

Association Between Gasdermin A and Gasdermin B Polymorphisms and Susceptibility to Adult and Childhood Asthma Among Jordanians

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Introduction: Gasdermin A (GSDMA) and gasdermin B (GSDMB) have been associated with childhood, and to a lesser extent with adult, asthma in many populations. **Aims:** In this study, we investigated the association between GSDMA and GSDMB variants and the incidence of adult and childhood asthma among Jordanians. **Methods:** Subjects were divided into two groups: adults and children. Within the adult group there were 129 asthma patients and 111 healthy controls. In the pediatric group there were 98 asthma patients and 112 healthy children. Gasdermin A (GSDMA) (rs7212938, T/G) and Gasdermin B (rs7216389, T/C) polymorphisms were genotyped using the PCR-RFLP method. Three analysis models were applied to the genotype data: co-dominant, dominant and recessive. **Results:** An association between the GSDMB T/C single nucleotide polymorphism (SNP) genotype and the incidence of childhood asthma was found ($p < 0.05$). GSDMB T/C SNP in children also showed a very high tendency toward significance with $p = 0.0532$ in the single locus analysis. In adults, no significant differences in the allelic frequencies of any of the SNPs analyzed were found between the case and control populations. At the haplotype level, GC haplotype was found to be associated with the risk of asthma in children while none of the tested haplotypes were found to be associated with asthma risk in adults. **Conclusions:** The findings of this study confirm the previously reported association between the GSDMB gene and the risk of childhood asthma.

Introduction

ASTHMA IS THE most common chronic disease and the most common medical emergency in children (Fanta, 2009). It is estimated to cost nearly 5.8 billion dollars yearly in the United States alone (Smith *et al.*, 1997). Despite its devastating impact, many aspects of the mechanism of asthma, and hence possible therapies, remain unknown. Asthma demonstrated 60% heritability in a twin study (duffy, 1990). Multiple genome-wide studies of asthma, culminating in the GABRIEL consortium (Moffat *et al.*, 2010) and EVE consortium (Torgerson *et al.*, 2011), showed an area of particular interest in chromosome 17q12-21, particularly the ORM1-like gene (ORMDL3), gasdermin B (GSDMB), and gasdermin A (GSDMA) (Bouzigon *et al.*, 2008; Sleiman *et al.*, 2008; Bisgaard *et al.*, 2009; Verlaan *et al.*, 2009; Wu *et al.*, 2009; Moffatt *et al.*, 2010; Akhabir and Sandford, 2011; Fang *et al.*,

2011; Lluís *et al.*, 2011; Ober and Yao, 2011; Torgerson *et al.*, 2011; Kang *et al.*, 2012; Li *et al.*, 2012; Marinho, *et al.*, 2012). The function of these genes is still under study, but there is evidence of ORMDL3 role in regulating endoplasmic reticulum (ER)-mediated calcium signaling and cellular stress (Cantero-Recasens *et al.*, 2010). The gasdermin family was found to be involved in regulation of epithelial apoptosis; GSDMA showing tumor suppressing characteristics while GSDMB demonstrating oncogenic traits (Saeki *et al.*, 2009). In fact, the gasdermin family was associated with gastric, hepatic, and breast cancers (Carl-McGrath *et al.*, 2008; Saeki *et al.*, 2009; Komiyama *et al.*, 2010; Edgren *et al.*, 2011). In several studies, the gasdermin family was found to be associated with autoimmune disease, including inflammatory bowel diseases (Christodoulou *et al.*, 2012), diabetes mellitus type I (Saleh *et al.*, 2011), primary biliary cirrhosis (Tanaka *et al.*, 2011), and rheumatoid arthritis (Kurreeman *et al.*, 2012).

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TABLE 1. GENE SNP ID, PRIMERS, AND RESTRICTION ENZYMES FOR GSDMA AND GSDMB POLYMORPHISMS

Gene	Reference SNP ID	Allele	Primers	Restriction enzyme	Length of digested fragments (bp)
GSDMA	rs7212938	T/G	(28)	Hpych4III	T: 213, 31 G: 125, 88, 31
GSDMB	rs7216389	C/T	5'-TGTCACATTTCCACCAGTTCC-3' 5'-TGTCACATTTCCACCAGTTCC-3'	NsiI	C: 489 T: 258, 231

SNP, single nucleotide polymorphism.

The gasdermin family has been associated with childhood asthma (Bouzigon *et al.*, 2008; Sleiman *et al.*, 2008; Bisgaard *et al.*, 2009; Ober and Yao, 2011; Kang *et al.*, 2012), yet its role in late-onset asthma remains controversial. In a study on an adult group, Fang *et al.* (2011) concluded that variants in 17q21 (ORMDL3 and GSDMB included) were significantly associated with adult-onset asthma. In contrast, Moffatt *et al.* (2010) compared childhood-onset to late-onset asthma and the results showed that variants at the ORMDL3/GSDMB locus are associated only with the childhood-onset disease. The gasdermin family relationship with atopy is also disputed, many studies deny a correlation with atopy and IgE levels (Bouzigon *et al.*, 2008 and Moffatt *et al.*, 2010), while a study by Yu *et al.* (2011) supported it.

The aim of this study is to investigate whether there is an association between gasdermin family GSDMB and GSDMA and the risk of asthma among Jordanians. Many studies have been done in several populations to verify this association but, to our knowledge, no previous study has examined this relationship in the Jordanian population. Accumulating evidence for this association is worth doing to strengthen the importance of GSDMB and/or GSDMA polymorphisms in asthma pathogenesis.

Material and Methods

Study population

This case-control study included 227 asthma patients and 223 healthy controls. Subjects were divided into two groups: adult subjects and children subjects. For asthma patients, 98 patients were asthma children and 129 patients were asthma adults, while for control subjects, 112 were healthy children and 111 were healthy adults. All subjects were recruited from the Jordan University Hospital (JUH) and Jordan Hospital (JH), Amman, Jordan, between August 2010 and January 2013. In adults, the diagnosis of asthma was according to the treating physician based on history, pulmonary tests, and physical examinations. While in children, the diagnosis of asthma was according to the treating physician based on the history and physical examination. The control subjects were all symptom free and had no history of atopic disorders or asthma. The study protocol was approved from the Institutional Review Board of University of Jordan. All patients and control subjects had signed an informed consent.

Total IgE level measurement

Serum total IgE levels were measured for all participants using the Total IgE ELISA Kit (IBL international GMBH.). The results were read using ELISA Reader (Sunrise-Tecan).

DNA isolation

Venous blood samples (5 mL) were collected in EDTA tubes. Genomic DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega). Isolated DNA was stored at -20°C till use.

Genotyping of the GSDMA and GSDMB polymorphisms by PCR-RFLP

The genetic polymorphisms of GSDMA (rs7212938) and GSDMB (rs7216389) were determined by the polymerase chain reaction/restriction fragment length polymorphism (PCR/RFLP) method. The SNP ID, primers (IDT), and restriction enzymes (New England Biolabs) used are described in Table 1. The PCR products were digested overnight by restriction endonuclease and were thereafter separated by electrophoresis on 2.5% agarose gel and stained with ethidium bromide.

Statistical analyses

SNP and haplotype frequencies across asthma and controls were computed using ordinal logistic regression analysis as implemented in SNPStats (Solé *et al.*, 2006). Linkage disequilibrium among the eight SNPs was calculated as D' (Lewontin, 1963), D' is the linkage disequilibrium coefficient standardized by the maximum value. The significant departure from the Hardy-Weinberg equilibrium (HWE) was assessed by the procedure described in Guo and Thompson, 1992, using a test analogous to Fisher's exact test as implemented in ARLEQUIN (Excoffier *et al.*, 2003). Analysis of association for each SNP with asthma and each haplotype with asthma was computed using the web tool SNPStats. p -Value adjusted as significant when $p \leq 0.05$. Statistical analyses were performed in a batch call to the R package (R Development Core Team, 2005, <http://www.R-project.org>).

Results

The 98 pediatric asthmatic cases included 33 females (32%) and 74 males (68%) with a median age of 5.64 ± 4.63 , while the 112 pediatric control subjects included 44 females (38%) and 71 males (62%) with a median age of 7.53 ± 4.83 (Table 2). The 129 asthmatic adults included 114 females (72%) and 46 males (28%) with a median age of 44.02 ± 14.95 . The 110 adult control subjects' median age was 38.74 ± 14.85 and included 52 (47%) females and 58 (53%) males (Table 2). The t-test and chi-square test showed significant difference between asthmatic patients and the control group, only in children, for gender and age ($p < 0.05$) (Table 2) and further statistical analysis was corrected toward these two confounders. No

TABLE 2. CHARACTERISTICS OF STUDY POPULATION

<i>Phenotype</i>	<i>Sample size</i>	<i>% Females/ % Males</i>	<i>Average age $\pm$ SD</i>	<i>T-test (p)</i>	<i>Mean IGE- level $\pm$ SD</i>	<i>T-test (p)</i>
Children asthma	98	0.32/0.68	5.64 $\pm$ 4.43	<0.05	188.05 $\pm$ 330.61	>0.05
Children control	112	0.38/0.62	7.58 $\pm$ 4.82		19.99 $\pm$ 21.89	
Adult asthma	129	0.72/0.28	44.02 $\pm$ 14.95	>0.05	134.14 $\pm$ 239.64	>0.05
Adult control	111	0.46/0.54	38.6 $\pm$ 14.85		17.22 $\pm$ 20.29	

SD, standard deviation.

TABLE 3. CLINICAL CHARACTERISTICS OF THE ADULT HEALTHY CONTROL AND ADULT ASTHMA

<i>Phenotype</i>	<i>No. of samples</i>	<i>FEV1 $\pm$ SD</i>	<i>FVC $\pm$ SD</i>	<i>Fratio $\pm$ SD</i>	<i>PEF $\pm$ SD</i>	<i>Smoking (%)</i>
Adult healthy control	111	95.48 $\pm$ 10.0	97.93 $\pm$ 10.5	101.55 $\pm$ 6.5	93.7 $\pm$ 16.1	26 (23.4)
Adult asthma	129	79.30 $\pm$ 19.8	79.25 $\pm$ 16.6	76.81 $\pm$ 10.4	75.3 $\pm$ 22.3	15 (11.6)

FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; Fratio, FEV1/FVC ratio in %; PEF, peak expiratory flow.

TABLE 4. MINOR ALLELE FREQUENCIES AND THEIR ASSOCIATION ANALYSES IN CHILDREN

<i>SNP name</i>	<i>Nucleotide change</i>	<i>Total frequency</i>	<i>Frequency in control</i>	<i>Frequency in asthma</i>	<i>Minor allelic association with asthma χ^2 (p)</i>	<i>HWE exact p</i>	<i>D'</i>
GSDMA children	G/T	0.44	0.41	0.47	0.731 (0.3927)	0.89	0.6384
GSDMB children	T/C	0.35	0.41	0.28	3.739 (0.0532)	0.29	

HWE, Hardy–Weinberg equilibrium.

TABLE 5. MINOR ALLELE FREQUENCIES AND THEIR ASSOCIATION ANALYSES IN ADULT

<i>SNP name</i>	<i>Nucleotide change</i>	<i>Total frequency</i>	<i>Frequency in control</i>	<i>Frequency in asthma</i>	<i>Minor allelic association with asthma χ^2 (p)</i>	<i>HWE exact p</i>	<i>D'</i>
GSDMA	G/T	0.38	0.38	0.38	0 (1)	0.076	0.5981
GSDMB	T/C	0.42	0.42	0.41	0.021 (0.89)	0.9	

TABLE 6. GENOTYPE FREQUENCY DISTRIBUTION AND ASSOCIATION STUDY BETWEEN ASTHMA AND CONTROL IN CHILDREN

			Frequency: No. (%)		Crude analysis		Adjusted by sex and age	
	Model	Genotype	Control (n=112)	Asthma (n=98)	p	Odds ratio (95% CI)	Odds ratio (95% CI)	p
GSDMA	Codominant	GG	38 (33.9)	28 (28.6)	0.37	1	1	0.11
		GT	57 (50.9)	48 (49)		1.14 (0.61–2.13)	1.18 (0.59–2.33)	
		TT	17 (15.2)	22 (22.4)		1.76 (0.79–3.91)	2.44 (1.02–5.83)	
	Dominant	GG	38 (33.9)	28 (28.6)	0.4	1	1	0.27
		GT+TT	74 (66.1)	70 (71.4)		1.28 (0.71–2.31)	1.44 (0.76–2.74)	
	Recessive	GG+GT	95 (84.8)	76 (77.5)	0.18	1	1	0.039
TT		17 (15.2)	22 (22.4)	1.62 (0.80–3.26)		2.21 (1.03–4.72)		
GSDMB	Codominant	TT	42 (37.5)	51 (52)	0.028 ^a	1	1	0.02
		CT	49 (43.8)	39 (39.8)		0.66 (0.36–1.18)	0.54 (0.28–1.03)	
		CC	21 (18.8)	8 (8.2)		0.31 (0.13–0.78)	0.3 (0.11–0.79)	
	Dominant	TT	42 (37.5)	51 (52)	0.034 ^a	1	1	0.012
		CT+CC	70 (62.5)	47 (48)		0.55 (0.32–0.96)	0.46 (0.25–0.85)	
	Recessive	TT+CT	91 (81.2)	90 (91.8)	0.024 ^a	1	1	0.04
		CC	21 (18.8)	8 (8.2)		0.85 (0.49–1.47)	0.4 (0.16–1)	

^aSignificant <0.05.

TABLE 7. GENOTYPE FREQUENCY DISTRIBUTION AND ASSOCIATION STUDY BETWEEN ADULT ASTHMA AND ADULT CONTROL

	<i>Model</i>	<i>Genotype</i>	<i>Frequency: No. (%)</i>		<i>Crude analysis</i>		<i>Adjusted by sex and age</i>	
			<i>Control (n = 111)</i>	<i>Asthma (n = 129)</i>	<i>p</i>	<i>Odds ratio (95% CI)</i>	<i>Odds ratio (95% CI)</i>	<i>p</i>
GSDMA	Codominant	GG	47 (42.3)	52 (40.3)	0.83	1	1	0.7
		GT	44 (39.6)	56 (43.4)		1.15 (0.66–2.01)	1.17 (0.65–2.11)	
		TT	20 (18)	21 (16.3)		0.95 (0.46–1.97)	0.86 (0.4–1.84)	
	Dominant	GG	47 (42.3)	52 (40.3)	0.75	1	1	0.81
		GT+TT	64 (57.7)	77 (59.7)		1.09 (0.65–1.82)	1.07 (0.62–1.83)	
	Recessive	GG+GT	91 (82)	108 (83.7)	0.72	1	1	0.52
		TT	20 (18)	21 (16.3)		0.88 (0.45–1.73)	0.79 (0.39–1.6)	
GSDMB	Codominant	TT	35 (31.5)	46 (35.7)	0.67	1	1	0.78
		CT	58 (52.2)	60 (46.5)		0.79 (0.45–1.39)	0.81 (0.45–1.46)	
		CC	18 (16.2)	23 (17.8)		0.97 (0.46–2.07)	0.89 (0.4–1.97)	
	Dominant	TT	35 (31.5)	46 (35.7)	0.5	1	1	0.51
		CT+CC	76 (68.5)	83 (64.3)		0.83 (0.48–1.42)	0.83 (0.47–1.45)	
	Recessive	TT+CT	93 (83.8)	106 (82.2)	0.74	1	1	0.98
		CC	18 (16.2)	23 (17.8)		1.12 (0.57–2.21)	0.84 (0.49–1.43)	

Significant <0.05.

significant difference in serum total IgE level was found between asthmatic patients and controls in both children and adults ($p > 0.05$), (Table 2). The clinical characteristics and the percentage of smoking subjects of the healthy adult control and the adult asthma are shown in Table 3. Regarding the family history, the adult asthma group scored 44.8%, while in the children asthma group it was 14%.

The allele frequencies of the GSDMA and GSDMB gene polymorphisms in patients and healthy controls are shown in Tables 4 and 5. All SNPs were in HWE as the null hypothesis for departure from HWE was rejected for all SNPs (Tables 4 and 5). No significant differences in the allelic frequencies of all SNPs tested between asthmatic patients and the control volunteers were found, although GSDMB T/C SNP in children showed a very high tendency toward significance with $p = 0.0532$. The two SNPs were not found to be in a linkage disequilibrium and D' was found to be 0.6384 (Table 4). On the genotype level, three analysis models were applied namely codominant, dominant, and recessive genotypes. In pediatric cases, the GSDMA genotype showed a significant association with asthma risk ($p < 0.05$) in the recessive model. This association was only found after applying the multiple variation correction with p -values of 0.039 (Table 6). GSDMB SNP results were different and the association with the risk of asthma was significant ($p < 0.05$) in all models before and after applying the multiple variation correction (Table 6).

In adults, the allele frequencies of the GSDMA and GSDMB in patients and healthy controls are shown in Table 5. No significant differences in the allelic frequencies of all SNPs between asthma patients and the control volunteers were found (Table 7). The linkage disequilibrium between the two SNPs was not found to be highly linked as the D' was 0.5981 (Table 5). Tables 8 and 9 show the frequencies of the haplotypes when the two tested SNPs are combined together. The association of these haplotypes and the risk of asthma was tested in both adults and children and the results are listed in Tables 10 and 11. In pediatrics, one haplotype was found to be associated with the risk of asthma, while in adults, none of the tested haplotypes was found to be associated with asthma risk, see Tables 10 and 11.

Discussion

In the current study, we have tested the association between GSDMA (rs7212938, T/G) and GSDMB (rs7216389, T/C) at locus 17q21.2 and risk of asthma in Jordanians. Strong association between GSDMB and childhood asthma was found. The association between GSDMB and childhood asthma was clear in the codominant, dominant, and recessive models, in which the p -values were 0.02, 0.012, and 0.04 after multiple variation correction, respectively. In case of GSDMA, an association was found in the recessive model only after multiple variation correction.

TABLE 8. HAPLOTYPE FREQUENCIES, CHILDHOOD

<i>Haplotype</i>	<i>Frequency in all samples</i>	<i>Frequency in control</i>	<i>Frequency in childhood asthma</i>
TT	0.3812	0.3219	0.4484
GC	0.2931	0.3219	0.2596
GT	0.2712	0.2718	0.271
TC	0.0545	0.0843	0.021

TABLE 9. HAPLOTYPE FREQUENCIES, ADULT

<i>Haplotype</i>	<i>Frequency in all samples</i>	<i>Frequency in control</i>	<i>Frequency in adult asthma</i>
GC	0.3532	0.3563	0.3506
TT	0.3157	0.3112	0.3196
GT	0.2677	0.2654	0.2696
TC	0.0635	0.0672	0.0603

TABLE 10. HAPLOTYPES ASSOCIATED WITH CHILDHOOD ASTHMA

Haplotype	Crude Analysis (n=210)			Adjusted analysis by sex and age (n=188)		
	Frequency	Odds ratio 95% CI	p	Frequency	Odds ratio 95% CI	p
TT	0.3812	1	–	0.38811	1	–
GC	0.2931	0.62 (0.38–1)	0.05 ^a	0.2924	0.53 (0.31–0.89)	0.017 ^a
GT	0.2712	0.73 (0.44–1.2)	0.22	0.2634	0.71 (0.41–1.23)	0.23
TC	0.0545	0.18 (0.05–0.7)	0.014 ^a	0.056	0.28 (0.07–1.11)	0.072

Haplotypes sequence as GSDMA, GSDMB.

^aSignificant $p < 0.05$.

Interestingly, the GSDMB CC homozygous minor genotype shows a protective effect (odds ratio of 0.3) as the genotype frequency was significantly higher in control subjects (18.8%) than asthmatic subjects (8.8%). Furthermore, such protective effect is clear in the single-locus analysis of asthma risk, as the GSDMB allelic frequency in the control was 0.41, while it was only 0.28 in the asthmatic patient. The strong association of childhood asthma with GSDMB was frequently concluded in several studies (Moffatt *et al.*, 2010; Kang *et al.*, 2012, and Yu *et al.*, 2011), including a review of genome-wide association studies on asthma by Akhabir (Akhabir and Sandford, 2011). However, the findings by Lluís *et al.* (2011) showed that GSDMB was not associated with childhood asthma, while GSDMA did correlate significantly with it. In case of GSDMA, the effect is opposite and the TT minor genotype has a risk effect with an odds ratio of 1.76 and 2.44 before and after the multiple variation correction, respectively. The GSDMA association is consistent with the previous work of Moffatt *et al.* (Moffatt *et al.*, 2010) and Jinho *et al.* (Yu *et al.*, 2011). Interestingly, our results show that adult asthma is more prevalent in female adults, a finding that has been linked to many factors, including hormonal fluctuations and changes in women (de Nijs *et al.*, 2013).

As multiple SNPs may increase the risk for asthma, four haplotypes were constructed and significant differences were observed in two haplotypes (GC and TC). Interestingly, both haplotypes have the C allele of the GSDMB and both display protective odds ratios of 0.53 for the GC and 0.28 for the TC. Taken together, despite the relatively small sample size in this study, these findings illustrate a clear evidence for the involvement of GSDMB and to lesser extent the GSDMA in the pathophysiology of childhood asthma among Jordanians.

The gasdermin family was found to be involved in the regulation of epithelial apoptosis; GSDMA showing tumor suppressing characteristics, while GSDMB demonstrating

oncogenic traits (Saeki *et al.*, 2009). In fact, the gasdermin family was associated with gastric, hepatic, and breast cancers (Carl-McGrath *et al.*, 2008; Saeki *et al.*, 2009; Komiya *et al.*, 2010 and Edgren *et al.*, 2011). It was also noted that ORMDL3 and GSDMA may be involved in immune maturation and T-cell development (Lluís *et al.*, 2011). Although the effect of rs7216389 in GSDMB is not clear, it was strongly associated in cis with transcript levels of ORMDL3 in EBV-transformed lymphoblastoid cell lines from children with asthma (Verlaan *et al.*, 2009). Interestingly, Verlaan *et al.* (2009) illustrated that the ZPBP/GSDMB/ORMDL3 locus was associated with asthma by identifying domain-wide cis-regulation through a combination of genetic and functional analysis (Verlaan *et al.*, 2009). One function of ORMDL3 is to inhibit Ca2p pump (SERCA) in the sarco-ER. Such inhibition reduces the ER Ca2p concentration and increases the unfolded-protein response, which is relevant in chronic inflammatory diseases such as asthma (Verlaan *et al.*, 2009).

Our results regarding the adult population showed no significant association of GSDMA/GSDMB with asthma. The relationship in adults has been less studied compared to the pediatric population. Moffatt *et al.* (2010) denied an association of GSDMA/GSDMB with adult asthma. However, other articles supported the association. Marinho *et al.* (2012) supported the association of both genes to adult asthma, while Fang *et al.* (2011) and Wan *et al.* (2012) only supported the relationship in GSDMB.

In conclusion, the finding of this study confirms the previously reported association between the GSDMB and to a lesser extent GSDMA and the risk of childhood asthma. No association was found between GSDMA and GSDMB with the risk of adult asthma. The differences in the outcome between the adults and children may point to the heterogeneity in the pathophysiology of asthma in children and adults.

TABLE 11. HAPLOTYPES ASSOCIATED WITH ADULT ASTHMA

Haplotype	Crude analysis (n=240)			Adjusted analysis by sex and age (n=240)		
	Frequency	Odds ratio 95% CI	p	Frequency	Odds ratio 95% CI	p
GC	0.3532	1	–	0.3532	1	–
TT	0.3157	1.042 (0.68–1.59)	0.85	0.3157	1.04 (0.67–1.61)	0.86
GT	0.2677	1.03 (0.61–1.75)	0.9	0.2677	1.21 (0.69–2.14)	0.5
TC	0.0635	0.91 (0.37–2.23)	0.84	0.0635	1.07 (0.41–2.81)	0.89

Haplotypes sequence as GSDMA, GSDMB.

Significant $p < 0.05$.

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Author Disclosure Statement

No competing financial interests exist.

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