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Hyperhomocysteinemia: Related genetic diseases and congenital defects, abnormal DNA methylation and newborn screening issues

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ABSTRACT

Homocysteine, a sulfur-containing amino acid derived from the methionine metabolism, is located at the branch point of two pathways of the methionine cycle, *i.e.* remethylation and transsulfuration. Gene abnormalities in the enzymes catalyzing reactions in both pathways lead to hyperhomocysteinemia. Hyperhomocysteinemia is associated with increased risk for congenital disorders, including neural tube closure defects, heart defects, cleft lip/palate, Down syndrome, and multi-system abnormalities in adults. Since hyperhomocysteinemia is known to affect the extent of DNA methylation, it is likely that abnormal DNA methylation during embryogenesis, may be a pathogenic factor for these congenital disorders. In this review we highlight the importance of homocysteinemia by describing the genes encoding for enzymes of homocysteine metabolism relevant to the clinical practice, especially cystathionine-β-synthase and methylenetetrahydrofolate reductase mutations, and the impairment of related metabolites levels. Moreover, a possible correlation between hyperhomocysteine and congenital disorders through the involvement of abnormal DNA methylation during embryogenesis is discussed. Finally, the relevance of present and future diagnostic tools such as tandem mass spectrometry and next generation sequencing in newborn screening is highlighted.

1. Introduction

Homocysteine (Hcy) is a sulfur-containing, non-proteinogenic amino acid derived from the methionine metabolism. In the homocysteine cycle, Hcy is located at the branch-point of two pathways: remethylation and transsulfuration pathways (Fig. 1) [1,2]. In the remethylation pathway, methionine is resynthesized through two reactions. In the first reaction, which occurs in all tissues, a methyl group is transferred to homocysteine from 5-methyl-tetrahydrofolate (5-methyl-THF) by the vitamin B12-requiring 5-methyl-THF-homocysteine-methyltransferase (folate cycle in all tissues). In the second reaction, which occurs mainly in liver and kidney, the methyl group is transferred from betaine to homocysteine by betaine–homocysteine methyl-transferase (BHMT). In the transsulfuration pathway, homocysteine is irreversibly condensed to serine to give cystathionine by the vitamin B6-dependent enzyme cystathionine β-synthase (CBS). Then, the vitamin B6-dependent enzyme cystathionine γ-lyase (CTH) breaks down cystathionine into cysteine, which could be a precursor of glutathione [3]. In liver, levels of methionine and homocysteine are tightly regulated. Abnormal accumulation of homocysteine results from inability to regulate this pathway and can be attributed to endogenous factors (polymorphisms of the genes coding for the main enzymes involved in homocysteine metabolism) or exogenous factors (dietary deficiency of folate, vitamin B6 or B12) [4]. Increased homocysteine is considered to be an independent risk factor for cardiovascular [5] and cerebrovascular diseases, like stroke or dementia [6], cancer [7], and is highly suspected to increase the risk of congenital defects, such as neural tube defects (NTD), congenital heart defects (CHD), nonsyndromic oral clefts (NOC), and Down syndrome (DS) [8]. However, mild to moderate accumulation of homocysteine due to homocysteine/folate cycle disorders are underdiagnosed.

This review summarizes the most important features related to the hyperhomocysteinemia (hHcy), particularly congenital defects, and highlights the importance of tandem mass spectrometry (MS/MS) and

Abbreviations: AHCY, adenosylhomocysteinase; BHMT, betaine–homocysteine methyl-transferase; Cbl-C, cobalamin C; CHD, congenital heart defects; CTH, cystathionine-γ-lyase; CBS, cystathionine-β-synthase; DS, Down syndrome; hHcy, hyperhomocysteinemia; Hcy, homocysteine; MAT, methionine adenosyltransferase; MS/MS, tandem mass spectroscopy; MTHFR, methylenetetrahydrofolate reductase; NGS, next generation sequencing; NOC, nonsyndromic oral clefts; NTD, neural tube defects; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; THF, tetrahydrofolate; tHcy, total Hcy; WES, whole exome sequencing; WGS, whole genome sequencing.

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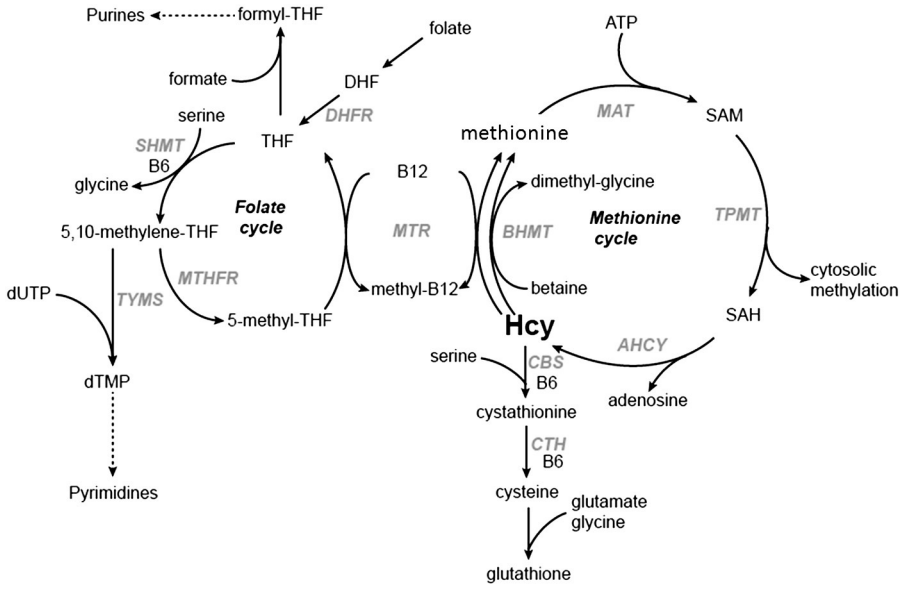

Fig. 1. Schematic representation of the homocysteine metabolism and relationship between folate and methionine cycles. AHCY, adenosylhomocysteinase; B6, vitamin B6; B12, vitamin B12; BHMT, betaine-homocysteinemethyltransferase; CBS, cystathionine-β-synthase; CTH, cystathionine-γ-lyase; DHF, dihydrofolate; DHFR, dihydrofolatereductase; Hcy, homocysteine; MAT, methionine adenosyltransferase; MTHFR, methylenetetrahydrofolate reductase; MTR, methionine synthase; 5-methyl-THF, 5-methyl-tetrahydrofolate; 5,10-methylene THF, 5,10-methylene-tetrahydrofolate; TPMT, thiopurine S-methyltransferase; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; SHMT, serine hydroxymethyltransferase; THF, tetrahydrofolate; TYMS, thymidylatesynthetase.

next generation sequencing (NGS) as fundamental tools for expanded newborn screening.

2. Hyperhomocysteinemia caused by inborn errors of metabolism

The reference values for total Hcy (tHcy) in plasma are 5–13 μM [6]. Circulating homocysteine is present in different forms: 80–90% as protein-bound, 10–20% as homocysteine-cysteine mixed disulfide and homocystine (dimer of homocysteine) and less than 1% as free reduced form [6]. Even though the relative composition of these three fractions of homocysteine can give some information [9], the measurement of total Hcy is considered in the clinical settings of great importance [10]. High Hcy levels are found in the remethylation and transulfuration pathways defetcs that are better clarified by determination of a second metabolite. For example, high Hcy and low methionine indicate a remethylation defect, whereas high Hcy and methionine levels suggest a transulfuration disorder. Evaluation of Hcy levels is also indicative of the folate status, since they are inversely correlated [11]. Epidemiological findings regarding the inverse relationship between Hcy and folate levels also stimulated the interest on Hcy as potentially modifiable risk factor [11]. The main indications for determining tHcy include: diagnosis of homocystinuria, identification of individuals with or at risk of developing folate or cobalamin deficiencies and identification of subjects at risk for cardiovascular disease [12]. Indeed, there is no general agreement on whether plasma homocysteine should be measured as part of any assessment of a patient with vascular occlusive event. It is likely that other measurements, such as B-vitamin, thyroid, and renal functional status, should be evaluated together with Hcy [13].

Several types of hHcy are classified in relation to the total plasma concentrations: moderate (15–30 μmol/L), intermediate (31–100 μmol/L) or severe (>100 μmol/L), respectively [14]. Homocystinuria is a term for a group of disorders characterized by elevated levels of homocysteine in plasma and urine. It is caused by genetic disorders in methionine, homocysteine and transcobalamin metabolism [15]. Dietary deficiencies in folic acid, vitamin B6, and/or vitamin B12 may also cause hHcy [16]. In general, hHcy is observed in approximately 5% of the population [14]. The most severe cases of homocystinuria are due to homozygous (or compound heterozygous) defects in the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene, cystathionine-beta-synthase (CBS) gene and cobalamin (vitamin B12) metabolism genes. Defects in other enzymes of homocysteine cycle cause less severe phenotypes (Table 1). Table 1 reports the mutations found in the genes coding the deficient enzymes and the corresponding ranges of plasma concentrations of methionine, Hcy, SAM and SAH. Often the range of variation is quite large. The extent of elevation depends on the specific mutation(s) causing the impairment of the homocysteine/folate cycle.

2.1. Methylenetetrahydrofolate reductase (MTHFR) deficiency

MTHFR is an important enzyme in the homocysteine metabolism (Fig. 1). So far 67 mutations have been identified in patients with MTHFR deficiency (http://www.hgmd.org). The frequency of mutations is quite common and varies tremendously among different ethnic groups and in different locations. Depending on the mutation, clinical manifestations could be different: acute neurological disturbance in early infancy; progressive encephalopathy in early childhood. However, most mutations are diagnosed in the first months of life. Two functional polymorphisms, C677T and A1298C, respectively, can be associated to a reduced activity. One copy of C677T variant reduces 40% of enzyme activity, whereas two copies result in about 70% decrease of the MTHFR enzyme activity [17]. The variant C677T gives rise to different problems concerning cardiovascular function, homocysteine regulation, DNA regulation, glutathione production, and low methylfolate levels. MTHFR polymorphism A1298C also affects MTHFR enzyme activity,reduces tetrahydrobiopterin levels, and is associated to nitric oxide production leading to aberrant regulation of neurotransmitters [18].

2.2. Cystathionine-β-synthase (CBS) deficiency

Cystathionine-β-synthase (CBS) catalyzes the conversion of homocysteine to cystathionine (Fig. 1). This reaction is the gateway (CBS pathway) for essential biochemical processes, such as glutathione synthesis, a critical component for normal detoxification and defense mechanisms in every cell. The CBS pathway contributes to removal of excess sulfur amino acids. Deficiency of CBS leads to accumulation of homocysteine, methionine, S-adenosylmethionine (SAM), S-adenosylhomocysteine (SAH), and sarcosine with reduced cystathionine and cysteine production (Table 1). Thus, the most effective

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