Elucidation of Genomic DNA Structures Distinctive of Patients with 46,XX Testicular DSD Using Genome-Wide Analyses

Introduction and Objective: Although it has been elucidated that several genes are involved in testicular differentiation including the SRY genes, the whole picture remains unclear. While functional analyses of candidate genes have been performed, genome-wide analyses have recently been used as alternative methods to specify regions involved in testicular differentiation. In the present study, we performed genome-wide analysis in patients with 46,XX testicular DSD in order to comprehensively elucidate the mechanisms of testicular differentiation.
Materials and Methods: Whole genomic DNA was extracted from the peripheral blood of 4 patients with 46,XX testicular DSD. All patients were SRY-negative. Genomic DNAs were digested by a restriction enzyme, amplified by PCR after adding specific adaptors, and hybridized to the GeneChip® Human Mapping 250K Array. Compared to normal female data, we detected common regions in 4 patients of (1) loss of heterozygosity (LOH) and (2) copy number variation (CNV) using Genotyping Console Software. This study was approved by the institutional review board.
Results: We allocated the probe number information to GenBank® sequence data and detected the loci of affected genomic DNA regions. (1) LOH was recognized in 19 regions of 11 chromosomes. Twenty-seven genes or vicinity areas were included in the applicable regions. (2) Copy number loss was recognized in 13 regions of 10 chromosomes, and these regions included 55 genes. Copy number gain was detected in 6 regions of 4 chromosomes, which included the upstream region of the SOX3 gene.
Conclusions: The LOH regions did not contain genes associated with testicular differentiation. However, the upstream area of the SOX3 gene was included in the region of copy number gain. Although mice and humans with SOX3 mutations do not show defects in sex determination, it was recently reported that Sox3 overexpression in the bipotential gonad led to complete XX male sex reversal. The area regulating SOX3 expression is presumably located in Xp27.1, which showed copy number gain. Subsequently, high expression of the SOX3 gene led to testicular differentiation despite SRY gene loss. Since this applicable area is not within a coding region, genome-wide analyses were valuable for detecting novel regions associated with testicular differentiation.