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7030

Molecular Analysis Of Congenital Adrenal Hyperplasia-X Syndrome And Development Of A Pilot Panel For Analyzing Structural Variations With Long-Read Sequencing

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Purpose: Genomic recombination events between the *CYP21A2* and *CYP21A1P* genes are frequent due to their high sequence homology. Deletions in both the *CYP21A2* and *TNXB* genes result in a chimeric *TNXA*/*TNXB* gene, leading to congenital adrenal hyperplasia (CAH)-X syndrome, characterized by a hypermobile form of Ehlers-Danlos syndrome. The aim of study was to determine the frequency of CAH-X syndrome and to develop a pilot panel for structural variation analysis using long-read sequencing technology. **Methods:** Among the 165 unrelated families with confirmed genetic mutations in the *CYP21A2* gene, 44 probands had large deletions in one or both alleles. Long-range PCR was performed in 12 patients out of 44 probands using a forward primer for the 5'-UTR of the *CYP21A2* and *CYP21A1P* and a reverse primer specific for exon 32 of *TNXB*. The amplified PCR products were then subjected to Sanger sequencing. We constructed a custom panel of 140 kb containing the RCCX modules arranged as *STK19-C4A-CYP21A1P-TNXA-STK19B-C4B-CYP21A2-TNXB*. In 4 patients with deletions in both the *CYP21A2* and *TNXB* genes, long-read sequencing was performed using the custom panel on the PacBio Sequel II platform. **Results:** Out of 12 probands, eight harbored *TNXA*/*TNXB* chimeras. One patient was homozygous for CAH-X CH1 with a 120 bp deletion in exon 35 and had a Beighton score of 6 for joint hypermobility and multiple diverticula in the ascending colon. Two patients were heterozygous for CAH-X CH1, while three were heterozygous for the CAH-X CH2 with an intact exon 35 and the c.P4058W in exon 40. Two patients carried heterozygous mutations p.S4175N in exon 43, which is an unclassified type. Using long-read sequencing with a custom panel, missense mutations and *TNXA*/*TNXB* chimeras were detected in four patients previously identified by conventional direct sequencing and MLPA. **Conclusions:** This study identified 8 patients with CAH-X syndrome carrying four different *TNXA*/*TNXB* chimeras by long-range PCR. Long-read sequencing emerged as a comprehensive and efficient method for the molecular analysis of CAH, especially for the

identification of structural variations. Biallelic deletion of *TNXB* was associated with severe clinical manifestations. Therefore, screening for CAH-X is essential for clinical management and prevention of the long-term consequences of CAH-X syndrome.

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