Molecular Biological Methods in the Diagnosis of Growth Disorders

K.K.P. Hartmann

Pediatric Endocrinology, Universitaets-Kinderklinik
Frankfurt/Main, Germany

The development of new techniques especially in the field of molecular biology and protein chemistry has led to a more profound understanding of the impact of different hormones. In addition to the hormones themselves, their specific receptors and binding proteins have become a focus of interest surrounding recent research into human growth disorders.

The mutation of the genes for growth hormone and growth hormone receptors as well as the regulation of the gene expression of these proteins has gained importance in the diagnosis of certain severe growth disorders.

Growth hormone (GH), human chorionic-somatomammotropine (hCS) and prolactin (PRL) are related molecules. The amino acid sequence of GH and PRL are 35% identical whereas the sequences of GH and hCS are 85% identical. The genes for GH and hCS are located on chromosome 17, the gene for PRL is located on chromosome 6. A gene cluster for GH and hCS, composed of 2 GH and 2 hCS genes plus 1 hCS-similar gene, has been described on chromosome 17 /1-3/. The first GH gene (GH-1) encodes for the circulating, biologically active GH; in contrast, the GH-protein of GH-2 is 13 amino acid residues smaller /4/. The GH gene is composed of 5 exons. Different splicing within the 3rd exon results in two different GH proteins with a molecular size of 22 and 20 kDa. The main GH fraction in the pituitary has a molecular size of 22 kDa. In addition to the 20 kDa GH variant described above, a GH aggregate with a molecular size of 45 kDa has been described. The GH gene is translated to a 24 kDa pre-GH precursor peptide which is transported into the endoplasmic reticulum and there secernized as the 22 kDa form.

Genetic diseases which lead to impaired growth hormone synthesis have been described as mutations in the GH-1 gene. For isolated growth hormone deficiency, type I (IGHD-I) various deletions have been described which lead to the elimination of the GH-1 gene /5-8/. The effect is severe hypoglycemia and detectable growth hormone deficiency within a short time of birth /9/. Patients with these conditions develop – possibly due to the absence of immune tolerance following therapy with biosynthetic growth hormone – specific antibodies directed against the submitted growth hormone /5,10/. The deletion of the GH-1 gene has been investigated by using restriction fragment polymorphism (RFLP) of the chromosomal DNA containing the GH-1 gene /6,7,11/. A new method in the study of growth hormone gene deletions is the analysis by polymerase chain reaction (PCR) /27/. In addition to IGHD-I, other isolated growth hormone deficiencies have been described with, in contrast to IGHD-I, higher growth hormone levels in the serum. In the case of these diseases, using RFLP analysis of the GH-1 gene, no deletion of the GH-1 gene could be found /12/. However, a different RFLP pattern of the GH-1 gene suggests the existence of point mutation inside the GH-1 gene /12/. The employment of PCR-analysis and denaturing DNA gel chromatography will be found useful in the detection of these mutations. In contrast to the GH genes the transcription or translation of the hCS genes is not necessary for growth.

For most patients with severe growth hormone disorders no genetic defect has been described. However, patients with Laron dwarfism have a defect of the growth hormone receptors due to a mutated gene of the growth hormone receptor /14/. Since the cloning of the gene for the growth hormone receptor GH-R /15/ many