Genetics of autoimmune hypoparathyroidism

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Summary

Primary hypoparathyroidism not only occurs as an isolated idiopathic autoimmune disease (idiopathic hypoparathyroidism) but also as a component disease within the scope of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED). Hypoparathyroidism constitutes the major endocrine component disease in APECED occurring in 80-90 % of patients and manifests during childhood. The inheritance of APECED, monogenic and autosomal-recessive, is due to a defect in a single gene, called autoimmune regulator gene (AIRE) which has been identified in 1997 and mapped to chromosome 21. So far, 45 different mutations have been detected throughout the entire coding region of the AIRE gene. Two mutations (exon 8 (R257X) and exon 8 (13-bp deletion), occur most frequently in European and North American populations, respectively. They are responsible for the expression of a truncated autoimmune regulator protein. There is evidence that the AIRE protein has a central role in maintenance of immune tolerance. It has multiple domains which are discussed to be involved in transcriptional activity, nuclear transport, DNA binding, and homomultimerization. Mutational analysis of AIRE gene allows to identify patients at risk for APECED. On the other hand, it can help to distinguish patients with APECED from those with isolated hypoparathyroidism, and thereby, avoiding family members not having APECED of unnecessary follow-up. However, the absence of a mutation in the AIRE gene does not exclude the APECED. Therefore, diagnosis is dependent on the determination of the clinical picture of the syndrome.

KEY WORDS: genetics, autoimmune hypoparathyroidism, AIRE gene.

Clinical aspects of hypocalcaemia

Hypoparathyroidism manifests with hypocalcaemia which is associated with reduced parathyroid hormone (PTH) secretion from parathyroid glands due to disease or surgical damage to the parathyroids. PTH may mobilize calcium from bone and increase the resorption over the kidney (Brandi et al., 1998). Hypocalcaemia leads to alterations in neuromuscular function. Major symptoms are paraesthesia around mouth and in fingers, muscle cramps, and seizures. In addition, tetany (involuntary muscle contraction) may occur in hands resulting in carpopedal spasm. Chronic hypocalcaemia leads to calcification of the basal ganglia of the brain and involves the development of cataracts. For anticipation of incipient tetany, the Chvostek's sign can be used which is elicited by tapping the facial nerve immediately after it exits from the auditory canal. Tetany can also be predicted by Trousseau's sign (Brandi et al., 1998). For this, a blood-pressure cuff is maintained for ten minutes at 3 mmHg above the systolic pressure. Patients with Trousseau's sign show spasmodic contraction of the small muscles of the hand (carpopedal spasm). The final diagnosis of hypocalcaemia comprises the consideration of the clinical setting as well as the measurements of serum calcium and phosphate concentrations. Successful treatment usually implies administration of calcium intravenously or orally, depending on the urgency for a rapid response as well as the treatment with a short-acting vitamin D metabolite, for the most part 1,25-dihydroxyvitaminD.

The extracellular calcium concentration is measured by the calcium sensing receptor (CaSR) which is widely expressed in several tissues and is located in the plasma membrane of the cell. It functions as a sensor for extracellular calcium concentration and regulates the secretion of the PTH in dependence on changing extracellular calcium values (Pearce et al., 1996). Thus, stable calcium concentration can be maintained. The CaSR was recently reported to be an autoantigen in hypoparathyroidism. Several mutations in the gene for the CaSR have been described. Some mutations inactivate CaSR causing familial hypocalciuric hypercalcemia, whereas other mutations activate CaSR resulting in autosomal dominant hypocalcemia (Thakker, 2001).

Hypoparathyroidism

Primary hypoparathyroidism occurs both as an isolated so-called idiopathic autoimmune disease (idiopathic hypoparathyroidism) as well as within the scope of the autoimmune polyglandular syndromes (APS). Idiopathic hypoparathyroidism is present in over 80% of patients. Most patients are characterized by acquired hypoparathyroidism due to surgery or autoimmune etiology. In addition, familial forms of congenital and acquired hypoparathyroidism have been described which arise from gene defects. These hereditary forms of hypoparathyroidism are of various genetic origin (Tab. I). They comprise familial syndromes with multiple organ system abnormalities or isolated familial syndromes with different modes of inheritance, all being accompanied by hypoparathyroidism (Ahn et al., 1986; Thakker et al., 1990; Parkinson and Thakker, 1992). With respect to multisystem abnormalities, the parathyroid gland abnormalities are not intrinsic to defects within the parathyroid gland but are otherwise secondary to other developments or regulatory abnormalities. For example, the DiGeorge syndrome (caused by a gene defect: 22q11 deletion) comprises several developmental disorders which are centered on the third and fourth branchial pouches. It is characterized by partial or complete hypoparathyreoidism as a result of congenital absence or hypoplasia of the parathyroid glands, a T-cell deficiency due to partial or incomplete development of the thy-

Table I - Overview of familial hereditary forms of hypoparathyroidism.

Hypoparathyroidism with multiple organ system abnormalities
• Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED)
• DiGeorge syndrome
• Renal dysgenesis /sensorineural deafness syndromes
Isolated non-autoimmune hypoparathyroidism syndromes
• X-linked hypoparathyroidism
• Autosomal recessive syndromes (PTH gene intron splice site mutation)
• Autosomal dominant syndromes (PTH signal peptide mutation; calcium receptor mutations)

PTH, parathyroid hormone.

mus gland, cardiac and conotruncal defects, and craniofacial abnormalities. There are both sporadic and inherited forms to be found. Hypoparathyroidism is further associated with another rare familial multiple organ system failure, called autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), also denominated as autoimmune polyendocrinopathy syndrome type 1 (APS1).

Hypoparathyroidism associated with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy

Clinical manifestation: Hypoparathyroidism is the most common endocrine component disease in APECED which is a combination of several distinct disorders affecting mainly endocrine glands being characterized by immune-mediated destruction of endocrine tissues. It is characterized by the concurrently presence of at least two of the following three features: hypoparathyroidism (failure of the parathyroid glands which control calcium), candidiasis (yeast infection), and adrenal insufficiency (failure of the adrenal gland) (Ahonen et al., 1990). Most patients with APECED develop hypoparathyroidism (Fig. 1). This autoimmune disease is characterized by the autoimmune destruction of the parathyroid gland and adrenal gland. T-cell abnormalities lead to mucocutaneous candidiasis. Highest incidence for developing hypoparathyroidism occurred between 10 and 15 years of age (Gylling et al., 2003). Further endocrinopathies such as type 1 diabetes, gonadal dysfunction, autoimmune thyroid disease or autoimmune hepatitis may be also present leading to variable combinations of component diseases (Dittmar and Kahaly, 2003, 2004 submitted; Kahaly and Dittmar, in press). Non-endocrine diseases such as dysplasias of ectodermal structures (keratopathy and dystrophy of dental enamel and nails), alopecia, vitiligo, autoimmune gastritis, and pernicious anemia are frequently present (Ahonen et al., 1990; Perheentupa, 1996). Hypoparathyroidism and mucocutaneous candidiasis are generally manifest during childhood, whereas the onset of autoimmune adrenal insufficiency occurs in early adolescence. Further component diseases may appear throughout adulthood. The phenotype of APECED varies widely. APECED patients show defective tolerance to certain self-antigens. Various other autoantigens have been observed (Uibo et al., 1994; Perniola et al., 2000; Betterle et al., 2002), but circulating autoantibodies against parathyroid gland have been difficult to demonstrate. Antibodies against CaSR have been observed in 35% of patients with APECED (Li et al., 1996), whereas other authors did not find differences versus controls (Gylling et al., 2003). APECED has a penetrance of 100% and does not display female preponderance.

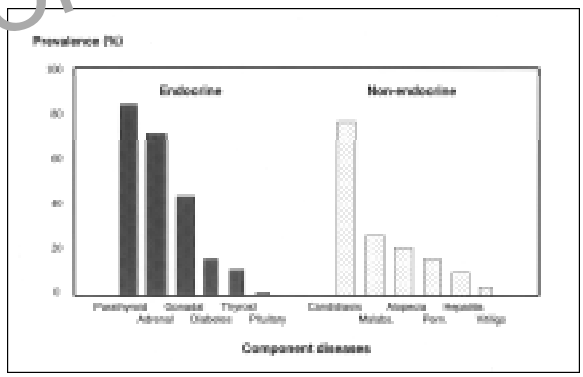

Figure 1 - Prevalence of endocrine and non-endocrine component diseases in patients with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED). Abbreviations: adrenal; Addisons's disease; candidiasis, mucocutaneous candidiasis; diabetes, type 1 diabetes mellitus; gonadal, gonadal failure; hepatitis; chronic active hepatitis; parathyroid, hypoparathyroidism; malabs., malabsorption syndromes; pern., pernicious anemia; pituitary, hypopituitarism; thyroid, thyroid disease.

Immunogenetics: Contrary to other autoimmune diseases, the genetic basis of APECED is monogenic with autosomal-recessive inheritance (Ahonen, 1985). It is due to a defect in a single gene which has been identified in 1997, mapped to chromosome region 21q22.3, and denominated as autoimmune regulator (AIRE) gene (Bjorses et al., 1996; Nagamine et al., 1997; Finnish-German APECED Consortium, 1997; Aaltonen et al., 1997; Chen et al., 1998). The AIRE gene has 14 exons spanning 11.9 kb of genomic DNA (Nagamine et al., 1997). So far, 45 different mutations have been published throughout the entire coding region of the AIRE gene (Pearce et al., 1998; Wang et al., 1998). These are deletions, insertions, and substitutions (Heino et al., 2001; Meloni et al., 2002). Many mutations are frameshift or nonsense mutations. Different mutations have varying effects on the *in vitro* function of the AIRE protein (Halonen et al., 2004). Nine mutations in the AIRE gene were found in patients with APECED (Finnish-German APECED Consortium, 1997; Nagamine et al., 1997; Scott et al., 1998; Rosatelli et al., 1998). Two mutation hotspots occur: R257X in exon 6 and 13-bp deletion (13-bpdel) in exon 8 (Fig. 2) which are the most frequent mutations in European populations. For both mutations, different haplotypes have been observed giv-

Figure 2 - Localisation of major mutations in the autoimmune regulator (AIRE) gene on chromosome 21 observed in patients with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED). The 14 exons of the gene are shown as vertical boxes. Most common mutations in European populations are R257X in exon 6 and 13-bp-del in exon 8. The mutation R139X has been observed in the Sardinian population.

ing evidence to independent origins. The R257X is a C to T transition being responsible for the expression of a truncated regulator protein. The 13-bp-del is characterized by a deletion of 13 nucleotides in exon 8 of the AIRE gene causing a frameshift mutation resulting in a truncated protein. R257X is the predominant mutation in Finnish and northern Italian patients. It has been found in 83% of alleles studied in Finnish patients with APECED (Nagamine et al., 1997) as well as in Swiss, northern Italian, and eastern European patients (Scott et al., 1998; Cihakova et al., 2001). In contrast, the 13-bpdel is the major mutation in Anglo-American and Norwegian patients, accounting for more than 70% of mutant alleles in United Kingdom and 50% of North American patients with APECED (Wang et al., 1998; Nagamine et al., 2000). In addition, specific mutations have been observed in geographically more isolated regions. For example, a single Sardinian mutation (R139X, arg139-to-term) in exon 5 of the AIRE gene was responsible for 19 of 21 Sardinian AIRE alleles in patients with APECED and has not been found in other populations (Rosatelli et al., 1998). There is evidence for some association between the type of mutation in the AIRE gene and the APECED phenotype. In this context, the frequency of hypoparathyroidism is lower in patients with the R257X allele in homozygous form than in patients without the allele (83% versus 94%) (Halonen et al., 2002).

The AIRE gene is expressed in immunologically relevant tissues, primarily in the thymus medulla, in lymph nodes and CD14-positive monocytes, but not in CD4-positive T cells (Pitkänen et al., 2001; Kogawa et al., 2002). It encodes the AIRE autoimmune regulator protein that contains 545 aminoacids (Nagamine et al. 1997). It is probably a central protein in the maintenance of immune tolerance and comprises multiple domains which might be involved in transcriptional activity, nuclear transport, DNA binding, and homomultimerization (Halonen et al., 2004). It contains two plant homeodomain (PHD) type zinc fingers, four nuclear receptor binding LXXLL motifs, a putative DNA-binding domain denominated SAND as well as a highly conserved N-terminal domain which is similar to the homogeneously staining region domain of the Sp100 protein (Pitkanen and Peterson, 2003). This important DNA binding molecule has structural domains that are characteristic for transcription regulators (Meloni et al., 2002). The AIRE expression in chondrocytes which were derived from human fetal growth plates as well as primary culture of human chondrocytes gave evidence for a potential impact of abnormal AIRE expression in the development of reversible metaphyseal dysplasia in APECED (Harris et al., 2003).

There is evidence that the phenotype of APECED is modified by other genetic factors (Halonen et al., 2002). However, recent data did not show any associations of the HLA class II DRB1, DQA1, and DQB1 alleles with hypoparathyroidism in patients with APECED (Gylling et al., 2003). The finding that most patients with hypoparathyroidism are males indicates that sex may modify the phenotype of APECED (Gylling et al., 2003).

The AIRE can be considered as a key toward the understanding of the molecular pathogenesis of APECED promoting the ability for molecular diagnosis. Mutational analysis of AIRE is important to identify patients at risk for APECED. In particular, genetic diagnosis of APECED is important to distinguish patients with isolated hypoparathyroidism from those who overlap with the phenotype of APECED, because it avoids family members not having APECED of unnecessary follow-up. However, the absence of a mutation in the AIRE gene does not exclude the APECED. Therefore, diagnosis is dependent on the determination of the clinical picture of the syndrome (Perheentupa, 2002). The etiology of hypoparathyroidism of APECED is still unknown. One should study whether the AIRE gene is expressed in the human parathyroid, because its absence could be a pathogenetic factor (search for AIRE mRNA in the parathyroid).

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