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The Effects of Polymorphisms in One-carbon Metabolism Genes on Manifestation of Ichthyosis Vulgaris

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Abstract

BACKGROUND: Ichthyosis vulgaris is the most common type of Mendelian disorders of cornification, caused by loss-of-function mutations in the gene encoding epidermal protein filaggrin (*FLG*), namely R501X and 2282del4. *FLG* 2282del4 mutation in heterozygotes is incompletely penetrant. Polymorphisms in one-carbon metabolism genes could be associated with clinical manifestation of ichthyosis vulgaris.

AIM: The purpose of the present study was to analyze the effects of *MTHFR*, *MTR,* and *MTRR* polymorphisms in patients with ichthyosis vulgaris.

METHODS: Thirty-one patients with ichthyosis vulgaris, 7 their *FLG* heterozygous relatives without symptoms of disorder, and 150 healthy controls were enrolled in the study. *FLG* null mutations — R501X (rs61816761) and 2282del4 (rs558269137) — and one-carbon metabolism gene polymorphisms — *MTHFR* C677T (rs1801133), *MTHFR* A1298C (rs1801131), *MTR* A2756G (rs1805087), and *MTRR* A66G (rs1801394) — were analyzed by a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay.

RESULTS: Among patients with ichthyosis, heterozygous for *FLG* 2282 del4 mutation, the distributions of genotypes for folate metabolism genes were: *MTHFR* C677T CC:CT:TT —29.4%:70.6%:0.0%; *MTHFR* A1298C AA:AC:CC — 52.9%:47.1%:0.0%; *MTR* A2756G AA:AG:GG — 70.3%:23.5%:5.9%; and *MTRR* A66G AA:AG:GG — 23.4%:52.9%:23.5%. The frequencies of *MTR* 2756AA and *MTRR* 66GG genotypes were 1.4–1.6 times higher in affected individuals heterozygous for 2282del4 than in patients with other *FLG* genotypes. In affected 2282del4 heterozygotes, the frequency of *MTR* 2756AA genotype was 1.6 times greater than in healthy controls ($p < 0.01$). The strongest association was found between *MTHFR* 677CT/*MTHFR* 1298AA/*MTR* 2756AA/*MTRR* 66AG genotype and ichthyosis — odds ratio (OR)=11.23 (95% confidence interval 2.51−50.21, $p = 0.002$).

CONCLUSIONS: Various genotypes of one-carbon metabolism genes increase the risk of ichthyosis in heterozygotes for the *FLG* 2282del4 mutation (OR 2.80–11.23). The most probable predisposing genotype is 677CT/1298AA/2756AA+AG/66AG.

Introduction

One of the largest groups of skin and subcutaneous tissue diseases is genodermatoses, which includes disorders of keratinization such as ichthyoses [1]. The prevalence of ichthyosis vulgaris (Q 80.0, OMIM 146700), which is regarded as the most common type of the disease, varies across countries [2], [3]. Ichthyosis vulgaris is caused by loss-of-function mutations in the gene encoding crucial epidermal protein filaggrin (*FLG*, 1q21.3, OMIM 135940). Lack of filaggrin results in keratin filament disorganization, abnormal architecture of lipid matrix, scaling, dry skin condition, and impaired skin barrier function [3], [4], [5], [6], [7].

Segregation analysis of ichthyosis vulgaris confirmed a monogenic (*Mendelian*) autosomal semidominant mode of inheritance and its association with *FLG* null mutations [8]. In populations of European ancestry, two most common mutations are 2282del4 (rs558269137) and R501X (rs61816761) [7]. Despite the fact that in heterozygotes, penetrance of the mutations ranges from 73% for 2282del4 to 96% for R501X [7], [9], any explanation of the difference between the frequencies of homo- and heterozygotes for *FLG* null mutations (13.0%) and prevalence of ichthyosis (1.3%) has not been proposed yet [2], [7], [10]. At the same time, epigenetic studies of eczema and atopic dermatitis demonstrated that the risk of the diseases is associated with methylation level of *FLG* gene [11], [12], [13]. It

might indicate the role of one-carbon metabolism in the regulation of *FLG* gene expression [1], [14].

Our previous research has shown that carriers of *FLG* 2282del4 mutation, who were heterozygous for C677T polymorphism in the *MTHFR* gene (1p36.22, OMIM 607093), were 7 times as likely to develop ichthyosis as subjects with wild-type 677CC genotype [15]. It was also found that an elevated plasma homocysteine level impaired not only the metabolism of sulfur-containing amino acids, methionine, and cysteine, but also keratin molecules [16], [17], [18] that might exacerbate the effects of FLG null mutations. Every of single nucleotide polymorphisms (SNPs) of one-carbon metabolism genes — *MTHFR* C677T (rs1801133) and A1298C (rs1801131), *MTR* A2756G (rs1805087) and *MTRR* A66G (rs1801394) — are associated with plasma homocysteine level [19], [20]. The only significant model for hyperhomocysteinemia is a four-locus model that includes SNPs of *MTHFR*, *MTR,* and *MTRR* [21].

Therefore, one-carbon metabolism genes could be considered as candidate regulatory genes of network for keratinization.

The aim of the present study was to analyze the effects of *MTHFR*, *MTR,* and *MTRR* gene polymorphisms in patients with ichthyosis vulgaris.

Materials and Methods

Patients with ichthyosis vulgaris were recruited from the Regional Clinical Dermatological and Venereological Dispensary no. 1 and dermatological and venereological dispensaries of Kharkiv region, Ukraine. Genomic DNA was isolated from blood samples of 31 patients with ichthyosis vulgaris and 7 their first-degree relatives without ichthyosis using salting-out method. The detection of *FLG* null mutations (R501X and 2282del4) and one-carbon metabolism gene polymorphisms (*MTHFR* C677T, *MTHFR* A1298C, *MTR* A2756G, and *MTRR* A66G) was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay with optimal primers (Metabion, Germany). The PCR products were digested with HinfI, MboII, HaeIII, and NdeI restriction endonucleases (Thermo Fisher Scientific, USA) [22], [23], [24], [25], [26], [27]. The digested PCR products were separated on 2.5% agarose gel (Amresco, USA).

The normality of distribution of continuous variables was tested by Shapiro–Wilk test. Correlations between groups were assessed by Pearson and Spearman correlation. The genotype frequencies were analyzed using Fisher's angular transformation. When multiple hypothesis tests were performed, a Bonferroni corrected p-value was used.

Differences in variables were statistically analyzed with Chi-square test with the values predicted by the assumption of Hardy–Weinberg equilibrium. Odds ratios (ORs) with 95% confidence interval (CI) were used to evaluate the association between one-carbon metabolism gene polymorphisms and risk of ichthyosis vulgaris.

All data analyses were performed using Statistica Basic Academic (version 13.3, TIBCO Software Inc., Palo Alto, CA, USA). The linkage disequilibrium (LD) parameters D' and r^2 were estimated and haplotype block analyses were performed in Haploview (version 4.2, Broad Institute, Cambridge, MA, USA).

Informed consent was obtained from all individuals involved in the study. The research was carried out in accordance with the basic bioethical principles of the World Medical Association's Declaration of Helsinki (2000, as amended in 2008), the Universal Declaration on Bioethics and Human Rights (1997), and the Convention on Human Rights and Biomedicine of the Council of Europe (1997). All procedures were approved by the local Ethics Committee of Kharkiv National Medical University.

Results

The results of a literature-based analysis of the geographical distribution of the *MTHFR* A1298C, *MTR* A2756G, and *MTRR* A66G alleles and genotypes frequencies in the northern hemisphere are reported in Table 1. The negative correlation was observed between latitude and frequency of *MTRR* 66AG genotype (Pearson r = -0.6523, p = 0.041). Previously, we found a negative relationship between the latitude and frequencies of *MTHFR* 677T allele and *MTHFR* 677CT genotype [53].

The geographic distribution of alleles and genotypes frequencies of one-carbon metabolism gene polymorphisms was also compared to plasma homocysteine levels across Europe using data provided in the related studies [52]. Homocysteine concentrations showed positive correlations with the frequencies of *MTR* 2756A allele and *MTR* 2756AA genotype (Pearson r = 0.689, p = 0.040 and Pearson r = 0.751, p = 0.020, respectively), and negative correlations with the frequencies of *MTR* 2756G allele and *MTRR* 66GG genotype (Pearson r = -0.737, p = 0.024 and r = -0.771, p = 0.015, respectively).

Based on our data, the penetrance of ichthyosis vulgaris in individuals with 2282del4/2282del4, 2282del4/R501X, and R501X/wt *FLG* genotypes would be considered a complete one, but in individuals with 2282del4/wt genotype, it was estimated at 67%.

The allele and genotype frequencies of one-carbon metabolism polymorphisms in patients with ichthyosis vulgaris and their relatives from Kharkiv region are reported in Table 2. Significant deviation from

Hardy–Weinberg equilibrium was detected for the *MTHFR* C677T genotypes in patients with ichthyosis vulgaris.

The frequencies of *MTR* 2756AA genotype and *MTRR* 66GG genotype were 1.4–1.6 times higher in affected individuals heterozygous for 2282del4 than in patients with other *FLG* genotypes (Table 2). In 2282del4 heterozygotes, the frequency of *MTR* 2756AA genotype in affected individuals was 1.6 times greater than in unaffected ones, but the frequency of *MTRR* 66GG genotype in the first group was 1.8 times lower than in the second one (Table 2). In affected 2282del4 heterozygotes, the frequency of *MTR* 2756AA genotype was 1.6 times greater than in healthy controls (Table 2).

To estimate the association between polymorphisms in one-carbon metabolism genes and ichthyosis vulgaris manifestation in individuals with 2282del4/wt genotype, we calculated OR for the different disease models representing from one to four variants of folate metabolism genes. Table 3 shows the only statistically significant results. In single-locus models, *MTHFR* C677T polymorphism was significantly associated with ichthyosis vulgaris in the overdominant genetic model (OR 3.600, 95% CI 1.207−10.712, p = 0.032). In two-locus models, a significant increase in disease manifestation was associated with *MTHFR* 677CT/*MTHFR* 1298AA + AC (OR 4.393; 95% CI 1.468−13.139, p = 0.008) and *MTHFR* 677CT/*MTR* 2756AA genotypes (OR 4.239, 95% CI 1.495−12.018, p = 0.007). The best three-locus model was one representing heterozygosity for polymorphisms *MTHFR* C677T and *MTRR* A66G, and homozygosity for the *MTHFR* A1298C polymorphism (OR 7.636, 95% CI 2.338−24.943, p = 0.001). The strongest association was found between *MTHFR* 677CT/*MTHFR* 1298AA/*MTR* 2756AA/*MTRR* 66AG genotype and ichthyosis (OR 11.231, 95% CI 2.512−50.209, p = 0.002). These results suggest that for the heterozygotes for *FLG* 2282del4 mutation the best model of clinical manifestation of ichthyosis is a four-locus model for folate metabolism genes.

The *MTHFR* 677CT/*MTHFR* 1298AA/*MTR* 2756AA+AG/*MTRR* 66AG genotype is most likely genotype associated with manifestation of ichthyosis vulgaris.

The *MTHFR*, *MTR*, and *FLG* genes are located on the chromosome 1, so LD might underlie this association. In individuals with *FLG* mutations, two LD blocks were revealed (Figure 1). The first one consisted

Table 1: Geographic distribution of genotype and allele frequencies of one-carbon metabolism single nucleotide polymorphisms in Europe

Country	*MTHFR*										*MTR*					*MTRR*					Source
	C677T					A1298C					A2756G					A66G					
	Genotype			Allele		Genotype			Allele		Genotype			Allele		Genotype			Allele		
	CC	*CT*	*TT*	*C*	*T*	*AA*	*AC*	*CC*	*A*	*C*	*AA*	*AG*	*GG*	*A*	*G*	*AA*	*AG*	*GG*	*A*	*G*	
Scotland	48.7	41.4	9.9	69.4	30.6	46.5	43.3	10.2	68.2	31.9	65.5	31.5	3.0	81.3	18.7	19.6	47.8	32.6	43.5	56.5	[28], [29]
Denmark	50.3	41.4	8.3	71.0	29.0	46.0	41.3	12.7	66.7	33.3	62.6	33.5	3.9	79.3	21.4	37.6	43.2	19.3	59.2	40.9	[30], [31], [32]
England	46.2	42.7	11.1	67.6	32.4	47.8	40.2	12.0	67.9	32.1	63.8	32.3	3.9	80.0	20.0	37.1	47.2	15.6	60.8	39.3	[32], [33]
Ireland	46.4	43.6	10.0	68.2	31.8	49.4	41.8	8.8	70.3	29.7	63.7	32.0	4.3	79.7	20.3	37.4	46.6	16.0	60.7	39.3	[34], [35], [36]
Poland	49.5	42.8	7.8	70.9	29.2	43.7	46.2	10.0	66.9	33.2	65.8	30.8	3.3	81.3	18.8	27.5	46.7	25.8	50.8	49.2	[37], [38], [39]
Germany	48.7	40.8	10.6	69.0	31.0	50.0	42.0	8.0	71.0	29.0	62.3	34.0	3.8	79.3	20.8	17.7	53.6	28.8	4.44	55.6	[40], [41], [42]
France	37.6	52.6	9.8	63.9	36.1	51.5	40.9	7.6	72.0	28.0	66.2	30.0	3.9	81.1	18.9	28.7	50.7	20.6	54.1	46.0	[34], [43], [44]
Austria	43.0	43.5	13.5	64.7	35.3	48.2	41.6	10.2	69.0	31.0	—	—	—	—	—	19.8	50.3	30.0	45.0	55.1	[45], [46]
Croatia	46.1	44.7	9.2	68.4	31.6	46.7	42.7	10.7	68.0	32.0	61.7	34.0	4.3	78.7	21.3	24.7	47.7	27.7	48.5	51.5	[47], [48]
Italy	29.0	54.8	16.1	56.5	43.6	47.7	35.5	16.8	65.5	34.5	67.5	29.2	3.3	82.1	17.9	26.3	52.5	21.2	52.5	47.5	[34], [49], [50], [51]
r	0.754	−0.717	−0.643	0.648	−0.648	−0.210	0.501	−0.335	0.107	−0.098	−0.281	0.334	−0.059	−0.221	0.271	0.383	−0.652	−0.149	0.286	−0.285	
p-value	0.012	0.020	0.045	0.043	0.043	0.561	0.141	0.344	0.769	0.788	0.464	0.379	0.880	0.568	0.480	0.275	0.041	0.682	0.423	0.424	

Table 2: Genotype and allele frequencies, and Hardy–Weinberg P values for one-carbon metabolism single nucleotide polymorphisms in ichthyosis vulgaris cases and controls

Filaggrin genotype	Phenotype	Ratio	*MTHFR* C677T					*MTHFR* A1298C					*MTR* A2756G					*MTRR* A66G				
			Genotype			Allele		Genotype			Allele		Genotype			Allele		Genotype			Allele	
			CC	CT	TT	C	T	AA	AC	CC	A	C	AA	AG	GG	A	G	AA	AG	GG	A	G
2282del4/2282del4, R501X/2282del4, R501X/wt (n=14)	Affected	Frequency (%)	26.7	66.7	6.7	60.0	40.0	57.1	35.7	7.1	75.0	25.0	50.0	42.9	7.1	71.4	28.6	35.7	50.0	14.3	60.7	39.3
		^{HWE}P	<0.001			1.000		0.893			1.000		0.882			1.000		0.891			1.000	
2282del4/wt (n=17)		Frequency (%)	29.4	70.6	0	64.7	35.3	52.9	47.1	0	76.5	23.5	70.3	23.5	5.9	83.9	16.1	23.4	52.9	23.5	50.0	50.0
		^{HWE}P	0.013			1.000		0.261			1.000		0.497			1.000		0.841			1.000	
		$^{R501X}P$	0.552			0.325		0.405			0.729		<0.001			<0.001		0.006			0.032	
2282del4/wt (n=7)	Unaffected	Frequency (%)	57.1	28.6	14.3	71.4	28.6	42.9	42.9	14.3	64.3	35.7	42.9	57.1	0	71.4	28.6	0	57.1	42.9	28.6	71.4
		^{HWE}P	0.011			1.000		0.801			1.000		0.103			1.000		0.265			1.000	
		$^{R501X}P$	<0.001			0.011		0.009			0.025		0.149			1.000		<0.001			<0.001	
		$^{2282IV}P$	<0.001			0.137		0.042			0.011		<0.001			0.006		<0.001			<0.001	
wt/wt (n=150)		Frequency (%)	54.7	40.0	5.3	74.7	25.3	42.7	44.7	12.7	65.0	35.0	42.7	38.7	18.7	62.0	38.0	25.3	37.3	37.3	44.0	56.0
		$^{R501X}P$	<0.001			<0.001		0.010			0.036		0.012			0.052		<0.001			0.001	
		$^{2282IV}P$	<0.001			0.022		0.038			0.016		<0.001			<0.001		0.003			0.227	
		$^{2282H}P$	<0.001			0.457		0.869			0.881		0.975			0.052		0.254			<0.001	

^{HWE}P value for the Hardy–Weinberg equilibrium test, $^{R501X}P$ value for the *FLG* homozygotes and compound heterozygotes with ichthyosis, $^{2282IV}P$ value for the *FLG* 2282del4 heterozygotes with ichthyosis, $^{2282H}P$ value for the *FLG* 2282del4 heterozygotes without ichthyosis

Table 3: Association between one-carbon metabolism polymorphisms and ichthyosis vulgaris risk in *FLG* 2282del4 heterozygotes

Genotype				*FLG*	Controls	Odds	confidence	p-value
MTHFR C677T	*MTHFR* A1298C	*MTR* A2756G	*MTRR* A66G	(n =17)	(n =150)	ratios	interval	
CT	—	—	—	12	60	3.600	1.207–10.712	0.032
—	—	AA	—	12	64	3.223	1.082–9.614	0.036
CT	AA	—	—	7	26	3.339	1.163–9.582	0.025
CT	AA+AC	—	—	12	53	4.393	1.468–13.139	0.008
CT	—	AA	—	6	26	4.239	1.495–12.018	0.007
CT	—	AA+AG	—	9	49	3.779	1.320–10.817	0,013
CT	—	—	AA+AG	9	37	3.436	1.236–9.549	0.018
CT	AA+AC	AA+AG	—	9	43	2.799	1.014–7.733	0.047
CT	AA	—	AG	6	10	7.636	2.338–24.943	0.001
—	AA	AA	AG	5	10	5.833	1.714–19.853	0.005
CT	—	AA	AG	5	10	5.833	1.714–19.853	0.005
CT	—	AA+AG	AA+AG	8	31	3.412	1.217–9.569	0.020
CT	AA	AA	AG	4	4	11.213	2.512–50.209	0.002

of SNPs of the *MTHFR* gene (rs1801133 and rs1801131) that demonstrated strong linkage (D'=1.00; LOD=2.32; r^2=0.195). The second block included mutations in the *FLG* gene (rs558269137 and rs61816761) with incomplete linkage (D'=1.00; LOD=1.53; r^2=0.109).

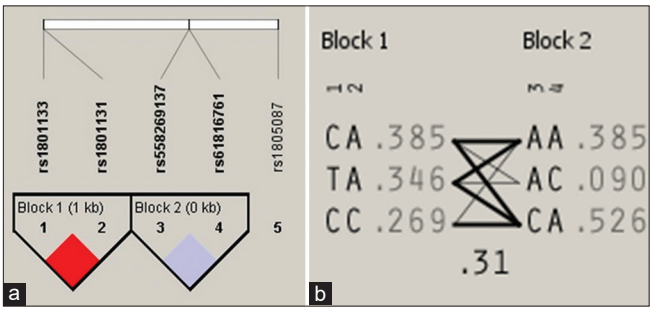

Figure 1: Linkage disequilibrium: Linkage disequilibrium blocks (a) and haplotypes (b) in individuals with filaggrin mutations. The color scheme shows the strength of linkage between markers: red – a strong linkage (D'=1, LOD >2), lilac – impossibility to calculate the linkage disequilibrium due to low frequency of the minor allele (D'=1, LOD <2). The connections between the LD blocks are shown as thick and thin lines for haplotypes with a frequency >10% and >1%, respectively

Discussion

The latitudinal features of the distribution of genotype and allele frequencies of one-carbon metabolism genes, as well as plasma homocysteine levels related to these polymorphisms, probably arose as adaptations to different climate conditions and had ethnoterritorial confinement [54], [55]. Thus, they need to be analyzed for each population separately.

In general, the penetrance of *FLG* 2282del4 mutation obtained in this research corresponds to our previous and literary data [7], [15]. We suggested that folate metabolism polymorphisms could be associated with clinical manifestation of ichthyosis vulgaris in individuals with 2282del4/wt genotype.

It is known that various genotypes for the *MTHFR*, *MTR,* and *MTRR* genes are related to cardiovascular, endocrine, reproductive disorders, certain cancer types, etc. [38], [56], [57], [58], [59], [60], [61], [62], [63], [64], [65], [66], [67], [68].

Polymorphisms of one-carbon metabolism genes and *FLG* null mutations are associated with the same disorders, including atopic dermatitis, eczema, inflammatory bowel disease, endocrine and gynecological diseases, skin permeability barrier dysfunction, and neoplasms [56], [68], [69], [70], [71], [72], [73]. These all suggest that other SNPs of folate metabolism genes, in addition to the *MTHFR* C677T variant, might affect *FLG* gene expression [73].

In our research, a strong association between homocysteine-raising polymorphisms of one-carbon metabolism genes and ichthyosis vulgaris was found in individuals with *FLG* null mutations.

LD blocks in chromosome 1 were not linked, perhaps because the distance between these loci exceeds 60 kb [74].

We tested a hypothesis about folate metabolism polymorphisms impact on the phenotypic expression of *FLG* null mutations in patients with ichthyosis vulgaris for England and Scotland only. This was because all the necessary data on the prevalence of the disease and frequencies of the *FLG* mutations and one-carbon metabolism polymorphisms were available for this region [2], [10], [75]. The frequencies of *FLG* heterozygotes and the predisposing genotype *MTHFR* 677CT/*MTHFR* 1298AA/*MTR* 2756AA+AG/*MTRR* 66AG are 0.13 and 0.092 in the region; thus, the combined probability of the clinical manifestation of ichthyosis vulgaris should be 0.012, that is not statistically different from the prevalence of the disease reported for the region — 0.013 (p = 0.857).

Conclusion

Various genotypes of one-carbon metabolism genes increase the risk of ichthyosis in heterozygotes for the *FLG* 2282del4 mutation (OR 2.799–11.231). The most probable predisposing genotype is 677CT/1298AA/2756AA+AG/66AG.

References

1. GBD 2017 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence,

and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990-2017: A systematic analysis for the global burden of disease study 2017. Lancet. 2018;392(10159):1789-858. https://doi.org/10.1016/S0140-6736(18)32279-7
PMid:30496104

2. Brown SJ, Relton CL, Liao H, Zhao Y, Sandilands A, Wilson IJ, *et al*. Filaggrin null mutations and childhood atopic eczema: A population-based case-control study. J Allergy Clin Immunol. 2008;121(4):940-6. https://doi.org/10.1016/j.jaci.2008.01.013
PMid:18313126
3. Brown SJ, McLean WH. One remarkable molecule: Filaggrin. J Invest Dermatol. 2012;132(3 Pt 2):751-62. https:/doi.org/10.1038/jid.2011.393
PMid:22158554
4. Hackl EV, Berest VP, Gatash SV. Effect of cholesterol content on gramicidin S-induced hemolysis of erythrocytes. Int J Pept Res Ther. 2012;18(2):163-70. https:/doi.org/10.1007/s10989-012-9289-9
5. Kezic S, Jakasa I. Filaggrin and skin barrier function. Curr Probl Dermatol. 2016;49:1-7. https://doi.org/10.1159/000441539
PMid:26844893
6. Popov M, Lyadova T, Volobuyeva O, Shepileva N, Kozlov A, Sorokina O. Cytokine production peculiarities in different forms of Epstein-Barr virus infection. Georgian Med News. 2017;2(263):55-9.
PMid:28452728
7. Thyssen JP, Maibach HI, editors. Filaggrin: Basic Science, Epidemiology, Clinical Aspects and Management. Heidelberg: Springer; 2014. p. 373.
8. Smith FJ, Irvine AD, Terron-Kwiatkowski A, Sandilands A, Campbell LE, Zhao Y, *et al*. Loss-of-function mutations in the gene encoding filaggrin cause ichthyosis vulgaris. Nat Genet. 2006;38(3):337-42. https:/doi.org/10.1038/ng1743
PMid:16444271
9. Palmer CN, Irvine AD, Terron-Kwiatkowski A, Zhao Y, Liao H, Lee SP, *et al*. Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. Nat Genet. 2006;38(4):441-6. https:/doi.org/10.1038/ng1767
PMid:16550169
10. Brown SJ, Relton CL, Liao H, Zhao Y, Sandilands A, McLean WHI, *et al*. Filaggrin haploinsufficiency is highly penetrant and is associated with increased severity of eczema: Further delineation of the skin phenotype in a prospective epidemiological study of 792 school children. Br J Dermatol. 2009;161(4):884-9. https:/doi.org/10.1111/j.1365-2133.2009.09339.x
PMid:19681860.
11. Ziyab AH, Karmaus W, Holloway JW, Zhang H, Ewart S, Arshad SH. DNA methylation of the filaggrin gene adds to the risk of eczema associated with loss-of-function variants. J Eur Acad Dermatol Venereol. 2013;27:e420-3. https:/doi.org/10.1111/jdv.12000
PMid:23003573
12. Bin L, Leung DY. Genetic and epigenetic studies of atopic dermatitis. Allergy Asthma Clin Immunol. 2016;12:52. https://doi.org/10.1186/s13223-016-0158-5
PMid:27777593
13. Lee J, Jang A, Seo SJ, Myung SC. Epigenetic regulation of filaggrin gene expression in human epidermal keratinocytes. Ann Dermatol. 2020;32(2):122-9. https://doi.org/10.5021/ad.2020.32.2.122
14. Crider KS, Yang TP, Berry RJ, Bailey LB. Folate and DNA methylation: A review of molecular mechanisms and the evidence for folate's role. Adv Nutr. 2012;3(1):21-38. https://doi.org/10.3945/an.111.000992
PMid:22332098
15. Fedota AM, Solodyankin AS, Ryzhko PP, Roshenyuk LV, Vorontsov VM, Solodyankina EA. C677T polymorphism of MTHFR gene ichthyosis patients. Bull Probl Biol Med. 2011;2(1):78-81.
16. Nazki FH, Sameer AS, Ganaie BA. Folate: Metabolism, genes, polymorphisms and the associated diseases. Gene. 2014;533(1):11-20. https://doi.org/10.1016/j.gene.2013.09.063
PMid:24091066
17. Borowczyk K, Suliburska J, Jakubowski H. Demethylation of methionine and keratin damage in human hair. Amino Acids. 2018;50:537-46. https://doi.org/10.1007/s00726-018-2545-3
PMid:29480334
18. Borowczyk K, Wróblewski J, Suliburska J, Akahoshi N, Ishii I, Jakubowski H. Mutations in homocysteine metabolism genes increase keratin N-homocysteinylation and damage in mice. Int J Genomics. 2018;2018:7570850. https://doi.org/10.1155/2018/7570850
PMid:30345292
19. Cristalli CP, Zannini C, Comai G, Baraldi O, Cuna V, Cappuccilli M, *et al*.·Methylenetetrahydrofolate reductase, MTHFR, polymorphisms and predisposition to different multifactorial disorders. Genes Genom. 2017;39:689-99. https://doi.org/10.1007/s13258-017-0552-5
20. Tinelli C, Di Pino A, Ficulle E, Marcelli S, Feligioni M. Hyperhomocysteinemia as a risk factor and potential nutraceutical target for certain pathologies. Front Nutr. 2019;6:49. https://doi.org/10.3389/fnut.2019.00049
PMid:31069230
21. Rossokha ZI, Kiryachenko SP, Gorovenko NJ. The role of MTHFR, MTRR, MTR1 intergenic interaction in the development of folate metabolism disturbance in patients with reproductive disorders. Ukrain Med J. 2018;2(3):1-5.
22. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, *et al*. A candidate genetic risk factor for vascular disease: A common mutation in methylenetetrahydrofolate reductase. Nat Genet. 1995;10(1):111-3. https://doi.org/10.1038/ng0595-111
PMid:7647779
23. van der Put NM, Gabreels F, Stevens EM, Smeitink JA, Trijbels FJ, Eskes TK, *et al*. A second common mutation in the methylenetetrahydrofolate reductase gene: An additional risk factor for neural tube defects? Am J Hum Genet. 1998;62(5):1044-51. https://doi.org/10.1086/301825
PMid:9545395
24. Matsuo K, Suzuki R, Hamajima N, Ogura M, Kagami Y, Taji H, *et al*. Association between polymorphisms of folate- and methionine-metabolizing enzymes and susceptibility to malignant lymphoma. Blood. 2001;97(10):3205-9. https://doi.org/10.1182/blood.v97.10.3205
PMid:11342450
25. Wilson A, Platt R, Wu Q, Leclerc D, Christensen B, Yang H, *et al*. A common variant in methionine synthase reductase combined with low cobalamin (Vitamin B12) increases risk for spina bifida. Mol Genet Metab. 1999;67(4):317-23. https://doi.org/10.1006/mgme.1999.2879
PMid:10444342
26. Blanchard C, Stucke EM, Burwinkel K, Caldwell JM, Collins MH, Ahrens A, *et al*. Coordinate interaction between IL-13 and epithelial differentiation cluster genes in eosinophilic esophagitis. J Immunol. 2010;184(7):4033-41. https://doi.org/10.4049/jimmunol.0903069
PMid:20208004
27. Chorna LB, Makukh HV, Akopyan HR, Zastavna DV, Prokopchuk NM. Analysis of MTHFR, MTR, MTRR genetic variations and FV and FII genesmutationsofcoagulation factors amongwomen with recurrent pregnancy losses. J VN Karazin Kharkiv Natl Univ Biol. 2011;13(947):118-24.
28. Yu L, Li T, Robertson Z, Dean J, Gu NF, Feng GY, *et al*. No association between polymorphisms of methylenetetrahydrofolate reductase gene and schizophrenia

in both Chinese and Scottish populations. Mol Psychiatry. 2004;9(12):1063-5. https://doi.org/10.1038/sj.mp.4001566
PMid:15289817

29. Theodoratou E, Farrington SM, Tenesa A, McNeill G, Cetnarskyj R, Barnetson RA, *et al*. Dietary Vitamin B6 intake and the risk of colorectal cancer. Cancer Epidemiol Biomarkers Prev. 2008;17(1):171-82. https://doi.org/10.1158/1055-9965.EPI-07-0621
PMid:18199722
30. Kokotas H, Grigoriadou M, Mikkelsen M, Giannoulia-Karantana A, Petersen MB. Investigating the impact of the Down syndrome related common MTHFR 677C>T polymorphism in the Danish population. Dis Markers. 2009;27(6):279-85. https://doi.org/10.3233/DMA-2009-0673
PMid:20075510
31. Bathum L, von Bornemann Hjelmborg J, Christiansen L, McGue M, Jeune B, Christensen K. Methylenetetrahydrofolate reductase 677C>T and methionine synthase 2756A>G mutations: No impact on survival, cognitive functioning, or cognitive decline in nonagenarians. J Gerontol A Biol Sci Med Sci. 2007;62A(2):196-201. https://doi.org/10.1093/gerona/62.2.196
PMid:17339646
32. Bethke L, Webb E, Murray A, Schoemaker M, Feychting M, Lönn S, *et al*. Functional polymorphisms in folate metabolism genes influence the risk of meningioma and glioma. Cancer Epidemiol Biomarkers Prev. 2008;17(5):1195-202. https://doi.org/10.1158/1055-9965.EPI-07-2733
PMid:18483342
33. Relton CL, Wilding CS, Laffling AJ, Jonas PA, Lynch SA, Tawn EJ, *et al*. Gene-gene interaction in folate-related genes and risk of neural tube defects in a UK population. Med Genet. 2004;41(4):256-60. https://doi.org/10.1136/jmg.2003.010694
PMid:15060097
34. Botto LD, Yang Q. 5, 10-methylenetetrahydrofolate reductase gene variants and congenital anomalies: A HuGE review. Am J Epidemiol. 2000;151(9):862-77. https://doi.org/10.1093/oxfordjournals.aje.a010290
PMid:10791559
35. Mills JL, Molloy AM, Parle-McDermott A, Troendle JF, Brody LC, Conley MR, *et al*. Folate-related gene polymorphisms as risk factors for cleft lip and cleft palate. Birth Defects Res A Clin Mol Teratol. 2008;82(9):636-43. https://doi.org/10.1002/bdra.20491
PMid:18661527
36. O'Leary VB, Mills JL, Pangilinan F, Kirke PN, Cox C, Conley M, *et al*. Analysis of methionine synthase reductase polymorphisms for neural tube defects risk association. Mol Genet Metab. 2005;85(3):220-7. https://doi.org/10.1016/j.ymgme.2005.02.003
PMid:15979034
37. Seremak-Mrozikiewicz A, Barlik M, Borowczak P, Kurzawińska G, Kraśnik W, Nowocień G, *et al*. The frequency of 677C>T polymorphism of MTHFR gene in the Polish population. Arch Perinat Med. 2013;19(1):12-8.
38. Nowak I, Bylińska A, Wilczyńska K, Wiśniewski A, Malinowski A, Wilczyński JR, *et al*. The methylenetetrahydrofolate reductase c.c.677 C>T and c.c.1298 A>C polymorphisms in reproductive failures: Experience from an RSA and RIF study on a Polish population. PLoS One. 2017;12(10):e0186022. https://doi.org/10.1371/journal.pone.0186022
PMid:29073227
39. Seremak-Mrozikiewicz A, Bogacz A, Deka-Pawlik A, Klejewski A, Wolski H, Drews K, *et al*. The polymorphisms of methionine synthase (MTR) and methionine synthase reductase (MTRR) genes in pathogenesis of preeclampsia. J Matern Fetal Neonatal Med. 2017;30(20):1-17. https://doi.org/10.1080/14767058.2016.1254183
PMid:27806663
40. Kloss M, Wiest T, Hyrenbach S, Werner I, Arnold ML, Lichy C, *et al*. MTHFR 677TT genotype increases the risk for cervical artery dissections. J Neurol Neurosurg Psychiatry. 2006;77(3):951-2. https://doi.org/10.1136/jnnp.2006.089730
PMid:16844951
41. Kurzwelly D, Knop S, Guenther M, Loeffler J, Korfel A, Thiel E, *et al*. Genetic variants of folate and methionine metabolism and PCNSL incidence in a German patient population. J Neurooncol. 2010;100(2):187-92. https://doi.org/10.1007/s11060-010-0154-4
PMid:20237949
42. Gast A, Bermejo JL, Flohr T, Stanulla M, Burwinkel B, Schrappe M, *et al*. Folate metabolic gene polymorphisms and childhood acute lymphoblastic leukemia: A case-control study. Leukemia. 2007;21(2):320-5. https://doi.org/10.1038/sj.leu.2404474
PMid:17136115
43. Niclot S, Pruvot Q, Besson C, Savoy D, Macintyre E, Salles G, *et al*. Implication of the folate-methionine metabolism pathways in susceptibility to follicular lymphomas. Blood. 2006;108(1):278-85. https://doi.org/10.1182/blood-2005-04-1567
PMid:16410450
44. Küry S, Buecher B, Robiou-du-Pont S, Scoul C, Colman H, Neel TL, *et al*. Low-penetrance alleles predisposing to sporadic colorectal cancers: A French case-controlled genetic association study. BMC Cancer. 2008;8:326. https://doi.org/10.1186/1471-2407-8-326
PMid:18992148
45. Födinger M, Buchmayer H, Heinz G, Papagiannopoulos M, Kletzmayr J, Rasoul-Rockenschaub S, *et al*. Effect of MTHFR 1298A→C and MTHFR 677C→T genotypes on total homocysteine, folate, and Vitamin B12 plasma concentrations in kidney graft recipients. J Am Soc Nephrol. 2000;11(10):1918-25.
PMid:11004224
46. Feix A, Winkelmayer WC, Eberle C, Sunder-Plassmann G, Födinger M. Methionine synthase reductase MTRR 66A>G has no effect on total homocysteine, folate, and Vitamin B12 concentrations in renal transplant patients. Atherosclerosis. 2004;174(1):43-8. https://doi.org/10.1016/j.atherosclerosis.2003.12.036
PMid:15135249
47. Lovricevic I, Franjic BD, Tomicic M, Vrkic N, De Syo D, Hudorovic N, *et al*. 5, 10-Methylenetetrahydrofolate reductase (MTHFR) 677 C→T genetic polymorphism in 228 croatian volunteers. Coll Antropol. 2004;28(2):647-54.
PMid:15666596
48. Jokić M, Brčić-Kostić K, Stefulj J, Ivković TC, Božo L, Gamulin M, *et al*. Association of MTHFR, MTR, MTRR, RFC1, and DHFR gene polymorphisms with susceptibility to sporadic colon cancer. DNA Cell Biol. 2011;30(10):771-6. https://doi.org/10.1089/dna.2010.1189
PMid:21438757
49. Motti C, Gnasso A, Bernardini S, Massoud R, Pastore A, Rampa P, *et al*. Common mutation in methylenetetrahydrofolate reductase. Correlation with homocysteine and other risk factors for vascular disease. Atherosclerosis. 1998;139(2):377-83. https://doi.org/10.1016/s0021-9150(98)00079-3
PMid:9712345
50. Saccucci P, Compagnone E, Verrotti A, Galasso C, Curatolo P. Lack of association between MTHFR C677T and MTHFR A1298C genetic polymorphisms and mental retardation. Nutr Neurosci. 2008;11(5):241-2. https://doi.org/10.1179/147683008X301595
PMid:18782485
51. Giusti B, Saracini C, Bolli P, Magi A, Martinelli I, Peyvandi F, *et al*. Early-onset ischaemic stroke: Analysis of 58 polymorphisms in 17 genes involved in methionine metabolism. Thromb Haemost. 2010;104(2):231-42. https://doi.org/10.1160/TH09-11-0748

PMid:20458436

52. Dhonukshe-Rutten RA, de Vries JH, de Bree A, van der Put N, van Staveren WA, de Groot LC. Dietary intake and status of folate and Vitamin B12 and their association with homocysteine and cardiovascular disease in European populations. Eur J Clin Nutr. 2009;63(1):18-30. https://doi.org/10.1038/sj.ejcn.1602897
PMid:17851461
53. Fedota OM, Roshchenyuk LV, Sadovnychenko IA, Merenkova IN, Gontar IV, Vorontsov VM. Analysis of one-carbon metabolism genes and epidermal differentiation complex in patients with ichthyosis vulgaris. Georgian Med News. 2017;264:90-7.
PMid:28480858
54. Binia A, Contreras AV, Canizales-Quinteros S, Alonso VA, Tejero ME, Silva-Zolezzi I. Geographical and ethnic distribution of single nucleotide polymorphisms within genes of the folate/homocysteine pathway metabolism. Genes Nutr. 2014;9(5):421. https://doi.org/10.1007/s12263-014-0421-7
PMid:25106483
55. Jones P, Beckett E, Yates Z, Veysey M, Lucock M. Converging evolutionary, environmental and clinical ideas on folate metabolism. Explor Res Hypothesis Med. 2016;1(3):34-41. https://doi.org/10.14218/ERHM.2016.00003b
56. Liew SC, Gupta ED. Methylenetetrahydrofolate reductase (MTHFR) T677T polymorphism: Epidemiology, metabolism and the associated diseases. Eur J Med Genet. 2015;58(1):1-10. https://doi.org/10.1016/j.ejmg.2014.10.004
PMid:25449138
57. Sun MY, Zhang L, Shi SL, Lin JN. Associations between methylenetetrahydrofolate reductase (MTHFR) polymorphisms and non-alcoholic fatty liver disease (NAFLD) risk: A meta-analysis. PLoS One. 2016;11(4):e0154337. https://doi.org/10.1371/journal.pone.0154337
PMid:27128842
58. Wang W, Jiao XH, Wang XP, Sun XY, Dong C. MTR, MTRR, and MTHFR gene polymorphisms and susceptibility to nonsyndromic cleft lip with or without cleft palate. Genet Test Mol Biomarkers. 2016;20(6):297-303. https://doi.org/10.1089/gtmb.2015.0186
PMid:27167580
59. Zhi X, Yang B, Fan S, Li Y, He M, Wang D, *et al*. Additive interaction of MTHFR C677T and MTRR A66G polymorphisms with being overweight/obesity on the risk of Type 2 diabetes. Int J Environ Res Public Health. 2016;13(12):1243. https://doi.org/10.3390/ijerph13121243
PMid:27983710
60. Khaligi K, Cheng G, Mirabbasi S, Khaligi B, Wu B, Fan W. Opposite impact of methylene tetrahydrofolate reductase C677T and methylene tetrahydrofolate reductase A1298C gene polymorphisms on systemic inflammation. J Clin Lab Anal. 2018;32(5):e22401. https://doi.org/10.1002/jcla.22401
PMid:29396861
61. Sorokina I, Myroshnychenko M, Sherstiuk S, Zubova Y, Nakonechna S, Panov S. The morphological picture of local immune responses in the kidneys, ureters and bladder of the foetuses and newborns, who developed in conditions of maternal preeclampsia. Georgian Med News. 2018;275:123-32.
PMid:29578438
62. Wan L, Li Y, Zhang Z, Sun Z, He Y, Li R. Methylenetetrahydrofolate reductase and psychiatric diseases. Transl Psychiatry. 2018;8:242. https://doi.org/10.1038/s41398-018-0276-6
PMid:30397195
63. Ma LM, Yang HP, Yang XW, Ruan LH. Methionine synthase A2756G polymorphism influences pediatric acute lymphoblastic leukemia risk: A meta-analysis. Biosci Rep. 2019;39(1):BSR20181770. https://doi.org/10.1042/BSR20181770
PMid:30559146
64. Ren ZJ, Zhang YP, Ren PW, Yang B, Deng S, Peng ZF, *et al*. Contribution of *MTR* A2756G polymorphism and MTRR A66G polymorphism to the risk of idiopathic male infertility. Medicine. 2019;98(51):e18273. c10.1186/s12958-020-00649-1
PMid:31860974
65. Tykhonova TM. Skin lesions in diabetes mellitus: Risk factors for development, clinical manifestations, prevention and treatment. Problemi Endokrinnoi Patologii. 2019;1:121-8. https://doi.org/10.21856/j-PEP.2019.1.15
66. Lupi-Herrera E, Soto-López ME, Lugo-Dimas AJ, Núñez-Martínez ME, Gamboa R, Huesca-Gómez C, *et al*. Polymorphisms C677T and A1298C of MTHFR gene: Homocysteine levels and prothrombotic biomarkers in coronary and pulmonary thromboembolic disease. Clin Appl Thromb Hemost. 2019;25:1076029618780344. https://doi.org/10.1177/1076029618780344
PMid:29916259
67. Zara-Lopes T, Galbiatti-Dias AL, Castanhole-Nunes MM, Padovani-Júnior JA, Maniglia JV, Pavarino EC, *et al*. Polymorphisms in MTHFR, MTR, RFC1 and CßS genes involved in folate metabolism and thyroid cancer: A case-control study. Arch Med Sci. 2019;15(2):522-30. https://doi.org/10.5114/aoms.2018.73091
PMid:30899306
68. Zhang Y, Zhan W, Du Q, Wu L, Ding H, Liu F, *et al*. Variants c.677 C>T, c.1298A>C in MTHFR, and c.66A>G in MTRR affect the occurrence of recurrent pregnancy loss in chinese women. Genet Test Mol Biomarkers. 2020;24(11):717-22. https://doi.org/10.1089/gtmb.2020.0106
PMid:33121283
69. Mahmud N, Molloy A, McPartlin J, Corbally RC, Whitehead AS, Scott JM, *et al*. Increased prevalence of methylenetetrahydrofolate reductase C677T variant in patients with inflammatory bowel disease, and its clinical implications. Gut. 1999;45:389-94. https://doi.org/10.1136/gut.45.3.389
PMid:10446107
70. Husemoen LL, Toft U, Fenger M, Jørgensen T, Johansen N, Linneberg A. The association between atopy and factors influencing folate metabolism: Is low folate status causally related to the development of atopy? Int J Epidem. 2006;35(4):954-61. https://doi.org/10.1093/ije/dyl094
PMid:16766537
71. Vasku V, Bienertova-Vasku J, Necas M, Vasku A. MTHFR (methylenetetrahydrofolate reductase) C677T polymorphism and psoriasis. Clin Exp Med. 2009;9(4):327-31. https://doi.org/10.1007/s10238-009-0054-0
PMid:19484352
72. van der Valk RJ, Kiefte-de Jong JC, Sonnenschein-van der Voort AM, Duijts L, Hafkamp-de Groen E, Moll HA, *et al*. Neonatal folate, homocysteine, Vitamin B12 levels and methylenetetrahydrofolate reductase variants in childhood asthma and eczema. Allergy. 2013;68(6):788-95. https://doi.org/10.1111/all.12146
PMid:23692062
73. Fedota AM. Genodermatoses in the Study of the Problems of Human Genetic Safety. Dissertation. Kiev; 2012.
74. Reich DE, Cargill M, Bolk S, Ireland J, Sabeti PC, Richter DJ, *et al*. Linkage disequilibrium in the human genome. Nature. 2001;411(6834):199-204. https://doi.org/10.1038/35075590
PMid:11346797
75. Gaughan DJ, Kluijtmans LA, Barbaux S, McMaster D, Young IS, Yarnell JW, *et al*. The methionine synthase reductase (MTRR) A66G polymorphism is a novel genetic determinant of plasma homocysteine concentrations. Atherosclerosis. 2001;157:451-6. https://doi.org/10.1016/s0021-9150(00)00739-5
PMid:11472746