Sanger sequencing of ILDR1 exon 7

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Abstract

Sanger sequencing was performed on available samples from all affected family members to determine whether the potential mutation in the causative gene co-segregated with the disease phenotype. In order to amplify exon 7 of the *ILDR1* gene, we designed the following primers: ILDR1-7F: TTGATGTCCTGATTCTGAGG and ILDR1-7R: CTCTGTGGTGGAATGAGAGG. The amplified products were then purified using Wizard SV Gel and PCR Clean-up system (Promega, USA) and were consequently sequenced using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Thermo Fisher Scientific, USA). The resulting sequencing reactions were then purified and precipitated using Ethanol/EDTA/Sodium acetate precipitation method. Capillary sequencing was performed in a Genetic Analyzer 3500 (Applied Biosystems, Thermo Fisher Scientific, USA) and the data were analyzed using Sequencing Analysis software. The sequences were aligned with the published sequence of the *ILDR1* gene (NM_001199799.1).

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