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AB096. Molecular diagnosis of aniridia or WAGR syndrome using a simple DHPLC-based semi-quantitative multiplex PCR for detection of *PAX6* large deletion

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Background: Wilms tumor, aniridia, genitourinary abnormalities, and mental retardation (WAGR) syndrome is a rare genetic disorder due to chromosomal deletions at 11p13 containing contiguous genes including *PAX6* and *WT1*. The size of the deletion may vary among individuals. Aniridia is almost always present in newborn with WAGR syndrome. However, not all individuals with aniridia are diagnosed with WAGR Syndrome. Mutation analysis by direct DNA sequencing in all *PAX6* coding exons was performed firstly in patients suspected to be aniridia. In case of the absence of *PAX6* point mutations, additional test for large deletion/duplication would be performed. Detection of chromosomal abnormalities associating with WAGR syndrome is technically challenging, being typically done using costly to perform, time-consuming and laborious techniques such as fluorescence in-situ hybridization (FISH), multiplex ligation-dependent probe amplification (MLPA) and array comparative genomic hybridization (array CGH) analyses.

Methods: To better facilitate the detection of such large deletion, we have developed a simple analysis of gross *PAX6* deletion based on semi-quantitative multiplex PCR using denaturing high-performance liquid chromatography (DHPLC) analysis. Relative chromatogram peak heights of exons 1, 2, 3, 4, 5, 6, 7, 8, 9, 12 and 13 of *PAX6* gene coupled with the reference genes on X-chromosome (*L1CAM* or *ACOT9*) and autosome (*TFF1* or *USP25*) in 4 different multiplex PCR sets of the tested samples were compared to those of the normal controls.

Results: Four patients under suspicion of aniridia regardless of the presence of WAGR without identifiable pathogenic *PAX6* point mutations were successfully diagnosed by this in-house method. A heterozygous whole *PAX6* gene deletion was found in three patients and a heterozygous *PAX6* deletion encompassing exons 1 was identified in one affected family.

Conclusions: The simplicity and reliability of this technique allow us to easily detect large genomic rearrangements in aniridia/WAGR-suspected patients.

Keywords: WAGR syndrome; aniridia; *PAX6* deletion; denaturing high-performance liquid chromatography (DHPLC); semi-quantitative PCR

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