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ORIGINAL ARTICLE PEDIATRICS

Association of childhood asthma with Gasdermin B (GSDMB) and Oromucoid-like 3 (ORMDL3) genes

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ABSTRACT

OBJECTIVE: Genome-length association studies have shown that Gasdermin B (GSDMB) and Orosomucoid-like 3 (ORMDL3) genes located on the long arm of chromosome 17 are associated with asthma. In this study, it was aimed to determine the possible relationship between asthma control test (ACT), exercise provocation test (ECT), and fractional nitric oxide (FENO) levels and GSDMB and ORMDL3 gene expressions.

METHODS: 59 asthmatic and 38 non-asthmatic children were included in the study. We divided the patient group into two subgroups as mild persistent asthma (29 patients) and moderate persistent asthma (30 patients). ORMDL3, GSDMB gene expression levels, ECT, total IgE levels, and eosinophil counts were measured in all cases. In addition, ACT and FeNO levels were measured in children with asthma. Afterward, the relationship of ORMDL3 and GSDMB gene expression coefficient changes with ECT, ACT, and FeNO was examined.

RESULTS: When patients with ACT ≤15 were compared with patients with ACT ≥20, ORMDL3 and GSDMB gene expressions were increased 6.74 and 11.74 times, respectively. Comparing patients with ACT ≥20 and ACT ≤15 in terms of coefficient changes (ΔCq), higher change values were observed for ΔCq ORMDL3 in patients with ACT ≤15 (p=0.015). Similarly, when patients with FENO ≤25 ppb were compared with patients with FENO >25 ppb, ORMDL3 and GSDMB gene expressions were increased by 2.93 and 3.56 times, respectively. When the coefficient changes were compared, no significant difference was found between FENO≤25 and FENO >25 patients. There was a slight negative correlation between ΔCq values and ACT score (p=0.003, r=-0.418 for ORMDL3, and p=0.016, r=-0.345 for GSDMB). In addition, we observed a statistically significant positive correlation between ORMDL3 and GSDMB gene expressions (r=0.80, p<0.001).

CONCLUSION: We showed that increased ORMDL3 and GSDMB gene expression levels may be associated with ACT scores, FeNO and ECT in asthma. These findings may encourage future studies with larger numbers of subjects that can use gene expression levels in various asthma phenotypes for prognostic prediction.

Keywords: Airway hyperresponsiveness; childhood asthma; exercise challenge test; GSDMB gene expression; ORMDL3 gene expression.

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As a chronic inflammatory disease, asthma has a strong genetic component. Thus, family and intragenome studies can helpto identify critical regions of genes that cause this disease. The findings from these genome-wide association studies (GWASs) show only weakly replicated asthma susceptibility genes and give insufficient information about the genes causing asthma. In recent years, as a result of candidate gene studies for complex diseases with the help of advances in technology, Moffatt et al. [1] identified in 2007 the first susceptibility locus for asthma on a chromosome, 17q12–21.1, containing Gasdermin B (GSDMB) and Orosomucoid-1-like-3 (ORMDL3) genes. The later GWASs along with a meta-analysis of GWASs have further shown that 17q is the most replicated asthma locus [2–4].

Previously, it has been shown that genotypes at SNPs found in the core region correlate with the expression of ORMDL3 and GSDMB that are two main candidate asthma genes at this locus [2–6]. Increased expression of ORMDL3 and GSDMB was also found to be related to the SNP-linking chromosome 17q21 to asthma [5, 7].

ORMDL3, which is located restrictedly in the endoplasmic reticulum (ER), is the regulator of downstream pathways of, remodeling genes, metalloproteases, sphingolipids as well as chemokines [8]. Furthermore, ORMDL3 plays a role in repressing serine palmitoyl-CoA transferase (the rate-limiting enzyme for sphingolipid biosynthesis) and inactivating the ATF6α branch of the unfolded protein response (UPR) which is the regulator of IL-6 and SERCA2b [8–12]. Especially, IL6 and SERCA2b are potentially important pathways leading to asthma [8–12]. Therefore, in this way, the concentration of Ca^{2+} in the ERreduces while the UPRelevates [5]. It is commonly believed that this effect is a factor in the development of chronic inflammatory diseases like asthma [2–5]. GSDMB is the regulator of 5-LO and TGF-β1 expressions, knowing as the pathway leading to the pathogenesis of asthma [11]. Furthermore, it was previously showed that mice have an asthma phenotype if expressing increased levels of human ORMDL3, or human GSDMB [13]. All these findings indicated the association between 17q12 and q21 locus and asthma. However, the functions of GSDMB and ORMDL3 genes and their effects on the development of asthma are still not fully understood.

Previously, the association between asthma and polymorphisms in GSDMB and ORMDL3 genes has been shown in various ethnic groups. Bouzigon et al. [6] reported that 17q21 variants can lead to an early predisposition to asthma. Moreover, Bisgaard et al. [7] reported that 17q12-q21 focus was related to recurrent wheezing, asthma, asthma exacerbation, and exercise provocation test (ECT) in children who are followedup throughout the school-age from early infancy.

Highlight key points

- The genetic structures of children with asthma may play an active role in the emergence of the disease.
- Genetic structure has an important effect on the course of asthma in children.
- Determination of genetic structure, which can also affect the treatment results of asthma, may be important in asthma management.

The relationship between the expression of ORMDL3 and GSDMB and the clinical features of asthma and asthma severity is relatively unclear. In this regard, we aimed to investigate the relationship between these genes and asthma-related clinical parameters. In childhood asthma, we investigated the effect of ACT, FENO, and ECT parameters on ORMDL3 and GSDMB gene expression.

MATERIALS AND METHODS

Subjects

Following the approval of the ethics committee of Gaziantep University (Decision no: February 19, 2013/no: 76), the subjects of this study were recruited among the patients from the Department of Pediatric Allergy and Immunology at Gaziantep University Faculty of Medicine between March 2013 and September 2013. According to the Helsinki Declaration, an informed consent form was signed for the parents of each child in the working group.

Ninety-seven children aged from 6 to 17 years old enrolled voluntarily in this study. Subjects were included in the asthmatic group if they had no chronic inflammatory diseases except asthma, were not diagnosed with lower and upper respiratory tract infection within the past 2 weeks, were not under the treatment of systemic and inhaled corticosteroids, and had no sign of any additional illness based on anamnesis and physical examination. Asthma and Allergies in Childhood (ISAAC) questionnaires were determined in patients with asthma and atopy in the control group [14]. Subjects were classified into two groups: 59 atopic asthmatics group

(29 mild and 30 moderate persistent) and 38 non-atopic non-asthmatic control groups according to GINA (Global Initiative for Asthma) criteria [15].

Determination of Immunoglobulin E (IgE) Levels and Total Eosinophil Counts

Total serum IgE levels were measured using the UniCAPfluoroenzyme immunoassay (Pharmacia Diagnostics, Uppsala, Sweden). The total number of blood eosinophils was determined using an automated hematology analyzer (Coulter-M, Beckman Coulter, Fullerton, CA).

Clinical Diagnosis of Asthma and Assessing Atopic Status

Asthma diagnosis and asthma control levels of the subjects were assessed by a physician according to the GINA criteria and these subjects were further subclassified as mild or moderate persistent asthma based on the ACS score [15]. ACT and childhood ACT (C-ACT) were also applied to the subjects to detectcontrollevel of asthma of the subjects. The International Study of ISAACquestionnaires were used to confirm the atopic status in the asthmatic group and non-atopic status in the control group.

FeNOMeasurement

Before the pulmonary function test, the FeNO level of asthmatic subjects was measured by using the single-breath online technique with the FeNO analyzer (NIOX-MINO; Aerocrine, Stockholm, Sweden) by the guidelines [16], and FeNO level was recorded as parts per billion (ppb) [17].

Pulmonary Function, Bronchodilator Reversibility, and Bronchial Hyperresponsiveness Challenge Tests

Spirometric evaluations were performed with a pulmonary function test device (SensorMedics, Yorba Linda, CA). We performed Bronchodilator Reversibility Test by measuring forced expiratory volume in 1 s (FEV1) 15 min after administration of 400 mcg salbutamol given by metered-dose inhaler using a spacer. An FEV1 increase of 200 mL or 12% concerning basal level was accepted as positive and bronchodilator response was recorded as a percentage (%) [18].

Bronchial hyperreactivity/hyperresponsiveness was assessed by ECT by the test protocol of the American Thoracic Society/European Respiratory Society [19]. It was assured that the subjects did not use any asthma medication at least 24 h before ECT. The ECT was accepted as positive if we observed an FEV1 decrease of 12% or more, and ECT was finalized, and the maximum FEV1 decrease was recorded as a percentage.

Measurement and Assessment of ORMDL3 and GSDMB Gene Expression Level

Blood samples storage and messenger-RNA (mRNA) isolation for genetic analysis were carried out in the Laboratory of Medical Biology Department at Gaziantep University, Faculty of Medicine. 2 mL blood samples within EDTA tube from the subjects were immediately transported to the laboratory with the appropriate condition. mRNA was isolated from the blood samples using mRNA Dual RNA Isolation Kit (GeneDirex, USA) according to the protocol provided by the manufacturer. The high-quality obtained mRNAs were confirmed for all samples with a NanoDrop measurement. The ideal amount of mRNA was determined as 1.5 μL mRNA per sample. ComplementaryDNA (cDNA) was synthesized from mRNA using the reverse transcription-polymerase chain reaction (RT-PCR) technique. We used the ABM EasyScript Plus™ cDNA Synthesis kit for cDNA reactions. For the determination of gene expression level, we first determine the suitability of primers, and the primers were made ready for quantitative polymerase chain reaction (qPCR).

During the optimization stage, the reactions were carried out in a thermal cycler (Bio-Rad C1000 Touch Thermal Cycler) with GeneDirex One PCR kit. GAPDH was selected as a housekeeping gene to determine the expression levels of ORDML3 and GSDMB genes. Sequence information of genes in Table 1 qPCR mixture was prepared with ABM EvaGreenqPCRMasterMix Low-ROX kit. PCR reactions were set up in 48 well-plates and performed using Illumina® Eco Real-Time PCR and pre-analyzed with Illumina Eco and Illumina Eco Study Softwares.

Fold Change (FC) in gene expression levels was calculated using the "differences of two groups normalize gene expression level" (ΔΔCq) method [20, 21]. In this method, the average gene expression levels (cycles of quantification; Cq) of each group subtract separately from the average reference gene expression levels and the "normalized gene expression level" (ΔCq) areobtained. Afterward, ΔCq levels of two group subtract from each

Table 1. Primers of GADPH, ORDML3, and GSDMB genes

Primer name	Forward primer (5′→3′)	Reverse primer (5′→3′)	Product length
GAPDH	GGGAGCCAAAAGGGTCATCATCTC	CCATGCCAGTGAGCTTCCCGTTCT	353bp
ORDML3	TCAGGCAGCCAAAGCACTTTAACC	ACCCATCCCACACTTGCTTCCATA	139bp
GSDMB	ACATGGAGCGAATGGGATAC	CTGAGGCACGAATTCTCTGT	234bp

GADPH: GAPDH was selected as a housekeeping gene to determine the expression levels of ORDML3 and GSDMB genes; ORDML:Oromucoid-like 3; GSDMB: Gasdermin B.

Table 2. Demographic, clinical, and laboratory characteristics of subjects included in the study

	Mild persistent asthma (n=29)	Moderate persistent asthma (n=30)	Control group (n=38)	p
Age (year)†	11.8±2.6	11.3±3.0	12.6±2.7	0.131*
Gender (male/female)	16/13	17/13	18/20	0.707**
Body mass index (kg/m²)†	19.7±3.0	18.5±3.2	18.7±2.3	0.222*
Eosinophil (%)‡	4.6 (3.7–6.7)	5.5 (4.2–6.8)	2.9 (1.5–3.6)	<0.001***
IgE (IU/ml)‡	180 (126–468)	349 (218–756)	50 (23–94)	<0.001***
Basal FEV1 (%)†	96.1±9.6	89.3±13.2	103.8±12.9	<0.001*
Basal FEV1/FVC (%)†	85.4±8.2	82.1±10.2	90.5±7.7	0.001*
BRT FEV1change (%)‡	6.0 (4.0–11.5)	9.5 (6.0–11.0)	2.0 (0.0–4.3)	<0.001***
FeNO (ppb)‡	21 (16–32)	46 (39–56)	–	<0.001****
ACT score‡	21 (19–21.5)	15.5 (11.5–18)	–	<0.001****
ORMDL3 ΔCq‡	-1.3 (-3.9; 1.0)	0.3 (-8.6; 2.0)	-0.6 (–3.2; 0.8)	0.462***
GSDMB ΔCq‡	-0.6 (-6.1; 2.5) (n=21)	1.3 (-3.9; 3.8) (n=27)	-0.01 (-3.0; 1.5) (n=35)	0.626***

FEV_1: Forced expiratory volume in 1 s; FVC: Functional vital capacity; BRT: Bronchodilator reversibility test; FeNO: Fractional concentration of exhaled NO; ppb: Parts per billion; ACT: Asthma control test, ORMDL3: Oromucoid-like 3; ΔCq: GSDMB: Gasdermin B; †: mean±SD; ‡: median (25–75 percentage); *: One-way ANOVA; **: Chi-square test; ***: Kruskal–Wallis test; ****: Mann–Whitney U-test.

other and the subtraction of subtractions (ΔΔCq) areobtained. Finally, the logarithm of ΔCqto the base of -2 value is calculated to assure that the border of subtraction does not adhere to its average value. In this way, the FC in the gene expression level of the two groups is calculated [10]. While higher ΔCq value indicates lower expression, a lower ΔCq value indicates higher expression. It can be calculated the FC to compare the normalized expression (ΔCq) of the two conditions. The FC is the expression ratio, and if it is positive, it means that the gene is upregulated. If the FC is negative, it means thatthe gene is downregulated [11].

Ethics Approval

All study procedures were done by a protocol previously approved by the Ethics Committee of Gaziantep University. All parents provided the written informed consent for the study procedures, and the children also gave the consent.

Statistical Analysis

All statistical analyses were performed using SPSS 18.0 (SPSS Inc., Chicago, Illinois). A $p<0.05$ was considered statistically significant. Non-parametric statistical analysis methods were used (one-way ANOVA, Chi-square test, Kruskal–Wallistest, Mann–Whitney U-test,and Spearman'scorrelation test) by evaluating the number and distribution of clinical parameters. Finally, the obtained laboratory results were evaluated with the clinical parameters.

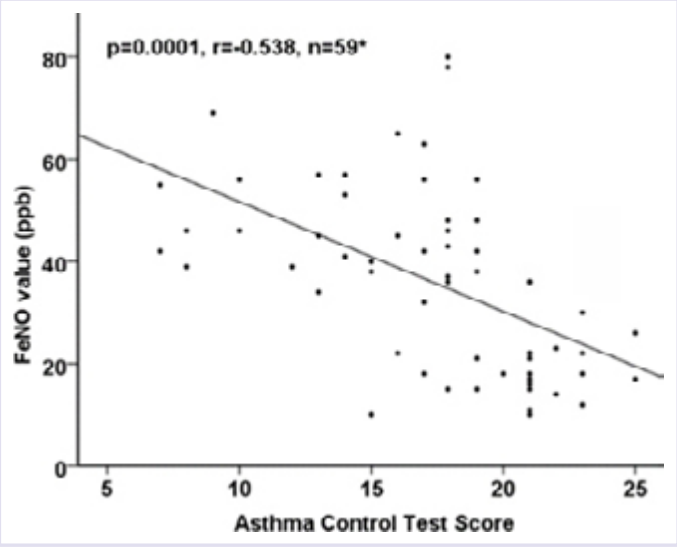

FIGURE 1. Comparison of FeNOwith ACT (*Spearmancorrelation test).

RESULTS

Characteristics of Cases

A total of 97 children with a mean age of 11.95±2.81 years (ranging from 6 to18 years) were evaluated. There was no significant difference among the groups in terms of age, gender, or body mass index (p>0.05). A comparison of the study population findings is presented in Table 2.

ORMDL3 and GDMB Gene Expression Levels in Asthmatic Children and Controls

ORMDL3 and GSDMB normalized gene expression values in the asthmatic group were 1.83 and 1.1 FC higher than those of the control group, respectively. However, this increase did not reach a statistically significant level (ORMDL3 vs. Control, p=0.786, and GSDMB vs. Control, p=0.671, Mann–Whitney U-test).

Relationship between FeNO and ACTScores

For the asthmatic group, we observed a significant negative correlation between FeNO level and ACT score (r=-0.56, p<0.0001, n=59) while there was also a positive correlation between FeNO level and bronchodilator response rate (r=0.302, p=0.02, n=59) (Fig. 1).

The Relationship between FeNOLevels and ORMDL3 and GSDMB Gene Expression Level

When ORMDL3 ΔCq values were compared based on the FeNO levels (FeNO levels >25 vs. ≤25 ppb), FeNO levels >25 ppb were2.93 FC higher than that of FeNO levels ≤25 ppb. This change was marginal statistically significant (p=0.051, Mann–Whitney U-test). In terms of GSDMB normalized gene expression values, there were 3.56 FC differences between the FeNo levels (FeNO>25 ppb vs. FeNO ≤25 ppb) though this difference was not statistically significant (p=0.241, Mann–Whitney U-test) (Fig. 2).

The Relationship between ACT Scores and ORMDL3 and GSDMB Gene Expression Level

We also found that ORMDL3 ΔCq values of ACT score ≤15 for asthmatic subjects were 6.74 FC higher than those of ACT score ≥20 and this increase in the ΔCq values was statistically significant (p=0.015, Mann–Whitney U-test). Moreover, GSDMB ΔCq values were also 11.71 FC higher for the same comparison (Fig. 3).

There were also significant negative correlations between ΔCq values of ORMDL3 and GSDMB genes in asthma patients and ACT score (p=0.003, r=-0.418,

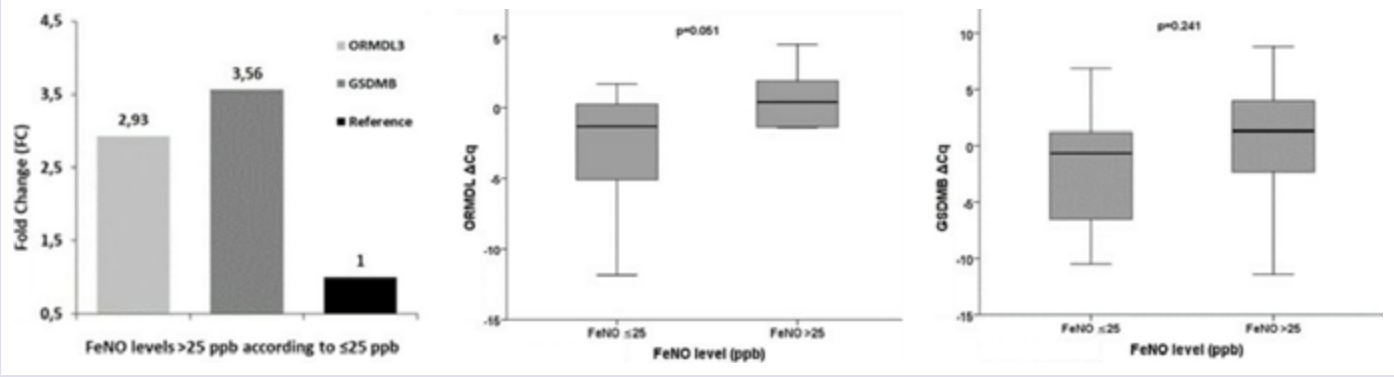

FIGURE 2. Comparison of ORMDL3/GSDMB genes FC and ΔCq values according to the FeNO level (*Mann–Whitney U-test).

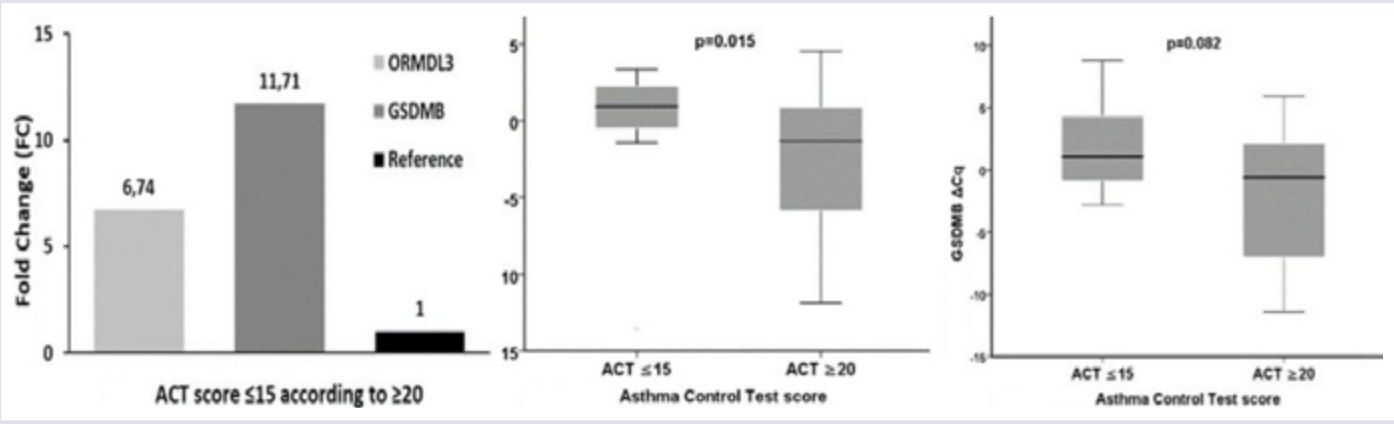

FIGURE 3. Comparison of ORMDL3/GSDMB genes FC and ΔCq values according to the ACT score (*Mann–Whitney U-test).

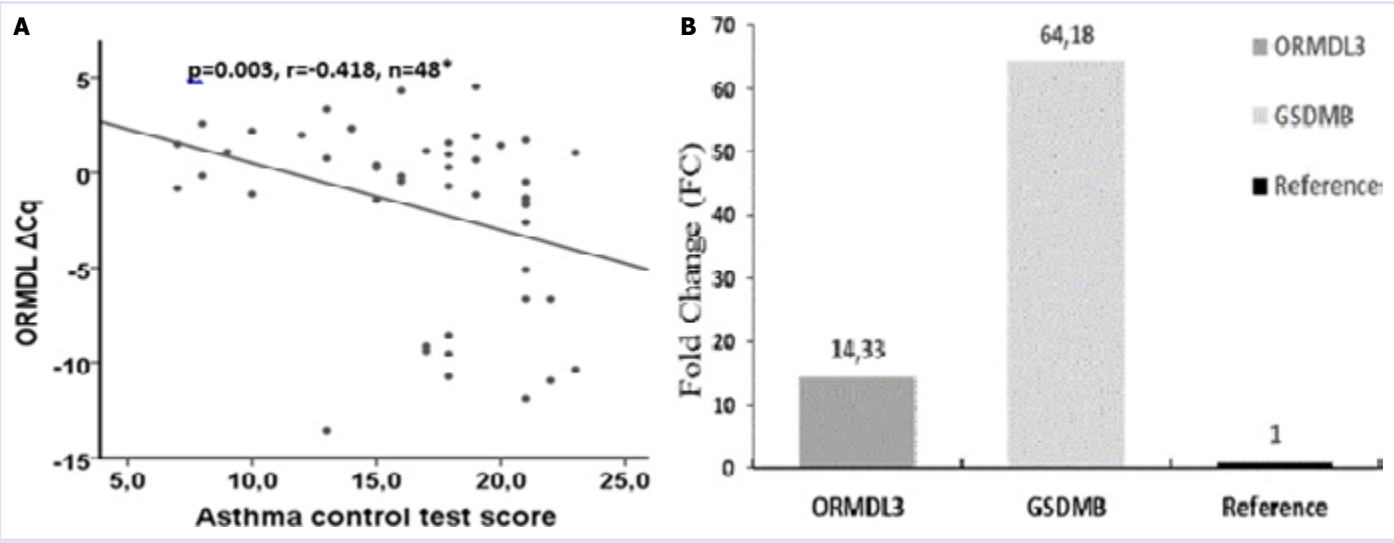

FIGURE 4. **(A)** Comparison of ORMDL3 genes ΔCq values according to the ACT score (*Spearman'scorrelation test). **(B)** Comparison of ORMDL3/GSDMB genes FC values according to ECT.

n=48, and p=0.016, r=-0.345, n=48, respectively, Spearman's correlation test) (Fig. 4A).

The Relationship between ECT Results and ORMDL3 and GSDMB Gene Expression Level

When only the patients diagnosed with asthma were evaluated, the GSDMB ΔCqlevel of the patients with ECT was 64.18 FC more than the negative ones (p=0.012, Mann–Whitney U-test) (Fig. 4B). On the other hand, for ORMDL3 ΔCq level, there was no statistically significant difference.

For asthmatic subjects, we observed statistically significant positive correlations between ORMDL3 ΔCq and max FEV1 decrease (%) (r=0.30, p=0.043, n=46) and between GSDMB ΔCq and max FEV1 decrease (%) (r=0.29, p=0.045, n=46, Spearman'scorrelation test).

There were no statistically significant differences between ORMDL3 and GSDMB normalized gene expression (ΔCq) in terms of the following clinical parameters: Absolute eosinophil count (≥400/<400 /mm^3), eosinophil percentage (% ≥4/<4), IgE level (median IgE: 132), FEV1 percentage (% ≥80/<80), FVC percentage (% ≥80/<80), FEF25–75 percentage (% ≥70/<70), and FEV1/FVC percentage (% ≥80/<80).

Relationship between ORMDL3 and GDMB Gene Expression Levels

When pooled all data, we observed a statistically significant positive correlation between ORMDL3 and GSDMB normalized gene expression (r=0.80, p<0.001, n=83, Spearman's correlation test).

DISCUSSION

Although many earlier studies focused on the gene polymorphism of ORMDL3 and GSDMB [22–24], the expression levels of these genes were evaluated in our study.

When we evaluated gene expression levels of GSDMB and ORMDL3, we identified several associations among the gene expression and the clinical parameters, including (a)ORMDL3 gene expression was found to be higher in uncontrolled asthmatics with low ACT scores. Moreover, negative correlations exist between ACT score and ORMDL3 and GSDMB gene expression levels,(b) ORMDL3 gene expression level in the asthmatic subjects was found to be higher in a group of FeNO>25 compared to FeNO ≤25 while the FeNO levels were also negatively correlated with ACT score and bronchodilator response rate, (c)GSDMB gene expression level of the subjects with positive ECT was higher than that of the subjects with negative ECT and we showed positive correlation between ORMDL3 and GSMB gene expression level and ECT level, (d)we found a significant correlation between ORMDL3 and GSDMB gene expression levels, evoking the common regulation of genes, and (e) we also observed a 1.83-fold increase in ORMDL3 gene expression level of the asthmatic group compared to the control group although this increase did not achieve statistical significance.

We observed a 64.18-fold increase in GSDMB gene expression for the asthmatic subjects who have positive ECT compared to that of negative ECT. Furthermore, we found a positive correlation between the GSDMB gene expression level and ECT degree. These findings indicate that GSDMB gene expression is associated with ECT, and the subjects who have ECT have also higher GSDMB gene expression. In fact, these results are in good agreement with the previous studies such that Yu et al. [25] also found a positive association between ECT and rs7216389 polymorphism, which is responsible for increased GSDMB gene expression. However, they did not find any association of GSDMB gene expression with either FEV1 change or eosinophil counts. Similarly, Tulah et al. [26], Moheimani et al. [27], and Nieuwenhuis et al. [28] showed that ECT is positively associated with rs7216389 polymorphism (the value of ECT tends to increase as the value of rs7216389 polymorphism increase). Das et al. [13]showed in a mice study that there is also a positive association between ECT and increased hGSDMBZp3-Cre expression which is the mice's version of GSDMD. Interestingly, Bisgaard et al. [7] reported that ECT is positively associated with rs7216389 polymorphism that is responsible for increased ORMDL3 gene expression for only 6-year-old children whereas such association does not exist for 1- and 4-year-old children. We also showed positive correlation between ORMDL3 gene expression level and ECT in our asthmatic patients older than 6 years old.

Ullemar et al. [29] and Tulah et al. [26] reported a positive association between asthma diagnosis and rs2305480 polymorphism leading to increased GSDMB gene expression. In our study, we found a 1.83-fold increase ORMDL3 gene expression level in asthmatic subjects though the increase did not achieve statistical significance. One of the reasons why we cannot find a meaningful difference is that our asthmatic patients are heterogeneous in terms of the age of onset of asthma. Hence, early-onset asthma was previously found to be related to the core 17q [4, 7, 30, 31]. As firstly reported by Bouzigon et al. [6], the associations between the onset of asthma symptoms for children before the age of 4 years and 17q genotypes were limited [6]. Furthermore, early-life exposures have been shown to modify the 17q genotype's effects on asthma risk and protection [8].

Although one GWAS on FeNO showed the associations between 17q SNPs [32], another study did not confirm this association [33]. Moreover, Van der Valk et al. [32] investigated the possible association between FeNO level and rs8069176 polymorphism that leads to an increase in ORMDL3 and GSDMB gene expression. They identified the association of rs8069176 polymorphism with FeNO level and ORMDL3 and GSDMB gene polymorphic expression. In our study, we showed a 2.92-fold increase ORMDL3 expression in the asthmatic subject whose FeNO level is <25 ppb compared to the asthmatic subjects with FeNO level is >25 ppb. Taking all into consideration, these findings indicate that the ORMDL3 gene may interact with NOS2 and may participate in one of the steps in eosinophilic inflammation [32].

Similar to the FeNO level, we identified significant associations of ORMDL3 and GSDMB gene expression with the ACT score. Especially, a statistically significant 6.74-fold increase in ORMDL3 gene expression was found in the asthmatic children with ACT score ≤15 when compared to the asthmatic children with ACT score ≥20. We also observed an 11.71-fold increase in GSDMB gene expression although it did not achieve a statistical significance. Furthermore, we reported negative correlations between ACT score and ORMDL3

and GSDMB gene expression. All these results show that ORMDL3 and GSDMB gene expression level is higher in the poorly controlled asthmatic patient. This is also supported by the study of Wan et al. [34] in such a way that they showed a strong relationship between ORMDL3/GSDMB locus on chromosome 17q12-21 (rs4794820) as a result of the development of severe asthma. Furthermore, Tang et al. [35] reported a negative association of rs2305480 polymorphism (leading to an increase in GSDMB gene expression) with FEV1. However, to date, there was no study in the literature showing the association of ACT score with ORMDL3 and GSDMB gene expression or polymorphisms.

In the 17q-focused study by Andiappan et al. [36], they showed the associations with blood eosinophilia whereas another GWAS by Gudbjartsson et al. [37] did not show such association. Likewise, any association between eosinophil counts and ORMDL3 and GSDMB gene expression level was not observed in our study.

Our new findings also support the notion that ORMDL3 and GSDMB genes are still important candidates for asthma sensitivity and uncontrolled asthma. Although the exact mechanism is not still known, several studies have continued to show promising results. For example, in one study, increased GSDMB gene expression was shown to inhibit TGFBeta1 expression which has an anti-inflammatory function in mice [13]. Allergy-induced ORMDL3 expression was also found to cause an increase in asthma-related chemokines in the airway epithelium [27]. Another study was reported that ORMDL3 is dramatically increased in the peripheral blood lymphocytes of infants with respiratory syncytial virus (RSV)-induced bronchiolitis compared to uninfected infants [36]. Luthers et al. [8] discussed growing evidence suggesting that increased ORMDL3 expressionspecifically in $CD4^+$ T lymphocytes constitutes a major underlying mechanism of asthma pathogenesis by skewing their differentiation and function.

Conclusion

In summary, we identified several associations among ORMDL3 and GSDMB genes and the clinical parametersrelated to asthma. The limited number of cases and possible errors in our measurements can be considered as limitations of our study. Further, future studies are needed to elucidate the potential role of these genes in asthma pathogenesis and in triggering an inflammatory response. Understanding the genetic association/mechanism causing atopic disease development and uncontrolled asthma is essential to develop potential new treatment methods and to reduce inflammatory response by directly targeting the genetic trigger instead of disease symptoms. In this way, new studies aiming to reduce the social and economic burden of asthma can be conducted.

Ethics Committee Approval: The Gaziantep University Clinical Research Ethics Committee granted approval for this study (date: 19.02.2013, number: 76).

Conflict of Interest: No conflict of interest was declared by the authors.

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Authorship Contributions: Concept – MA, OK, MYO, SOB, EK, SP, MK; Design – MA, OK, MYO, SOB, EK, SP, MK; Supervision – MA, OK, MYO, SOB, EK, SP, MK; Fundings – MA, OK, MYO, SOB, SP; Materials – MA, OK, MYO, SOB, SP; Data collection and/or processing – MA, OK, MYO, SOB, SP; Analysis and/or interpretation – MA, OK, MYO, SOB, SP; Literature review – MA, OK, MYO; Writing – MA, OK, MYO; Critical review – MA, OK, MYO.

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