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Case Report

A rare case report on novel pathogenic mutation of TSC2 gene explained at molecular level

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ABSTRACT

Tuberous sclerosis is a neurocutaneous genetic syndrome inherited as autosomal dominant pattern. This disease is caused by mutations of either of the tumor suppressor genes named TSC1 or TSC2 gene. It encodes for hamartin and tuberin which modulates mTOR pathway and regulate cell growth and proliferation. We report a case of a 7 year old child positive for pathogenic variant of TSC2 mutation having multiple seizures, angiofibromas, shagreen patch. Imaging studies are indicative of multiple calcified nodules in sub ependymal region, abnormal subcortical white matter suggestive of tuberous sclerosis. Molecular tests suggested that the mutation occurred results in alteration of splicing mechanism. Due to such alteration, the incomplete TSC2 gene encodes an altered tuberin protein i.e., unable to interact with Ras homologue enriched in brain (Rheb), leading to dysregulation of mammalian target of rapamycin (mTOR) signalling causing tuberous sclerosis disease.

Keywords: Tuberous sclerosis, Neurocutenous, Hamartin, Tuberin, Rapamycin

INTRODUCTION

Tuberous sclerosis complex (TSC) is a rare neuro-cutaneous syndrome with an autosomal dominant genetic inheritance caused by mutations in two tumor-suppressor genes TSC1 (chr 9q34) or TSC2 (chr16p13.3) gene with the prevalence of one in 6000 live birth, affecting both sexes.[1] TSC is marked primarily by the formation of hamartomas in a wide variety of organs including the brain, heart, lungs, kidneys, and skin which becomes apparent only in late childhood and also associated with neurological manifestations resulting in epilepsy, learning difficulties, behavioural problems, and renal failure, among other complications.[2,3] No molecular link has been established between the genes responsible for the hamartoma syndromes.[4] Loss of heterozygosity of either of the TSC1 and TSC2 or dysregulation of mTOR is an essential causative factor in the disease TSC.

Structure associated with tuberous sclerosis complex

TSC1 and TSC2 gene encodes for hamartin and tuberin that forms heterodimers and work together to regulate cell growth and size. TBC1D7 is associated with Tuberous Sclerosis Complex as it binds through TSC1 in the absence of TSC2.[5] Loss of TBC1D7 partially disrupts the association between TSC1 and TSC2, resulting in a decrease in Rheb-GAP activity.

TSC1 consists of 23 exons, 21 contain coding sequence and two are alternatively spliced. Hamartin protein comprised of 1164 amino acids (130kDa) and had no significant homology to tuberin. The amino acid residues 145–510 of hamartin contain the function for activation of Rho GTPase.

TSC2 gene consists of 41 exons of which exon 25, 26 and 31 are subjected to alternative splicing.[6] Tuberin has a

full-length isoform of 1807 amino acids (198 kDa) of which 1517-1674 encoded by exon 34-38 has a homology to GTPase activating protein Rheb (Ras homolog enriched in brain). Hamartin interact with tuberin within 302-430 (domain). First 418 amino acids of tuberin contain the binding site for hamartin. Hamartin stabilizes tuberin by inhibiting its interaction with the HERC1 ubiquitin ligase. Rheb is a direct target of TSC2 and shares 30% sequence identity with GAP domain.[2] Frequent point mutation cause change in a single base pair in the TSC2 gene or create a premature stop signal in the instructions for making tuberin.

Association of tuberous sclerosis complex with Rheb

TSC is involved in AMPK, AKT, and ERK1/2 pathway that leads to mTOR pathway. TSC2 inhibits Rheb activity by hydrolysing GTP to GDP. Within the TSC2, there is an active site residue called N1601 which stimulate Rheb GTP hydrolysis. Rheb activates mTORC1 and hence mTORC1 acts as a signalling hub. mTOR is activated through a couple of steps. AMPK activates TSC2 that inhibits Rheb, TSC2 is associated with AKT pathway which is activated using growth factors. AKT inhibits TSC2, leading to activation of mTOR. ERK1/2 is involved in Ras pathway and it also inhibits TSC2 which in turn activates mTORC1. p53 activates TSC2 which is activated by cellular stress like DNA damage. There are regulations at Rag sites. Rag isoforms are inhibited by GATOR1, GATOR1 is inhibited by GATOR2, GATOR2 is inhibited by CASTOR1 and CASTOR1 is inhibited by arginine. GATOR2 can be inhibited by sestrin 2 which is inhibited by leucine. Hence, arginine and leucine are activators of mTORC1 (Figure 1).

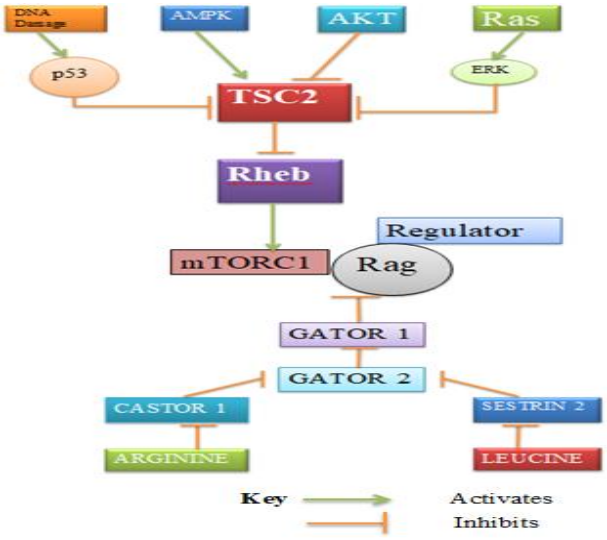

Figure 1: TSC2 is associated with Rheb and further have its effect on other components.

AMPK: AMP-activated protein; AKT: protein kinase B; ERK: extracellular-signal-regulated kinase; TSC2: tuberous sclerosus 2; Rheb: Ras homologous enriched inBrain; mTORC1, mammalian target of rapamycin 1; Rag; Ras-related GTP binding protein.

mTOR signalling pathway

Mammilian (mechanistic) target of rapamycin or mTOR, a 289- serine/threonine kinase protein belongs to the phosphoinositide 3-kinase (PI3K)-related kinase family, found in two functionally distinct multi-protein complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2).[7] mTORC1 phosphorylates and activates p70S6K that allows pyrimidine synthesis within the cell. It phosphorylates and activates CAD that is indispensable for pyrimidine synthesis. p70S6K inhibits eEF2K (eukaryotic elongation factor kinase) which inhibits eEF2 through phosphorylation which is necessary for elongation of protein polypeptide within the ribosome. p70S6K activates eIF4B (eukaryotic initiation factor 4B). p70S6K can directly phosphorylate the S6 ribosomal subunit and activate the ribosome. mTORC1 also phosphorylates and inhibits 4EBP1 (eukaryotic initiation factor 4E (eIF4E)- binding protein 1) which is an inhibitor of eIF4E (eukaryotic initiation factor 4E). It inhibits by binding to eIF4E by destabilizing its ability to bind to eIF4G. So, 4EBP1 inhibition allows activation of eIF4G and it allows protein synthesis. mTORC1 controls autophagy through the regulation of a protein complex consisting unc-51-like kinase 1 (ULK1), autophagy-related gene 13 (ATG13) and focal adhesion kinase family-interacting protein of 200 kDa (FIP200).[8] mTOR also inhibits TFEB (Transcription factor EB) by phosphorylation which is a master regulator of lysosomal biogenesis and also a mass regulator of many of the different autophagy proteins. So, mTOR inhibiting TFEB can finally reduce autophagy (Figure 2).

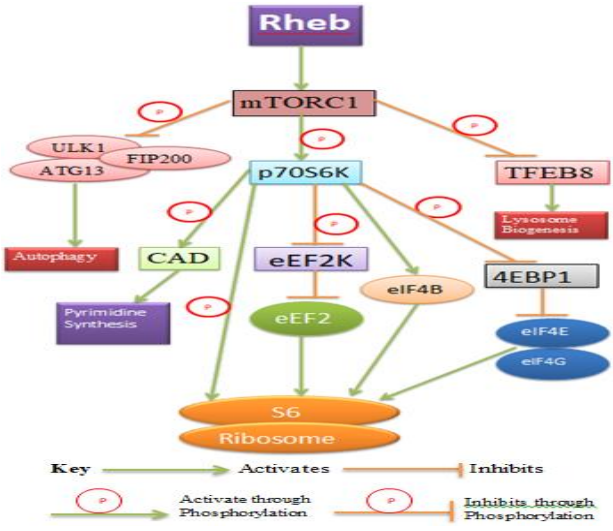

Figure 2: mTOR pathway at a glance.

Rheb, Ras Homologous enriched inBrain; mTORC1, mammalian target of rapamycin 1; FIP200, Focal adhesionkinase family –interacting protein; ULK1, unc-51-kinase 1; ATG13, autophagy related gene 13; p70S6K, p70 ribosomal S6 Kinase; eEF2K, eukaryotic elongation factor kinase; eEF2, eukaryotic elongation factor; eIF4B, eukaryotic initiation factor 4B; 4EBP1, eukaryotic initiation factor 4E-binding protein; eIF4E, eukaryotic initiation factor 4E;eIF4G, eukaryotic initiation factor 4G; TFEB8, Transcription factor EB.

Our work focuses on the clinical exome sequencing test conducted to identify the exact mutation related to the disease. Most of the cases reported earlier, doesn't include any confirmatory genetic test, but we are presenting a case report with detailed clinical diagnosis along with confirmatory genetic test.

CASE REPORT

A 7 year old female child from Bankura, West Bengal has been referred to "Ultraclinic-Fetal Medicine and Fertility Centre" with history of multiple episodes of generalized seizure. She was born of non-consanguineous marriage with no problems during birth. In past, at the age of 4 months she encountered fever with multiple seizures and multiple hospital admissions. She was on antiepileptic drug since the age of 6 months with poor control. Lately, she was diagnosed to have tuberous sclerosis on the basis of clinical and radiological tests. The child had multiple hyper-pigmented papules over the nasolabial region called angiofibromas (Figure 3) along with a shagreen patch over the back (Figure 4).

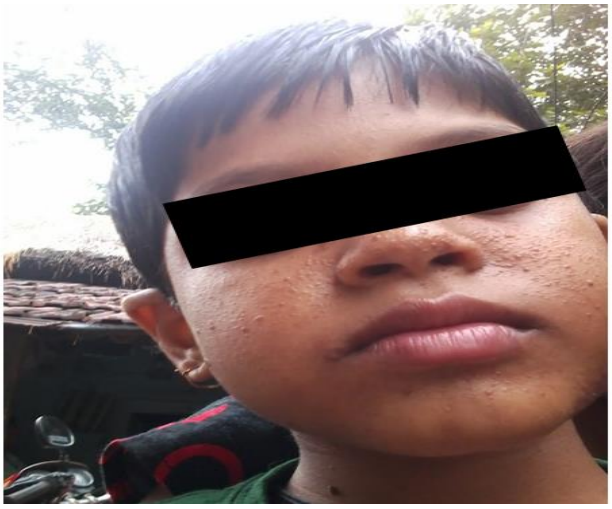

Figure 3: Facial angiiofibroma.

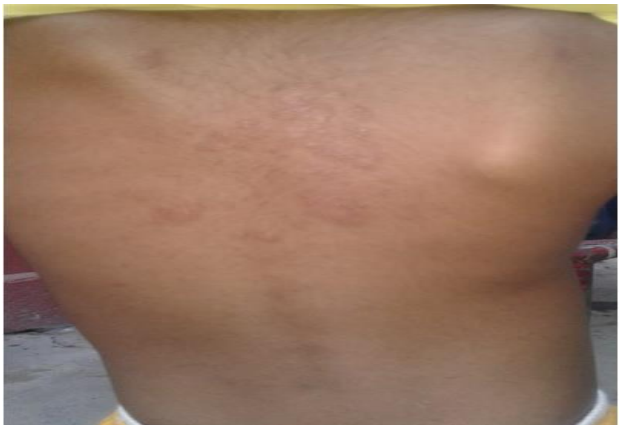

Figure 4: Shagreen patch is an area of thickened elevated pebbly skin found on the back.

For investigations, the child was subjected to the electroencephalogram (EEG) followed by CT scan (computed tomography) of brain. Afterwards, multiplanar images of brain were obtained on T1 and T2 weighted and FLAIR sequences using magnetic resonance imaging (MRI). For genetic investigations, the child was prescribed clinical exome sequencing. This test analyses the TSC1 and TSC2 genes associated with tuberous sclerosis. The Strand® clinical exome test is a laboratory developed test (LDT) to detect variations in genes associated with known inherited diseases. In addition to, the 'pathogenic' or 'likely pathogenic' variants in all other genes were investigated for associated and secondary findings. The test covers ~4500 genes and is a comprehensive test for inherited disorders. The step in the test includes:

Library preparation

Genomic DNA is isolated from blood. DNA is quantified using Qubit and 50 ng is taken for library preparation by incorporation of adapters, platforms-specific tags and barcodes. The genomic DNA has been sheared and tagmented using transposons given by TruSight One.

Target enrichment

500 ng of tagged and amplified sample libraries are pooled into a single tube and set up for enrichment using biotinylated, target specific probes checked for qualitative and quantitative analysis. Target libraries are amplified using limited PCR steps including extension and denaturation. Flow cells are densely coated with primers complimentary to DNA Library fragments. Repetition of this process leads to clonal clusters of localised identical strands. Afterwards it is subjected to next-generation sequencing (NGS) on the illumina NGS platforms (MiSeq and NextSeq).

FASTQ files were generated using MiSeq reporter from illumina for analysis. The reads were aligned against the whole genome using STRAND NGS (http://www.strand-ngs.com). Variants are then imported into StrandOmicsv5.0 interface for identifying variants of interest.

DISCUSSION

EEG investigation showed paroxysmal burst of generalized and synchronous spike, sharp and slow wave discharges are seen often in a pseudoperiodic manner with intermittent burst suppression pattern in the tracing giving the impression of seizure disorder. CT scan of brain showed multiple calcified nodules in sub-ependymal region along with sub-cortical hypodense area at both frontal, right occipital and parietal region. It also indicates small non-enhancing cystic changes in cortical sulci at bifrontal and parieto-occipital region bilaterally. MRI scan suggests abnormal subcortical white matter

signal intensity associated with sub-ependymal nodules in the lateral ventricles suggestive of tuberous sclerosis.

Clinical exome test results shows that child harbors one copy (heterozygous) of a pathogenic variant in the TSC2 gene which may be associated with the tuberous sclerosis complex. The identified heterozygous variant (c.1974A>C) lies in the essential splice acceptor site, in intron 18 of the TSC2 gene (chr16:2121783A>C) (Figure 5). The insilico splice prediction tool (SplicePort and NNSPLICE) suggests, this variant might affect splicing due to the loss of constitutive splice site and introduction of a new splice site which may leads to a frameshift and consequent premature termination of the protein; likely result in the loss of function. Moreover, due to introduction of premature stop codon, this aberrant transcript will likely be targeted by nonsense mediated mRNA decay mechanism.[9] The identified variant seems to be a novel variant, as it has not been previously reported in literature. It lies in the vicinity of other pathogenic variants associated with TSC thus labelled as "pathogenic".

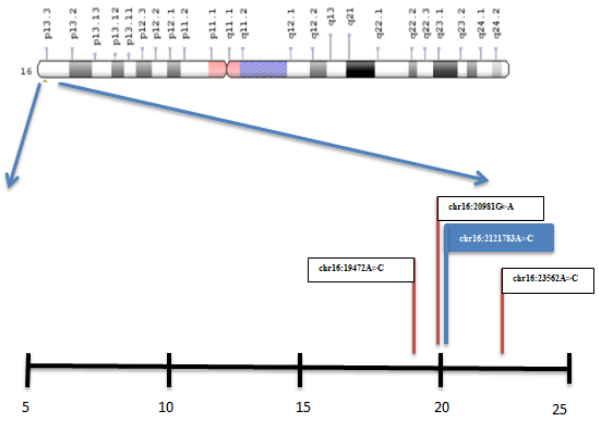

Figure 5: Position of TSC2 gene in chromosome 16p 13.3, where the de novo mutation took place at position chr16:2121783A>C along with chr16:1947-2A>G, chr16:20981G>A chr16:23562A>C variant.

The exon-intron junction must be conserved for specific and proper splicing. Some exons are constitutively spliced and many are alternatively spliced to generate variable forms of mRNA from a single pre-mRNA species. Abnormal splicing of the pre-mRNA is one of the reasons for occurrence of much human disease.[10] Nuclear pre-mRNA splicing is catalysed by the spliceosome, a multi-megadalton ribonucleoprotein (RNP) complex. Over 300 different proteins are associated with human spliceosomes, majority having specific RNA recognition activities, while others have NTPases which drives the overall process forward and ensure its consistency. Splicing is an essential step in gene expression. Human genetic disorders are caused by a mutation that can add/remove a single splice site altering the splicing mechanism.[10] In most cases, use of any unnatural splice sites or intron retention introduces premature termination codons (PTCs) into the mRNA, typically resulting in degradation by nonsense-mediated decay (NMD) and loss of function of the mutated allele.

CONCLUSION

Due to mutation in the TSC2 gene (chr16:2121783A>C) at c1947A>C, at intron 18, that has been positive for a heterozygous 'pathogenic' variant is detected in the essential splice acceptor site. It is suggestive that this variant might affect splicing due to the loss of constitutive splice site and introduction of a new splice site. As discussed earlier, the TSC2 gene plays an integral part within many molecular pathways of which mTOR is the most important. In TSC2, exon 34-exon 38 encodes amino acid residue 1517-1674 has the homology to Rheb. Since, the de novo mutation that has occurred at intron 18 that will likely result in the loss of function, therefore it might lead to a frameshift and consequent premature termination of the protein. It is possible due to introduction of a premature stop codon. This aberrant transcript will likely be targeted by nonsense mediated mRNA decay mechanism (as suggested by the clinical exome test). Thus, we may conclude that the tuberin protein encoded by the incomplete TSC2 gene is being unable to interact with Rheb, leading to dysregulation of mTOR signalling and causing tuberous sclerosis disease.

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