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 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 (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 homo- and heterozygotes for FLG 2282del4 mutation, the frequencies of MTR 2756AA and MTRR 66G 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/MTRR 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 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, MTHFR A1298C, 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 HinfI, MbolI, HaellII, and Ndel 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 $D^2$ 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
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 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 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 677CT/MTHFR 1298AA + AC (OR 4.399; 95% CI 1.468–13.139, p = 0.008) and MTHFR 677CT/MTRR 2756AA genotypes (OR 4.239, 95% CI 1.945–12.018, 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/MTRR 1298AA/MTRR 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/MTRR 1298AA/MTRR 2756AA+AC/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 | FLG |
|---------|-------|-----|------|-----|
|         | A1298C | A2756G | A66G | Source |
|         | C677T | 677CT | 66GG |        |
| Scotland |       |       |      |        |
| Denmark |       |       |      |        |
| England |       |       |      |        |
| Ireland |       |       |      |        |
| Poland |       |       |      |        |
| Germany |       |       |      |        |
| France |       |       |      |        |
| Austria |       |       |      |        |
| Croatia |       |       |      |        |
| Italy |       |       |      |        |

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

| Flagrnn genotype | Phenotype | Ratio | MTHFR C677T | MTHFR A1298C | MTR A2756G | MTRR A66G | Source |
|-----------------|-----------|-------|-------------|--------------|-------------|-----------|-------|
|                 | Allele    | C    | T           | AA           | AC          | AG        |       |
|                 | CC        | TT    |              |              |             |           |       |

p value for the Hardy–Weinberg equilibrium test, "p" value for the FLG homozygous and compound heterozygous with ichthyosis, "p" value for the FLG 2282del4 heterozygous without ichthyosis.
Table 3: Association between one-carbon metabolism polymorphisms and ichthyosis vulgaris risk in FLG 2282del4 heterozygotes

| Genotype   | FLG       | Controls | Odds ratios | confidence interval | p-value |
|------------|-----------|----------|-------------|---------------------|---------|
|            | C677T     | A1298C   | A2756G     | MTHR0               |         |
| CT         | —         | —        | —           | 12                  | 60      | 3.600 | 1.207– | 0.032 |
|            | —         | AA       | —           | 12                  | 64      | 3.223 | 1.082– | 0.036 |
| CT         | AA+AC     | —        | —           | 12                  | 53      | 4.393 | 1.468– | 0.008 |
| CT         | —         | AA       | —           | 6                   | 26      | 4.239 | 1.495– | 0.007 |
| CT         | —         | AA+AG    | —           | 9                   | 49      | 3.779 | 1.320– | 0.013 |
| CT         | —         | AA+AG    | AA+AG      | 9                   | 37      | 3.436 | 1.238– | 0.018 |
| CT         | AA+AC     | AA+AG    | —           | 9                   | 43      | 2.799 | 1.014– | 0.047 |
| CT         | AA+AG     | AA       | —           | 6                   | 10      | 7.636 | 2.338– | 0.001 |
| CT         | —         | AA       | AA+AG      | 5                   | 10      | 5.833 | 1.714– | 0.005 |
| CT         | —         | AA+AG    | AA+AG      | 8                   | 31      | 3.412 | 1.217– | 0.020 |
| CT         | AA       | AA+AG    | —           | 4                   | 4       | 11.213 | 2.512– | 0.002 |

Figure 1: Linkage disequilibrium: Linkage disequilibrium blocks (a) and haplotypes (b) in individuals with filagrin 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 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 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 MTHFR 677CT/MTHFR 1298AA/MTR 2756AA+AG/MTRR 66AG.

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