Chapter 1 : Dopamine - Wikipedia

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Mutation screening was performed using single-strand conformation polymorphism analysis followed by direct sequencing of the presumably mutated exons; in patients whose results showed a normal pattern on single-strand conformation polymorphism analysis, the entire coding region of the GCH-I gene was sequenced. All these mutations are predicted to cause amino acid changes in the highly conserved regions of the gene. In patients from 3 other families and in both patients with sporadic DRD, no alterations in the translated portion of the GCH-I gene were observed. None of the mutations in the GCH-I gene described so far were detected more than once, which precludes the possibility of creating simple DNA testing procedures for routine clinical practice. HEREDITARY dystonias are a clinically and genetically heterogeneous group of disorders characterized primarily by sustained muscle contractions, twisting and repetitive movements, and abnormal postures. One clinical form of hereditary dystonia, originally described as "hereditary dystonia with marked diurnal fluctuations" HPD 1 , 2 or "rigid form of torsion dystonia," 3 , 4 is now increasingly recognized as a distinct nosologic entity within a spectrum of familial dystonic syndromes. The hallmark of this disorder is a "dramatic" and sustained response to relatively small doses of levodopa. In most familial DRD cases, autosomal-dominant transmission of the trait has been reported. Using a candidate gene approach, Ichinose et al 12 studied the gene for GTP cyclohydrolase I GCH-I , the rate-limiting enzyme in biosynthesis of tetrahydrobiopterin, which, in turn, serves as an essential cofactor for tyrosine hydroxylase TH and is involved in converting L-tyrosine to levodopa. In view of these findings, the pathogenesis of dystonia in patients with DRD may be related to the secondary dopamine deficiency in the nigrostriatal dopaminergic pathways, resulting from low GCH-I activity, the lack of tetrahydrobiopterin, and the disturbed function of TH. Therefore, studies of additional families with DRD are of crucial importance for clarifying the molecular genetics of DRD. Patients and methods We examined 6 large, multigenerational families comprising 54 affected members and 2 patients with sporadic DRD. The main criterion for inclusion was a marked and sustained response to low doses of levodopa. Such a response was observed in all the familial cases examined, as well as in 1 patient with a negative family history; in 1 sporadic case, levodopa treatment caused only a moderate improvement. Thirty-seven patients, including 35 familial cases, were examined by 2 of us E. Blood samples were obtained with informed consent from 16 individuals, and genomic DNA was extracted by standard procedures. Our strategy of mutation screening was based on single-strand conformation polymorphism SSCP analysis, followed by direct sequencing of the presumably mutated exons. Six exons of the GCH-I gene, including splicing junctions, were amplified using a polymerase 1 were as follows: For exons 2 through 6, primers were used as reported elsewhere. Polymerase chain reaction products were diluted 1: One microliter of each sample was loaded onto a 0. The second set of polymerase chain reactions was carried out for direct sequencing of exons that showed abnormally migrating bands on SSCP analysis or in several cases for direct sequencing of the entire coding region. Polymerase chain reaction products were purified and concentrated with SUPREC Takara, Tokyo, Japan and sequenced directly with the same primers as for amplification. Sequences were confirmed on identical and complementary strands. Results Three new heterozygote mutations identified in our families are shown in Table 1. The presence of these mutations was presumed on the basis of altered bands on SSCP analysis and was confirmed by direct sequencing of the corresponding exons in one affected member from each family not shown. This mutation destroys a restriction site for the enzyme NlaIII, and restriction analysis confirmed that all the affected family members are heterozygous carriers of the mutation Figure 2. All these sequence alterations are located within highly conserved regions of the gene 25 and are likely to disturb activity of the GCH-I. Moreover, because their presence was confirmed by either SSCP or restriction analysis in several patients in each family including an obligate carrier in family DRD-1 , this may serve as additional evidence for the pathogenic role of the mutations identified. In patients from 3 families, and in both patients with sporadic DRD, no abnormally

migrating bands were observed on SSCP analysis; direct sequencing of the entire coding region and splicing junctions of the GCH-I gene in these patients revealed no variations compared with the control sequence. We identified 3 new point mutations distributed throughout the coding region of the GCH-I gene, all being missense mutations resulting in protein amino acid sequence alterations. Several previous groups also observed disease-causing changes in GCH-I.

Chapter 2 : Top shelves for Victorian Embroidery

Age-Related Dopamine-Dependent Disorders International Symposium on Age-Related Monoamine-Dependent Disorders and Their Modulation by Gene and Gender, Tokyo, November

Dopa-responsive dystonia DRD and tetrahydrobiopterin BH4 defects are inherited disorders characterized by monoamine neurotransmitter deficiency with decreased activity of one of the BH4-metabolizing enzymes. The aim of the study was to determine the utility of cultured skin fibroblasts for the diagnosis of these diseases. Neopterin production was very low and biopterin production was reduced in both disorders. Reference values of all enzyme activities and pterin production were measured in fibroblasts and also in amniocytes for prenatal diagnosis. Cultured skin fibroblasts are a useful tool in the diagnosis of BH4 deficiencies. Intracellular neopterin and biopterin concentrations and GTPCH activity in cytokine-stimulated fibroblasts are particularly helpful in diagnosing patients with DRD. Tetrahydrobiopterin BH4 1 is the essential cofactor for aromatic amino acid mono-oxygenases Fig. Phenylalaninehydroxylase is responsible for hydroxylation of phenylalanine to tyrosine in the liver. Tyrosinehydroxylase and tryptophanhydroxylase are the rate-limiting enzymes in the biosynthesis of the neurotransmitters dopamine and serotonin. BH4 is also a cofactor for the different forms of nitric oxide synthase. Open in new tab Figure 1. The enzyme converts GTP to the intermediate 7,8-dihydroneopterin triphosphate Fig. It is constitutively expressed in many cell types, including fibroblasts, and catalyzes the synthesis of 6-pyruvoyltetrahydropterin from 7,8-dihydroneopterin triphosphate. Two enzymes are responsible for the regeneration of BH4 following oxidation by the amino acid mono-oxygenases: At least DHPR is widely distributed in animal tissues and constitutively expressed in fibroblasts 4. Most of these deficiencies are characterized by neonatal hyperphenylalaninemia, developmental delay, progressive neurological deterioration, hypokinesia, hypersalivation and drooling, swallowing difficulties, truncal hypotonia, increased limb tone, myoclonus, and temperature instability, with onset in the first months of life 8. More than patients are tabulated in the international database 7. Dopa-responsive dystonia DRD is a clinical syndrome characterized by childhood-onset dystonia usually before 12 years of age , with dramatic clinical normalization that is sustained with low doses of l-Dopa 9. The dystonia typically affects the legs, causing gait disturbance and postural instability. Signs and symptoms usually are progressive and worsen later in the evening or after exertion diurnal fluctuation Elements of Parkinsonism rigidity, bradikynesia, and rest tremor commonly occur, especially in adulthood Early motor development usually is normal, and hyperphenylalaninemia is absent. Reduced penetrance and gender-related vulnerability are reported, with females being more affected than males 2. The clinical phenotypic expression is quite diverse 15 , and many cases appear to be sporadic Today, the diagnosis of BH4 deficiencies is based on neonatal screening for phenylketonuria and urinary pterin analysis The examination of pterins and neurotransmitter metabolites in cerebrospinal fluid CSF requires an invasive procedure, but it is the only way to make a reliable diagnosis of DRD 10 Definitive diagnosis of BH4 deficiencies is achieved by enzyme activity determinations and DNA analysis 8. In mononuclear blood cells, GTPCH can be induced by phytohemagglutinin 26 , and reference values have been reported recently by Hibiya et al. However, this test is quite complex and must be performed within 20 h after sample collection In previous studies, fibroblasts were used for indirect measurement of pterin metabolism 28 , based on the induction of GTPCH expression with cytokines, but no data are available about nonpathological and pathological values. In the present study, we used a similar stimulation procedure for measurement of both pterins and GTPCH activity. To our knowledge, fibroblasts have not been systematically tested for the other BH4-metabolizing enzymes. Cultured skin fibroblasts are easily used and practical for the diagnosis of many metabolic disorders. The samples can be transported at room temperature and stored for long periods after early passages for later analysis. The aim of the present study was to demonstrate the utility of cultured skin fibroblasts as a diagnostic tool for DRD and BH4 deficiencies. Particularly for DRD, the enzyme activity measurement is essential for final diagnosis. BH4 metabolism was studied in fibroblasts from a large number of controls and in patients with different forms of BH4 deficiency. Materials and Methods subjects Primary skin fibroblasts were obtained from 21 patients and

35 controls. Eight patients four males, four females , 10â€”45 years of age at the time of the skin biopsy, were affected by DRD McKusick Thirteen patients, 2 months to 11 years of age at the time of the skin biopsy, were affected by BH4 deficiencies. The diagnosis was confirmed by mutation analysis in all patients. Control value biopsies were obtained from 17 subjects 10 males, 7 females between birth and 1 year of age and 18 subjects 11 males, 7 females between 1 and 45 years of age. No neurological abnormalities were detected in any of the controls. To establish reference values for prenatal diagnosis, 14 amniocyte samples collected between 15 and 18 weeks of gestational age were also studied. The activities of all of the BH4-metabolizing enzymes, as well as neopterin and biopterin production after stimulation with cytokines, were measured in all samples. The procedures used were in accordance with the current revision of the Helsinki Declaration of Falcon cell culture plasticware was from Becton-Dickinson. Pteridine derivatives 6,7-dimethyltetrahydropterin and sepiapterin were from Schircks Laboratories. Enzymatic reactor for synthesis of dihydroneopterin triphosphate. All other chemicals were from Fluka. Amniocytes were cultured in AmnioMax C medium. Cells were passaged by trypsinization and studied at low passage numbers 3â€” All cells were Mycoplasma negative, as checked by the Hoechst staining method After incubation for 24 h, cells were harvested by trypsinization, washed with phosphate-buffered saline, and immediately lysed for neopterin and biopterin measurement and for GTPCH activity assay. Dephosphorylation of neopterin triphosphate was achieved by hydrolysis with alkaline phosphatase The reaction was stopped by acidification to pH 2. The intracellular concentrations of neopterin and biopterin after h stimulation with cytokines are expressed as pmol per mg of protein. The assay monitors the conversion of the substrate GTP under saturating conditions to neopterin triphosphate, which is detected as neopterin, the oxidized and dephosphorylated product Fig. Fibroblasts were analyzed immediately after h incubation with cytokines. The mixture was adjusted to pH 8. The assay was modified according to Shintaku et al. BH4 is then measured as the oxidized product, biopterin. The same procedure was used for the blanks. Samples were diluted 1: The assay monitors the conversion of sepiapterin to BH2, which is then measured as the oxidized product, biopterin. Unstimulated cells from one confluent cm2 plate were suspended in 1 mL of lysis buffer 0. The pterin derivative 6,7-dimethyltetrahydropterin was oxidized in situ by peroxidase in the presence of H2O2 to q-6,7-dimethyldihydropterin and thus was recycled for the DHPR reaction. The assay mixture contained 8. The consumption of NADH was measured against corresponding blanks at nm for 5 min. The activities of the various enzymes are expressed as units per mg of protein. View popup Table 1. View popup Table 2. Neopterin and biopterin production and enzyme activity in fibroblasts of patients with DRD and autosomal recessive BH4 deficiencies. No significant difference was observed at different ages 0â€”1 year, 1. A decrease in enzyme activity was observed in cells collected after the first year of age 0â€”1 year, 0. No differences were observed in other age groups after 1 year. Enzyme activities in males 0. DHPR activity was also similar in amniocytes and fibroblasts, as well as in males and females males, 5â€”7. A decrease in activity was observed after 1 year of age 0â€”1 year, 5. Cytokine-stimulated GTPCH-deficient cells autosomal recessive form, three cases showed extremely low concentrations of neopterin and biopterin Fig. Cells from DRD patients eight cases also showed reduced neopterin and biopterin concentrations compared with controls Fig. In DRD patients, the median enzyme activity was 0. Open in new tab Figure 2. Discussion Autosomal recessive BH4 deficiencies are characterized by the presence of neonatal hyperphenylalaninemia with abnormal urinary pterin excretion. The differential diagnosis between phenylketonuria and BH4 deficiencies is extremely important because BH4-deficient patients do not respond to low phenylalanine dietary treatment. However, they benefit from early substitution therapy with BH4 and neurotransmitter precursors l-Dopa and 5-hydroxytryptophan 36 Hyperphenylalaninemia usually is not present in these patients; however, the oral phenylalanine loading test is consistent with a partial deficiency of BH4 in the liver. Low CSF concentrations of homovanillic acid and 5-hydroxyindoleacetic acid, metabolites of dopamine and serotonin, respectively, are found in all variants of BH4 deficiencies. CSF examinations in DRD patients revealed low concentrations of homovanillic acid, indicating a lack of dopamine synthesis, and low neopterin and biopterin concentrations are consistent with a GTPCH deficiency The final diagnosis of BH4 deficiencies is achieved by enzyme activity determinations and, with some limitations, by detection of mutations. PTPS and DHPR activities are measurable in many

different tissues and cells such as erythrocytes 21 22 , fibroblasts 23 24 , and liver 19 Dried blood spots are routinely used for DHPR deficiency screening among hyperphenylalaninemic newborns Measurement of GTPCH activity is rather difficult because this enzyme is not expressed in blood cells and fibroblasts. The test must be performed with fresh blood samples, within 20 h after sample collection Therefore, measurement of enzyme activity is extremely important for the diagnosis of DRD. Recent studies demonstrated that cytokine-stimulated fibroblasts may be used for investigating BH4 pathway integrity 28 , by measuring intracellular neopterin and biopterin production patterns, and for GTPCH activity measurement Here, we adapted these methods to investigate in cytokine-stimulated fibroblasts both pterin production patterns and GTPCH activity, with a diagnostic purpose. We first determined the reference values of intracellular neopterin and biopterin in fibroblasts and amniocytes after stimulation with cytokines. GTPCH activity was slightly but not significantly higher when cells were collected during the first year of life.

Chapter 3 : Age-Related Dopamine-Dependent Disorders - CORE

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Chapter 4 : - NLM Catalog Result

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