Abstract

The differentiation of the embryonic gonads into either testes or ovaries according to the genetic makeup is the key step in mammalian sex-determination. Although several genes involved in human sex determination have been identified; existence of several XY females and XX males (sex reversals) with the normal sequence of the known genes suggests the existence of additional gene(s) involved in sex-determination in human. Therefore, this study was aimed at analyzing a large number of sex reversed female (93 individuals), male (1 individual) and intersex (1 individual) cases with known sex-determining genes, and search for additional regions(s) linked with sex reversal in the cases with unexplained genetic etiology. Most of the cases classified as ‘primary amenorrhea’ or ‘XY females’ by clinicians in fact confirmed to androgen insensitivity syndrome (AIS) upon detailed clinical and genetic analyses. To understand the genetic basis of AIS, we undertook direct sequencing of androgen receptor (AR) gene in all the AIS cases. The identification of the mutations in AR gene explained genetic etiology of AIS in a significant number of cases (~20%), and also indicated the frequent occurrence of AR mutations in these cases. In vitro assays using mutant AR alleles indicated that the mutation resulted either in the loss of ligand binding to the receptor, or the loss of transactivation function of the ligand-AR complex.

In an effort to understand the genetic basis of varying penetrance of AIS, we undertook sequence analysis of complete coding and untranslated region (UTR) regions of SRD5A2 gene and triplet repeat length polymorphisms in AR gene. We observed no mutation in
SRD5A2 gene in these cases, however, we observed putative role of AR-CAG repeat in affecting the overall AIS phenotype. On the basis of the smaller size of the CAG repeats among individuals with partial androgen insensitivity (PAIS) in comparison to individuals with complete androgen insensitivity syndrome (CAIS), we proposed that the loss of AR function due to the molecular defects causing partial loss of androgen sensitivity may be compensated partially by the presence of smaller CAG repeats length in the background. Similarly, the presence of longer CAG repeats stretch in the background may enunciate the overall extent of androgen insensitivity.

Among the other category of the sex reversals were the gonadal dysgenesis cases. Sequencing of the major sex-determining genes in these cases could explain the etiology of the disorder in just 10% of the familial cases (1 out of 10). Therefore genome wide scan was undertaken on the other nine families to search for the additional genes linked with sex reversed phenotype. The genetic linkage analysis revealed a LOD score of 5.77 at the chromosomal locus Xp11.21-11.23, which covers a region of 3.41 cM (~3.41 Mb). Human genome database had 53 transcript entries for this region of X-chromosome. In silico analysis of the above region indicated certain candidate genes namely; UBE1, SSX2, MAGED1, GAGEC1, ZNF741, ZNF21 and WDR13, NUDT10, NUDT11. However, direct sequencing of the coding regions of the above genes did not show any mutation. One of the limiting factors in selecting the right candidate gene(s) was the lack of the information on the expression of these genes in the embryonic gonad.
The study of the unique cases of sex reversal included a sex reversed male (46,XX), who was completely masculinized, and had almost normal male genitalia despite the absence of SRY gene. The development of external genitalia and testicles despite the absence of SRY gene, indicated the existence of other potential candidates, which may initiate the testicular differentiation in the absence of SRY gene. It is also possible that the alternation of the dosage of a gene, which is normally up-regulated by SRY gene, might have resulted in the initiation of the male pathway in this individual. Another unique case was a SRY negative 46,XX intersex individual. The real-time PCR analyses for the gene copy number identified four copies of WNT4 gene in this individual. Taking into consideration this observation and the evidences from previous studies, we hypothesized that the WNT4 gene may be placed at the crossroads of the sex-determination pathway and the switch may be decided at the level of WNT4 gene.

On the whole, this study identified several pathogenic novel mutations in the AR gene of sex reversed (46,XY) individuals. This finding has helped in providing genetic counseling to improve the lifestyle of the patients and avoid transmission of mutant alleles to the coming generations. Identification of a novel chromosomal locus on Xp11.21-23 with high LOD score suggested the presence of another X-linked gene responsible for sex-determination in human. The genetic analysis of 46,XX male indicated the existence of a gene, mutations in which may give rise to 46,XX male individuals despite the absence of SRY gene. Additional insights into the genetic cascade of sex-determination have been provided by the identification of WNT4 gene duplication in an intersex case.

Abstract