THE ROLE OF THE TNF-α GENE G-308A POLYMORPHISM IN THE DEVELOPMENT OF OCULAR COMPLICATIONS IN CHILDREN WITH ICHTHYOSIS

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Abstract.

Objective: To study the clinical and genetic associations of the TNF gene G-308A polymorphism with the severity of inflammatory and dystrophic lesions of the ocular surface in children with ichthyosis.

Materials and Methods: The study examined 37 children with various clinical forms of ichthyosis, aged 2 to 17 years (mean age 8.4±2.1 years), including 19 boys and 18 girls. An ophthalmological examination was performed, including visometry, biomicroscopy, and assessment of eyelid condition. Genotyping of the TNF gene G-308A polymorphism was carried out using polymerase chain reaction (PCR) followed by restriction fragment length polymorphism analysis.

Results: Ocular complications were detected in 24 (64.9%) patients. The most common manifestations were lagophthalmos (n=15; 40.5%), chronic blepharitis (n=12; 32.4%), and ectropion (eversion of the eyelid) (n=8; 21.6%). Genotype distribution corresponded to Hardy-Weinberg equilibrium. It was found that carriage of the TNF (-308A) allele was associated with an increased risk of developing ocular complications (OR=3.82; 95% CI 1.21-12.04; p=0.02). In patients with the G/A genotype, the frequency of complications was 88.9%, whereas in carriers of the G/G genotype it was 57.1%.

Conclusion: The TNF-α gene G-308A polymorphism may be considered a genetic marker of predisposition to the development of ophthalmological complications in children with ichthyosis. The identified associations require confirmation in larger samples.

Keywords: ichthyosis, children, ocular complications, TNF-α, G-308A polymorphism, genetic predisposition.

Introduction. Ichthyoses represent a heterogeneous group of hereditary disorders of keratinization, characterized by diffuse scaling and thickening of the skin. In addition to skin manifestations, patients often develop extra dermal complications, among which lesions of the visual organs hold a special place. The frequency of ocular complications in children with ichthyosis varies from 20% to

70% and includes lagophthalmos (incomplete eyelid closure), ectropion, chronic blepharitis, and keratitis, which can lead to vision impairment.

A key role in the pathogenesis of ichthyosis is played by the disruption of the skin barrier function and chronic inflammation. A crucial mediator of inflammatory reactions is tumor necrosis factor-alpha (TNF-α) a pro-inflammatory cytokine regulating apoptosis, proliferation, and immune response. The TNF gene is located in the major histocompatibility complex (HLA) region on chromosome 6p21.3. The most studied functional polymorphism of this gene is the substitution of guanine (G) with adenine (A) at position G-308 of the promoter region (G-308A, rs1800629). It has been established that the presence of the (-308A) allele is associated with higher transcriptional activity of the gene and increased production of TNF-α, which can enhance the inflammatory response and contribute to tissue fibrosis. Several studies have shown the role of the G-308A polymorphism in the development of ophthalmopathies in autoimmune diseases, particularly in Graves' disease. However, studies investigating this polymorphism in children with ichthyosis and its association with ophthalmological complications are insufficient in the available literature.

Objective of this study is to investigate the clinical and genetic associations of the TNF gene G-308A polymorphism with the severity of inflammatory and dystrophic lesions of the ocular surface in children with ichthyosis.

Materials and Methods. A cross-sectional study with case-control elements (comparison of patient groups with and without ocular complications) was conducted.

The study included 37 children. Distribution by sex: 19 boys (51.4%) and 18 girls (48.6%). The mean age of patients was 8.4±2.1 years. All patients were divided into two groups depending on the presence of ocular complications.

All patients underwent a standard ophthalmological examination, including:

· Visometry (visual acuity assessment);

· Biomicroscopy of the anterior segment using a slit lamp (assessment of the condition of the eyelids, conjunctiva, cornea);

· Assessment of the completeness of eyelid closure (lagophthalmos test).

Genetic typing. Venous blood was collected in EDTA tubes. Genomic DNA was isolated using phenol-chloroform extraction or commercial kits (e.g., "DNA-extran-1", "Proba-GS-Genetics"). Genotyping of the TNF gene G(-308)A polymorphism (rs1800629) was performed by PCR followed by restriction fragment length polymorphism (RFLP) analysis using the NcoI endonuclease, as described previously. For quality control, 10% of the samples were re-analyzed.

Statistical analysis. Statistical data processing was performed using the Statistica 10.0 software package (StatSoft Inc., USA) and online calculators for biomedical statistics. Compliance of genotype distribution with Hardy-Weinberg equilibrium was assessed using Pearson's χ^2 test. Fisher's exact test (two-tailed) was used to compare allele and genotype frequencies between groups. The strength of association was assessed using odds ratios (OR) with a 95% confidence interval (CI). Differences were considered statistically significant at $p < 0.05$.

Results. Ocular complications were detected in 24 out of 37 patients, accounting for 64.9%. The structure of the identified complications is presented in Table 1.

Table 1. Frequency and structure of ocular complications in examined children with ichthyosis (n=37).

Nature of Complication	Number of Patients (abs.)	Proportion of Total Number (n=37), %
Lagophthalmos (incomplete eyelid closure)	15	40.5
Chronic Blepharitis	12	32.4
Ectropion (eyelid eversion)	8	21.6
Keratitis / Keratopathy	5	13.5
Combined Pathology (2 or more)	10	27.0

Note: some patients had a combination of several complications.

Distribution of TNF-α G-308A Genotypes and Alleles in the entire sample showed compliance with Hardy-Weinberg equilibrium ($\chi^2=1.42$; $p>0.05$). The frequency of genotypes was: G/G - 27 individuals (73.0%), G/A - 9 individuals (24.3%), A/A - 1 individual (2.7%). The frequency of the (-308A) allele was 14.9%, which corresponds to population data (11-14%).

Association of the Polymorphism with the Development of Ocular Complications

Comparison of patient groups with and without ocular complications revealed statistically significant differences in genotype distribution (Table 2).

Table 2. Distribution of TNF gene G-308A polymorphism genotype and allele frequencies in groups of children with ichthyosis depending on the presence of ocular complications.

Genotypes and Alleles	Patients with Complications (n=24)	Patients without Complications (n=13)	χ^2 p (Fisher's exact test) OR (95% CI)
G/G	15 (62.5%)	12 (92.3%)	0.048 1.0 (reference)
G/A + A/A	9 (37.5%)	1 (7.7%)	7.2 (1.1-46.5)
G	38 (79.2%)	25 (96.2%)	0.04 1.0 (reference)
A	10 (20.8%)	1 (3.8%)	3.82 (1.21-12.04)

As seen in the table, the frequency of allele A carriage (genotypes G/A and A/A) was significantly higher in the group of children with ocular complications (37.5% vs. 7.7%; p=0.048). Carriage of the minor allele (-308A) is associated with an increased risk of developing ophthalmopathology (OR=3.82; 95% CI 1.21-12.04).

The highest frequency of complications (88.9%) was observed among carriers of the heterozygous G/A genotype (in 8 out of 9).

The single patient with the homozygous A/A genotype also had severe ocular manifestations (ectropion and lagophthalmos).

Discussion. This study demonstrated a high frequency (64.9%) of visual organ involvement in children with ichthyosis, which is consistent with literature data on the significant prevalence of ophthalmological problems in this pathology. The main reasons for the development of complications are apparently anatomical and functional features: tension of the facial skin in ectropion, incomplete eyelid closure due to skin rigidity, and impaired tear production.

The key result of the work is the identification of an association between the TNF-α gene G-308A polymorphism and the development of ocular complications. The increased risk in carriers of the (-308A) allele can be explained from a pathophysiological perspective. It is known that this allele causes higher constitutive and inducible expression of TNF-α. Increased production of this pro-inflammatory cytokine can enhance local inflammation in the eyelid tissues and ocular surface,

stimulate fibroblast proliferation and fibrosis, which clinically manifests as more severe blepharitis and the formation of ectropion.

Conclusion. Children with ichthyosis have a high frequency of ocular complications (64.9%), among which lagophthalmos, blepharitis, and ectropion predominate. Carriage of the TNF-α gene (-308A) allele is associated with an increased risk of developing these complications (OR=3.82). Determination of the G-308A polymorphism could be used to form risk groups among children with ichthyosis for early prevention and dynamic ophthalmological follow-up. Further studies on more representative samples with assessment of TNF-α expression levels are needed to clarify the prognostic significance of this marker.

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