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

Olena Fedota¹, Iuri Sadovnychenko¹,²*, Lilia Chorna³, Larysa Roshchenyk⁴,⁵, Vitalii Vorontsov⁵, Pavlo Ryzhko⁵, Ivanna Haiboniuk¹, Sergei Belyaev¹, Igor Belozorov¹, Halyna Makukh⁷

¹Department of Obstetrics and Gynecology, School of Medicine, V.N. Karazin Kharkiv National University, Kharkiv, Ukraine; ²Department of Medical Biology, 5th Faculty of Foreign Students Training, Kharkiv National Medical University, Kharkiv, Ukraine; ³Department of Genetics Research Laboratory, Institute of Hereditary Pathology, National Academy of Medical Sciences of Ukraine, Lviv, Ukraine; ⁴Department of Dermatology, Venereology and AIDS, 2nd Medical Faculty, Kharkiv National Medical University, Kharkiv, Ukraine; ⁵Regional Clinical Dispensary for Skin and Venereal Diseases no. 1, Kharkiv, Ukraine; ⁶Department of Genetics, Obstetrics, Gynecology and Fetal Medicine, Faculty of General Practice–Family Medicine, Kharkiv Medical Academy of Postgraduate Education, Kharkiv, Ukraine; ⁷Department of Surgical Diseases, Operative Surgery and Topographical Anatomy, School of Medicine, V.N. Karazin Kharkiv National University, Kharkiv, Ukraine

Abstract

BACKGROUND: Ichthyosis vulgaris is the most common type of Mendelian disorders of comification, 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 of their FLG heterozygous relatives without symptoms of disorder, and 150 healthy controls were enrolled in the study. FLG null mutations — R501X (rs18161761) 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 C677TT 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 2282 del4 mutation (OR 2.80–11.23). The most probable predisposing genotype is FLG 2282del4 heterozygotes, the frequency of 2282del4 mutation ranges from 73% for 2282del4 to 96% for R501X (rs18161761). Despite the fact that in heterozygotes, penetrance of the mutations ranges from 73% for 2282del4 to 96% for R501X [7], 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].

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 (rs18161761) [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].
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 MTRRA66G (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, MTHFRA1298C, MTRA2756G, and MTRRA66G) was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay with optimal primers (Metabion, Germany). The PCR products were digested with Hinfl, Mbol, 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–Wilks 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 r² were estimated and haplotype block analyses were performed in Haplovie (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/R501X, 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 MTHFR 2282AA 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 dominant 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 2757CT/MTRR 1298AA + AC (OR 4.393; 95% CI 1.468–13.139, p = 0.008) and MTHFR 277CT/MTRR 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 homogyzosity for the MTHFR A1298C polymorphism (OR 7.636, 95% CI 2.338–24.943, p = 0.001). The strongest association was found between MTHFR 277CT/MTRR 1298A/AA MTHFR 2756AA + AG/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 277CT/MTRR 1298AA/MTRR 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

<table>
<thead>
<tr>
<th>Country</th>
<th>MTHFR C677T</th>
<th>MTHFR A1298C</th>
<th>MTR A2756G</th>
<th>MTRR A66G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allele</td>
<td>Genotype</td>
<td>Allele</td>
<td>Genotype</td>
<td>Allele</td>
</tr>
<tr>
<td>Scotland</td>
<td>48.7</td>
<td>41.4</td>
<td>16.3</td>
<td>81.3</td>
</tr>
<tr>
<td>Germany</td>
<td>48.7</td>
<td>41.7</td>
<td>16.3</td>
<td>81.3</td>
</tr>
<tr>
<td>France</td>
<td>37.6</td>
<td>52.6</td>
<td>15.1</td>
<td>81.3</td>
</tr>
<tr>
<td>Austria</td>
<td>43.0</td>
<td>36.8</td>
<td>15.1</td>
<td>81.3</td>
</tr>
<tr>
<td>Croatia</td>
<td>64.7</td>
<td>36.8</td>
<td>15.1</td>
<td>81.3</td>
</tr>
<tr>
<td>Italy</td>
<td>72.0</td>
<td>14.3</td>
<td>15.1</td>
<td>81.3</td>
</tr>
</tbody>
</table>

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 dominant 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 277CT/MTRR 1298AA + AC (OR 4.393; 95% CI 1.468–13.139, p = 0.008) and MTHFR 277CT/MTRR 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 homogyzosity for the MTHFR A1298C polymorphism (OR 7.636, 95% CI 2.338–24.943, p = 0.001). The strongest association was found between MTHFR 277CT/MTRR 1298A/AA MTHFR 2756AA + AG/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 277CT/MTRR 1298AA/MTRR 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 2: Genotype and allele frequencies, and Hardy–Weinberg P values for one-carbon metabolism single nucleotide polymorphisms in ichthyosis vulgaris cases and controls

<table>
<thead>
<tr>
<th>Flaggrn genotype</th>
<th>Phenotype</th>
<th>MTHFR C677T</th>
<th>MTHFR A1298C</th>
<th>MTR A2756G</th>
<th>MTRR A66G</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Genotype</td>
<td>Allele</td>
<td>Genotype</td>
<td>Allele</td>
<td>Genotype</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>TT</td>
<td>AA</td>
<td>AG</td>
<td>AA</td>
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<tr>
<td></td>
<td>p-value</td>
<td>p-value</td>
<td>p-value</td>
<td>p-value</td>
<td>p-value</td>
</tr>
</tbody>
</table>

*p value for the Hardy–Weinberg equilibrium test, *p value for the FLG homozygotes and compound heterozygotes with ichthyosis, **p value for the FLG 2282del4 heterozygotes without ichthyosis, ***p value for the FLG 2282del4 heterozygotes without ichthyosis.
of SNPs of the MTHFR gene (rs1801133 and rs1801131) that demonstrated strong linkage (D’=1.00; LOD=2.32; r²=0.195). The second block included mutations in the FLG gene (rs558269137 and rs61816761) with incomplete linkage (D’=1.00; LOD=1.53; r²=0.109).

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 ethnonterritorial 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 were 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/MTR 2756AA+AG/MTRR 66AG. The most probable predisposing genotype is 677CT/1298AA/MTR 2756AA+AG/MTRR 66AG.

References

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PMid:19681860.

PMid:23003573

PMid:27777593


PMid:22332098


PMid:24091066

PMid:29480334

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PMid:31069230


PMid:76477779

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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.
The paper discusses the frequency of 5, 10-methylenetetrahydrofolate reductase (MTHFR) 677C>T polymorphism in the Polish population. It mentions various studies that have investigated the role of MTHFR and other folate-related genes in the pathogenesis of different conditions, including preeclampsia, meningioma, glioma, neural tube defects, and colorectal cancer. The paper also highlights the impact of MTHFR polymorphisms on cognitive decline and risk of vascular disease.

Key findings include:

- The frequency of MTHFR 677C>T polymorphism in Polish patients with preeclampsia compared to controls.
- The association between MTHFR polymorphisms and the risk of meningioma and glioma.
- The role of MTHFR polymorphisms in neural tube defects risk association.
- The relationship between MTHFR polymorphisms and the risk of cognitive decline in nonagenarians.
- The impact of MTHFR polymorphisms on the risk of cervical artery dissections.
- The effect of MTHFR polymorphisms on homocysteine and other risk factors for vascular disease.
- The correlation between MTHFR polymorphisms and the risk of follicular lymphomas.

The paper concludes by emphasizing the importance of understanding the genetic basis of these diseases and the potential implications for preventive strategies and personalized medicine.