Genes Genom (2017) 39:689–699
DOI 10.1007/s13258-017-0552-5

Online ISSN 2092-9293
Print ISSN 1976-9571

REVIEW

Methylenetetrahydrofolate reductase, MTHFR, polymorphisms and predisposition to different multifactorial disorders

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Received: 30 January 2017 / Accepted: 24 April 2017 / Published online: 31 May 2017

Abstract Gene polymorphisms involved in homocysteine-methionine pathway result in hyperhomocysteinemia, a predisposing condition to several diseases. Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in folate and homocysteine metabolism. The two known functional polymorphisms of *MTHFR* gene, 677C>T and 1298A>C have been implicated in a variety of multifactorial diseases: cardio-cerebrovascular and neurodegenerative disorders, autoimmune diseases, birth defects, diabetes, neuropsychiatric disorders, cancer and renal disease. C667T, and to a lesser extent A1298C polymorphisms, have been also reported to have a pharmacogenetic role in predicting drug toxicity in cancer and rheumatoid arthritis treatment. We review here the principal effects of the *MTHFR* gene variations in different clinical conditions.

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Keywords Folate pathway · Homocysteine · Hyperhomocysteinemia · Methylenetetrahydrofolate reductase · Single nucleotide polymorphism

Introduction

Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in folate and homocysteine metabolism and catalyses the conversion of 5,10-methylenetetrahydrofolate into 5-methyltetrahydrofolate (5-MTHF), the predominant circulating form of folate (Brustolin et al. 2010). MTHFR is involved in folate-dependent homocysteine remethylation carried out by methionine synthase. The first link between MTHFR and diseases was discovered in 1972 when Mudd et al. reported several cases of homocystinuria, characterised by increased homocysteine excretion in the urine, that could not be explained by deficiencies in other enzymes known to cause this condition. The enzyme activity assays showed a severe deficiency in MTHFR, which determines an impaired ability to convert homocysteine to methionine, resulting in significantly increased homocysteine plasma levels (Mudd et al. 1972).

It has been demonstrated that several polymorphisms in genes involved in the homocysteine–methionine pathway cause hyperhomocysteinemia, suggesting that such genetic variants may play a role in several multifactorial disorders associated with hyperhomocysteinemia of high prevalence in the general population.

Individuals with very elevated blood levels of total homocysteine (tHcy >100 mmol/L) caused by congenital metabolism errors experience above all rapidly progressing atherosclerosis and thrombo embolic events. This observation has led to the “homocysteine hypothesis”, which suggests that even a moderate increase in tHcy concentration

may cause cardiovascular disease (McCully 1996). Furthermore functional MTHFR polymorphisms, have been associated to different multifactorial diseases.

However, besides genetic defects in the enzymes involved in homocysteine metabolism, an elevation in plasma homocysteine levels can also arise from nutritional deficiencies in vitamin cofactors, or from other factors including some chronic medical conditions and drugs (Brustolin et al. 2010).

However, whatever the clinical setting considered, the fallout of gene variants can not be solely attributed to increase in homocysteine levels (Blom and Smulders 2011) and remains, still today, a matter of discussion whether any beneficial effects of folic acid therapy are to be referred to its direct effect or to a reduction of hyperhomocysteinemia.

Here we have reviewed the current knowledge on MTHFG gene structure and function, its main implications on homocysteine pathway and the impact of MTHFR gene variations in different multifactorial diseases conditions (namely cardio-cerebrovascular disease, neurodegenerative diseases, autoimmune disorders, birth defects, diabetes, neuropsychiatric disorders, cancer, and renal disease) and on pharmacokinetic or pharmacodynamic properties of several drugs.

MTHFR gene

The gene *MTHFR* (OMIM #607093) encodes for the enzyme methyltetrahydrofolate reductase and was firstly described by Goyette et al. in 1998 (Goyette et al. 1998). The gene, located on chromosome 1 (1p36.3), contains 11 exons, ranging from 102 to 432 bp. The analysis of the complete genomic structure suggests that both alternative initiation and alternative splicing can occur (Homberger et al. 2000). In the GRCh38p. 7 release, the *MTHFR* gene is described with nine different transcript variants, but only four are protein–coding (ENST00000376592.5, ENST00000376590.7, ENST00000376583.7 and ENST00000376585.5), as described in Fig. 1 (http://www.ensembl.org/). The 5′ region of human *MTHFR* gene reveals some interesting characteristics: it contains consensus promoter regions for CAAT and GC boxes, as well as for several other known promoter elements (SP1, AP1, AP2). Despite the presence of these regulatory regions, the gene lacks the most common regulatory region, the consensus TATA-box, present in the majority of eukaryotic genes. Some other genes involved in homocysteine metabolism display a similar pattern. It has been demonstrated that gene polymorphisms involved in the homocysteine-methionine pathway result in hyperhomocysteinemia, suggesting that such genetic variants may play a role in several multifactorial disorders associated with hyperhomocysteinemia of high prevalence in the general population (Brustolin et al. 2010). Although several *MTHFR* gene variants have been identified, the most characterized and understood are the single nucleotide polymorphisms (SNPs) at position 677 (*MTHFR* 677C>T), at position 1298 (*MTHFR* 1298A>C), at position 1317 (*MTHFR* 1317T>C) and at position 1793 (*MTHFR* 1793G>A) (Böttiger et al. 2007). *MTHFR* 677C>T (rs1801133) is located at the folate binding site, changing an alanine into a valine residue (p.A222V). *MTHFR* 1298A>C (rs1801131) is located within a presumptive regulatory domain, changing glutamic acid into an alanine residue (p.E429A), whereas *MTHFR* 1317T>C is a silent mutation. *MTHFR* 1793G>A results in an amino acid substitution (p.R594Q), that does not seem to affect the functional activity of the enzyme (Rady et al. 2002). To date, among the known *MTHFR* gene polymorphisms, only the 677C>T and 1298A>C SNPs are functional. Genetic testing can be requested for the *MTHFR* gene alone, or in the context of broader molecular genetic profiles as part of preventive medicine. Polymorphism analysis is generally performed by direct sequencing of the entire gene or a fragment thereof, using restriction fragment length polymorphisms (RFLPs) or through microarray techniques (Böttiger et al. 2007). Tests on MTHFR in vitro expression and enzyme activity performed in bacterial extracts revealed that the homozygous condition for 1298CC and 677TT resulted in an activity decreased by 45 and 68%, respectively, while the mutant enzyme containing both SNPs had

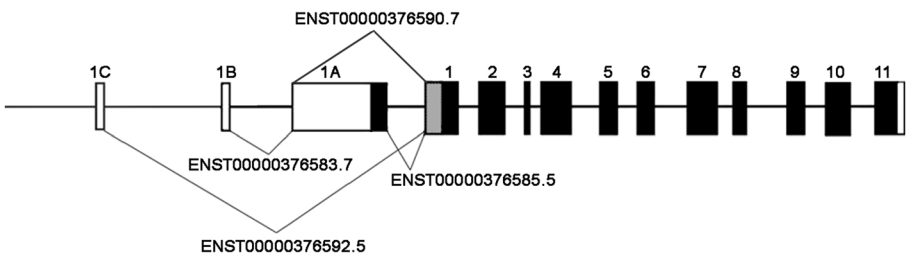

Fig. 1 *MTHFR* gene structure. The figure shows a schematic representation of the gene, with four protein coding transcript variants. *Dark rectangles* represent exons encoding the open reading frame (ORF), *open rectangles* represent exons that encode untranslated region (UTR) and the *cross-hatched rectangle* represents coding region depending on the slice pattern

42% residual activity (Weisberg et al. 2001). Bagley and Selhub showed that in red blood cells of *MTHFR* 677CC individuals, folate is totally represented by 5-MTHF. Conversely, the carriers of the 677TT genotype accumulate formylated folates, which points to a disruption of the folate cycle (Bagley and Selhub 1998). Functional analysis of recombinant expressed human *MTHFR* proved that 677TT genotype results in an enhanced propensity to dissociate into monomers and to lose its flavin adenine dinucleotide cofactor, with a consequent decreased enzyme activity (Yamada et al. 2001).

The 677C>T substitution is the most common missense variation of *MTHFR*, with a global prevalence of 40% (Böttiger et al. 2007). The frequency of this variant differs across the races: the TT homozygote genotype is approximately 1.45% in African blacks, 9.6% in Turkish, 12% in Indians, 41% in Chinese and 23% in Italians (Yun at al 2015; Sazci et al. 2005).

Homocysteine pathway

Homocysteine, an amino acid not involved in protein synthesis, is an intermediate in methionine metabolism. Plasmatic homocysteine levels are determined by several factors, such as genetic alterations of enzymes of methionine metabolism and the deficiency of vitamin B12, vitamin B6 and folic acid (Vijayan et al. 2016). Folic acid is not biologically active until converted into folate by the enzyme MTHFR. Folic acid is a substrate for cellular production of tetrahydrofolate (THF), a precursor to 5-MTHF, necessary for normal methionine synthase (MTR) enzyme activity, in addition to being the natural circulating form of folate. Folate transfers 1-carbon moieties to various organic compounds by increasing S-adenosylmethionine (SAM) levels. There are two main strategies that can be used to lower homocysteine: oral administration of high doses of folates or 5-MTHF. Besides folate, both vitamin B6 and vitamin B12 are necessary cofactors in homocysteine metabolism (Cianciolo et al. 2008).

The 80–90% of circulating homocysteine is protein-bounded, 10–20% is present as homocysteine–cysteine mixed disulphide and homocysteine (dimer of homocysteine), and less than 1% the free reduced form (Mansoor et al. 1995). Homocysteine is located at a branch-point of metabolic pathways: it is irreversibly degraded via the trans-sulphuration pathway to cysteine or it is remethylated back to methionine (Fig. 2).

Trans-sulphuration

Trans-sulphuration is facilitated by the action of two vitamin B6-dependent enzymes: cystathionine β-synthase (CBS) and cystathionine γ-lyase (CTH). CBS catalyses the condensation of homocysteine and serine to cystathionine, and CTH subsequently catalyses the hydrolysis of cystathionine to cysteine and α-ketobutyrate. Human CBS is expressed in liver, kidneys, muscle, brain and ovary and also during early embryogenesis in the neural and cardiac systems (Quéré et al. 1998).

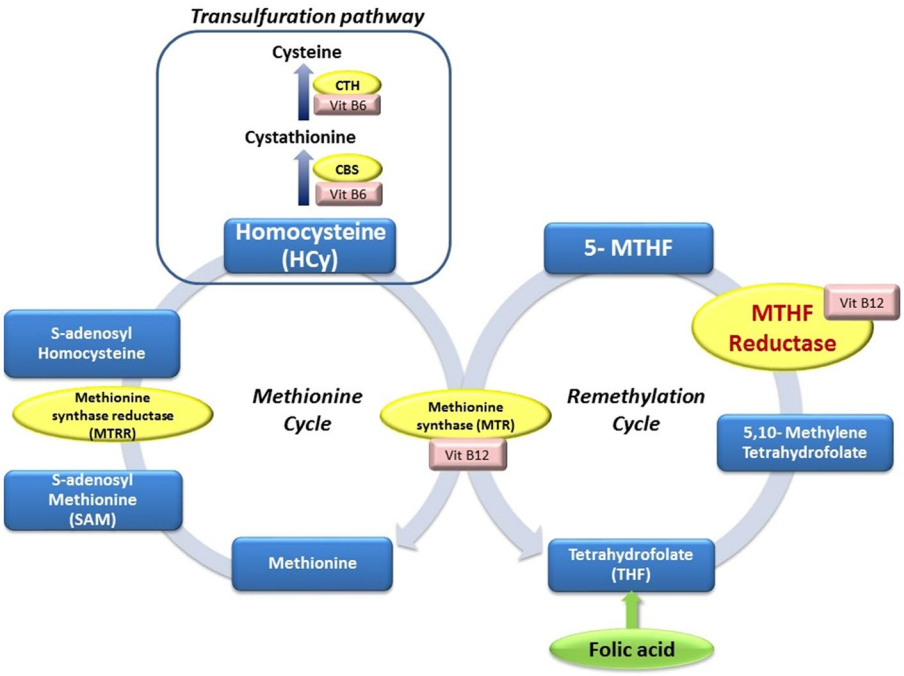

Fig. 2 Homocysteine metabolic pathway

Remethylation

Homocysteine remethylation to methionine is catalysed by the MTR enzyme (Li et al. 1996) and links the folate cycle with homocysteine metabolism. MTR requires vitamin B12 as a cofactor. Methionine synthase reductase (MTRR) reactivates the complex by reductive methylation, using SAM as a methyl donor. While the MTR enzyme is ubiquitous, another homocysteine remethylation system, betaine-homocysteine methyltransferase (BHMT) is mainly expressed in the liver and kidneys. The function of the MTHFR enzyme is of great importance in the regulation of available 5-MTHF for homocysteine remethylation (Blom and Smulders 2011).

MTHFR and disease association

MTHFR enzyme regulates the availability of 5-MTHF for homocysteine remethylation, but the pathological consequences of MTHFR gene variants cannot be attributed solely to the increase in homocysteine levels (Blom and Smulders 2011). However, it is a matter of debate whether the cause of the underlying mechanisms is the rise in homocysteine levels or folic acid deficiency or both. Hyperhomocysteinemia is observed in 5% of the general population and is associated with increased risk for many pathological conditions, including cardio-cerebrovascular diseases, neurodegenerative disorders, autoimmune diseases, birth defects, diabetes, neuropsychiatric disorders, cancer and renal disease (Brusolin et al. 2010). Here we reviewed the current literature, including meta-analyses, case–control and association studies, focused on the impact of the two functional *MTHFR* polymorphisms, 677C>T and 1298A>C on different multifactorial diseases (Table 1).

Cardio-cerebrovascular disease

Genetic variants of *MTHFR* gene have been extensively studied among the candidate genetic factors for cardiovascular risk, given that hyperhomocysteinemia is regarded as an important non-traditional risk factor for atherosclerosis (Brusolin et al. 2010). Furthermore, endothelial cells can eliminate homocysteine only by the folic acid and vitamin B12-dependent remethylation pathway regulated by MTHFR and methionine synthase. For this reason, normal activity of both enzymes is essential to prevent the increase in homocysteine to a pathological level in vascular endothelial cells (Debreceni and Debreceni 2014). Several studies have associated *MTHFR* C677T and A1298C polymorphisms with an increased susceptibility to myocardial infarction (MI), although the data are in some cases inconsistent (Kozieradzka et al. 2012; Angeline et al. 2007; Zee et al. 2007; Kaul et al. 2006). A recent meta-analysis by Alizadeh S et al., consisting of a comprehensive review of MEDLINE and EMBASE databases and including a total of 47 studies, investigated the possible impact of C667T and A1298C polymorphisms on MI risk. When considering the overall patient population, the results lacked finding statistically significant associations between *MTHFR* genotypes and MI risk. Conversely, in subgroup analysis by race, the carriage of the T allele of C677T polymorphism increased the risk of MI by 63% in Africans, while the heterozygous CT genotype was associated with a 19% decreased risk of MI compared with the CC genotype in North Americans. No similarly significant associations were found in European and Asian populations. In the stratified analysis by age, C677T polymorphism proved to be protective against MI risk in patients aged more than 50 years (Alizadeh et al. 2016).

Stroke has emerged as the second commonest cause of mortality worldwide and is a major public health problem, affecting about 15 million people. The global prevalence of stroke is estimated to be about 400–800/100,000 people (WHO 2011). The contribution of genetic factors to stroke pathogenesis is supported by the association of specific gene variants and environmental factors. Several studies have indicated *MTHFR* 677C>T polymorphism as a potential risk factor for stroke in different ethnicities (Vijayan et al. 2016; WHO 2011; Biswas et al. 2009; Trabetti 2008). Vijayan et al. evaluated the influence of polymorphism *MTHFR* 677C>T on the risk for ischemic stroke in South Indian population and found a significantly higher frequency of *MTHFR* T allele in the patients with ischemic stroke compared to the healthy subjects (Vijayan et al. 2016). Nevertheless, evidence from a cumulative meta-analysis by Cronin et al. reported a lack of association of *MTHFR* 677TT genotype with risk of ischemic stroke in North and Central Europe, but a surprisingly high association in Italy and Japan. This discrepancy might be traced back to a dose-dependent effect due to the presence of an allele frequency gradient in addiction to folate intake, folate status variability and differences between the populations (Cronin et al. 2005). About this issue, a large meta-analysis of genetic studies and clinical trials suggested that the effect of MTHFR C677T gene variants on stroke risk can be modified by folate status (Holmes et al. 2011). Therefore, the efficacy of folic acid therapy in stroke prevention should be evaluated and interpreted in the light of combined effects of baseline folate levels and MTHFR gene C677T polymorphism. Indeed, the authors showed that the effect of MTHFR genotype on stroke risk is subjected to modification by population dietary folate levels. The China Stroke Primary Prevention Trial (CSPPT), a large randomized trial among adults with hypertension in China without a history of stroke or MI, was designed using individual measures of MTHFR genotype and baseline folate

Table 1 Descriptive information for meta-analyses, case–control and association studies of MTHFR polymorphisms in multifactorial diseases

Study endpoint	MTHFR SNPs		Hcy levels	Ethnicity or setting	Study type	Sample size	Association	References
	C677T	A1298C						
Cardio-cerebrovascular disease								
Ischemic stroke	✓	n.a	n.a	South India	Case–control	200/193	Strong association of *MTHFR* 677 CT genotype/T allele with ischemic stroke-large vessel disease	Vijayn et al. (2016)
Venous thromboembolism	✓	n.a	✓	France and Switzerland	Association	150	Association of *MTHFR* 677 T allele with concentrations of methylfolate in red-blood cells and risk of venous thromboembolism	Quéré et al. (1998)
Myocardial infarction	✓	n.a	n.a	Poland	Association	637	No	Kozieradzka et al. (2012)
Myocardial infarction	✓	✓	✓	India	Case–control	100/100	No	Angeline et al. (2007)
Cardiovascular disease	✓	n.a	✓	USA	Association	24,968	No	Zee et al. (2007)
Myocardial infarction	✓	✓	n.a	Caucasian and Asian	Meta-analysis	13,151/16,275	No	Alizadeh et al. (2016)
Ischemic stroke	✓	✗	✓	India	Case–control	120/120	Association of *MTHFR* 677 C/T polymorphism with a 4.5-fold increased risk for ischemic stroke	Biswas et al. (2009)
Stroke/TIA	✓	n.a	n.a	Caucasian and Asian	Meta-analysis	4933/7082	Risk of stroke/TIA associated with the *MTHFR* 677 T allele increased in a dose-dependent manner	Cronin et al. (2005)
Stroke	✓	✗	n.a	Asia, America, Australia, New Zealand	Meta-analysis	59,995	No	Holmes et al. (2011)
Stroke and myocardial infarction	✓	n.a	n.a	China	Association	20,702	No	Huo et al. (2015)
Neurodegenerative disorders								
Parkinson's disease	✓	✓	n.a	China	Case–control	512/512	Protective effect of the T allele of *MTHFR* 677 C/T variant and of A–T haplotype of *MTHFR* 677 C/T and A1298C variants	Yuan et al. (2016)
Alzheimer and vascular dementia	✓	✓	✓	Italy	Case–control	122/72	No	Ravaglia et al. (2004)

Table 1 (continued)

Study endpoint	MTHFR SNPs		Hcy levels	Ethnicity or setting	Study type	Sample size	Association	References
	C677T	A1298C						
Autoimmune diseases	✓	✓						
Rheumatoid arthritis	✓	✓	n.a	Caucasian and Asian	Meta-analysis of case–control studies	1762/1610	Association of *MTHFR* 677 C/T polymorphism with rheumatoid arthritis in Asians, and of *MTHFR* 1298 A/C polymorphism in the overall population	Cen et al. (2016)
MTX response in rheumatoid arthritis patients	✓	✓	✓	India	Association	322	Association of MTHFR 677 C/T and A1298C polymorphisms with MTX efficacy	Ghodke-Puranik et al. (2015)
Birth defects	✓	✓						
Neural tube defects	✓	✓	✗	Brazil	Case–control	41/44	No	Félix et al. (2004)
Cleft lip	✓	✓	✗	Thailand	Case–control	109/202	No	Shotelersuk et al. (2003)
Orofacial clefts and congenital heart defects	✗	✗	✓	Brazil	Meta-analysis of cohort studies	37,076	No	Verkleij-Hagoort et al. (2007)
Diabetes								
Type 2 diabetes mellitus	✗	✗	n.a	United Arab Emirates	Case–control	169/209	No	El Hajj Chehadeh et al. (2016)
Type 2 diabetes mellitus	n.a	✓	n.a	Caucasian and Asian	Case–control	897/852	Significant association of *MTHFR* A1298C polymorphism with diabetes risk in Asian population, no association in Caucasians	Yan et al. (2014)
Cancer								
Breast cancer	✗	✗	n.a	Caucasian and Asian	Meta-analysis of case–control studies	15,351/19,340	No	Lissowska et al. (2007)
Renal cell carcinoma	✓	✗	n.a	China	Case–control	81/80	No	Lv et al. (2015)
Renal cell carcinoma	✓	✓	n.a	Caucasian	Case–control	1097/1555	Risk of renal cell carcinoma associated with the *MTHFR* 677T allele	Moore et al. (2008)

ESRD end-stage kidney disease, *n.a* not available, *MTX* methotrexate, *TIA* transient ischemic attack

✓ association

✗ no association

level, with the aim to test the hypothesis of a possible larger effect of folic acid intervention in Asiatic region without a folic acid fortification program. The CSPPT, found that the ACE inhibitors plus folic acid therapy, compared with ACE inhibitors alone, significantly reduced the relative risk of first stroke by 21%. Further adjustment for important covariables, including baseline homocysteine levels, did not substantially change the results. Poor folate intake is prevalent in most countries without mandatory folic acid food supplementation. Among individuals with CC or CT genotypes, the highest risk of stroke and the greatest benefit of folic acid therapy were in those with the lowest baseline folate levels. In addition, individuals with the TT genotype may require a higher dosage of folic acid supplementation to overcome biologically insufficient levels. The results suggested that in some countries with folic acid fortification and widespread use of folic acid supplements, namely United States and Canada, there might be room for further reduction of stroke incidence using more targeted folic acid therapy, in particular among the carriers of the TT genotype and low or moderate folate levels (Huo et al. 2015).

Neurodegenerative diseases

Low folate and raised homocysteine concentrations in blood have been associated with poor cognitive performance in the general population (Brusolin et al. 2010). Elevated homocysteine levels result in neurotoxic and vasotoxic effects in dementia and Alzheimer's disease suggesting that homocysteine is a direct marker for early cognitive decline (Ravaglia et al. 2005). There is some evidence to suggest that changes in homocysteine levels may be also neurotoxic to dopaminergic neurons. A recent case–control study to assess the impact of *MTHFR* 677C>T and *MTHFR* 1298A>C variants on Parkinson's disease, demonstrated that the T allele of the first variant and AA/TT haplotype were protective against the risk of developing the disease (Yuan et al. 2016).

Autoimmune disorders

Autoimmune diseases are chronic inflammatory conditions, associated with premature mortality, severe morbidity and functional impairment, with a consequent high financial burden for both patients and society. Since disease progression and complications can considerably differ between the patients, genetic markers are of potential relevance for identifying those individuals at a higher risk of more severe presentation (Rodriguez–Rodriguez et al. 2015). Rheumatoid arthritis is an autoimmune disease that affects between 0.5 and 1% of the population. Again, the impact of *MTHFR* polymorphisms on the risk of rheumatoid arthritis is variable across different ethnicities, and poor data are currently available in non-Asian populations. A recent meta-analysis to investigate the combined effects of C677T and A1298C polymorphisms and genetic susceptibility to rheumatoid arthritis highlighted a significant association of *MTHFR* C677T polymorphism T allele in Asians, and of *MTHFR* A1298C polymorphism A allele in the overall population (Cen et al. 2016).

Birth defects

Neural tube defects constitute a major health burden (0.5–2/1000 pregnancies worldwide), and still remain a preventable cause of birth, neonatal, and infant death, or significant lifelong handicaps. The malformations derive from failure of the neural folds to fuse in the midline, and to form the neural tube between the third and the fourth week of embryonic development. Most neural tube defects are sporadic, and either genetic or non-genetic environmental factors are involved in its aetiology. Consanguinity was suggested to contribute to the high incidence of neural tube defects in several countries. Genetic predisposition constitutes the major underlying risk factor, with a strong implication of genes that regulate folate one-carbon metabolism and planar cell polarity (Salih et al. 2014), even if some studies on small patient samples lacked finding significant correlations between the 677C>T and 1298A>C polymorphisms of the *MTHFR* gene and neural tube defects (Félix et al. 2004). Orofacial clefts are one of the most common birth defects in humans, with dissimilar prevalences across geographical regions and ethnic groups. These diseases have a complex, multifactorial inheritance involving genetic and environmental factors. Wilcox et al. have proposed that periconceptional use of folate could prevent the occurrence of cleft lip and palate, but not of cleft palate alone (Wilcox et al. 2007). Other authors reported a significant increase in the risk of having a child with cleft palate in mothers heterozygous for both functional *MTHFR* polymorphisms (Shotelersuk et al. 2003). The risk of a child with cleft palate is significantly increased if mothers, carrying *MTHFR* 677TT or *MTHFR* 1298CC genotype, also had a low periconceptional intake of dietary folate and/or folic acid supplements, suggesting that the two *MTHFR* polymorphisms are independent risk factors for cleft palate (Verkleij-Hagoort et al. 2007).

Diabetes

Type 2 diabetes mellitus is the most common form of diabetes with clinical consequences giving rise to chronic multiple organ complications. *MTHFR* polymorphisms have been implicated in diabetes and micro-/macrovascular complications. The link between *MTHFR* genotype and diabetes however is strongly dependent on the race. Recently, El

Hajj Chehadeh and colleagues observed no significant differences in genotype and haplotype distributions between 169 diabetes patients and 209 healthy controls in an Emirati population (El Hajj Chehadeh et al. 2016). An extensive meta-analysis from a Chinese group focused on the relationship between the *MTHFR* A1298C genotypes and the susceptibility of diabetes confirmed the differences related to ethnicity. Using a systematic review of articles available in PubMed, Embase, Chinese Biomedical Literature Database (CBM, Chinese), China National Knowledge Infrastructure (CNKI), and Wangfang Database (Chinese), the authors found that *MTHFR* A1298C polymorphism significantly affected diabetes susceptibility in Asian population, but this association was not observed in Caucasians (Yan et al. 2014).

Neuropsychiatric disorders

A wide range of diseases, including neuropsychiatric disorders and autism, has been associated with increased homocysteine levels in biological fluids, even if this correlation is debated (Kałużna-Czaplińska et al. 2013). An Italian study found no significant relationship between *MTHFR* polymorphisms and age, cognitive status and type of dementia in a cohort of 72 subjects with cognitive disorders and 122 healthy controls. Plasma total homocysteine did not differ significantly in relation to *MTHFR* genotypes, but subjects of all genotypes with low serum folate (<12 nmol/L) had higher plasma total homocysteine compared to subjects with high serum folate (>12 nmol/L). The study suggests that 677C>T and 1298A>C polymorphisms are common in Northern Italian population, but do not significantly affect plasma total homocysteine levels of elderly individuals, even under conditions of low folate status (Ravaglia et al. 2004).

Cancer

Folate deficiency was suggested to increase the risk of cancer through impaired DNA repair synthesis and disruption of DNA methylation that may lead to proto-oncogene activation (Duthie 1999). It is biologically plausible that polymorphisms or gene-environment interactions rather than the folate intake alone might affect breast cancer risk, since functional polymorphisms in folate-related genes contribute to the alteration of folate metabolism. Nevertheless, *MTHFR* polymorphisms have been intensively studied in breast cancer and the results are inconsistent (Lewis et al. 2006; Lissowska et al. 2007). A recent meta-analysis, performed by pooling 20 eligible studies indicated a moderately significant effect of 677C>T polymorphism on breast cancer risk. On the other hand, subgroup analysis based on ethnicity showed a strong association between TT genotype and breast cancer in Asian population, but not in Caucasians (Lissowska et al. 2007). The influence of *MTHFR* 677C>T polymorphism on the renal cell carcinoma (RCC) are also contrasting. In a recent study by Lv et al., *MTHFR* 677C>T polymorphism appeared to be protective against the occurrence of RCC, even if no association was found between *MTHFR* 677C>T genotypes with RCC susceptibility (Lv et al. 2015). In spite of the paucity of data about this topic, previous evidence had indicated a significant influence of *MTHFR* 677C>T polymorphism genotypes and T allele on renal cancer risk, but this disagreement may be due from the differences in population race and sample size (Moore et al. 2008).

Renal disease

Chronic kidney disease (CKD) represents an increasing burden on the worldwide healthcare system leading to poor outcomes and high costs. CKD is above all a risk factor for cardiovascular disease; likewise, cardiovascular disease may promote CKD, resulting in a vicious cycle. This association between cardiovascular disease and CKD is present from the earliest stages of CKD, therefore delaying the evolution to end-stage renal disease (ESRD) remains a primary goal for CKD patients, since at present there are no specific treatments to avoid cardiovascular disease in this population (Turner et al. 2012). This evidence has generated interest for hyperhomocysteinemia and folic acid deficiency as risk factors for cardiovascular disease and their role in CKD progression (Marti et al. 2011). The renal and the vascular damage could be related to the effects on the cells of the vascular wall of the hyperhomocysteinemia and reduced availability of folic acid might be so amplifying the chronic inflammation of CKD patients (Akchurin and Kaskel 2015; Colì et al. 2011; Udeanu et al. 2014).

Although some studies seem to exclude a direct association between *MTHFR* 677C>T genotype and long–term kidney outcomes (Rady et al. 2002), *MTHFR* 677C>T polymorphism has been shown to contribute to increased cardiovascular risk in ESRD patients (Wrone et al. 2004), similarly to the general population (Zee et al. 2007). On the other hand, a recent study on 630 Italian Caucasian population found a lower frequency of *MTHFR* 677C>T and A1298A>C polymorphisms among dialysis patients in end-stage kidney failure compared to subjects without or with slight-moderate renal impairment, suggesting a protective role of both polymorphisms on renal function (Trovato et al. 2015). These results are in agreement with a recent investigation where the *in silico* analysis for nine SNPs commonly associated with ESRD, including the *MTHFR* 677C>T, performed with eight different algorithms (PROVEAN, SIFT, Mutation Assessor, Polyphen 2, PhD-SNP, SNAP, MutationTaster, PMUT), revealed that

677C>T was not classified as deleterious (Prakash et al. 2016). A prespecified renal substudy of the CSPPT examines the effects of the combination of ACE inhibitors and folic acid with ACE inhibitors alone in reducing the risk of renal function decline in a hypertensive population (Xu et al. 2016). The distinctiveness of this study lies in the fact that it has been conducted in a population without folic acid fortification, including participants across a spectrum of renal function at baseline from normal to moderate CKD, and that cyanocobalamin was not used in the therapy. The authors found that treatment with ACE inhibitors plus folic acid, compared with ACE inhibitors alone, reduced the risk of progression of CKD by 21% and the rate of eGFR decline by 10% in hypertensive patients. Patients with CKD benefited most from the folic acid therapy, with a 56 and 44% reduction in the risk for progression of CKD and the rate of eGFR decline, respectively. In the ACE inhibitors plus folic acid group, both the size of the increase in serum folate and the drop in homocysteine were greater in the participants with CKD than in those without CKD. In particular, the greatest drop in serum homocysteine was in TT homozygotes of MTHFR C677T polymorphism, while the magnitude of the homocysteine decrease in those with CC/CT genotypes was relatively small. Similarly to the stroke risk found in the CSPPT study, the effect of MTHFR genotype on CKD progression is expected to be subject to modification by population dietary folate levels (Huo et al. 2015; Xu al. 2016).

In kidney transplant recipients, time of dialysis prior to transplantation, anemia, and chronic immunosuppression are likely to trigger a combination of immunologic responses, prothrombotic state, dysmetabolic alterations, and inflammatory abnormalities, which lead to a substantially increased cardiovascular risk in comparison with the general population (La Manna et al. 2010). To the best of our knowledge, there are no studies that have analysed the impact of MTHFR polymorphism on cardiovascular risk in kidney transplant recipients. Besides, treatment of stable kidney transplant recipients with a multivitamin containing high-dose folic acid, B6, and B12 lowers tHcy levels but does not reduce cardiovascular disease outcomes or total mortality in these patients (Bostom et al. 2011). In opposite, Oetting et al. reported a correlation with the TT genotype of the *MTHFR* 677C>T polymorphism and the only risk of acute rejection (Oetting et al. 2012).

Pharmacogenetics

Genetic variants can affect pharmacokinetic or pharmacodynamic properties of several drugs and account for interindividual differences in treatment response and adverse events among the patients. Ghodke-Puranik et al. evaluated the pharmacogenetic influence of gene polymorphisms in folate pathway genes in rheumatoid arthritis patients receiving methotrexate, proving that the SNPs 677C>T and 1298A>C were able to increase methotrexate treatment efficacy (Ghodke-Puranik et al. 2015). Recently, Zhao et al. described a significant association between *MTHFR* 677C>T polymorphism and increased risk of methotrexate hepatic and gastrointestinal toxicity, independent of methotrexate dosage in Caucasians. *MTHFR* 1298A>C polymorphism does not seem to affect significantly hepatic and hematological toxicity, and it might have a protective effect on mucositis and gastrointestinal toxicity (Zhao et al. 2016).

Conclusion

Homocysteine levels and *MTHFR* polymorphisms have been investigated in association with a variety of diseases with a multifactorial etiology. *MTHFR* 677C>T SNP seems to be also as a predictor for methotrexate toxicity in a pharmacogenetic approach. Conflicting results have been reported about the association between *MTHFR* gene variants with diabetes, breast cancer, renal cell carcinoma and kidney failure. The neural tube defects and cognitive disorders have not been proven to correlate with the carriage of *MTHFR* genotypes. Despite the well-established associations described above, long follow–up studies in larger samples size are necessary to definitively confirm the role of *MTHFR* genotypes in several pathological conditions.

Furthermore, the correlation between the bioinformatics tools (e.g. PROVEAN, SIFT, Mutation Assessor, Polyphen 2, PhD-SNP, SNAP, MutationTaster, PMUT, ExAc) and functional assay (e.g. in vitro and in vivo models) might unravel many unsolved queries that will be useful to disclose the genetic landscape for multifactorial diseases.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

Ethical approval This article does not contain any studies with human subjects or animals performed by any of the authors.

References

Akchurin OM, Kaskel F (2015) Update on inflammation in chronic kidney disease. Blood Purif 39:84–92

Alizadeh S, Djafarian K, Moradi S, Shab-Bidar S (2016) C667T and A1298C polymorphisms of methylene tetrahydrofolate reductase gene and susceptibility to myocardial infarction: a systematic review and meta-analysis. Int J Cardiol 217:99–108

Angeline T, Jeyaraj N, Tsongalis GJ (2007) MTHFR gene polymorphisms, B-vitamins and hyperhomocystinemia in young and

middle-aged acute myocardial infarction patients. Exp Mol Pathol 82:227–233

Bagley PJ, Selhub J (1998) A common mutation in the methylenetetrahydrofolate reductase gene is associated with an accumulation of formylated tetrahydrofolates in red blood cells. Proc Natl Acad Sci USA 95:13217–13220

Biswas A, Ranjan R, Meena A, Akhter MS, Yadav BK, Munisamy M, Subbiah V, Behari M, Saxena R (2009) Homocystine levels, polymorphisms and the risk of ischemic stroke in young Asian Indians. J Stroke Cerebrovasc Dis 18:103–110

Blom HJ, Smulders Y (2011) Overview of homocysteine and folate metabolism. With special references to cardiovascular disease and neural tube defects. J Inherit Metab Dis 34:75–81

Bostom AG, Carpenter MA, Kusek JW, Levey AS, Hunsicker L, Pfeffer MA, Selhub J, Jacques PF, Cole E, Gravens-Mueller L et al (2011) Homocysteine-lowering and cardiovascular disease outcomes in kidney transplant recipients: primary results from the folic acid for vascular outcome reduction in transplantation trial. Circulation 123:1763–1770

Böttiger AK, Hurtig-Wennlöf A, Sjöström M, Yngve A, Nilsson TK (2007) Association of total plasma homocysteine with methylenetetrahydrofolate reductase genotypes 677C>T, 1298A>C, and 1793G>A and the corresponding haplotypes in Swedish children and adolescents. Int J Mol Med 19:659–665

Brustolin S, Giugliani R, Félix TM (2010) Genetics of homocysteine metabolism and associated disorders. Braz J Med Biol Res 43:1–7

Cen H, Huang H, Zhang LN, Liu LY, Zhou L, Xin XF, Zhuo RJ (2016) Associations of methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C polymorphisms with genetic susceptibility to rheumatoid arthritis: a meta-analysis. Clin Rheumatol 36:287–297

Cianciolo G, La Manna G, Colì L, Donati G, D'Addio F, Persici E, Comai G, Wratten M, Dormi A, Mantovani V et al (2008) 5-methyltetrahydrofolate administration is associated with prolonged survival and reduced inflammation in ESRD patients. Am J Nephrol 28:941–948

Colì L, Donati G, Cappuccilli ML, Cianciolo G, Comai G, Cuna V, Carretta E, La Manna G, Stefoni S (2011) Role of the hemodialysis vascular access type in inflammation status and monocyte activation. Int J Artif Org 34:481–488

Cronin S, Furie KL, Kelly PJ (2005) Dose-related association of MTHFR 677 T allele with risk of ischemic stroke: evidence from a cumulative meta-analysis. Stroke 36:1581–1587

Debreceni B, Debreceni L (2014) The role of homocysteine-lowering B-vitamins in the primary prevention of cardiovascular disease. Cardiovasc Ther 32:130–138

Duthie SJ (1999) Folic acid deficiency and cancer: mechanisms of DNA instability. Br Med Bull 55:578–592

Félix TM, Leistner S, Giugliani R (2004) Metabolic effects and the methylenetetrahydrofolate reductase (MTHFR) polymorphism associated with neural tube defects in southern Brazil. Birth Defects Res A Clin Mol Teratol 70:459–463

Ghodke-Puranik Y, Puranik AS, Shintre P, Joshi K, Patwardhan B, Lamba J, Niewold TB, Chopra A (2015) Folate metabolic pathway single nucleotide polymorphisms: a predictive pharmacogenetic marker of methotrexate response in Indian (Asian) patients with rheumatoid arthritis. Pharmacogenomics 16:2019–2034

Goyette P, Pai A, Milos R, Frosst P, Tran P, Chen Z, Chan M, Rozen R (1998) Gene structure of human and mouse methylenetetrahydrofolate reductase (MTHFR). Mamm Genome 9:652–656

El Hajj Chehadeh SW, Jelinek HF, Al Mahmeed WA, Tay GK, Odama UO, Elghazali GE, Al Safar HS (2016) Relationship between MTHFR C677T and A1298C gene polymorphisms and complications of type 2 diabetes mellitus in an Emirati population. Meta Gene 9:70–75

Holmes MV, Newcombe P, Hubacek JA, Sofat R, Ricketts SL, Cooper J, Breteler MM, Bautista LE, Sharma P, Whittaker JC et al (2011) Effect modification by population dietary folate on the association between MTHFR genotype, homocysteine, and strokerisk: a meta-analysis of genetic studies and randomised trials. The Lancet 378:584–594

Homberger A, Linnebank M, Winter C, Willenbring H, Marquardt T, Harms E, Koch HG (2000) Genomic structure and transcript variants of the human methylene tetrahydrofolate reductase gene. Eur J Hum Genet 8:725–729

Huo Y, Li J, Qin X, Huang Y, Wang X, Gottesman RF, Tang G, Wang B, Chen D, He M et al (2015) Efficacy of folic acid therapy in primary prevention of stroke among adults with hypertension in China: the CSPPT randomized clinical trial. JAMA 313:1325–1335

Kałużna-Czaplińska J, Żurawicz E, Michalska M, Rynkowski J (2013) A focus on homocysteine in autism. Acta Biochim Pol 60:137–142

Kaul S, Zadeh AA, Shah PK (2006) Homocysteine hypothesis for atherothrombotic cardiovascular disease: not validated. J Am Coll Cardiol 48:914–923

Kozieradzka A, Pepinski W, Waszkiewicz E, Olszewska M, Maciorkowska D, Skawronska M, Niemcunowicz-Janica A, Dobrzycki S, Musial WJ, Kaminski KA (2012) The rs1801133 polymorphism of methylenetetrahydrofolate reductase gene- the association with 5-year survival in patients with ST-elevation myocardial infarction. Adv Med Sci 57:106–111

La Manna G, Cappuccilli ML, Cianciolo G, Conte D, Comai G, Carretta E, Scolari MP, Stefoni S (2010) Cardiovascular disease in kidney transplant recipients: the prognostic value of inflammatory cytokine genotypes. Transplantation 89:1001–1008

Lewis SJ, Harbord RM, Harris R, Smith GD (2006) Meta-analyses of observational and genetic association studies of folate intakes or levels and breast cancer risk. J Natl Cancer Inst 98:1607–1622

Li YN, Gulati S, Baker PJ, Brody LC, Banerjee R, Kruger WD (1996) Cloning, mapping and RNA analysis of the human methionine synthase gene. Hum Mol Genet 5:1851–1858

Lissowska J, Gaudet MM, Brinton LA, Chanock SJ, Peplonska B, Welch R, Zatonski W, Szeszenia-Dabrowska N, Park S, Sherman M et al (2007) Genetic polymorphisms in the one-carbon metabolism pathway and breast cancer risk: a population-based case-control study and meta-analyses. Int J Cancer 20:2696–2703

Lv C, Bai Z, Liu Z, Luo P, Zhang J (2015) Renal cell carcinoma risk is associated with the interactions of APOE, VHL and MTHFR gene polymorphisms. Int J Clin Exp Pathol 8:5781–5786

Mansoor MA, Bergmark C, Svardal AM, Lønning PE, Ueland PM (1995) Redox status and protein binding of plasma homocysteine and other aminothiols in patients with early-onset peripheral vascular disease. Homocysteine and peripheral vascular disease. Arterioscler Thromb Vasc Biol 15:232–240

Marti F, Vollenweider P, Marques-Vidal PM, Mooser V, Waeber G, Paccaud F, Bochud M (2011) Hyperhomocysteinemia is independently associated with albuminuria in the population-based CoLaus study. BMC Public Health 11:733

McCully KS (1996) Homocysteine and vascular disease. Nat Med 2:386–389

Moore LE, Hung R, Karami S, Boffetta P, Berndt S, Hsu CC, Zaridze D, Janout V, Kollarova H, Bencko V et al (2008) Folate metabolism genes, vegetable intake and renal cancer risk in central Europe. Int J Cancer 122:1710–1715

Mudd SH, Uhlendorf BW, Freeman JM, Finkelstein JD, Shih VE (1972) Homocystinuria associated with decreased methylene tetrahydrofolate reductase activity. Biochem Biophys Res Commun 46:905–912

Oetting WS, Zhu Y, Brott MJ, Matas AJ, Cordner GK, Pan W (2012) Validation of genetic variants associated with early acute

rejection in kidney allograft transplantation. Clin Transplant 26:418–423
Prakash S, Sarangi AN, Tripathi G, Sharma RK, Agrawal S (2016) Prediction of susceptible biomarkers for end stage renal disease among North Indians. Nephrology 21:592–600
Quéré I, Wutschert R, Zittoun J, Bellet H, Reber G, Gris JC, Janbon C, Schved JF, de Moerloose P (1998) Association of red-blood methylfolate but not plasma folate with C677T MTHFR polymorphism in venous thromboembolic disease. Thromb Haemost 80:707–709
Rady PL, Szucs S, Grady J, Hudnall SD, Kellner LH, Nitowsky H, Tyring SK, Matalon RK (2002) Genetic polymorphisms of methylenetetrahydrofolate reductase (MTHFR) and methionine synthase reductase (MTRR) in ethnic populations in Texas; a report of a novel MTHFR polymorphic site, G1793A. Am J Med Genet 107:162–168
Ravaglia G, Forti P, Maioli F, Scali RC, Arnone G, Talerico T, Pantieri T, Nativio V, Mantovani V, Bianchin M (2004) Common polymorphisms in methylenetetrahydrofolate reductase (MTHFR): relationships with plasma homocysteine concentrations and cognitive status in elderly northern italian subjects. Arch Gerontol Geriatr Suppl 9:339–348
Ravaglia G, Forti P, Maioli F, Martelli M, Servadei L, Brunetti N, Porcellini E, Licastro F (2005) Homocysteine and folate as risk factors for dementia and Alzheimer disease. Am J Clin Nutr 82:636–643
Rodriguez-Rodriguez L, López-Mejias R, Fernández-Gutiérrez B, Balsa A, González-Gay MA, Martín J (2015) Rheumatoid arthritis: genetic variants as biomarkers of cardiovascular disease. Curr Pharm Des 21:182–201
Salih MA, Murshid WR, Seidahmed MZ (2014) Classification, clinical features, and genetics of neural tube defects. Saudi Med J 35:S5–S14
Sazci A, Ergul E, Kaya G, Kara I (2005) Genotype and allele frequencies of the polymorphic methylenetetrahydrofolate reductase gene in Turkey. Cell Biochem Funct 23:51–54
Shotelersuk V, Ittiwut C, Siriwan P, Angspatt A (2003) Maternal 677CT/1298AC genotype of the MTHFR gene as a risk factor for cleft lip. J Med Genet 40:e64
Trabetti E (2008) Homocysteine, MTHFR gene polymorphisms, and cardio-cerebrovascular risk. J Appl Genet 49:267–282
Trovato FM, Catalano D, Ragusa A, Martines GF, Pirri C, Buccheri MA, Di Nora C, Trovato GM (2015) Relationship of MTHFR gene polymorphisms with renal and cardiac disease. World J Nephrol 4:127–137
Turner JM, Bauer C, Abramowitz MK, Melamed ML, Hostetter TH (2012) Treatment of chronic kidney disease. Kidney Int 81:351–362
Udeanu M, Guizzardi G, Di Pasquale G, Marchetti A, Romani F, Dalmastri V, Capelli I, Stalteri L, Cianciolo G, Rucci P et al (2014) Relationship between coronary artery disease and C-reactive protein levels in NSTEMI patients with renal dysfunction: a retrospective study. BMC Nephrol 15:152
Verkleij-Hagoort A, Bliek J, Sayed-Tabatabaei F, Ursem N, Steegers E, Steegers-Theunissen R (2007) Hyperhomocysteinemia and MTHFR polymorphisms in association with orofacial clefts and congenital heart defects: a meta-analysis. Am J Med Genet A 143A:952–960
Vijayan M, Chinniah R, Ravi PM, Sivanadham R, Mosses Joseph AK, Vellaiappan NA, Krishnan JI, Karuppiah B (2016) MTHFR (C677T) CT genotype and CT-apoE3/3 genotypic combination predisposes the risk of ischemic stroke. Gene 591:465–470
Weisberg IS, Jacques PF, Selhub J, Bostom AG, Chen Z, Curtis Ellison R, Eckfeldt JH, Rozen R (2001) The 1298A–>C polymorphism in methylenetetrahydrofolate reductase (MTHFR): in vitro expression and association with homocysteine. Atherosclerosis 156:409–415
WHO (2011) The atlas of heart disease and stroke. Retrieved 19 Oct 2011 from http://www.who.int/cardiovascular_diseases/resources/atlas/en/
Wilcox AJ, Lie RT, Solvoll K, Taylor J, McConnaughey DR, Abyholm F, Vindenes H, Vollset SE, Drevon CA (2007) Folic acid supplements and risk of facial clefts: national population based case-control study. BMJ 334:464
Wrone EM, Hornberger JM, Zehnder JL, McCann LM, Coplon NS, Fortmann SP (2004) Randomized trial of folic acid for prevention of cardiovascular events in end-stage renal disease. J Am Soc Nephrol 15:420–426
Xu X, Qin X, Li Y, Sun D, Wang J, Liang M, Wang B, Huo Y, Hou FF (2016) Efficacy of folic acid therapy on the progression of chronic kidney disease: the renal substudy of the china stroke primary prevention trial. JAMA Intern Med 176:1443–1450
Yamada K, Chen Z, Rozen R, Matthews RG (2001) Effects of common polymorphisms on the properties of recombinant human methylene tetrahydrofolate reductase. Proc Natl Acad Sci USA 98:14853–14858
Yan Y, Liang H, Yang S, Wang J, Xie L, Qin X, Li S (2014) Methylenetetrahydrofolate reductase A1298C polymorphism and diabetes risk: evidence from a meta-analysis. Ren Fail 36:1013–1017
Yuan L, Song Z, Deng X, Xiong W, Yang Z, Deng H (2016) Association of the MTHFR rs1801131 and rs1801133 variants in sporadic Parkinson's disease patients. Neurosci Lett 616:26–31
Yun L, Xu R, Li G, Yao Y, Li J, Cong D, Xu X, Zhang L (2015) Homocysteine and the C677T gene polymorphism of its key metabolic enzyme MTHFR are risk factors of early renal damage in hypertension in a Chinese Han population. Medicine 94:e2389
Zee RY, Mora S, Cheng S, Erlich HA, Lindpaintner K, Rifai N, Buring JE, Ridker PM (2007) Homocysteine, 5,10-methylenetetrahydrofolate reductase 677C>T polymorphism, nutrient intake, and incident cardiovascular disease in 24,968 initially healthy women. Clin Chem 53:845–851
Zhao M, Liang L, Ji L, Chen D, Zhang Y, Zhu Y, Ongaro A (2016) MTHFR gene polymorphisms and methotrexate toxicity in adult patients with haematological malignancies: a meta-analysis. Pharmacogenomics 17:1005–1017