Abstract

Epilepsy is a genetically heterogeneous neurological disorder with a disproportionately high prevalence in consanguineous populations due to increased homozygosity. As part of an ongoing investigation of inherited epilepsies in Pashtun families from Khyber Pakhtunkhwa, Pakistan, six multiplex pedigrees (EP-36, EP-72, EP-97, EP-101, EP-114, and EP-120) were clinically and genetically characterized. Comprehensive phenotyping included neurological examination, electroencephalography (EEG), and brain magnetic resonance imaging (MRI). Genomic DNA isolated from affected individuals and available family members was analyzed by Exome sequencing (ES), followed by variant prioritization and Sanger sequencing for segregation analysis.

In family EP-36, WES identified a rare homozygous KCNQ2 variant (c.1232C>A; p.Pro411Gln) in three affected siblings with early-onset generalized epilepsy. Structural analyses predicted impaired Kv7.2 channel function, and segregation analysis supported autosomal recessive inheritance. In family EP-72, a novel homozygous GNAO1 variant (c.943C>A; p.Pro315Thr) was detected in two siblings with childhood-onset generalized tonic–clonic seizures. The variant was absent from population databases and completely co-segregated with the disease phenotype. In family EP-97, four affected brothers with neonatal-onset epilepsy harbored a homozygous LAMA5 variant (c.9448G>A; p.Gly3150Ser). Neuroimaging demonstrated left mesial temporal sclerosis in the proband, while structural modeling predicted disruption of laminin α5 stability and extracellular matrix interactions. The variant co-segregated with disease and was absent in 100 ethnically matched controls.

Molecular docking demonstrated mutation-dependent alterations in ligand binding across all three proteins. In KCNQ2 (p.Pro411Gln), the trifluoromethyl-phenyl moiety remained embedded within the hydrophobic pocket; however, the interacting residues shifted to Val182, Leu183, Gly186, Leu197, and Leu126, accompanied by electrostatic rearrangements involving Lys120, Arg201, Glu123, and Glu130. A novel hydrogen bond with Thr194 was observed in the mutant complex. These changes resulted in slightly reduced binding affinity (S = –5.67 to –6.43 kcal.mol^{-1} vs –6.45 to –6.83 kcal.mol^{-1} in the wild type), increased pose variability (RMSD_refine = 1.39–2.53 Å), and less favorable placement and refinement energies, suggesting modest destabilization of ligand binding.

In GNAO1 (p.Pro315Thr), GDP retained strong binding affinity (S = –9.37 to –8.61 kcal·mol^{-1}) with stable docking conformations (RMSD_refine = 1.7–2.4 Å). The nucleotide remained anchored through conserved interactions with Arg177, Arg179, Lys46, and Lys271, while Asp273, Asn270, Thr327, and Asn150 stabilized the guanine and ribose moieties. Although Pro315Thr substitution introduced additional hydrogen-bonding potential and local flexibility adjacent to Thr327, the overall GDP-binding architecture remained largely preserved.

Docking of N-acetyl-D-glucosamine (NAG) to the LAMA5 p.Gly3150Ser variant produced highly consistent binding poses (S = –5.13 to –4.98 kcal·mol^{-1}; RMSD_refine <1.3 Å), indicating preservation of the carbohydrate-binding mode. Key interactions with Arg1341 and Cys1340 were maintained, while Glu1201 contributed additional polar stabilization. Although occasional increases in ligand conformational energy suggested minor structural adjustments, the overall binding remained energetically favorable.