BRIEF REVIEW
ΒΡΑΧΕΙΑ ΑΝΑΣΚΟΠΗΣΗ

Clinical, classical and molecular genetics of 21-hydroxylase deficiency
Current concepts

Congenital adrenal hyperplasia due to deficiency of the enzyme 21-hydroxylase (21-OH) is divided into the classical and non-classical forms. It is one of the most common autosomal recessive inherited diseases in humans. The classical form appears at a rate of between 1:5,000 and 1:15,000 among the live neonates of North America and Europe. The rate of the non-classical form is especially high among Ashkenazi Jews and some Mediterranean populations (i.e. Italians, Hispanics). Three alleles are associated with the 21-OH locus and can combine in various ways to give rise to individuals who are either unaffected, heterozygote carriers, or affected with the classical or non-classical disease. Variable signs and symptoms of hyperandrogenemia (hirsutism, acne, virilization, short stature, menstrual irregularities) are common in both forms of the disorder. Between the genes responsible for the synthesis of the enzyme 21-OH and the antigens of the HLA system there exist both a proven genetic linkage and a proven genetic linkage disequilibrium. The most common haplotypes usually observed in the classical form are HLA-B_{w47}, HLAB_5 and HLA-B_{35} and in the non-classical form of the disease the haplotype HLA-$B_{14}DR_1$. The great progress in molecular biology during recent years has resulted in the development of new sensitive methods of DNA analysis and study, such as polymerase chain reaction and southern blot analysis. The synthesis of 21-OH is controlled by two genes, the active CYP21B gene and the CYP21A pseudogene. All forms of the disease have known sequences of gene changes due to mutations in isolated proteins or to translocations or deletions of genetic material whole series of genes.

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ΑΡΧΕΙΑ ΕΛΛΗΝΙΚΗΣ ΙΑΤΡΙΚΗΣ 2002, 19(5):534–538

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Κλινική, κλασική και μοριακή γενετική της ανεπάρκειας της 21-υδροξυλάσης

Περίληψη στο τέλος του άρθρου

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Congenital adrenal hyperplasia (CAH) caused by a deficiency of adrenal 21-hydroxylase (21-OH) is an autosomal recessive inherited disorder of steroid metabolism and is divided in to the classical (C-CAH) and the non-classical (NC-CAH) form.[1,2] 21-OH block is the most common form of CAH (90% of cases), the most frequent cause of sexual ambiguity and the most frequent endocrine cause of neonatal death. The prevalence of these disorders is high and CAH has been estimated to affect approximately in 1,000 non-Jewish Caucasian1 women.[3–5] Heterozygocity for 21-OH deficiency has been estimated to affect approximately 1 in 60 of the general non-Jewish Caucasian population, although it may affect as many as 1 in 3 Ashkenazi Jews.[6,7] Close genetic linkage between the HLA complex located on the short arm of chromosome6 and 21-OH deficiency was first described by Dupont at al.[8] Studies of families with informative recombinations clearly indicate that the 21-OH deficiency gene segregates with HLA-B, constituting genetic linkage with this locus.[9,10] The great progress in molecular biology during recent years has resulted in the development of new sensitive methods of DNA analysis and study, such as the polymerase chain reaction (PCR) and southern blot analysis. The synthesis of 21-hydroxylase is controlled by two genes, the active CYP21B gene and the CYP21A pseudogene.[11]

CLINICAL GENETICS

Forms of the disease

There are two forms of the disease: (a) The classical form of which the most prominent features are virilization of the external genitalia and/or the body (simple

virilizing form) or virilization of the external genitalia and/or the body with renal salt wasting as defined by hyponatremia, hyperkalemia, inappropriate natriuresis and low serum and urinary aldosterone (salt-wasting form of CAH).[9] (b) The non-classical form characterized by virilization, menstrual disturbances, acne, obesity, oily skin, hirsutism and sterility. Oligospermia and subfertility have been reported in men with 21-OH deficiency.[9]

Classification

The scheme of classification which was proposed by Kohn et al has been accepted by the international scientific community in general. This scheme recognises three alleles for 21-OH deficiency: 21-OHd^{normal}, 21-OHd^{mild}, and 21-OHd^{severe}. The classical disorder results when a person is homozygous for 21-OHd^{severe}. In the non-classical disorder the following phenotypes exist: 21-OHd^{mild}/21-OHd^{mild} or 21-OHd^{severe}/21-OHd^{mild}. Heterozygotes have the mildest enzyme deficiency and may be clinically asymptomatic. Their phenotype is 21-OHd^{mild}/21-OHd^{normal} or 21-OHd^{severe}/21-OHd^{normal}. Cryptic 21-OH deficiencies are biochemically indistinguishable from those with the non-classical form and have the same genotype.[13]

Incidence

Speiser et al found the frequency of NC-CAH and its heterozygous forms to be respectively 1 in 27 and 1 in 3 for Ashkenazi Jews, 1 in 53 and 1 in 4 for Hispanics, 1 in 63 and 1 in 5 for Yugoslavs, 1 in 333 and 1 in 9 for Italians and 1 in 1000 and 1 in 14 in other Caucasians.[3] NC-CAH is one of the most common autosomal recessive disorders in humans. Based on the 17-OHP response to acute ACTH stimulation, the incidence of 21-OH deficiency among hirsute Greek women has been estimated to be 3.4%.[14]

Clinical presentation

In the classical form of the disease, with almost complete deficiency of the enzyme, 0–3% of the normal, there may be virilization of the external genitalia of a female fetus at birth (CAH due to 21-OH deficiency is the most common cause of ambiguous genitalia in the newborn and results in female pseudohermaphroditism).[8] About one third of patiens with 21-OH deficiency have the simple virilizing form.[15] If untreated the female with CAH will develop signs of progressive virilization. Pubic hair will appear by age 2–4 years, followed by axillary hair. Bone age is advanced by age 2 years, and because of early epiphyseal closure, height in childhood is achieved at the expense of shortened stature in adulthood. Progressive masculinization continues, with development of the male habitus, acne, deep voice, amenorrhea and infertility. An electrolyte imbalance of the salt-losing type occurs in approximately one-third of patients with the virilizing form and is usually apparent within a few days of birth. The infant with this disorder goes on to develop an Addisonian-like crisis with hyponatremia, hyperkalemia and acidosis.

Rapid diagnosis and treatment are necessary to save these infants. The non-classical form of 21-OH deficiency, with enzyme deficiency of about 30–50% of normal, is a disease of extreme phenotypic diversity. Individuals with the disease, while normal at birth, may present peri- or post-pubertally with symptoms such as precocious adrenarche, acne, hirsutism, menstrual irregularity, clitoromegaly, amenorrhea, short adult height and infertility. Cryptic patients may develop symptoms and symptomatic non-classical patients have been observed to undergo spontaneous improvement.[12,16]

CLASSICAL GENETICS

The major histocompatibility complex (MHC)

The Human Leucocyte Antigens (HLA) complex, coding for a large number of leucocyte surface antigens important in human organ transplantation, is comprised of several closely linked loci. Four of these, HLA-A, HLA-B, HLA-C and HLA-D/DR can be defined by serologic testing and have been subdivided on the basis of the specific tissue antigen produced by each of their multiple alleles. One allele from each of the HLA loci A, B, C, and D/DR constitutes a haplotype. Each person carries two halpotypes, one from the father and one from the mother. From studies of families the 21-OH gene was localized between HLA-A and glyoxalase I, probably between HLA-B and DR.[17]

Genetic linkage with HLA and genetic linkage diseqiuilibrium

Close genetic linkage between the HLA complex located on the short arm of chromosome 6 and 21-OH deficiency was first described by Dupont et al in 1977.[10] In this study, HLA genotyping of parents and children in six families with one or more child affected with CAH due to 21-OH deficiency was performed. Each individual

inherits one chromosome 6 from his father and one from his mother. In five of the six families, all of the affected offspring were HLA identical, and all were HLA different from their unaffected sibs. In the sixth family, the two affected sibs were HLA-B identical. At the Eighth International Histocompatibility Workshop, it was calculated by statistical methods of genetic analysis that the Lod score for linkage between HLA and 21-OH deficiency CAH was 15.65. The Lod score is a statistical index of the genetic linkage and a value ≥3 means that linkage exists.[17] In genetic studies, it is important to distinguish between genetic linkage and genetic linkage disequilibrium, which is the non-random association of alleles of different genetic loci. Thus, not only are 21-OH deficiency and HLA genetically linked, but there is genetic linkage disequilibrium between 21-OH deficiency and HLA alleles. For classical 21-OH deficiency, the most significant association is with HLA B_{47}.[3] Genetic linkage disequilibrium also has been reported for the nonclassical form of 21-OH deficiency and a significantly increased frequency of HLA B_{14}, DR_1 has been observed.[18] The HLA antigens B_5, B_{35} and B_{40} are also associated with increased risk of classical 21-OH deficiency.[3] The other significant HLA-B antigen association with NC-CAH is the HLA B_{35}.[13]

MOLECULAR GENETICS

The new sensitive methods of DNA analysis and study such as the polymerase chain reaction (PCR) and southern blot analysis have been applied to the investigation of 21-OH deficiency.

By hybridizing a human 21-complementary DNA (cDNA) probe to genomic digests, White and colleagues detected two genes encoding 21-OH hydroxylase enzyme, the active CYP21B gene and the CYP21A pseudogene. Both genes are located at the site of the genes HLA and are closely related to the C_4A and C_4B genes encoding the components of complement.[11] The mutations, translocations or deletions which have been observed in the classical and non-classical form are shown in table 1.

Classical form

The deletion of CYP21B and C_4B genes which is related with the $A_3BW_{47}DR_7$ haplotype was the first mutation reported.[19] The mutation of adenine or cytosine at intron 2 (A or C→G) was the most frequent genetic change related with the classical form of the disease.[20,21] The other mutations related with complete deficiency of 21-OH enzyme are the mutation in codon 318, the deletion of eight pairs of bases in exon 3, the mutation Arg→^{356}Trp and four mutations in the cluster: lle-Val-Clu-Met^{236,239}→Asn-Glu-Glu-Lys.[22] The mutation lle^{172} →Asn is related specifically with the simple virilizing form of the disease, with enzyme activity of 3–7% of the normal activity.[23]

Non-classical form

The mutation Val^{281}→Leu was observed in patients with the HLA-$B_{14}DR_1$ haplotype.[24] In some populations,

Table 1. Genetic changes which resulted/deletion or deficiency of 21 hydroxylase enzyme.

Genetic change	Mapping	Phenotype	Enzyme activity
A or C→*G*	Intron 2	Sv/sw	minimal (≈1)
deletion of pairs and bases	Exon 3	sw	0
lle^{172} → Asn	Exon 4	sv	3–7
lle^{236}→Asn	Exon 6	sw	0
Val^{237}→Clu			
Clu→Clu			
Met^{239} → Lys			
Clu^{292} → Ser	Exon 7	sw	0
Arg^{356} → Trp	Exon 8	sv/sw	2
Pro^{30} → Leu	Exon 1	NC/SV	30–60
Val^{281} → Leu	Exon 7	Nc	20–50
Pro^{453}→Ser	Exon 10	Nc	20–50

sw: Classical form with salt wasting, sv: Simple virilizing form, Nc: Non-classical form, } Cluster

such as Ashkenazi Jews, this mutation was a common genetic polymorphism with a gene frequency of more than 10%. The mutation $Pro^{30} \rightarrow Leu$ is detected in one third of alleles of patients with the non-classical form of the disease.[25] Other mutations are the mutation $Pro^{30} \rightarrow Ser$ which was observed later.[26] Further investigations are in progress to elucidate the complete molecular genetics of this disease.

ΠΕΡΙΛΗΨΗ

Κλινική, κλασική και μοριακή γενετική της ανεπάρκειας της 21-υδροξυλάσης

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Η συγγενής υπερπλασία των επινεφριδίων, που οφείλεται σε ανεπάρκεια του ενζύμου 21-υδροξυλάση (21-OH), διακρίνεται στην κλασική και μη κλασική, όψιμης εμφάνισης μορφή, και αποτελεί ένα από τα συχνότερα, κατά αυτοσωματικό υπολειπόμενο τύπο, μεταδιδόμενα νοσήματα του ανθρώπου. Η συχνότητα της κλασικής μορφής κυμαίνεται μεταξύ 1:5.000 και 1:15.000 στα νεογνά στους πληθυσμούς της Β. Αμερικής και Ευρώπης, ενώ η συχνότητα της μη κλασικής μορφής της νόσου είναι ιδιαίτερα υψηλή στους Εβραίους-Aschkenazi και σ' ένα μέρος των μεσογειακών λαών. Τα τρία αλλήλια της 21-OH μπορούν να συνδυαστούν με διάφορους τρόπους στους ανθρώπους και να προκύψουν φυσιολογικά άτομα, ετεροζυγώτες και κλινικώς πάσχοντες με την κλασική και μη κλασική μορφή. Τα συμπτώματα και σημεία υπερανδρογοναιμίας (δασυτριχισμός, ακμή, αρρενοποίηση, βραχύ ανάστημα, διαταραχές της εμμηνορρυσίας) είναι κοινά σε αμφότερους τους τύπους της ενζυμικής διαταραχής. Μεταξύ των γονιδίων του ενζύμου 21-OH και των αντιγόνων του συστήματος HLA υφίσταται γενετική σύνδεση και γενετική σύνδεση ανισορροπίας. Οι συνηθέστεροι απλότυποι που συναντώνται στην κλασική μορφή της νόσου είναι οι HLA B_{w47}, HLA-B_5 και HLA-B_{35}, ενώ στη μη κλασική μορφή ο απλότυπος HLA-$B_{14}DR_1$. Η μεγάλη πρόοδος της Μοριακής Βιολογίας των τελευταίων ετών είχε ως αποτέλεσμα την ανάπτυξη ευαίσθητων μεθόδων ανάλυσης και μελέτης της δομής του DNA, όπως η αλυσιδωτή αντίδραση της πολυμεράσης και η ανάλυση Southern blot. Η σύνδεση του ενζύμου 21-OH ελέγχεται από δύο γονίδια, το ενεργό γονίδιο *CYP21B* και το ψευδογονίδιο *CYP21A*. Και στις τρεις μορφές της νόσου έχουν αναφερθεί γονιδιακές μεταβολές, που οφείλονται σε μεταλλάξεις του γονιδίου της 21-OH, διαμεταθέσεις ή ελλείψεις γενετικού υλικού.

Λέξεις ευρετηρίου: Γενετική, Συγγενής υπερπλασία επινεφριδίων

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