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P445

Beyond NGS: Detailed Analysis of Sanger, MLPA and RNA Confirms Tuberous Sclerosis Complex in Clinically Diagnosed Patients

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INTRODUCTION

Tuberous sclerosis complex (TSC) is a well-known genetic condition, characterized by growth of non-cancerous tumors in various organs, as well as an increased risk for renal and brain malignancies. Despite established diagnostic criteria and molecular testing, 10-25% of clinically diagnosed TSC patients have negative genetic testing. This is attributed to deep intronic or low frequency mosaic variants that standard sequencing and deletion/duplication analysis may miss [1].

We report two adult female patients with clinical diagnoses of TSC but negative conventional NGS testing, who sought further evaluation to inform reproductive decision making. Utilizing Multiplex Ligation-dependent Probe Amplification (MLPA), Sanger sequencing, and RNA studies, both patients had novel low-frequency mosaic variants in TSC2, confirming their molecular diagnoses.

CASE PRESENTATION

Patient 1 had a clinical diagnosis of TSC since infancy including rhabdomyomas, cortical tubers, renal angiomyolipomas and cysts, facial angiofibromas and learning difficulties. She also had congenital lymphedema and neuronal intestinal dysplasia. Her initial genetics workup at 13 years old included a microarray, TSC panel and exome sequencing which were non-diagnostic. She returned for follow up at 18 years old when she was 11 weeks pregnant.

Patient 2 had a clinical diagnosis of TSC since 10 years old when she was found to have a subependymal giant cell astrocytoma and subsequent evaluations identified cortical tubers, renal angiomyolipomas and cysts, hypomelanotic macules, and facial angiofibromas. She presented for genetic testing at 30 years old when she was undergoing an infertility evaluation. A TSC panel yielded non-diagnostic results.

DIAGNOSTIC WORKUP

A conclusive result was identified for both patients by manually reviewing RNA, Sanger, and MLPA data from a custom cancer panel with TSC1 and TSC2 plus RNA.

Patient 1 had a pathogenic variant in TSC2, specifically a tandem duplication of exons 32-37, detected at levels indicative of a low frequency mosaic variant. This copy gain was first detected by MLPA (Figure 1), and confirmed in the RNA data, where junctional reads supported tandem duplication of these exons (Figure 3).

Patient 2 had a pathogenic variant in TSC2, specifically c.690C>A p.C230*, with a low variant allele frequency of <10% in DNA-based reads. This was detected through more sensitive analysis of the NGS reads, followed by confirmation by Sanger sequencing and identification of the variant in the RNA data as well (Figure 2).

TREATMENT AND MANAGEMENT

Both patients were counseled about the reproductive implications of the molecular diagnosis and the low frequency variants. There were no changes in the clinical management of their TSC related symptoms.

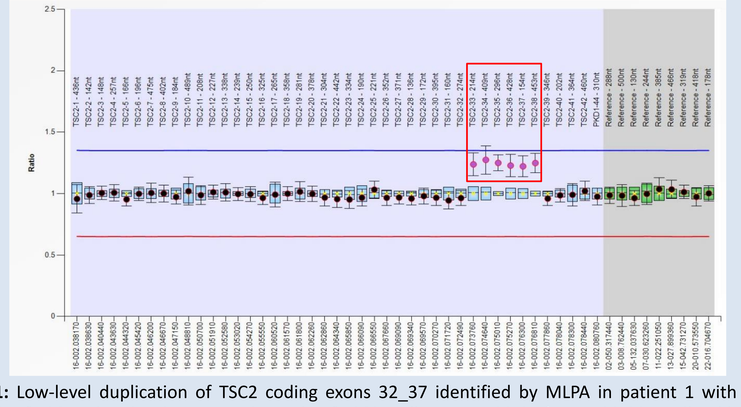

Figure 1: Low-level duplication of TSC2 coding exons 32_37 identified by MLPA in patient 1 with a clinical diagnosis of TSC. A low-level copy number gain of TSC2 coding exons 32-37 (exons 33-38, outlined in red) was identified by MLPA. If in tandem, this duplication is predicted to result in a frameshift and premature termination codon, resulting in an NMD-prone transcript.

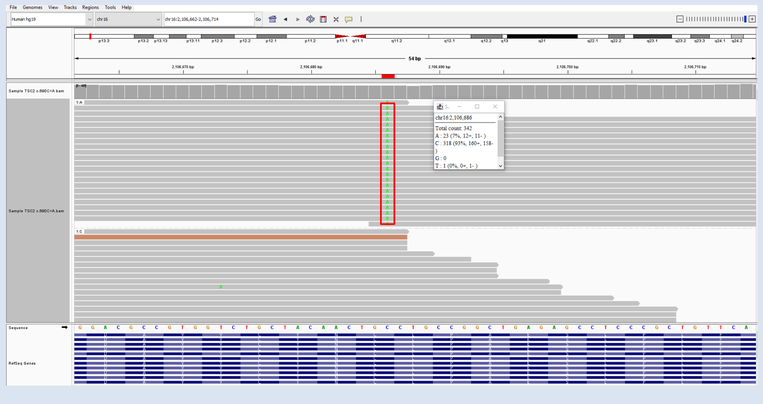

Figure 2. TSC2 c.690C>A p.C230* identified in 7% of NGS reads in patient 2 with a clinical diagnosis of TSC. Screenshot from Integrated Genomics Viewer (IGV) identifies a single nucleotide substitution (C>A) in ~7% of NGS reads at genomic position 2106686 in Chromosome 16. This low-level substitution (outlined in red) is predicted to result in a premature termination codon at p.C230.

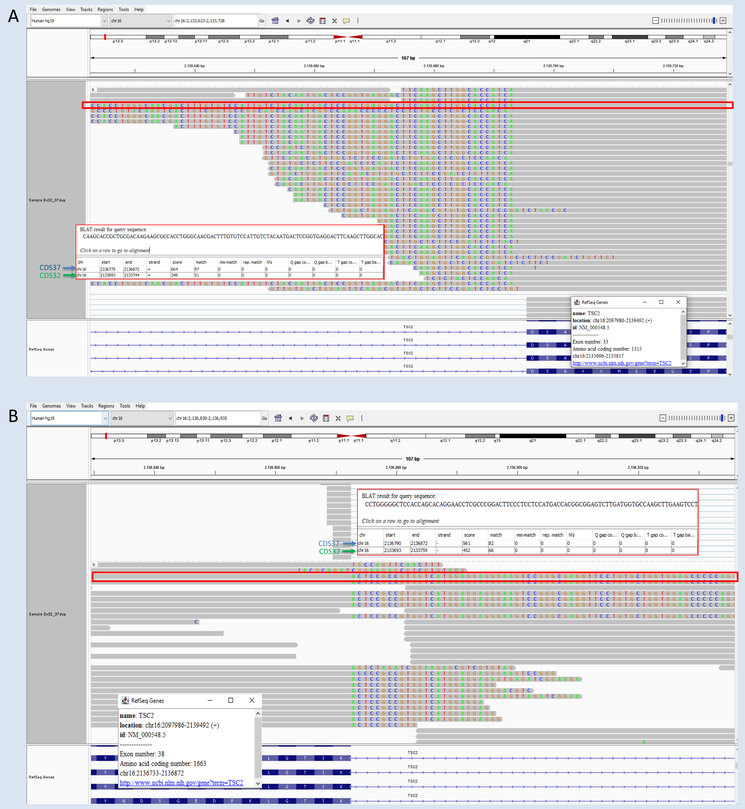

Figure 3: Soft-clipped RNA reads supporting tandem duplication of TSC2 coding exons 32 to 37 in patient 1. Screenshots from IGV of RNA reads identified to have a low-level duplication of coding exons 32_37 in TSC2 by MLPA confirm that this alteration is in tandem, and thus predicted to result in a frameshifted transcript leading to a premature termination codon and NMD-prone transcript. A) Multiple soft-clipped reads on the left side of CDS32 (exon 33) align to the coding sequence of CDS37 (exon 38), as shown in BLAT (BLAST-like alignment tool, UCSC) query of the sequence outlined in red. B) Multiple soft-clipped reads on the right side of CDS37 (exon 38) align to the coding sequence of CDS32 (exon 33), as shown in BLAT query of the sequence outlined in red.

OUTCOME AND FOLLOW UP

Patient 1 declined prenatal diagnostic testing and chose to pursue postnatal genetic testing. Patient 2 brought the results to her fertility team to determine how it may impact their recommendations. Both patients were glad to have found a genetic cause after so long.

DISCUSSION

We present two adult, female patients with clinical diagnoses of TSC without molecular confirmation following DNA-only analysis. Additional scrutiny of MLPA, Sanger, and RNA data successfully identified low frequency pathogenic variants in TSC2. These data suggests that additional testing modalities beyond conventional NGS should be considered for patients with clinically diagnosed TSC who have negative DNA testing results.

An important caveat is that these low frequency variants may not be causal and there could be other disease-causing alterations that were not detected due to technical limitations. The low frequency variants could be expressed in relevant tissues and be causing TSC or it could not be causal and the true underlying variant is undetected.

Challenges include that RNA testing requires a blood sample, whereas most DNA analyses can be performed on buccal or saliva-based samples. In addition, current RNA testing for TSC1 and TSC2 is performed as part of a cancer panel, and associated risk estimates for those reports are cancer-focused, which may not be the primary concern for individuals with TSC.

CONCLUSIONS

These cases highlight the importance of including additional testing modalities, such as MLPA, Sanger and RNA analysis in the molecular diagnosis of TSC, particularly when no significant variants are identified through conventional NGS testing. This is especially important for TSC which has mosaicism in 10-15% of cases [2]. By including additional testing methodologies, a greater chance of molecular diagnosis is achieved. Successfully identifying a causative variant enhances patient care, enabling TSC patients and their families to make informed decisions regarding preventive screening, healthcare decision-making, and family-planning.

REFERENCES

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