Clinical and Genetics Approaches to Hypogonadotropic Hypogonadism

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Idiopathic hypogonadotropic hypogonadism (IHH) is often defined when a girl or a boy reaches the age 13 and 14 respectively, with a bone age of at least 11, who lack secondary sexual characteristics in the presence of low serum sex steroids as well as low gonadotropins. In an individual with pubertal delay, to establish a diagnosis of IHH frequently requires a battery of tests and a long follow-up period. Increasingly popular use of whole exome sequencing made extremely important contributions in making timely diagnosis of IHH and understanding the human reproductive biology.

Key words: Idiopathic hypogonadotropic hypogonadism, clinical and genetics approach

Genetics in Pituitary Short Stature

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Studies on animal model about the developmental phases of pituitary gland have contributed to the understanding of the functional roles of the signal molecules responsible for the embryologic changes, development, growth and maturation and the transcription factors interacting with these molecules. The genes related to congenital pituitary deficiencies are evaluated in two groups according to their temporal and spatial expression during the developmental phases. The mutations of genes taking place in the early phase of development (*GLI2, HESX1, FGF8, FGF1, PROK2, PROKR2, OTX2, SOX2, SOX3, PITX2, ARNT2, LHX3, LHX4*) result in more complex and heterogeneous phenotypes such as pituitary anomalies, various craniofacial and limb malformations in addition to pituitary hormone deficiencies. On the other hand, mutations of genes playing a role in the late development (*PROP1* and *POU1F1*) are usually associated with multiple pituitary hormone deficiency (MPHD) phenotype. In 1992, the *POU1F1* gene has been described. It is the human homologue of the mapped *PIT1* gene identified in 1990 in dwarf mouse models and it has been screened in MPHD cases; the mutations have been shown and in this way, the first gene related to the phenotype has been described. In 1996, the human homologue of the *PROP1* gene mapped in dwarf mouse model has been reported and *PROP1* gene mutations have been shown by which the second gene of the disease has been determined. The first development of pituitary gland starts with the external stimulus. These are signal proteins like SHH, BMP, FGF, WNT and SHH, which function at early stages and even though they do not have any direct role in the development of Rathke's pouch, they are important for the development of midline, forebrain, brain lobes and eyes. SHH plays a role in the expression of GLI factors, which are expressed in the early stages of pituitary development. It has been shown that the heterozygous mutations in *GLI2* gene are related to pituitary hormone deficiency as well as to holoprosencephaly, ectopic hypophysis, hypophyseal hypoplasia, mild hypophyseal anomalies, polydactyly and corpus callosum agenesis. *HESX1*, the first pituitary transcriptional factor during embryologic development, has influence on expression of other transcriptional factors (LHX1, LHX2, LHX3) together with the formation of midline and Rathke's pouch. In the developing hypophysis, the *HESX1* expression should be decreased in order to express *PROP1* firstly and then *PIT1* at the right time. The essential clinical finding for *HESX1* mutations, showing both autosomal dominant and

recessive inheritance in human, is the optic nerve hypoplasia seen in 30% of the cases. Growth hormone deficiency is detectable in all the cases carrying mutation and in 50% of cases carrying mutation have other deficiencies in various pituitary hormones. *HESX1* mutations are detectable in nearly 1% of cases with general septo-optic dysplasia. *FGF8* and *FGFR1* genes, which are expressed in the ventral diencephalon, are necessary factors for Rathke's pouch development. The previously described mutations in *FGF8* and *FGFR1* genes related to Kallman syndrome and normosmic hypogonadotropic hypogonadism have been associated with septo-optic dysplasia and MPHD in 2012 and the prevalence was given as 4%. In a study held in 2012, prokinesis, previously associated with portal angiogenesis, neuronal development and migration, was considered to be associated with pituitary stalk transection syndrome and *PROK2* and *PROKR2* genes as well as with other genes related to hypopituitarism and pituitary stalk transection syndrome (*LHX4, HESX1, OTX2, SOX3*) were screened with the approach to candidate gene in 72 cases with pituitary stalk transection syndrome. In the same study, heterozygous mutations related to hypogonadotropic hypogonadism in three of the cases were detected in three *PROKR2* genes-one of them was new and the other two were isolated previously. In this study, one of them was heterozygous and the other one was homozygous. In total, 2 mutations were detected in *HESX1* genes. The gene frequency of *PROKR2* in hypophyseal cut and septo-optic dysplasia is given as 3%. *OTX2*, which is not expressed in the hypophysis but important in brain development as a TF, is seen with a large spectrum of syndromes such as with various hypophyseal deficiency findings from isolated growth hormone deficiency to panhypopituitarism, as well as hypoplastic and ectopic hypophysis and Chiari syndromes. The function losses in these gene mutations are shown in MPHD and additionally in cases with anophthalmia, small adenohypophysis and ectopic neurohypophysis. The other genes that play an important role in the hypophyseal development are *SOX1, SOX2* and *SOX3*. *SOX2* mutations in humans are associated with hypophyseal deficiencies and eye anomalies. *SOX3* gene is a gene which needs an expression continuing at a steady pace and thus the increase or decrease in expression could cause hypophyseal deficiency and central nervous system (CNS) malformations. As a gene on X chromosome, *SOX3* is associated with mental retardation, panhypopituitarism and brain anomalies (corpus callosum hypoplasia, hypoplastic hypophysis stalk and ectopic neurohypophysis) with its X-linked inheritance. *PITX1* and *PITX2* genes play a role in the later stages of pituitary development. These genes are important for CNS development and the other TFs playing a role in the hypophyseal development coordinate with these, especially with *PITX1*. *PITX2* is a TF expressed in the CNS, upper arms, lungs, liver and tongue. It plays a vital role in determining right/left axis. *PITX2* mutations have been associated with Axenfeld-Rieger syndrome described in 1996 which is characterized by rare ocular anomalies, craniofacial dysmorphism, teeth and umbilical anomalies in humans. The other gene related to this syndrome, *FOXC1*, was identified in 2009. For a big family associated with hypophyseal deficiency, eye anomalies, diabetes insipidus, epilepsy, CNS and kidney anomalies, the *ARNT2* gene was described in 2013. This gene is a helix-loop-helix motif carrying a TF. *LHX3* is expressed lifelong from embryologic stages through adult stage in Rathke's pouch, developed hypophysis, motor neurons, spinal cord, hindbrain, retina and epiphysis; *LHX4* expression starts in Rathke's pouch, shows limited expression in adenohypophysis and finishes at birth becoming progressively lower. *LHX4* is needed for the expression of *LHX3* at the right time. In addition, *LHX3* is needed for the expression of *HESX1, NOTCH2, SF1, TBX19* (corticotroph cell development), GnRH, FSHβ and *POU1F1*. LHX3 is responsible for autosomal recessive MPHD and 70% of cases have limited rotation in the neck. *LHX4* mutations are associated with a wide range of syndromes such as autosomal dominant isolated growth hormone deficiency as well as hypophysis hypoplasia, ectopic neuroectoderm, sellar hypoplasia, corpus callosum hypoplasia, or Chiari syndrome. The prevalence of this gene in cases with pituitary stalk transection syndrome is 2.4%. Although many genes associated with hypophyseal development have been characterized recently with the help of improvements in molecular genetics, 80-90% of the etiology of congenital MPHD was not able to be enlightened yet. The studies held in Caucasians have shown that the prevalence of short stature is 1: 4000. Because almost 43-63% of these cases are MPHD, the prevalence of MHPD is reported as 1: 8000. MHPD is defined as the short stature occurring as a result of the deficiency of one of the five other hormones of adenohypophysis (ACTH, TSH, PRL, LH and FSH) in addition to deficiency of growth hormone. Generally, these cases refer to endocrine clinics with findings like prolonged jaundice, hypoglycemia, micropenis which occur as a result of deficiencies of these hormones. According to the related gene, MPHD was classified into six groups: *POU1F1*-related type 1 (MIM: 613038, CPHD#1), *PROP1*-related type 2 (MIM: 262600, CPHD#2), LHX3-related type 3 (MIM: 221750, CPHD#3), *LHX4*-related type 4 (MIM: 262700, CPHD#4), *HESX1*-related type 5 (MIM: 182230, CPHD#5) and *OTX2*-related type 6 (MIM: 613986, CPHD#6). 30 mutations in *PROP1* gene were notified after the relationship between autosomal recessive MPHD was shown in 1998. This gene is important for the *POU1F1* gene expression and for the formation of pituitary precursors. Even though the frequency of the mutation shows variability depending on ethnicity, the frequency is 30% in familial

cases, whereas it is 1-2% in families with one case only. However, in a study that has been held in Turkey since 2012 in a series of 51 MPHD cases, sequence analysis of the *PROP1* gene was performed and one case and the affected brother were found to have homozygous 'stop' codon and other cases were found to have no mutations. *POU1F1* gene is necessary for the change and permanence of thyrotroph cells, somatotroph cells and lactotroph cells. The gene can both show dominant and recessive inheritance and also the heterozygous mutations, which can result in disease and cause a function loss in the gene product by dominant negative effect. Between 2007 and 2009, a project entitled 'the analysis of *PROP1, PIT1, HESX1* and *LHX3* mutations in multiple pituitary hormone deficiencies' was carried in nine Pediatric Endocrinology Clinics from Turkey with cooperation of İstanbul University Pediatric Endocrinology Clinic and Medical Genetics Department. The aim of this project was to investigate the mutations in the *PROP1, PIT1, HESX1* and *LHX3* genes which are unique to the Turkish population, to examine the relationship between phenotype and genotype, to evaluate the contribution of these mutations to early diagnosis and treatment and to complete the preliminary studies in indication of new genes in cases and families which are found to have no mutations. In 55 cases, 38 sporadic and 17 familial, clinical examination, hormone values, neuro-radiologic evaluation and pedigree were completed. The DNA samples from cases and their families have been kept in a bank. Firstly, DNA sequencing method and secondly, the multiplex ligation-dependent probe amplification (MLPA) procedure, for investigation of all gene/exon deletions in related genes in cases that were found to have no mutations, have been performed as molecular study gradually. A mutation was determined in total of 30.9% of cases: in 21.8% of cases-*PROP1* mutation, in 7.3% of cases-*POU1F1* mutation and in 1.8% of cases-*HESX1* mutation. In this study, one new mutation in the *PROP1* gene and three new mutations in the *POU1F1* gene were identified. The 66% of the mutations present in *PROP1* gene were whole gene mutations and this result showed the contribution of MLPA test to diagnosis in molecular genetics approach.

Key words: Genetics, pituitary, short stature

Clinical Findings of Osteoporosis

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Osteoporosis is one of the skeletal diseases which result in increase of fracture risk because of declined bone strength. In these days, it is necessary to evaluate osteoporosis as a disease related to multiple genetic, physical, hormonal and nutritional factors. Bone strength has two major components: 1) Bone density and 2) Bone quality. Osteoporosis causes fracture formation with minimal traumas during daily activities. The loss of bone density and deterioration of bone quality is an inevitable consequence of aging. In women, trabecular bone loss accelerates after menopause. With aging, the plaques forming vertebrae attenuate and the connections between trabeculae decrease. Moreover, horizontal trabeculae decrease more than the vertical trabeculae. The most important result of osteoporosis is fractures and most important of those is hip fractures. All around the world, as the number of old population increases, the incidence of hip fractures also increases. The prevalence of osteoporosis increases with the aging population, as well. Over age 50, one in every four women and one in every 8 men are found to be osteoporotic. Again, every white woman over age 50 has 40% risk of fracture in the hip, vertebrae, or wrist. The prevalence of vertebral fracture in postmenopausal women is estimated to be 20%. As a result, fractures related to osteoporosis can be seen in one in every two women and one in eight men in Caucasians. For indicating how dangerous osteoporosis is, one in every six women pass though the risk of hip fractures, whereas one in every 9 women has the risk of catching breast cancer. Besides, the mortality of hip fracture is way higher than the mortality of breast cancer. 50-60% of patients passing through a hip fracture cannot reach their previous functional capacity and 20% of those need long-term care. Along with the high mortality and morbidity rates of fractures depending on osteoporosis, they also bring an enormous economic burden on countries. Merely one third of osteoporotic patients get the right diagnosis, solely one seventh of osteoporotic patients get the right treatment.

More than one factor should be taken into consideration in the development of osteoporosis and these are:

1) Peak bone mass: the maximum bone mass reached at ages 25-30.

2) The rate of bone turnover.

3) The micro-architecture of bone and bone quality: The differences occurring in organic matrix of bone.

4) The macro-architecture of bone: the massiveness of bone, the length of femur and the ratio of the periosteal bone formation over endosteal bone turnover.