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Development of semiquantitative PCR (sqPCR) assays for determining the extent of the Williams syndrome microdeletion among affected Sri Lankan patients

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Background: Williams Beuren Syndrome (WBS) is associated with congenital heart defects, developmental delay, hypercalcaemia and characteristic facial dysmorphisms. A variable chromosome 7q11.23 microdeletion of 1.5 to 1.8 Mb with loss of 28 genes including elastin (*ELN*) is implicated in its aetiology.
Objective: To determine the extent of the WBS microdeletion using semiquantitative PCR (sqPCR).
Methods & Methods: Suspected WBS cases (n=24) were recruited following informed consent. Genomic DNA was extracted from blood samples and spectrophotometrically quantified. Twelve target regions within and flanking the WBS region were used for sqPCR amplification and dosage determination. The *CFTR* gene was used as the reference gene for normalization. Twelve separate duplex sqPCR assays were developed, each involving the simultaneous amplification of the target and control regions of the patient sample (P). Positive and negative control samples (N) were also amplified using the same target and control primers. Following agarose gel electrophoresis, the amplified products were quantified using the Bio-Rad, Molecular Imager, Gel DocTM XR+ with Image LabTM software. A ratio of P: N of 0.5 indicated a deletion while a ratio of 1 indicated absence of a deletion. Assay results were validated against whole exome sequencing (WES).
Results: Nineteen patients were found to have a deletion including 17 with the typical 1.5Mb deletion (reported in around 95% of WBS cases) and two had an atypical larger size deletion. Five patients did not have the deletion. These results were validated by WES.
Conclusion: The sqPCR is a low-cost assay and using only two markers around 0.64Mb apart from each other, the assay is able to detect 99.7% of the described deletions. Using multiple duplex sqPCR assays, the extent of deletion can be determined and used in patients suspected of having atypical deletions that may be missed by other investigations.
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