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SLC19A1 Pharmacogenomics Summary

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Folate is a crucial nutrient that supports important physiological functions such as DNA synthesis, cell division and substrate methylation. Low folate level caused by suboptimal intake, transport and cellular utilization of folate, is a risk factor for diseases such as spina bifida and cardiovascular diseases [1]. Intracellular uptake of folate is in part mediated by the reduced folate carrier protein 1 (RFC1), encoded by the human solute carrier family 19, member 1 (*SLC19A1*) gene. RFC1 is a high-capacity, bi-directional transporter of 5-methyl-tetrahydrofolate and thiamine monophosphate. RFC1 also actively transports antifolate chemotherapeutic agents, such as methotrexate (MTX), into cells [2–9]. In addition to its role in folate uptake, RFC1 plays critical role in folate homeostasis of mammalian cells, where it is down regulated in response to folate deficiency [10].

SLC19A1 is found on chromosome 21 (21q22.3). Several groups of investigators have cloned cDNAs (approximately 2.7 kb in length) encoding the 591-amino acid human *SLC19A1* from lymphoblast, placenta, and small intestine cDNA libraries [2–5]. The *SLC19A1* gene from human lymphoblasts was shown to contain five exons (exon 2 to exon 6), which code for RFC1 protein [11–13]. There are at least four 5-prime alternative exons, which are used in the production of RFC1 mRNA transcript in lymphoblast cells. These are spliced in a mutually exclusive manner to exon 2, which contains the translational start site for the RFC1 protein. Semi-quantitative PCR shows that exon 1 is preferentially incorporated in the transcript [11]. A separate study showed that RFC1 exhibits alternative splicing [14]. Specifically, three splice variants of RFC1 were identified from a human liver genomic library, and resulted from the incorporation of three alternatives of exon 1 and different 3' sequences. Functional deletion analysis of the region upstream of the transcriptional start site of the *SLC19A1* gene led to the identification of two TATA-less promoters, each of which showed significant differences in the efficiency of transcription.

A humanized mouse model for the reduced-function folate carrier has been created [15]. Expressing human *SLC19A1* in transport-deficient Chinese hamster ovary cells results in

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Additional information, including detailed mapping information for *SLC19A1* gene (PA327), variants and lists of linked drugs and diseases is presented at http://www.pharmgkb.org/search/annotatedGene/slc19a1/index.jsp.

restoration of MTX transport and MTX sensitivity [4]. Several point mutations have been identified in *SLC19A1* and down regulation of *SLC19A1* mRNA has also been associated with impaired MTX transport and MTX resistance [16–19]. A reverse correlation between the RFC promoter methylation and its mRNA level in cancer cell lines has been described [20]. These constitute important factors in the development of resistance to anti-folate chemotherapeutic agents. A recent study in polymorphism and expressions of folate pathway genes showed that *SLC19A1* expression correlated with the sensitivity of several drugs (antifolates, thiopurines, nitrosoureas, and DACH-platinum drugs) in the NCI-60 cancer cell lines [21]. Furthermore, some groups have also found association between the expression of RFC1 and accumulation of methotrexate in Acute Lymphocytic Leukemias (ALL) cells [22,23]. The *SLC19A1* mRNA expression from ALL blasts isolated from newly diagnosed children were higher in certain lineage ALL such as the hyperdiploid B-lineage compared to nonhyperdiploid ALL. In addition, the accumulation of methotrexate polyglutamates was highest in the hyperdiploid B-lineage which indicated that higher *SLC19A1* expression plays important role in MTX accumulation [22,23].

Sequencing of SLC19A1 in healthy subjects

The *SLC19A1* gene is highly polymorphic in humans. The proximal promoter region, exons 3 to 5 and their flanking intronic regions of the *SLC19A1* gene has been resequenced in an ethnically diverse population of 276 individuals as part of the Pharmacogenetics of Membrane Transporters (PMT) project (http://pharmacogenetics.ucsf.edu/cgi-bin/Study.py). This cohort includes unrelated healthy individuals from the San Francisco Bay Area (80 African Americans, 80 European Americans, 60 Asian Americans, 50 Mexican Americans and 6 Pacific Islanders). A total of 6 non-synonymous SNPs were found in exons 3 to 5 of *SLC19A1* gene in this cohort. (https://www.pharmgkb.org/do/serve?objId=PA327&objCls=Gene#tabview=tab2 and in http://pharmacogenetics.ucsf.edu/). Among the non-synonymous SNPs, there are 4 singletons (only found on one chromosome from the sequenced SOPHIE cohort) and they are Leu338Phe (1012C>T, rs59638403, 0.6% in African American); Gly341Asp (1022G>A; rs56822323, 0.6% in European American); Cys458Gly (1372T>G; rs58227024, 0.6% in European American) and Asp522Asn (1564G>A; rs58836581, 0.6% in African American) and two rare variants with minor allele frequency of 1% (Arg456Gln; 1367G>A; rs59841046 and Ala469Val; 1406C>T; rs7278825). One common non-synonymous variant (Ala558Val; 1792C>T; rs35786590) is reported in the dbSNP (dbSNP 130) with total allele frequency of 49.9% and it is found across the four HapMap populations (CEU, HCB, JPT and YRI) (http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=35786590). A frameshift as a result of deletion of 52bp in Exon 3 and give rise to a synonymous SNP (Ala324Ala; 972G>A; rs56138890) is found in the Asian American (12.7 %) and in Mexican American (3 %) populations of the SOPHIE cohort (http://pharmacogenetics.ucsf.edu/) and also reported in the Indian population in India (dbSNP Build 131, http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=56138890). A common non-synonymous SNP in Exon 2 of the gene, Arg27His (80G>A; rs1051266), which was not sequenced in the PMT project, but it is reported in the dbSNP, have total minor allele frequency of 44% and is found across all ethnic groups. In addition, the sequencing of *SLC19A1* promoter region in 72 healthy individuals in Australia revealed a 61-bp insertion polymorphisms in 78% of these individuals. Functional studies conducted by this group showed that this 61-bp insertion resulted in higher luciferase activity as a result from the additional binding sites for AP-2 and Mzf-1 transcription factors in this 61-bp insertion region [13].

Sequencing of SLC19A1 in resistant cell lines and tumor specimens

Several *SLC19A1* gene mutations leading to antifolate-resistant phenotype are observed in rodent and human cell lines [6,16,24]. In one study, L1210 mouse leukemia cells were selected

for resistance to the anti-folate (6R)-5,10-dideazatetrahydrofolate [16]. The resistant mouse leukemia cells were shown to have two point mutations in *SLC19A1*, I48F and W105G, which contributed to the drug-resistant phenotype. Since these mutations dramatically altered the kinetics of folate transport, they were reported as participating in substrate interaction and likely constitute the substrate recognition domain of RFC1. Since the L1210/D3 resistant cells were shown to express the mutant transporter but the wild-type allele was still present in the genomic DNA, it seems that the wild-type allele was silenced during the development of resistance. Another point mutation in *SLC19A1* was identified in a methotrexate transport-defective human T-ALL cell line known as CCRF-CEM [24]. This cell line has a lysine to glutamic acid substitution at codon 45 (E45K) and has been identified to be resistant to other anti-folate [16,25]. Although this nonsynonymous variant could lead to methotrexate resistance due to decreased membrane transport, and have altered binding affinities and transport of folate substrates, there was no evidence that this mutation occurred in ALL samples from children [24]. Other point mutations have been discovered by sequence analysis in human CEM leukemia cells which is resistant to a thymidylate synthase inhibitor (GW1843) and methotrexate. The mutations appeared to be Val29Leu, Glu45Lys and Ser46Ile in the first transmembrane domain of the *SLC19A1* and conferred resistance to GW1843 in cells transfected with these mutations [26]. Similarly, other point mutations in *SLC19A1* have also been reported in human leukemia cells after cellular exposure to gradually increasing PT523, a potent dihydrofolate reductase inhibitor. DNA sequencing of these cells revealed mutations in *SLC19A1*: Leu143Pro, Ala147Val, Arg148Gly, Gln150Stop and these mutations resulted in decreased RFC protein levels and showed impaired methotrexate transport [27]. On the other hand, a few studies have been conducted where RFC1 were resequenced in tumor samples. In one study, 203 B-precursor ALL specimens, 32 T-lineage ALL specimens and 11 AML specimens were resequenced for its RCF1. Only 3 B-precursor ALL specimens were found to have nonsynonymous variants, i.e. Asp56His and Asp522Asn [28]. The study showed that the methotrexate uptake is lower in the blast cells from B-precursor ALL patient with the Asp522Asn mutation compared with cells from the wild type carriers. In another study, RFC1 was resequenced in 162 osteosarcoma samples, and the study has identified new variants, such as Ser46Asn, Glu21Lys, Ala7Val and Ser4Pro, in addition to the common variant Arg27His. [29]. In addition, the clinical significance of these genetic polymorphisms in terms of methotrexate plasma level and resistance has not been determined.

Table 1 summarizes the functional effect of genetic variants in SLC19A1 gene identified through sequencing project in healthy subjects, in patients and in antifolate resistant cells.

Important variants

(For full mapping information, see http://www.pharmgkb.org/search/annotatedGene/slc19a1/variant.jsp) The *SLC19A1* gene is polymorphic in humans. The effect of the variants in *SLC19A1* has been widely studied in various clinical conditions where folate transport, synthesis and metabolism pathways are involved.

1. *SLC19A1*: Arg27His; 80G>A (rs1051266)
2. *SLC19A1*: 5’-UTR variant, −43T>C, (rs1131596)
3. *SLC19A1*: Pro232Pro; 6318C>T (rs12659)
4. *SLC19A1*: 3’-UTR; 2606G>T (rs1051296)
5. *SLC19A1*: 3'UTR, 2522C>T (rs1051298)

1) *SLC19A1*: Arg27His; 80G>A (rs1051266)

The most extensively studied variant of *SLC19A1* gene is 80G>A (rs1051266), a common non-synonymous polymorphism in exon 2 that results in substitution of a histidine for an arginine at residue 27 in the protein sequence. The estimated genotype frequencies among Caucasians were found to be: GG=0.29, GA=0.473, AA=0.237 [30]. The frequency of allele A was 0.473 in Caucasians, 0.564 in African Americans, and 0.472 in Hispanics. This variant has been widely studied for its function in transport uptake as well as its association with risk to diseases and drug response and toxicity. Below we summarize examples of these associations with this important *SLC19A1* variant, Arg27His.

Association with plasma folate levels—This common nonsynonymous variant of *SLC19A1* 80G>A was associated with plasma folate levels in which individuals who were homozygous AA had higher plasma folate levels in comparison to individuals who carried the G allele [31]. This association was not observed in other studies [32,33]. One study observed an association of SLC19A1 80G>A and folate levels in red blood cells, and this association is greater in women than in men [33].

Association with risk of common birth defects—Since folic acid and vitamin B12 are very important in reducing the occurrences of common birth defects such as neural tube defects, or NTD, the genetic variants in *SLC19A1* and other folate pathway genes have been investigated for their roles in common birth defects. One study showed that in Italians, the G allele of variant G80A was observed at higher frequencies in affected children with neural tube defects (NTD), their mothers, and their fathers [34]. Another study investigated the interaction between use of periconceptional folate supplementation in mothers and the risk of spina bifida in their infants as a function of the genotype at nucleotide position 80 of *SLC19A1* in the infants [35]. It was found that infants born from mothers who did not take folate during periconception were at higher risk of having spina bifida if they carried the GG genotype (OR=2.4) compared to infants with the AA genotype. On the other hand, infants whose mothers took folate during periconception had a decreased risk of spina bifida if they carried the GG genotype compared to infants with the AA genotype. Although the findings did not reach statistical significance, this study reveals a potentially interesting gene-nutrient interaction involving folate use in mothers and *SLC19A1* genotype at nucleotide position 80 in infants and risk of spina bifida. The G80A variant has also been studied in congenital defects, such as congenital heart disease (CHD) and cleft palate. One study showed that infants born from mothers who did not use folate supplementation were at significantly higher risk of CHD (OR=2.94) compared to infants whose mothers took folate during periconception. In addition, the CHD risk was significantly higher in infants with the GG (OR=4.03) and GA (OR=4.14) genotypes compared to infants with the AA genotype, if their mothers did not use folate supplementation. No significant association was found between *SLC19A1* G80 genotype or maternal folic acid supplementation and the risks of cleft palate [36].

Association with other risks affected by folate levels—There are several biosynthetic pathways in humans that require folate. As a result, any changes to the folate bioavailability could influence human health. A study involving 156 patients showed that the A allele had a significant protective effect against thrombosis (OR = 0.56) [37].

Association with methotrexate transport—The functional effect of the G80A polymorphism was assessed in transport-impaired K562 cells transfected with the reference Arg27 or variant His27-RFC1 proteins [38]. The variant protein, Arg27His, transported methotrexate and 5-formyl tetrahydrofolate similarly compared to reference RFC1. Although the kinetics of methotrexate transport by Arg27-RFC1 protein were not significantly different compared to K562 cells transfected with His27-RFC1, minor differences (~ 2-fold) were

detected between the Arg27-RFC1 and His27-RFC1 proteins in the interaction affinity (Ki) of other anti-folates, including Tomudex, 5,10-dideazatetrahydrofolate, GW1843U89, 10-ethyl-10-deazaaminopterin, and 5-formyl tetrahydrofolate. The Ki for these other anti-folates in His27-RFC1 were ~ 2-fold lower although there were no significant differences between the K562 cells transfected with Arg27-RFC1 and His27-RFC1 with their sensitivities to growth inhibition by methotrexate and other anti-folates. The results suggested that there were at most minor functional differences between Arg27- and His27-RFC1 in terms of substrate affinities and/or transport efficiencies.

Association with methotrexate plasma levels and response—Altered cellular uptake of methotrexate may have an effect on methotrexate plasma level and thus hamper the efficacy and toxicities of methotrexate. A clinical study assessed whether there was an association of this variant Arg27His with methotrexate plasma level and clinical outcome in childhood acute lymophoblastic leukemias [39]. The result showed that children with the AA genotype had more adverse events and worse overall prognosis than patients with the GG genotype. In addition, it was determined that patients carrying the AA genotype had higher methotrexate plasma levels (P =0.004) than patients with either the GG or GA genotypes. Another clinical study was conduced in patients treated with weekly low-dose methotrexate for rheumatoid arthritis [40]. The result showed that the AA genotype was associated with 3.4-fold higher level of methotrexate polyglutamates (the active metabolites of methotrexate) compared to those with the 80GG and 80GA (OR 95% CI 1.4 – 8.4; p=0.007). This result is supported by another study, where they showed that patients with AA genotype have a 3.32-fold higher probability of remission of rheutmatoid arthritis symptoms [41]. Genetic variants in the candidate genes of the folate pathway have also been examined for their role in drug response. One of the studies describes the association of polymorphisms in folate pathway with the response of methotrexate and sulfasalazine combination regimens in early rheumatoid arthritis patients [42]. In this study (n=98 Caucasians), a gene-gene interaction between *SLC19A1* 80A allele and *MTR* (5-methyltetrahydrofolate-homocysteine methyltransferase) 2756A (rs1805087) is observed with response to the therapy. The result showed that the allele combinations associated with responders are MTR2756A allele in combination with *SLC19A1* 80A allele (multivariate analysis, p=0.0002). Recently, Gregers et al. reported the influence of this variant G80A on the risk of methotrexate relapse and toxicity [43]. This study consisting of 500 children with ALL found that the AA variant was associated with a 50% better chance of staying in remission compared with GG or GA variants.

Association with methotrexate toxicities—Given the importance of *SLC19A1* G80A in methotrexate plasma level and response as discussed above, additional studies have been carried out to assess the effect of this variant with methotrexate toxicities in ALL patients who are treated with high-dose methotrexate. A large retrospective candidate gene study involving 240 ALL patients, this variant G80A is associated with methotrexate gastrointestinal toxicity [44]. In addition, a recent study by Gregers et al. reported that children with ALL (a total of 182 children with toxicity phenotype information) who have AA variant has higher degree of bone marrow toxicity and a higher degree of liver toxicity [43].

Association with risk of cancer—Genes involved with folate uptake and distribution have been investigated for different types of cancer risk including breast, prostate, colorectal and lymphoma [45–48]. Most of them have found no significant association of this variant with the cancer risk. However, a recent case-control study involving Chinese population revealed significant association of 80AA with increased risk of esophageal cancer (n=216) and gastric cancer (n=633) with adjusted odds ratio (OR) of 1.80 (95% confidence interval = 1.29–2.51) and 1.59 (95% confidence interval = 1.25–2.02) respectively [49]. In addition, a case-control study involving 245 pediatric acute lymphobastoid leukemia patients and 500 controls has

found a gene-gene interaction between *SLC19A1* and nicotinamide N-methyltransferase (*NNMT*) [50], where subjects with *SLC19A1* 80AA/*NNMT* IVS – 151CT + TT genotype had a 4.2 fold increase in ALL risk (p=0.001).

2) *SLC19A1*: 5'-UTR variant, −43T>C, (rs1131596)

This common variant in the 5'-UTR region of *SLC19A1* is found in all HapMap populations. A clinical study involving 106 rheumatoid arthritis patients who received methotrexate treatment has found this variant is associated with lower RFC1 protein expression [51] and it is predicted that this regulatory region shows putative consensus sequence of the AP1 transcription factor. This variant is also associated with red blood cell folate levels, with the C allele associated with low red blood cell folate levels. However, this regulatory variant is not sufficient to predict patient response to MTX therapy or toxicity [51].

3) *SLC19A1*: Pro232Pro; 6318C>T (rs12659)

In a study which involved 122 lung cancer cases and 122 matched controls in Xuan Wei, China, where the incidence of lung cancer mortality is high due to indoor smoky coal emissions [52], the group found that the synonymous variant in SLC19A1 Pro232Pro (rs12659) is associated with an increased risk of lung cancer in this population (OR=1.83, 95% CI = 1.02 – 3.28). The effect of this variant Pro232Pro, as well as other polymorphisms in genes involved in one-carbon metabolic pathway which controls nucleotide synthesis and DNA methylation [53], was assessed for their effect on non-Hodgkin lymphoma (NHL) among over 1000 cases and 949 population-based controls. However, the variant Pro232Pro did not show any protective association. A positive association of this variant Pro232Pro is implicated in a study involving cervical carcinoma patients where platinum-based chemotherapy was used for their treatment [54]. In this study, the probability of response was higher in patients with the variant allele T (P=0.032).

4) *SLC19A1*: 3'-UTR ; 2606G>T (rs1051296)

This variant in the 3'-UTR region of *SLC19A1* showed high allele frequencies (40 – 50%) in all HapMap populations. Potentially, a SNP located within the 3'-untranslated region (UTR), which contains regulatory sequences and binding sites for other molecules that could alter the stability of the mRNA transcript of the gene. However, this SNP has not been functionally characterized although this SNP has been genotyped and assessed for its effect on non-Hodgkin lymphoma (NHL) among over 1000 cases and 949 population-based controls [53]. There is no significant association of this variant with risk of NHL.

5) *SLC19A1*: 3'UTR, 2522C>T (rs1051298)

Similarly to rs1051296, this variant in the 3'-UTR region of *SLC19A1* (rs1051298) showed high allele frequencies (40 – 50%) in all HapMap populations. An analog of methotrexate called pemetrexed, which is transported into the cells by SLC19A1, has recently been approved for its indication in non-small cell lung cancer. Forty-eight patients enrolled in the Phase II clinical trial (NCCTG and SWOG study N0426) for pemetrexed plus bevacizumab in advanced non-small cell lung cancer, were genotyped for the this SNP in *SLC19A1* as well as other drug metabolizing enzymes [55]. The result from this study showed that the variant T allele is correlated with 3-month progression-free status (P=0.01) and with median progression-free survival (CC v CT v TT: 5.7 v 2.8 v 4.7 months, respectively; log-rank test, P=0.05).

Conclusion

SLC19A1 is ubiquitously expressed in human tissues and is recognized for its role in the transport of folates and anti-folates drugs. Its role together with other genes that are involved

in folate synthesis and metabolism have important implications in governing folate homeostasis and thus in preventing diseases such as neural tube defects and other birth defects. Variants in the *SLC19A1* gene have been found to be correlated with various cancers and with variable response to methotrexate and related compounds for rheumatoid arthritis and cancer. The common non-synonymous variant Arg27His has been widely studied and some studies have showed significant correlation of the variants with disease risk and/or cancer. The significant associations have failed to be replicated in some studies, suggesting that the overall contributions to the phenotype may not be largely affected and cannot be explained by this variant alone. Besides disease risk, the effect of this common variant has been investigated in pharmacogenomics studies related to methotrexate response and toxicity. The overall evidence suggests that the A allele of this variant Arg27His (G80>A) is associated with protective effect in MTX response yet higher toxicity to high dose MTX treatment [42–44]. However, this variant did not reach genomewide significant in the recent genomewide association study on methotrexate clearance and toxicity [56], suggesting that genes other than *SLC19A1* gene may be the major contributing factor for methotrexate response and toxicity. Large prospective study will be required to determine the effect of genetic variant in S*LC19A1* on risk of methotrexate treatment failure as well as determining whether dosing adjustment to methotrexate according to *SLC19A1* genotype can improve overall treatment efficacy are important questions to be addressed. Although some rare non-synonymous variants (≤1% MAF) have been reported through sequencing effort in healthy subjects, resistant cell lines and tumor samples, the effects of these variants in clinical outcome are yet to be determined.

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Table 1

The functional effect of genetic variants in *SLC19A1* gene identified through sequencing project in healthy subjects, in patients and in antifolate resistant cells.

Summary of SLC19A1 variants in healthy subjects (germline polymorphisms)			
SNP	**Source**	**Function/Effect**	**Allele Frequency**
rs1051266 (Arg27His)	HapMap database and dbSNP (Build 131)[c]	**i.** Association with plasma folate levels [31–33] **ii.** Association with risk of common birth defects [35,36] **iii.** Association with methotrexate transport [38] **iv.** Association with methotrexate plasma level and response [40–42] **v.** Association with methotrexate toxicities [57] **vi.** Association with risk of cancer [46–50]	G allele: 55% (European); 50% (Han Chinese); 43.2% (Japanese); 25.8% (Sub-Saharan African)
rs59638403 (Leu338Phe)	PMT sequencing project in unrelated healthy individuals[a, b]	Not characterized	0.6% in African American
rs56822323 (Gly341Asp)	PMT sequencing project in unrelated healthy individuals[a, b]	Not characterized	0.6% in African American
rs58227024 (Cys458Gly)	PMT sequencing project in unrelated healthy individuals[a, b]	Not characterized	0.6% in African American
rs58836581 (Asp522Asn)	PMT sequencing project in unrelated healthy individuals[a, b]	Not characterized	0.6% in African American
rs59841046 (Arg456Gln)	PMT sequencing project in unrelated healthy individuals[a, b]	Not characterized	1.7% in Asian American
rs7278825 (Ala469Val)	PMT sequencing project in unrelated healthy individuals[a, b]	Not characterized	1% in Mexican and 0.8% in Asian
rs56138890 (Ala324Ala)	PMT sequencing project in unrelated healthy individuals[a, b] and in Indian population in India (dbSNP Build 131)[c]	Not characterized	12.7% Asian American; 3% in Mexican American; 14.9% Indian
61bp insertion in -715 and -714 of SLC19A1 basal promoter (GenBank: AF046920.1)	In 72 healthy subjects	Increase in promoter activity [13].	78% with this insertion. The ethnicity of the subjects was not mentioned.
Summary of SLC19A1 variants identified in tumor specimens[e]			
SNP	***Source***	***Function/Effect***	***Allele Frequency***
Asp56His and Asp522Asn (rs58836581)	3 B-precursor ALL out of 203 B-precursor ALL, 32 T-lineage ALL specimens and 11 AML specimens[d,e]	The uptake of methotrexate in blast cells from patients with Asp522Asn variant is significantly lowered compared to other samples containing reference SLC19A1 gene [28].	Asp167His: 1 patient; Asp522Asn: 1 patient
Ser46Asn, Glu21Lys, Ala7Val, Ser4Pro and Arg27His (rs1051266)	162 osteosarcoma samples	The effect of these variants with methotrexate plasma level and resistance cannot be ruled out [29].	Arg27/Arg27: 30.2%; His27/His27: 22.8%; His27/Arg27: 37.6%; Ser46Asn: 4.32%; Glu21Lys: 1.85%; Ala7Val: 1.23%; Ser4Pro: 1.23%;

Summary of SLC19A1 variants in healthy subjects (germline polymorphisms)			
SNP	**Source**	**Function/Effect**	**Allele Frequency**
Summary of point mutations of SLC19A1 identified by acquired resistance to various antifolates in sublines[e]			
SNP	***Source***	***Function/Effect***	***Allele Frequency***
Ile46Phe and Trp105Gly	Point mutations in L1210 mouse leukemia cells.	Transfection of the *SLC19A1* cDNA containing these mutants in L1210 cells conferred resistance to 5,10-dideazatetrahydrofolate compound [16,25].	Not determined
Arg27His (rs1051266), Val29Leu, Glu45Lys and Ser46Ile, Leu143Pro, Ala147Val, Arg148Gly, Gln150Stop, Ser225Stop, Gly239Stop, Lys393Stop, Leu203Stop, Gly44Arg, Glu257Stop	Point mutations in human CCRF-CEM leukemia cells.	Human CCRF-CEM leukemia cells resistant to methotrexate and other anti-folates (e.g. GW1843, PT523, pemetrexed, trimetrexate, edatrexate) **i.** Glu45Lys: Folic acid influx doubled in the transfected L1210 cells with Glu45Lys. Whereas methotrexate and 5-formyltetrahydrofolate influx markedly decreased in the murine cells transfected with Glu45Lys. In addition, this study conducted in CEM cells demonstrated that E45K transfected cells have an initial rate of methotrexate influx ~ 0.5-fold that of reference cells [24,58]. **ii.** Val29Leu, Glu45Lys, Ser46Ile: Transfection of the SLC19A1 cDNA containing these mutants in CEM cells conferred resistance to GW1843 [26]. **iii.** Gly44Arg: Transfection of this mutant into K562 and Chinese Hamster Ovary cells resulted in 12-fold increase in the transport Km for MTX [19,59]. **iv.** Leu143Pro, Ala147Val, Arg148Gly, Gln150Stop: These mutations were found in CEM cells exposed to increasing PT523 concentrations. The resulted sublines displayed up to 3500 fold resistance to antifolates compounds, have decreased SLC19A1 protein levels and have impaired methotrexate transport [27]. **v.** Stop condon: Ser225Stop, Gly239Stop, Lys393Stop, Leu203Stop, Glu257Stop: The SLC19A1 protein is not expressed in these nonsense mutations (stop codon). The SLC19A1 mRNA is expressed in these resistance sublines except for the sublines resistance to ZD9331 and PT523 antifolates compounds where no SLC19A1 mRNA is detected [59]. **vi.** Arg27His: This common SNP is also found in CEM cells exposed to methotrexate and edatrexate and it is present together with another point mutation (e.g. Glu45Lys and Ser225Stop). The functional effect is likely due to be the point mutation that occurs rather than Arg27His, since other studies have shown similar methotrexate uptake and kinetic transport between K562 cells transfected with Arg27 and His27 [38].	Not determined

Source:

[a] http://pharmacogenetics.ucsf.edu;

[b] https://www.pharmgkb.org/search/annotatedGene/slc19a1/;

[c] http://www.ncbi.nlm.nih.gov/snp

Note:

[d] These leukemia samples were collected during the time of diagnosis and approximately 10% of the samples were at the time of disease recurrence.

[e] These variants are likely from somatic cells although the possibility that the variants are from normal cell DNA (germline DNA) that contaminated the tumor sample cannot be ruled out.