Atlas of Genetics and Cytogenetics in Oncology and Haematology

OPEN ACCESS JOURNAL

INIST-CNRS

Gene Section
Review

DHFR (dihydrofolate reductase)

Maja Krajinovic, Rachid Abaji, Bahram Sharif-Askari

Research Center, CHU Sainte-Justine, Montreal, QC, Canada (MK, RA, BSA); Department of Pediatrics, University of Montreal, Canada (MK); 3-Department of Pharmacology, University of Montreal, Canada (MK, RA, BSA) Maja.krajinovic@umontreal.ca; rasheed3000@hotmail.com; bahram_sharif@hotmail.com

Published in Atlas Database: December 2015
Online updated version : http://AtlasGeneticsOncology.org/Genes/DHFRID40303ch5q14.html
Printable original version : http://documents.irevues.inist.fr/bitstream/handle/2042/66069/12-2015-DHFRID40303ch5q14.pdf
DOI: 10.4267/2042/66069

Abstract

Dihydrofolate reductase (DHFR) is a member of the reductase enzyme family, which is ubiquitously expressed in all organisms. Levels of this enzyme peak at the G1/S cell cycle boundary. Autoregulation, through DHFR-RNA interactions, has also been reported. DHFR catalyzes the NADPH dependent reduction of dihydrofolate (DHF) to tetrahydrofolate (THF) needed for several one-carbon transfer reactions in purine and pyrimidine synthesis (Jensen et al 1997, Klon et al 2002). It is also the only enzyme that reduces folic acid, a synthetic vitamin not found in nature, to dihydrofolate (Banka et al. 2011). Reduction of DHFR enzymatic activity diminishes the THF pool inside the cell which slows DNA synthesis and cell proliferation eventually leading to cell death (Assaraf et al 2007, Klon et al 2002, Morales et al 2009). DHFR inhibition is essential to the action of antifolate medications used to treat cancer and some inflammatory diseases. Changes in DHFR expression can affect susceptibility to a variety of diseases dependent on folate status such as spina bifida and cancer. Likewise, human DHFR (hDHFR) has become a major drug target in anticancer therapy (Klon et al 2002, Sharif-Askari et al 2010).

Keywords
Dihydrofolate (DHF), Tetrahydrofolate (THF), Folate-dependent enzymes, DNA synthesis.

Identity

Other names: DHFRP1, DYR, EC 1.5 1.3

HGNC (Hugo): DHFR

Location : 5q14.1

Location (base pair)
Start at 80,626,226 and ends at 80,654,981 bp from pter (according to UCSC17 Dec. 2015). Total bp: 28,756bp.

DNA/RNA

Description

DHFR encoded on chromosome 5 and has 6 exons which are separated by 5 introns.
Chen et al (1984) determined that the DHFR gene is about 30 kb long and consists of 6 exons separated by 5 introns.
The full length transcript is 28756 bp long and length of 3'-UTR of some human DHFR mRNA molecules was found to be 2900 nucleotides (Chen et al 1984).

Transcription

The human DHFR contains two promoters, the minor transcript which represents only 1% of DHFR mRNA molecules and the major transcript which codes for 99% DHFR mRNA.
Independent regulation, low prevalence, nuclear enrichment and low translational efficiency suggest that the DHFR minor transcript may function in

vivo to regulate the transcriptional activity of the major promoter (Blume et al 2003 and Martianov et al 2007).

Pseudogene

The DHFR gene family includes the functional DHFR gene and four other intronless pseudogenes, dihydrofolate reductase pseudogene (DHFRP1-4), based on human-rodent somatic cell hybridization. Pseudogene-4 (DHFRP4) is assigned to chromosome 3, pseudogene-1 to chromosome 18 with two transcripts and pseudogene-2 (DHFRP2) to chromosome 6 with one transcript (Anagnou et al. 1984 and Anagnou et al. 1985). Interestingly, according to Anagnou et al. 1988 report, pseudogene-1 (DHFRP1) was found to be present in some individuals while completely absent in others with an interethnic variation in frequency which might implicate a recent origin in the evolutionary process (Anagnou et al., 1988).
Recent studies suggest that DHFRP4, now known as dihydrofolate reductase-like 1 (DHFRL1) is expressed giving a functional protein product which shows a similar but less specific activity to that of DHFR enzyme (McEntee et al 2011).

Protein

Description

Sequence length: 187 AA. Craik et al. (1983) in DHFR protein study reported that altered surface structures can account for functional differences among the members of a family. Also they pointed out that 'sliding' of the intron-exon junctions may design a mechanism for generating length polymorphisms and divergent sequences.

Expression

DHFR is extensively expressed in the fetal and adult tissues such as heart, liver, skeletal muscle, thymus, kidney, brains and whole blood with higher expression in adult brain in compare to fetal brain (Banka et al 2011).

Localisation

While the dihydrofolate reductase enzyme is thought to be present in multiple cellular compartments, it is most particularly localized in the cytosol and the nucleoplasm.

Function

DHFR is a key enzyme in folate metabolism as it is involved in 5,10-methylene tetra hydro folate (THF) generation from 7,8-dihydrofolate (DHF). The generated 5,10-methylene THF is used for the conversion of deoxyuridylate (dUMP) to deoxythymidylate (dTMP) in a reaction catalyzed by thymidylate synthase (TS). The regenerated THF starts subsequent rounds of thymidylate biosynthesis. Moreover DHFR contributes to the de novo mitochondrial thymidylate biosynthesis pathway and catalyzes de novo glycine and purine synthesis as well as DNA precursor synthesis (Anderson et al 2011 and Assaraf et al 2007).

Homology

Funanage et al (1984) assigned the DHFR gene to chromosome 5 and further narrowed the assignment to 5q11-q22. Based on their evidences, there is a homology between both the short and long arm of hamster chromosome 2 and human chromosome 5. Also, the sequences of the human and mouse DHFR proteins studied by Chen et al was shown to differ in only 21 of 186 amino acids, which reflects an 89% homology in the DNA coding sequences of the genes (Funanage et al 1984 and Chen et al 1984).

Mutations

Germinal

Germline polymorphisms

1. Location: Intron 1

Polymorphism: 19-bp insertion /deletion (rs70991108

Impact: Low-serum folate/ high homocysteine, change in mRNA levels

Related disorders: Neural tube defects and breast cancer

Note: 19 base pair deletion in intron 1 which elevated risk of developing breast cancer, neural-tube defects (NTD) and may be a risk factor for low birth weight and preterm delivery (Xu et al 2007, Vander linden 2006 and Johnson et al 2005). In contrast, Parle-McDermott et al (2007), demonstrated that 19-bp deletion allele (D) may be a protective genetic factor against NTD by increasing DHFR mRNA levels in pregnant women. Moreover, a study by Rafighdoost also suggests that DHFR 19-bp D/D genotype reduce the risk of Nonsyndromic cleft lip with or without cleft palate (NS-CL/P) in Iranian subjects (Rafighdoost et al. 2015).
Ongaro et al (2009) and Vagace et al (2011) reported that homozygosity for DHFR 19 bp deleted allele polymorphism has been associated with increased hepatotoxicity in leukemia patients treated with MTX.

2. Location: 3'-UTR

Polymorphism: C829T, A721T, A1171T

Impact: MTX resistance

Related disorder: NTD and Rheumatoid Arthritis

Note: C829T: Goto et al., (2001), by analyzing 3' untranslated region (UTR) of the human DHFR gene transcript discovered C829T substitution located 223 base pairs downstream from the stop codon and positioned between the first and second polyadenylation site. It interferes with miR-24 function leading to higher DHFR mRNA and protein levels. Present in 14.2% of the Japanese populations, the 829T/T mRNA expression level was found to be higher than 829C/C due to the higher stability of the T/T mRNA (Goto et al, 2001).

A721T did not have and significant association with NTD, but was found to be in complete linkage disequilibrium (LD) with the 19-bp indel polymorphism. (Parle-McDermott et al 2007).
Sharma et al (2009) demonstrated that DHFR A1171T (rs7387) polymorphism located in 3'UTR is considered as putative predictor for MTX response in rheumatoid arthritis patients.

3. Location: Downstream to 3'UTR
Polymorphism: A35289G (rs1232027)
Related disorder: MTX efficacy in patients with psoriatic arthritis.
Note: Chandran et al (2010) found an association of the A allele of A35289G polymorphism with MTX efficacy in patients with psoriatic arthritis.

4. Location: Minor promoter
Polymorphism: C-1610G or T (rs1650694) and A-317/G (rs408626)
Impact: Higher DHFR expression
Related disorder: Higher risk of relapse in ALL
Note: Three polymorphisms in DHFR promoter in the 2 kb region upstream of the first or minor transcription of DHFR gene, (C-1610G/T, C-680A, and A-317G) were found associated with treatment responses in children with acute lymphoblastic leukemia (ALL). Haplotype 1 contains both the A-317 and C-1610 alleles and conferred higher transcriptional activity, as shown by reporter gene assay and quantitative mRNA analysis, likely explaining a worse prognosis in patients carrying this haplotype. The ALL patients who were carriers of this haplotype had reduced event free survival (EFS) (Dulucq et al 2008).

5. Location: Major promoter
Polymorphism: G308A (rs1105525), C35T (rs1650697), Length polymorphism 63/91: 9-bp insertion deletion/ 9-bp repeat (rs3045983/ -)
Impact: Higher DHFR expression
Related disorder: Higher risk of relapse in ALL
Note: Six polymorphisms including five SNPs, C35T, C304T, G308A, G319A, and A413G substitutions, along with one length polymorphism composed of two sequence motifs (i.e. insertion/deletion at position 63 and variable number of 9-bp elements at position 91), were identified in the major promoter of DHFR which participate in regulation as both a major promoter and a noncoding minor transcript. Haplotype 1b was identified as a haplotype responsible for the lower relapse-free survival observed in ALL patients. This haplotype is defined by C-1610, C-680, A-317 in the minor promoter and three alleles (T35, A308 and compound length polymorphisms composed of 9-base pair (bp) insertion at position 63 and triple 9bp element at position 91) in the major promoter (Dulucq et al 2008 and Al-Shakfa, et al 2009).

6. Location: Intron 3
Polymorphism: A10372C (rs1677693) and A8890G (rs1643659)
Related disorder: Colorectal cancer (Levine AJ et al 2010).
Polymorphism: 79940143T>C (rs1643650)
Related disorder: Rheumatoid Arthritis
Note: According to Salazar et al, this polymorphism was significantly associated with response to MTX in rheumatoid arthritis patients; patients with C/C and C/T genotypes showed a better response to treatment that those with T/T (Salazar et al 2014).

Mutations
Location: 458A>T (Asp153Val)
Related disorder: Megaloblastic Anemia.
Note: Cario et al., 2011 reported a homozygous DHFR mutation, 458A>T (Asp153Val) that leads to DHFR deficiency which in turn results in a complex hematological and neurological disease that can be successfully resolved with folic acid or folinic acid replacement (Banka et al 2011 and Cario et al., 2011).

Implicated in

Megaloblastic anemia

Dihydrofolate reductase deficiency is an autosomal recessive metabolic disorder characterized by the hematologic findings of megaloblastic anemia and variable neurologic symptoms.
A germline missense mutation in DHFR was identified causing subsequent extensive enzyme deficiency and resulting in an inborn error of metabolism which is characterized by megaloblastic anemia and/or pancytopenia, severe cerebral folate deficiency, and cerebral tetrahydrobiopterin deficiency (Banka et al 2011).

Neural tube defects (NTD)

Neural tube closure occurs during a period of rapid cellular proliferation and DHFR activity may be a crucial factor in maintaining optimal DNA synthesis during this time. Changes in the activity of the folate cycle enzymes may affect the folate levels and affect NTD development. The most extensively studied DHFR polymorphism is a 19bp insertion to deletion in the first intron and two polymorphisms within the 3' untranslated region (721A>T and 829C>T) of the DHFR gene (Parle-McDermott et al 2007).

Rheumatoid Arthritis (RA)

Response to treatment with Methotrexate in RA treatment was found to be influenced by the genotypes of the DHFR polymorphisms rs7387 (Sharma et al 2009) and rs1643650 (Salazar et al 2014).

Breast cancer

The DHFR 19-bp deletion polymorphism affects the transcription of DHFR gene in humans which can modify the risk of breast cancer in multivitamin

supplement users. A multivitamin supplement has adverse effects in patients carrying the 19-bp insertion allele (Xu et al 2007).

Colorectal cancer (CRC)

Levine et al, 2010 demonstrated significant associations between two DHFR tagSNPs (rs1677693 and rs1643659, located on the third intron of the gene) and CRC risk only in individuals not using multivitamin supplements.

Retinoblastoma

Risk of retinoblastoma was significantly elevated among children of mothers homozygous for the 19bp deletion allele taking prenatal synthetic folic acid supplements (Orjuela, et al 2012).

Nasopharyngeal carcinoma (NPC)

DHFR has a significantly higher expression in NPC and is involved in NPC progression through the nucleotide biosynthetic process (Lee et al 2013).

Acute lymphoblastic leukemia (ALL)

MTX is an important component of maintenance therapy in ALL, it exerts its cytotoxicity function by depletion of reduced folates due to interfering with folate metabolism. Changes in DHFR expression level has been found to correlate with MTX efficacy in ALL (Ongaro et al 2009). Polymorphisms in DHFR gene may affect therapeutic responses to antifolates, leading to lower treatment efficacy or higher adverse drug event frequency. Particular haplotype (1b) increases mRNA levels of DHFR and was associated with a higher risk of ALL relapse.

References

Myoda, T. T. and Funanage, V. L.. Personal Communication Wilmington, Del. 10/7/1983.

Al-Shakfa F, Dulucq S, Brukner I, Milacic I, Ansari M, Beaulieu P, Moghrabi A, Laverdière C, Sallan SE, Silverman LB, Neuberg D, Kutok JL, Sinnett D, Krajinovic M. DNA variants in region for noncoding interfering transcript of dihydrofolate reductase gene and outcome in childhood acute lymphoblastic leukemia Clin Cancer Res 2009 Nov 15;15(22):6931-8

Anagnou NP, Antonarakis SE, O'Brien SJ, Nienhuis AW. A novel form of human polymorphism involving the hDHFR-psi 1 pseudogene identifies three RFLPs Nucleic Acids Res 1987 Jul 10;15(13):5501

Anderson DD, Quintero CM, Stover PJ. Identification of a de novo thymidylate biosynthesis pathway in mammalian mitochondria Proc Natl Acad Sci U S A 2011 Sep 13;108(37):15163-8

Askari BS, Krajinovic M. Dihydrofolate reductase gene variations in susceptibility to disease and treatment outcomes Curr Genomics 2010 Dec;11(8):578-83

Assaraf YG. Molecular basis of antifolate resistance Cancer Metastasis Rev 2007 Mar;26(1):153-81

Banka S, Blom HJ, Walter J, Aziz M, Urquhart J, Clouthier CM, Rice GI, de Brouwer AP, Hilton E, Vassallo G, Will A, Smith DE, Smulders YM, Wevers RA, Steinfeld R, Heales S, Crow YJ, Pelletier JN, Jones S, Newman WG. Identification and characterization of an inborn error of metabolism caused by dihydrofolate reductase deficiency Am J Hum Genet 2011 Feb 11;88(2):216-25

Blume SW, Meng Z, Shrestha K, Snyder RC, Emanuel PD. The 5'-untranslated RNA of the human dhfr minor transcript alters transcription pre-initiation complex assembly at the major (core) promoter J Cell Biochem 2003 Jan 1;88(1):165-80

Cario H, Smith DE, Blom H, Blau N, Bode H, Holzmann K, Pannicke U, Hopfner KP, Rump EM, Ayric Z, Kohne E, Debatin KM, Smulders Y, Schwarz K. Dihydrofolate reductase deficiency due to a homozygous DHFR mutation causes megaloblastic anemia and cerebral folate deficiency leading to severe neurologic disease Am J Hum Genet 2011 Feb 11;88(2):226-31

Chandran V, Siannis F, Rahman P, Pellett FJ, Farewell VT, Gladman DD. Folate pathway enzyme gene polymorphisms and the efficacy and toxicity of methotrexate in psoriatic arthritis J Rheumatol 2010 Jul;37(7):1508-12

Chen MJ, Shimada T, Moulton AD, Cline A, Humphries RK, Maizel J, Nienhuis AW. The functional human dihydrofolate reductase gene J Biol Chem 1984 Mar 25;259(6):3933-43

Craik CS, Rutter WJ, Fletterick R. Splice junctions: association with variation in protein structure Science 1983 Jun 10;220(4602):1125-9

Dulucq S, St-Onge G, Gagné V, Ansari M, Sinnett D, Labuda D, Moghrabi A, Krajinovic M. DNA variants in the dihydrofolate reductase gene and outcome in childhood ALL Blood 2008 Apr 1;111(7):3692-700

Funanage VL, Myoda TT, Moses PA, Cowell HR. Assignment of the human dihydrofolate reductase gene to the q11----q22 region of chromosome 5 Mol Cell Biol 1984 Oct;4(10):2010-6

Goto Y, Yue L, Yokoi A, Nishimura R, Uehara T, Koizumi S, Saikawa Y. A novel single-nucleotide polymorphism in the 3'-untranslated region of the human dihydrofolate reductase gene with enhanced expression Clin Cancer Res 2001 Jul;7(7):1952-6

Jensen DE, Black AR, Swick AG, Azizkhan JC. Distinct roles for Sp1 and E2F sites in the growth/cell cycle regulation of the DHFR promoter J Cell Biochem 1997 Oct 1;67(1):24-31

Johnson WG, Scholl TO, Spychala JR, Buyske S, Stenroos ES, Chen X. Common dihydrofolate reductase 19-base pair deletion allele: a novel risk factor for preterm delivery Am J Clin Nutr 2005 Mar;81(3):664-8

Klon AE, Héroux A, Ross LJ, Pathak V, Johnson CA, Piper JR, Borhani DW. Atomic structures of human dihydrofolate reductase complexed with NADPH and two lipophilic antifolates at 1 09 a and 1 05 a resolution

Lee SW, Chen TJ, Lin LC, Li CF, Chen LT, Hsing CH, Hsu HP, Tsai CJ, Huang HY, Shiue YL. Overexpression of thymidylate synthetase confers an independent prognostic indicator in nasopharyngeal carcinoma Exp Mol Pathol 2013 Aug;95(1):83-90

Levine AJ, Figueiredo JC, Lee W, Conti DV, Kennedy K, Duggan DJ, Poynter JN, Campbell PT, Newcomb P, Martinez ME, Hopper JL, Le Marchand L, Baron JA, Limburg PJ, Ulrich CM, Haile RW. A candidate gene study of folate-associated one carbon metabolism genes and colorectal cancer risk Cancer Epidemiol Biomarkers Prev 2010 Jul;19(7):1812-21

Martianov I, Ramadass A, Serra Barros A, Chow N, Akoulitchev A. Repression of the human dihydrofolate reductase gene by a non-coding interfering transcript Nature 2007 Feb 8;445(7128):666-70

McEntee G, Minguzzi S, O'Brien K, Ben Larbi N, Loscher C, O'Fágáin C, Parle-McDermott A. The former annotated human pseudogene dihydrofolate reductase-like 1 (DHFRL1) is expressed and functional Proc Natl Acad Sci U S A 2011 Sep 13;108(37):15157-62

Morales C, García MJ, Ribas M, Miró R, Muñoz M, Caldas C, Peinado MA. Dihydrofolate reductase amplification and sensitization to methotrexate of methotrexate-resistant colon cancer cells Mol Cancer Ther 2009 Feb;8(2):424-32

Ongaro A, De Mattei M, Della Porta MG, Rigolin G, Ambrosio C, Di Raimondo F, Pellati A, Masieri FF, Caruso A, Catozzi L, Gemmati D. Gene polymorphisms in folate metabolizing enzymes in adult acute lymphoblastic leukemia: effects on methotrexate-related toxicity and survival Haematologica 2009 Oct;94(10):1391-8

Orjuela MA, Cabrera-Muñoz L, Paul L, Ramirez-Ortiz MA, Liu X, Chen J, Mejia-Rodriguez F, Medina-Sanson A, Diaz-Carreño S, Suen IH, Selhub J, Ponce-Castañeda MV. Risk of retinoblastoma is associated with a maternal polymorphism in dihydrofolatereductase (DHFR) and prenatal folic acid intake Cancer 2012 Dec 1;118(23):5912-9

Parle-McDermott A, Pangilinan F, Mills JL, Kirke PN, Gibney ER, Troendle J, O'Leary VB, Molloy AM, Conley M, Scott JM, Brody LC. The 19-bp deletion polymorphism in intron-1 of dihydrofolate reductase (DHFR) may decrease rather than increase risk for spina bifida in the Irish population Am J Med Genet A 2007 Jun 1;143A(11):1174-80

Rafighdoost F, Rafighdoost A, Rafighdoost H, Rigi-Ladez MA, Hashemi M, Eskandari-Nasab E. The 19-bp deletion polymorphism of dihydrofolate reductase (DHFR) and nonsyndromic cleft lip with or without cleft palate: evidence for a protective role J Appl Oral Sci 2015 May-Jun;23(3):272-8

Salazar J, Moya P, Altés A, Díaz-Torné C, Casademont J, Cerdà-Gabaroi D, Corominas H, Baiget M. Polymorphisms in genes involved in the mechanism of action of methotrexate: are they associated with outcome in rheumatoid arthritis patients? Pharmacogenomics 2014 Jun;15(8):1079-90 doi: 10

Sharma S, Das M, Kumar A, Marwaha V, Shankar S, Singh P, Raghu P, Aneja R, Grover R, Arya V, Dhir V, Gupta R, Kumar U, Juyal RC, K TB. Purine biosynthetic pathway genes and methotrexate response in rheumatoid arthritis patients among north Indians Pharmacogenet Genomics 2009 Oct;19(10):823-8

Vagace JM, Caceres-Marzal C, Jimenez M, Casado MS, de Murillo SG, Gervasini G. Methotrexate-induced subacute neurotoxicity in a child with acute lymphoblastic leukemia carrying genetic polymorphisms related to folate homeostasis Am J Hematol 2011 Jan;86(1):98-101

Xu X, Gammon MD, Wetmur JG, Rao M, Gaudet MM, Teitelbaum SL, Britton JA, Neugut AI, Santella RM, Chen J. A functional 19-base pair deletion polymorphism of dihydrofolate reductase (DHFR) and risk of breast cancer in multivitamin users Am J Clin Nutr 2007 Apr;85(4):1098-102

van der Linden IJ, Afman LA, Heil SG, Blom HJ. Genetic variation in genes of folate metabolism and neural-tube defect risk Proc Nutr Soc 2006 May;65(2):204-15

This article should be referenced as such:

Krajinovic M, Abaji R, Sharif-Askari B. DHFR (dihydrofolate reductase). Atlas Genet Cytogenet Oncol Haematol. 2016; 20(9):468-472.