

Association Between ADAM33 Polymorphisms and Susceptibility with Adult and Childhood Asthma Among Jordanians

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Disintegrin and metalloprotease 33 (*ADAM33*) have been associated with childhood and adult asthma in many populations. *ADAM33* mutations might predispose to altered lung function in early infancy. In this study, we investigated the association between single-nucleotide polymorphisms in *ADAM33* and the incidence of adult and childhood asthma among Jordanians. One hundred seven pediatric asthmatic patients, 115 healthy pediatric patient controls, 160 adult asthmatic patients, and 110 healthy adults were enrolled in this study. *ADAM33* polymorphisms were genotyped using the polymerase chain reaction/restriction fragment length polymorphism method. A strong association between the V4 genotype and incidence of childhood asthma was found. In the single-locus analyses of asthma risk, V4 C/G single nucleotide polymorphism (SNP) showed a trend toward significance with $p=0.07$. Interestingly, the CC homozygous mutant genotype frequency was significantly higher in asthmatic subjects (15.9%) than in control subjects (2.6%), resulting in an odds ratio of 7.05. In adult cases, S2, the F+1 and Q–1 genotype showed a significant association ($p\leq 0.05$) with the incidence of asthma. Two haplotypes also exhibited a significant association with asthma ($p\leq 0.05$). In conclusion, the findings of this study confirm the already reported association between V4 SNP and the incidence of childhood asthma as well as between S2, F+1, and Q–1 SNPs and the incidence of adult asthma in several populations.

Introduction

ASTHMA IS ONE of the most common chronic disorders of childhood. Overall, asthma affects nearly 155 million individuals worldwide. Studies have shown that asthma is linked with genetic changes and gene–environment interactions (Koppelman, 2006). More than 100 genes have been identified to be associated with asthma, but none with so great a statistical significance as the disintegrin and metalloproteinase 33 gene (*ADAM33*). The role of the *ADAM33* gene, which is located on chromosome 20p13, remains unclear in the development of asthma. The accumulating data indicate that *ADAM33* may be involved in airway remodeling and may determine lung function throughout life (Postma and Howard, 2004). After the first report of association between *ADAM33* polymorphisms and asthma in two Caucasian populations from the United Kingdom and United States of America, a number of replication studies have been published

with very diverse results. The association between *ADAM33* single nucleotide polymorphisms (SNPs) and asthma has been confirmed in Caucasian, African, and Hispanic Americans, as well as in the Netherland, German, Korean, and Japanese populations (Howard *et al.*, 2003; Cheng *et al.*, 2004; Lee *et al.*, 2004; Werner *et al.*, 2004). Interestingly, a study in Puerto Rican and Mexican populations failed to show an association between *ADAM33*, asthma, asthma severity, and atopy (Lind *et al.*, 2003). Simpson *et al.* (2005) linked *ADAM33* with early-life origins of asthma and suggested that *ADAM33* mutations might predispose to altered lung function in early infancy. Furthermore, epidemiological studies support *ADAM33*'s role as a susceptibility gene for more severe and progressive disease. Investigation of nine SNPs of *ADAM33* in 200 asthmatics in the Netherlands showed that four SNPs (S–2, T–1, T–2, and V–4) were significantly associated with a progressive decline in FEV1 when compared with normal controls (Jongepier *et al.*,

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2004). A recent meta-analysis has shown that polymorphisms in the *ADAM33* gene are risk factors for bronchial asthma (Liang *et al.*, 2013). Importantly, this meta-analysis revealed a profound interethnic variation, as it illustrated that the *ADAM33* gene and risk of bronchial asthma was more significant in Asian populations. Haplotypes of *ADAM33* were also associated with asthma in different populations (Howard *et al.*, 2003; Lind *et al.*, 2003; Wang *et al.*, 2006).

We have previously shown that polymorphisms in the *ADAM33* gene are associated with allergic rhinitis among Jordanians (Zihlif *et al.*, 2013). Different genotypes and haplotypes were found to be associated with the risk of allergic rhinitis in adults ($p \leq 0.05$). In this study, we meant to determine whether *ADAM33* SNPs are associated with the development of asthma among adults and children. The chosen SNPs were previously associated with the susceptibility of asthma in different populations.

Materials and Methods

Study population

This case-control study included 107 asthmatic pediatric patients, 115 healthy pediatric patient controls, 160 asthmatic adult patients, and 110 healthy adult controls (Table 1). The study protocol was approved by the Institutional Review Board at the University of Jordan. The patients and control subjects were recruited for the study between August of 2010 and January of 2013 at the University of Jordan Hospital and Jordan Hospital in Amman, Jordan. In adults, the diagnosis of asthma was according to the treating physician based on history, pulmonary tests, and physical examinations. All the control subjects were symptom free and had no history of atopic disorders or asthma. In children, the diagnosis of asthma was according to the treating physician based on the history and physical examination. The pulmonary tests were not used to diagnose asthma in children, as spirometry is usually impossible under 5 years old according to most Guidelines on the Management of Asthma (Townshend *et al.*, 2007). All the control subjects were symptom free and had no history of atopic disorders and asthma. All patients and control subjects signed an informed consent form.

Total immunoglobulin E level measurement

Serum total immunoglobulin E (IgE) levels were measured for all participants using a Total IgE ELISA kit (IBL International). The results were read using ELISA Reader. According to the kit ranges, subjects with IgE levels below 100 IU/mL were considered as having normal IgE levels.

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 until further use.

Genotyping of *ADAM33* gene by polymerase chain reaction/restriction fragment length polymorphism

The genetic polymorphism analysis of eight SNPs for the *ADAM33* gene was determined by the polymerase chain reaction (PCR)/restriction fragment length polymorphism method. The primers and restriction enzymes were similar to those used in our previous publication (Zihlif *et al.*, 2013). The PCR products were digested overnight by restriction endonuclease, thereafter separated by electrophoresis on agarose gel, and stained with ethidium bromide. For adults, eight SNPs were tested, namely, T1, T2, T+1, V4, S1, S2, and Q-1, while in the pediatric patients, we excluded the F+1 because of the small amounts of DNA that were yielded from the children's samples.

Statistical analysis

SNP and haplotype frequencies across asthmatic and control patients were computed using ordinal logistic regression analysis as implemented in SNPStats (Sole *et al.*, 2006). Linkage disequilibrium among the eight SNPs was calculated as D' (Lewontin, 1963), where D' is the linkage disequilibrium coefficient standardized by the maximum value. The significant departure from 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.*, 2005). Analysis of association for each SNP with asthma and each haplotype with asthma was computed using the web tool SNPStats. p -Values were adjusted as significant when $p \leq 0.05$. The statistical analyses are performed in a batch call to the R package (R Development Core Team, 2005, <http://R-project.org>).

Results

The 107 pediatric asthmatic cases included 33 women (31%) and 74 men (69%) with a median age of 5.96 ± 4.63 , while the 115 pediatric control subjects included 44 women (38%) and 71 men (62%) with a median age of 7.53 ± 4.83 (Table 1). The 160 asthmatic adults included 114 women (71%) and 46 men (29%) with a median age of 44.73 ± 15.41 . The 110 adult control subjects' median age was 38.74 ± 14.85 .

TABLE 1. CHARACTERISTICS OF STUDY POPULATION

Phenotype	Sample size	% Females	% Males	Av. age $\pm$ SD	p-Value t-test	Mean IgE-level $\pm$ SD	p-Value t-test	Mean FEV1 (%)
Adult asthma	160	0.71	0.29	44.73 ± 15.41	> 0.05	134.96 ± 236.68	> 0.05	81
Adult control	110	0.47	0.53	38.74 ± 14.85		17.37 ± 20.33		96
Children asthma	107	0.31	0.69	5.96 ± 4.63	< 0.05	214.09 ± 350.21	> 0.05	NA ^a
Children control	115	0.38	0.62	7.53 ± 4.83		19.83 ± 21.86		NA ^a

^aNA was not performed.

SD, standard deviation; IgE, immunoglobulin E.

TABLE 2A. MINOR ALLELE FREQUENCIES AND THEIR ASSOCIATION ANALYSES IN ADULTS

<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 rhinitis χ^2 (p-value)</i>	<i>HWE exact p</i>
T1	A/G	0.2	0.18	0.22	0.5 (0.48)	0.45
T2	G/A	0.21	0.19	0.23	0.482 (0.487)	0.36
T+1	G/A	0.21	0.18	0.23	0.767 (0.381)	0.72
V4	C/G	0.2	0.19	0.2	0.032 (0.858)	0.68
S1	G/A	0.06	0.05	0.06	0.096 (0.756)	0.6
S2	C/G	0.37	0.32	0.41	1.747 (0.186)	0.9
F+1	T/C	0.4	0.34	0.45	2.532 (0.1116)	0.61
Q-1	G/A	0.16	0.12	0.19	1.87 (0.1714)	0.073

Boldface in the second column indicates the minor allele.

SNP, single nucleotide polymorphism; HWE, Hardy-Weinberg equilibrium.

TABLE 2B. 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 rhinitis χ^2 (p-value)</i>	<i>HWE exact p</i>
T1	A/G	0.19	0.19	0.2	0.032 (0.858)	0.81
T2	G/A	0.22	0.22	0.22	0 (1)	0.85
T+1	G/A	0.2	0.2	0.21	0.031 (0.861)	0.22
V4	C/G	0.25	0.2	0.31	3.185 (0.0743)	0.049
S1	G/A	0.08	0.07	0.09	0.272 (0.6022)	0.37
S2	C/G	0.41	0.41	0.41	0 (1)	1
Q-1	G/A	0.2	0.2	0.21	0.031 (0.861)	0.84

Boldface in the second column indicates the minor allele.

and included 52 (47%) women and 58 (53%) men (Table 1). The *t*-test and Chi-square test showed a significant difference between asthmatic patients and the control group only in children with regard to gender and age ($p < 0.05$) (Table 1), and further statistical analysis was corrected toward those two confounders. No significant difference in serum total IgE level was found between asthmatic patients and controls in both children and adults ($p > 0.05$) (Table 1).

The allele frequencies of the *ADAM33* gene polymorphisms in patients and healthy controls are shown in Table 2A and B. All SNPs were in HWE, as the null hypothesis for departure from HWE was rejected for all SNPs (Table 2A, B). No significant differences in the allelic frequencies of all SNPs tested between asthmatic patients and the control volunteers were found, although V4 C/G SNP in children showed a tendency toward significance with $p = 0.07$. At the genotype level, three analysis models were applied, namely co-dominant, dominant, and recessive genotypes. Those models were applicable in all cases except in case of S1 SNPs in children, as S1 is made only of two genotypes: (GG: 187 and GA: 35). In pediatric cases, the V4 genotype showed a significant association ($p \leq 0.05$) with asthma risk in both co-dominant and recessive models with p values of 0.002 and < 0.001 before multiple-variation correction and 0.02 and 0.005 after multiple-variation correction, respectively (Table 3A). Q-1 SNP genotype was found to be associated with asthma risk only in the recessive model after the correction toward multi variant with a p value of 0.02 (Table 3A). The linkage disequilibrium between the SNPs was tested, and T4c most of the SNPs were found to be highly linked (Table 4A,

4B). The haplotypes result when we combine the seven SNPs T1, T2, T+1, V4, S1, S2, and Q-1. The GAAGGGG haplotype was found to be significantly associated with asthma incidence ($p \leq 0.05$) both before and after multi-variant correction (Table 5A).

In the case of adults, the allele frequencies of the *ADAM33* gene polymorphisms in patients and healthy controls are shown in Table 2B. No significant differences in the allelic frequencies of all SNPs between asthma patients and the control volunteers were found. The linkage disequilibrium between the tested SNPs were tested and all SNPs were found to be highly linked except in S1 with V4, F+1, and Q-1 cases as the D' are 0.2484, 0.24, and 0.1413, respectively (Table 4B). At the genotype level, the S2, F+1, and Q-1 genotype showed a significant association ($p \leq 0.05$) with asthma risk at least in one of the three analysis models used. The strongest associations were found in the F+1 SNP case, in which the associations were clear for the co-dominant and dominant models both before and after correction against gender and age (Table 3B) with p values of 0.01 and 0.03 before correction against gender and age and 0.021 and 0.042 in the co-dominant and dominant models after correction, respectively (Table 3B). In the case of Q-1, strong associations were found in the recessive model both before and after correction against gender and age with p -values of 0.016 and 0.021, respectively (Table 3B). Q-1 also showed a significant association in the co-dominant model ($p = 0.044$); however, such association was lost after correction toward gender and age ($p = 0.06$) (Table 3). S2 SNP showed a significant association in the dominant model both before and

TABLE 3A. GENOTYPE FREQUENCY DISTRIBUTION AND ASSOCIATION STUDY BETWEEN ASTHMA AND CONTROL IN ADULTS

	<i>Model</i>	<i>Genotype</i>	<i>Frequency: No. (%)</i>		<i>Crude analysis</i>		<i>Adjusted by age and sex</i>	
			<i>Control (n = 110)</i>	<i>Asthma (n = 160)</i>	<i>p-Value</i>	<i>Odds ratio (95% CI)</i>	<i>Odds ratio (95% CI)</i>	<i>p-Value</i>
T1	Co-dominant	AA	75 (68.2)	99 (61.9)	0.51	1	1	0.47
		AG	31 (28.2)	52 (32.5)		1.27 (0.74–2.17)	1.4 (0.8–2.47)	
		GG	4 (3.6)	9 (5.6)		1.7 (0.51–5.75)	1.37 (0.39–4.8)	
	Dominant	AA	75 (68.2)	99 (61.9)	0.29	1	1	0.22
		AG + GG	35 (31.8)	61 (38.1)		1.32 (0.79–2.2)	1.4 (0.82–2.39)	
	Recessive	AA + AG	106 (96.4)	151 (94.4)	0.45	1	1	0.74
T2	Co-dominant	GG	73 (66.4)	97 (60.6)	0.41	1	1	0.47
		GA	33 (30)	52 (32.5)		1.19 (0.7–2.02)	1.3 (0.74–2.27)	
		AA	4 (3.6)	11 (6.9)		2.07 (0.63–6.76)	1.75 (0.52–5.92)	
	Dominant	GG	73 (66.4)	97 (60.6)	0.34	1	1	0.26
		GA + AA	37 (33.6)	63 (39.4)		1.28 (0.77–2.13)	1.36 (0.8–2.31)	
	Recessive	GG + GA	106 (96.4)	149 (93.1)	0.24	1	1	0.42
T + 1	Co-dominant	GG	74 (67.3)	95 (59.4)	0.39	1	1	0.45
		GA	32 (29.1)	56 (35)		1.36 (0.8–2.32)	1.41 (0.81–2.45)	
		AA	4 (3.6)	5 (5.6)		1.75 (0.52–5.92)	1.38 (0.39–4.86)	
	Dominant	GG	74 (67.3)	95 (59.4)	0.19	1	1	0.21
		GA + AA	36 (32.7)	65 (40.6)		1.41 (0.85–2.34)	1.41 (0.83–2.39)	
	Recessive	GG + GA	106 (96.4)	151 (94.4)	0.45	1	1	0.74
V4	Co-dominant	CC	75 (68.2)	101 (63.1)	0.2	1	1	0.19
		CG	28 (25.4)	54 (33.8)		1.43 (0.83–2.47)	1.54 (0.87–2.74)	
		GG	7 (6.4)	5 (3.1)		0.53 (0.16–1.74)	0.6 (0.17–2.06)	
	Dominant	CC	75 (68.2)	101 (63.1)	0.39	1	1	0.27
		CG + GG	35 (31.8)	59 (36.9)		1.25 (0.75–2.09)	1.36 (0.79–2.33)	
	Recessive	CC + CG	103 (93.6)	155 (96.9)	0.21	1	1	0.29
S1	Co-dominant	GG	99 (90)	141 (88.1)	0.56	1	1	0.4
		GA	11 (10)	18 (11.2)		1.15 (0.52–2.54)	1.14 (0.5–2.62)	
		AA	0 (0)	1 (0.6)		NA (0–NA)	NA (0–NA)	
	Dominant	GG	99 (90)	141 (88.1)	0.63	1	1	0.6
		GA + AA	11 (10)	19 (11.9)		1.21 (0.55–2.66)	1.24 (0.55–2.83)	
	Recessive	GG + GA	110 (100)	159 (99.4)	0.31	1	1	0.19
S2	Co-dominant	CC	52 (47.3)	53 (33.1)	0.065	1	1	0.072
		CG	45 (40.9)	83 (51.9)		1.81 (1.07–3.07)	1.86 (1.07–3.24)	
		GG	13 (11.8)	24 (15)		1.81 (0.83–3.93)	1.76 (0.78–3.96)	
	Dominant	CC	52 (47.3)	53 (33.1)	0.02	1	1	0.022
		CG + GG	58 (52.7)	107 (66.9)		1.81 (1.1–2.98)	1.84 (1.09–3.10)	
	Recessive	CC + CG	97 (88.2)	136 (85)	0.45	1	1	0.55
F + 1	Co-dominant	TT	50 (45.5)	48 (30)	0.03	1	1	0.042
		TC	45 (40.9)	81 (50.6)		1.87 (1.09–3.21)	1.94 (1.10–3.41)	
		CC	15 (13.6)	31 (1.4)		2.15 (1.03–4.48)	2.05 (0.96–4.42)	
	Dominant	TT	50 (45.5)	48 (30)	0.01	1	1	0.012
		TC + CC	60 (54.5)	112 (70)		1.94 (1.17–3.22)	1.97 (1.16–3.35)	
	Recessive	TT + TC	95 (86.4)	129 (80.6)	0.21	1	1	0.32
Q – 1	Co-dominant	CC	15 (13.6)	31 (19.4)	0.044	1.52 (0.78–2.98)	1.42 (0.71–2.86)	0.06
		TT	84 (76.4)	110 (68.8)		1	1	
		CT	25 (22.7)	40 (25)		1.22 (0.69–2.17)	1.17 (0.64–2.13)	
	Dominant	CC	1 (0.9)	10 (6.2)	0.17	7.64 (0.96–60.81)	7.67 (0.92–64.01)	0.24
		TT	84 (76.4)	110 (68.8)		1	1	
	Recessive	CT + CC	26 (23.6)	50 (31.2)	0.016	1.47 (0.85–2.55)	1.41 (0.79–2.51)	0.021
		TT + CT	109 (99.1)	150 (93.8)		1	1	
		CC	1 (0.9)	10 (6.2)		7.27 (0.92–57.59)	7.39 (0.89–61.33)	

CI, confidence interval.

TABLE 3B. GENOTYPE FREQUENCY DISTRIBUTION AND ASSOCIATION STUDY BETWEEN ASTHMA AND CONTROL IN CHILDREN

	<i>Model</i>	<i>Genotype</i>	<i>Frequency: No. (%)</i>		<i>Crude analysis</i>		<i>Adjusted by age and sex</i>	
			<i>Control (n = 222)</i>	<i>Asthma (n = 160)</i>	<i>p-Value</i>	<i>Odds ratio (95% CI)</i>	<i>Odds ratio (95% CI)</i>	<i>p-Value</i>
T1	Co-dominant	AA	77 (67)	69 (64.5)	0.93	1	1	0.87
		AG	33 (28.7)	33 (30.8)		1.12 (0.62–2)	0.95 (0.43–2.09)	
		GG	5 (4.3)	5 (4.7)		1.12 (0.31–4.02)	1.47 (0.3–7.17)	
	Dominant	AA	77 (67)	69 (64.5)	0.7	1	1	0.96
		AG + GG	38 (33)	38 (35.5)		1.12 (0.64–1.94)	1.02 (0.48–2.14)	
	Recessive	AA + AG	110 (95.7)	102 (95.3)	0.91	1	1	0.61
T2	Co-dominant	GG	70 (60.9)	66 (61.7)	0.88	1	1	0.54
		GA	40 (34.8)	35 (32.7)		0.93 (0.53–1.63)	0.79 (0.37–1.7)	
		AA	5 (4.3)	6 (5.6)		1.27 (0.37–4.37)	1.85 (0.41–8.35)	
	Dominant	GG	70 (60.9)	66 (61.7)	0.9	1	1	0.78
		GA + AA	45 (39.1)	41 (38.3)		0.97 (0.56–1.66)	0.9 (0.44–1.86)	
	Recessive	GG + GA	110 (95.7)	101 (94.4)	0.67	1	1	0.35
T + 1	Co-dominant	AA	5 (4.3)	6 (5.6)	0.92	1.31 (0.39–4.41)	2.02 (0.46–8.81)	0.83
		GG	76 (66.1)	68 (63.5)		1	1	
		GA	33 (28.7)	33 (30.8)		1.12 (0.62–2)	0.95 (0.43–2.08)	
	Dominant	AA	6 (5.2)	6 (5.6)	0.69	1.12 (0.34–3.63)	1.5 (0.37–6.08)	0.93
		GG	76 (66.1)	68 (63.5)		1	1	
	Recessive	GA + AA	39 (33.9)	39 (36.5)	0.9	1.12 (0.64–1.94)	1.03 (0.5–2.15)	0.55
V4	Co-dominant	GG + GA	109 (94.8)	101 (94.4)	0.9	1	1	0.55
		AA	6 (5.2)	6 (5.6)		1.08 (0.34–3.45)	1.52 (0.38–6.06)	
		CC	72 (62.6)	58 (54.2)		1	1	
	Dominant	CG	40 (34.8)	32 (29.9)	0.002*	0.99 (0.56–1.77)	0.92 (0.44–1.94)	0.02
		GG	3 (2.6)	17 (15.9)		7.03 (1.97–25.18)	7.51 (1.4–40.24)	
		CC	72 (62.6)	58 (54.2)		1	1	
S1	Co-dominant	CG + GG	43 (37.4)	49 (45.8)	0.2	1.41 (0.83–2.42)	1.29 (0.65–2.59)	0.46
		CC + CG	112 (97.4)	90 (84.1)		1	1	
		GG	3 (2.6)	17 (15.9)		7.05 (2–24.82)	7.73 (1.48–40.48)	
	Dominant	CC	72 (62.6)	58 (54.2)	<0.001*	1	1	0.005
		CG + GG	43 (37.4)	49 (45.8)		1.41 (0.83–2.42)	1.29 (0.65–2.59)	
		CC + CG	112 (97.4)	90 (84.1)		1	1	
S2	Co-dominant	GG	3 (2.6)	17 (15.9)	0.25	1	1	0.35
		GA	15 (13)	20 (18.7)		1.53 (0.74–3.18)	1.54 (0.62–3.81)	
		CC	72 (62.6)	58 (54.2)		1	1	
	Dominant	CG	40 (34.8)	32 (29.9)	0.99	0.96 (0.53–1.72)	0.7 (0.32–1.51)	0.47
		GG	19 (16.8)	18 (16.8)		1 (0.46–2.18)	1.17 (0.42–3.22)	
		CC	72 (62.6)	58 (54.2)		1	1	
Q – 1	Co-dominant	CG + GG	43 (37.4)	49 (45.8)	0.91	0.97 (0.56–1.68)	0.79 (0.38–1.65)	0.54
		CC + CG	96 (83.5)	89 (83.2)		1	1	
		GG	19 (16.5)	18 (16.8)		1.02 (0.5–2.07)	1.45 (0.59–3.58)	
	Dominant	GG	75 (65.2)	65 (60.8)	0.22	1	1	0.05
		GA	34 (29.6)	40 (37.4)		1.36 (0.77–2.39)	1.4 (0.66–2.97)	
		AA	6 (5.2)	2 (1.9)		0.38 (0.08–1.97)	0 (0–Inf)	
Q – 1	Co-dominant	GG	75 (65.2)	65 (60.8)	0.49	1	1	0.66
		GA + AA	40 (34.8)	42 (39.2)		1.21 (0.7–2.09)	1.18 (0.57–2.45)	
		GG + GA	109 (94.8)	105 (98.1)		1	1	
	Recessive	AA	6 (5.2)	2 (1.9)	0.17	0.35 (0.07–1.75)	0 (0–Inf)	0.02
		GG	75 (65.2)	65 (60.8)		1	1	
		GA + AA	40 (34.8)	42 (39.2)		1.21 (0.7–2.09)	1.18 (0.57–2.45)	

*Significant $p < 0.05$.

after correction against gender and age with p values of 0.02 and 0.022, respectively (Table 3B). The S2 genotype in the dominant model was very close to the significance both before and after correction against gender and age.

In terms of haplotypes, 2 haplotypes GAACGGCG and AGGGGCCG exhibited significant association with asthma ($p < 0.05$) before correcting against gender and age (Table 5B). However, and after correcting against gender and age, only AGGGGCCG kept the significant association.

Discussion

In our previous publication, we reported a strong association between ADAM33 SNP genotypes and allergic rhinitis among Jordanians (Zihlif *et al.*, 2013). Similar SNPs were tested in adults, and only F + 1 was excluded in children because of the low amount of DNA in some samples. In the single-locus analyses of adult asthma risk, the allele frequencies of the eight SNPs were not significantly different

TABLE 4A. PAIRWISE LINKAGE DISEQUILIBRIA (D') OF ADAM33 SEVEN SINGLE NUCLEOTIDE POLYMORPHISMS IN ADULTS

	<i>T2</i>	<i>T+1</i>	<i>S1</i>	<i>S2</i>	<i>V4</i>	<i>F+1</i>	<i>Q-1</i>
T1	0.9997	0.9883	0.9989	0.9941	0.9996	0.904	0.9978
T2		0.955	0.9982	0.9944	0.9328	0.9101	0.9979
T+1			0.9983	0.9944	0.966	0.8721	0.9037
S1				0.6752	0.2484	0.24	0.1413
S2					0.8314	0.805	0.9135
V4						0.8858	0.9765
F+1							0.9466
Q-1							

TABLE 4B. PAIRWISE LINKAGE DISEQUILIBRIA (D') OF ADAM33 SEVEN SINGLE NUCLEOTIDE POLYMORPHISMS IN CHILDREN

	<i>T2</i>	<i>T+1</i>	<i>S1</i>	<i>S2</i>	<i>V4</i>	<i>Q-1</i>
T1	0.9848	0.9853	0.5414	0.5919	0.9758	0.7542
T2		0.9854	0.5511	0.9707	0.8712	0.8167
T+1			0.5584	0.7292	0.9312	0.8174
S1				0.5034	0.0097	0.1871
S2					0.7628	0.6598
V4						0.9515
Q-1						

TABLE 5A. HAPLOTYPES ASSOCIATED WITH ASTHMA IN ADULTS

<i>Haplotype</i>	<i>Crude analysis (n=270)</i>			<i>Adjusted analysis by sex and age (n=269)</i>		
	<i>Frequency</i>	<i>Odds ratio 95% CI</i>	<i>p-Value</i>	<i>Frequency</i>	<i>Odds ratio 95% CI</i>	<i>p-Value</i>
AGGCGCTG	0.4367	1	—	0.4347	1	—
AGGGGCTG	0.1263	1.08 (0.6–1.95)	0.79	0.1268	1.11 (0.98–2.74)	0.73
AGGCGGCA	0.0862	1.97 (0.97–4.04)	0.063	0.086	1.75 (0.83–3.69)	0.14
GAACGGCG	0.1874	1.66 (1.02–2.7)	0.042*	0.1885	1.64 (0.98–2.74)	0.059
AGGGAGCA	0.0432	1.71 (0.69–4.25)	0.25	0.0434	1.92 (0.76–4.87)	0.17
AGGCGCCG	0.0296	1.10 (0.39–3.09)	0.86	0.0297	1.09 (0.38–3.13)	0.87
AGGGGCCG	0.0118	1.66 (0.25–11.16)	0.6	0.0119	1.34 (0.2–9.12)	0.76
GAACGGTG	0.0091	0 (NA–NA)	1	0.0088	0 (NA–NA)	1
AGGCAGCA	0.0097	0.72 (0.09–5.62)	0.75	0.0098	0.6 (0.08–4.73)	0.62

Haplotypes sequence such as T1, T2, T+1, V4, S1, S2, F+1, and Q-1.

*Significant $p < 0.05$.

NA, not applicable.

TABLE 5B. HAPLOTYPES ASSOCIATED WITH ASTHMA IN CHILDREN

<i>Haplotype</i>	<i>Crude analysis (n=222)</i>			<i>Adjusted analysis by sex and age (n=200)</i>		
	<i>Frequency</i>	<i>Odds ratio 95% CI</i>	<i>p-Value</i>	<i>Frequency</i>	<i>Odds ratio 95% CI</i>	<i>p-Value</i>
AGGCGCG	0.4364	1	—	0.4587	1	—
GAACGGG	0.154	1.06 (0.61–1.83)	0.84	0.1528	1.19 (0.57–2.49)	0.64
AGGGGCG	0.1243	1.69 (0.9–3.15)	0.1	0.1128	1.29 (0.55–3.01)	0.56
AGGCGGA	0.0834	0.83 (0.35–1.96)	0.67	0.0812	0.66 (0.21–2.09)	0.48
AGGGGGA	0.0435	1.12 (0.4–3.11)	0.83	0.0343	0.59 (0.08–4.06)	0.59
AGGGAGA	0.031	2.12 (0.54–8.43)	0.29	0.0336	2.22 (0.48–10.32)	0.31
AGGCAGA	0.0245	1.79 (0.45–7.1)	0.41	0.0178	1.37 (0.22–8.56)	0.74
GAAGGGG	0.0189	69,528 (69,527–69,530)	<0.0001*	0.0216	3.3e+7 (3.2e+7–3.2e+7)	<0.0001*

Haplotypes sequence such as T1, T2, T+1, V4, S1, S2, and Q-1.

*Significant $p < 0.05$.

between the asthmatic and control subjects ($p > 0.05$). At the genotype level, the S2, F+1, and Q-1 genotypes showed a significant association ($p < 0.05$) with asthma risk in at least one of the three analysis models used in this study. The strongest association was found in the F+1 SNP case, in which the association was observed in the co-dominant and dominant models. Interestingly, the association between F+1 and asthma has been reported in many populations, including Chinese, Northern Indian, German, and Icelandic populations (Werner *et al.*, 2004; Blakey *et al.*, 2005; Awasthi *et al.*, 2011; Jie *et al.*, 2011). Furthermore, the meta-analysis performed by Liang *et al.* (2013) confirmed such associations. In this meta-analysis, the association between F+1 SNP and asthma was stronger in the Asian populations. The F+1 locus SNP was also found to be associated with lung function loss in early life at both 3 and 5 years of age (Simpson *et al.*, 2005).

In the Q-1 case, the association was significant in the co-dominant and recessive models. Clearly, the minor allele genotype of CC was more abundant in asthmatic patients, scoring 6.2%, while in the control patients it scored only 0.9%. This significant association between Q-1 and the risk of asthma was previously reported in the Chinese Han population and was associated with childhood asthma in Caucasians (Van Eerdewegh *et al.*, 2002; Jie *et al.*, 2011). Importantly, van Diemen *et al.* (2005) demonstrated that individuals homozygous for minor alleles of SNPs Q-1 (CC) had a significantly accelerated decline in FEV₁ of 9.6 mL/year compared with the wild type (van Diemen *et al.*, 2005). Q-1 SNP is the intrinsic region and studies suspected that Q-1 may influence the splicing of *ADAM33* to produce a variant protein (van Diemen *et al.*, 2005). One possible explanation for the role of Q-1 SNP in asthma has been proposed by van Diemen *et al.* (2005), who proposed that the close location of Q-1 to exons Q, P, and R, the site that comprises the epidermal growth factor (EGF) domain, may affect the EGF signaling that is known to be important to lung morphogenesis (van Diemen *et al.*, 2005).

The genotype of SNP S2 was also found to be associated with the incidence of asthma. This association was found in the dominant model with a p value of 0.02 and a tendency toward association in the co-dominant model with a p value of 0.065. S2 has been associated with asthma in British, black Americans, white Americans, Hispanic Americans, and Thai populations (Howard *et al.*, 2003; Hongngarm *et al.*, 2008). The S2 SNP is a silent mutation, and a biological explanation for the effect of this SNP is that it may be in linkage disequilibrium with the true causative SNP.

A comparison of the findings in adult samples with the outcome of our allergic Rhinitis study reveals some similarities and some differences (Zihlif *et al.*, 2013). Importantly, the Q-1, F+1, and S2-positive association is common between both studies. Interestingly, Q-1 minor allele genotype of CC has shown a very close p -value of 0.02 and a close odds ratios of 7.27, which, indeed, may both indicate that this genotype has an important contribution to the incidence of diseases and re-enforce the suggestion that those two diseases have a similar pathophysiological origin. In contrast, the reported linkage between T1, T2, and V4 genotype and allergic rhinitis are not seen in this study.

In children, we identified a strong association between V4 genotype and incidence of asthma. This association was clear in both co-dominant and recessive models in which the

p values were 0.002 and < 0.001 before multiple-variation correction and 0.02 and 0.005 after multiple-variation correction, respectively. Interestingly, the CC homozygous mutant genotype frequency was significantly higher in asthmatic subjects (15.9%) than in control subjects (2.6%), resulting in an odds ratio of 7.03 and 7.05 before multiple-variation correction and 7.53 and 7.73 after multiple-variation correction in the co-dominant and recessive models, respectively. Furthermore, in the single-locus analyses of asthma risk, V4 allelic frequency showed a tendency toward significance with $p = 0.07$. The V4 was found to be significantly associated with asthmatic children in United States/United Kingdom combined, United Kingdom-only, Dutch White, and Chinese Han populations (Van Eerdewegh *et al.*, 2002; Howard *et al.*, 2003; van Diemen *et al.*, 2005). More recently, V4 homozygous mutant genotypes were also found to be associated with asthma risk in Indian children and north Chinese children (Su *et al.*, 2008; Qu *et al.*, 2011). The V4 SNP is located in the 3'UTR of *ADAM33* and suspected to have an effect on the transcription of *ADAM33* (van Diemen *et al.*, 2005).

Importantly, the strong V4 SNP association with asthma reported in this study was not found in adults. It is also important to clarify that the association between the V4 genotype and allergic rhinitis risk reported by us among Jordanians was found with the heterozygote genotype (CG) and not with the homozygous mutant genotype (CC) (Zihlif *et al.*, 2013). Furthermore, the frequency of the minor allele in the three patient groups, namely asthmatic, allergic rhinitis, and childhood asthmatic groups, scores 18%, 20%, and 31%, respectively.

The other SNPs tested in this study were not found to be significantly associated with asthma incidence in children except for the Q-1, which only showed an association after the multi-variant correction in the recessive model. A larger size study may reveal whether this significant association is real or not. The haplotype analysis outcome suggests that the GAACGGTG and GAACGGCG haplotypes may be risk factors for childhood asthma with p values of 0.043 and 0.0001.

In conclusion, the finding of this study confirms the already reported association between the *ADAM33* gene and the incidence of asthma in different populations. Similar to our previous study on the allergic rhinitis, Q-1, F+1, and S'2 showed positive associations with the risk of asthma in adults. In children, a strong association between the V4 SNP in the *ADAM33* gene and the incidence of childhood asthma was found. The differences in the frequency and in the associations with the incidence of asthma and/or allergic rhinitis point out the heterogeneity between the role of *ADAM33* in the pathophysiology of asthma in children and adults on the one hand and the heterogeneity of such a role between asthma and allergic rhinitis on the other hand.

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Author Disclosure Statement

The authors have no conflicts of interest.

References

- Awasthi S, Tripathi P, Ganesh S, Husain N (2011) Association of ADAM33 gene polymorphisms with asthma in Indian children. *J Hum Genet* 56:188–195.
- Blakey J, Halapi E, Bjornsdottir US, *et al.* (2005) Contribution of ADAM33 polymorphisms to the population risk of asthma. *Thorax* 60:274–276.
- Cheng L, Enomoto T, Hirota T, *et al.* (2004) Polymorphisms in ADAM33 are associated with allergic rhinitis due to Japanese cedar pollen. *Clin Exp Allergy* 34:1192–1201.
- Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: an integrated software package for population genetics data analysis. *Evol Bioinform Online* 1:47–50.
- Guo S, Thompson E (1992) Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics* 48:361–372.
- Hongngarm T, Jameekornrak A, Limwongse C, *et al.* (2008) Association between ADAM33 polymorphisms and asthma in a Thai population. *Asian Pac J Allergy Immunol* 26:205–211.
- Howard TD, Postma DS, Jongepier H, *et al.* (2003) Association of a disintegrin and metalloprotease 33 (ADAM33) gene with Asthma in ethnically diverse populations. *J Allergy Clin Immunol* 112:717–722.
- Jie Z, Hu Z, Bai C, Jin M (2011) ADAM33 gene polymorphisms associate with asthma susceptibility and severity in East China Han population. *J Asthma* 48:979–985.
- Jongepier H, Boezen HM, Dijkstra A, *et al.* (2004) Polymorphisms of the ADAM33 gene are associated with accelerated lung function decline in asthma. *Clin Exp Allergy* 34:757–760.
- Koppelman GH (2006) Review gene by environment interaction in asthma. *Curr Allergy Asthma Rep* 6:103–111.
- Lee JH, Park HS, Park SW, *et al.* (2004) ADAM33 polymorphism: association with bronchial hyper-responsiveness in Korean asthmatics. *Clin Exp Allergy* 34:860–865.
- Lewontin RC (1963) The interaction of selection and linkage. I General considerations; heterotic models. *Genetics* 49:49–67.
- Liang S, Wei X, Gong C, *et al.* (2013) A disintegrin and metalloprotease 33 (ADAM33) gene polymorphisms and the risk of asthma: a meta-analysis. *Hum Immunol* 74:648–657.
- Lind DL, Choudry S, Ung N, *et al.* (2003) ADAM33 is not associated with asthma in Puerto Rican or Mexican populations. *Am J Respir Crit Care Med* 168:1312–1316.
- Postma DS, Howard T (2004) ADAM33 gene: confirming a gene without linkage. *Clin Exp Allergy* 34:1–5.
- Qu S, Sun D, Wang Y, *et al.* (2011) Association of ADAM33 polymorphisms with childhood asthma in a northern Chinese population. *Exp Mol Pathol* 91:775–779.
- R Development Core Team (2005) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria, ISBN 3-900051-07-0.
- Solé X, Guinó E, Valls J, *et al.* (2006) SNPStats: a web tool for the analysis of association studies. *Bioinformatics* 22:1928–1929.
- Simpson A, Maniatus N, Jury F, *et al.* (2005) Polymorphism in a disintegrin and metalloprotease 33 (ADAM 33) predict impaired early-life lung function. *Am J Respir Crit Care Med* 172:55–60.
- Su D, Zhang X, Sui H, *et al.* (2008) Association of ADAM33 gene polymorphisms with adult allergic asthma and rhinitis in a Chinese Han population. *BMC Med Genet* 9:82–87.
- Townshend J, Hails S, McKean M (2007) Diagnosis of asthma in children. *BMJ* 335:198–202.
- van Diemen CC, Postma DS, Vonk JM, *et al.* (2005) A disintegrin and metalloprotease 33 polymorphisms and lung function decline in the general population. *Am J Respir Crit Care Med* 172:329–333.
- Van Eerdewegh P, Little RD, Dupuis J, *et al.* (2002) Association of the ADAM33 gene with asthma and bronchial hyperresponsiveness. *Nature* 418:426–430.
- Wang P, Liu QJ, Li JS, *et al.* (2006) Lack of association between ADAM33 gene and asthma in a Chinese population. *Int J Immunogenet* 33:303–306.
- Werner M, Herbon N, Gohlke H, *et al.* (2004) Asthma is associated with single-nucleotide polymorphisms in ADAM33. *Clin Exp Allergy* 34:26–31.
- Zihlif M, Mahafza T, Obeidat NM, *et al.* (2013) Frequency of genetic polymorphisms of ADAM33 and their association with allergic rhinitis among Jordanians. *Gene* 531:462–466.

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