Supplementary information

S1. WRN gene sequencing report. Description of the methodology and interpretation of the pathogenic variant found.

Genetix - Research center for human and reproductive genetics

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WRN gene sequencing

Methodology

DNA extraction was initially performed, and then construction of the libraries was done by Nextera XT kit (Illumina). After that, the sequencing of the libraries (2x150) was done through MiSeq sequencer (Illumina). Finally, bioinformatic analysis of the *WRN* gene was carried out.

Regarding to quality control of genomic data, it meets the following requirements: 100% representativeness of all gene exposures, minimum coverage of 20x, sequencing by Sanger of those regions of interest with a coverage less than 20x, and analysis of small deletions/insertions and point mutations in the coding region and splicing sites using NGS (Next Generation Sequencing). This study does not allow us to detect large deletions/duplications.

Interpreting

The coding region of the *WRN* gene was analyzed and the variant NM_ 000553.4:c.2581C>T (NP_000544.2:p.Gln861Ter) in homozygosis was identified. This truncated protein affects the helicase domain of the protein and is unable to enter the cell nucleus. It has been previously reported in the literature in the homozygous state in a patient with premature aging (Hum Mutat. Jun:27(6):558-67).

Several cell defects have been identified in cultured somatic cells derived from individuals with Werner syndrome (WS), including a much shorter replicative life span compared to controls of the same age.

The WS cell genome is highly unstable, with an increase in chromosomal rearrangements and large deletions. WS cells are unable to optimally repair DNA, and telomeric length is rapidly shortened. WS cells also appear to be deficient in DNA replication and certain DNA repair pathways being hypersensitive to DNA damaging agents. In addition, WS cells are affected in RNA polymerase II transcription and p53-mediated apoptosis. These alterations could explain the pathogenesis of WS.

Conclusion

The variant NM_000553.4:c.2581C>T (NP_000544.2:p.Gln861Ter) is identified in homozygosis. This variant generates a stop codon at position 861 and has been classified by the predictors as pathogenic.

It is recommended to perform the analysis of this variant in relatives at risk, because it is an autosomal recessive condition.

This result must be interpreted by your treating physician.

Genetic counseling is recommended.

Note

This study does not exclude mutations in unrealized regions such as exon/intron limits beyond those analyzed, or in splicing recognition sites.

The efficiency of the method used is close to 99%, however, diagnostic errors can occur to have mutations at the site of union of the primers, which would generate preferential allelic amplification in the PCR assay.

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