

Genetic Markers for Asthmatic Saudis



Eman Al-Abdulsalam

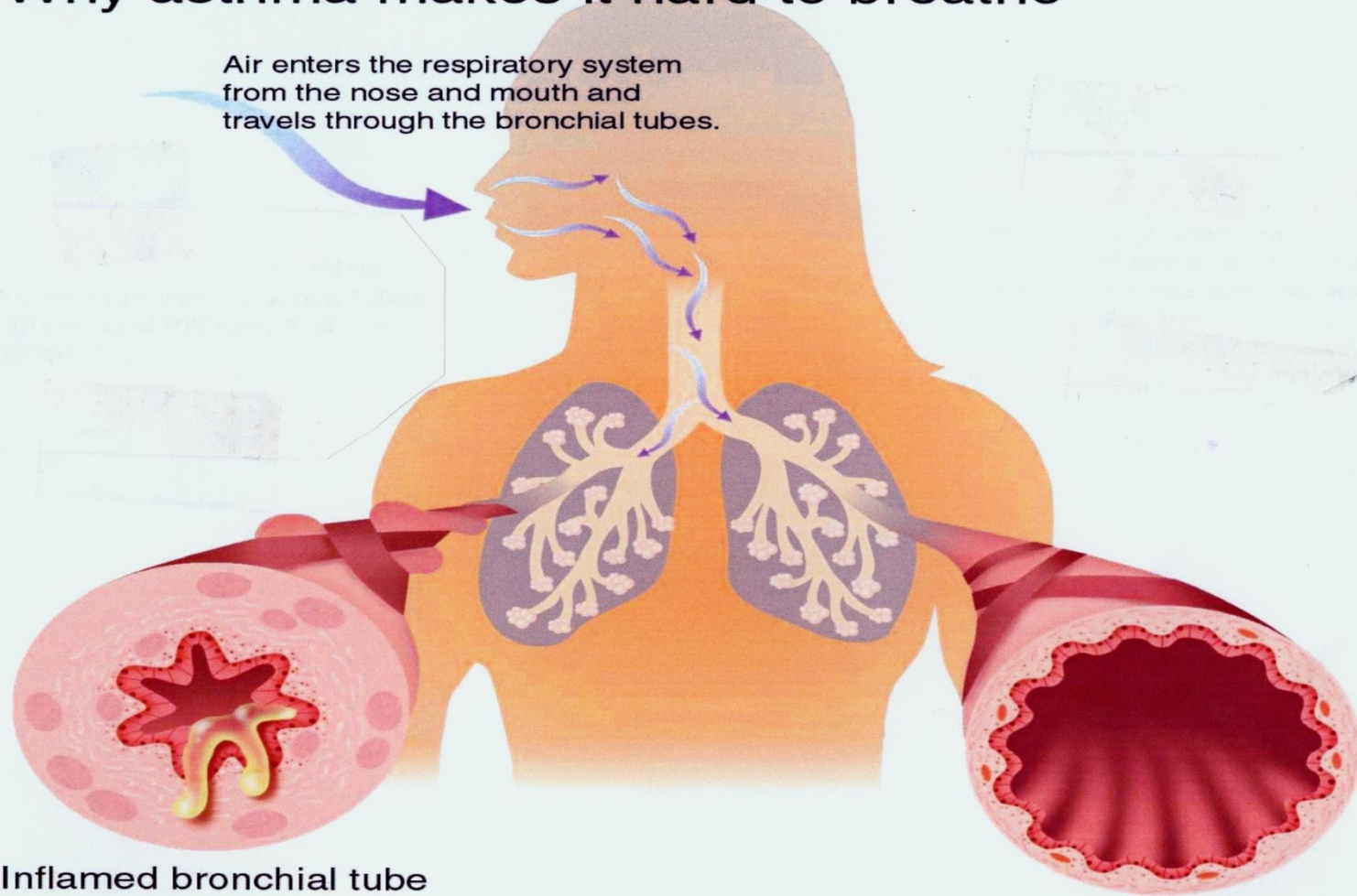


King Saud University
College of Science
Biochemistry Department

Asthma

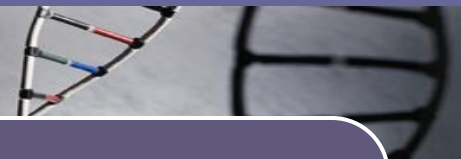
Why asthma makes it hard to breathe

Air enters the respiratory system from the nose and mouth and travels through the bronchial tubes.



**Inflamed bronchial tube
of an asthmatic**

Normal bronchial tube

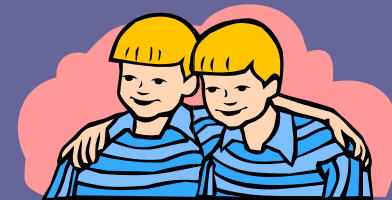


IT IS A COMMON CHRONIC DISEASE OF CHILDREN
AGED LESS THAN 5 YEARS AND IT IS THE MOST
FREQUENT CAUSE FOR THEIR HOSPITAL ADMISSIONS

Asthma as a genetic disease

Twin studies :

With asthma, a large twin study of 7000 twins was performed and showed that the concordance in monozygote was higher than dizygote



Association studies



Allele 1

5' -GTCGTACGTCAGTCCG-3'

3' -CAGCATGCAGTCAGGC-5'

Single Nucleotide Polymorphism
Allele 2

5' -GTCGTACTTCAGTCCG-3'

3' -CAGCATGAAGTCAGGC-5'





Research Aims

Common variants of three different genes (*IL13*, *MS4A2* and *IL4R α*) will be assayed in the asthmatic Saudi population to fulfill the following objectives:

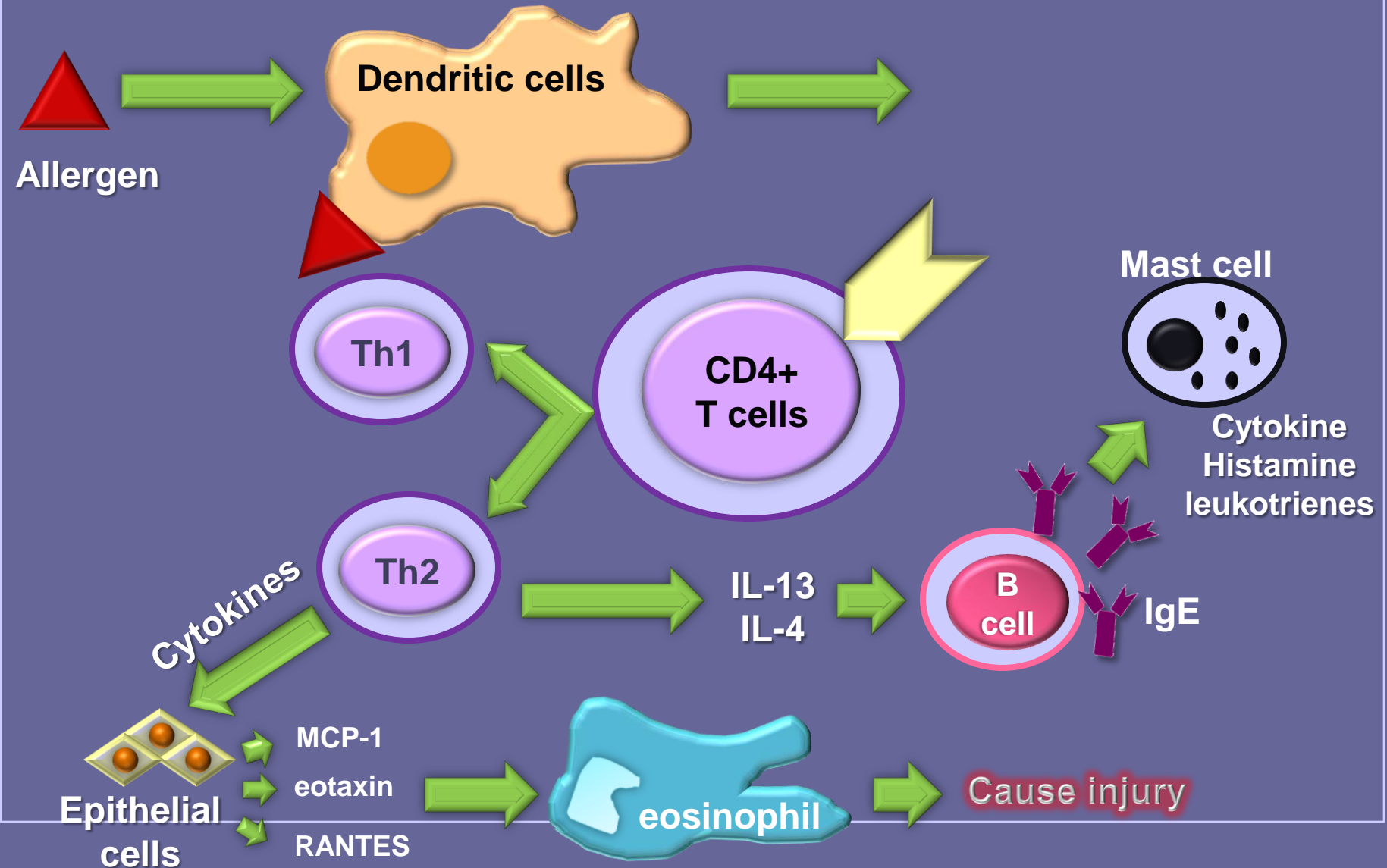
1. To determine the common variants in the asthmatic Saudis.
2. To determine allele frequencies of each variant in both asthmatic and normal Saudi individuals and compare it with that in other populations.
3. To identify genetic markers for asthma in the Saudi population.
4. To develop and optimize a high throughput assay for screening for known mutations in the Saudis.

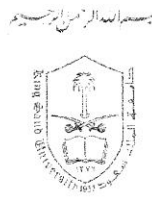


Study SNPs

Chromosome	Gene	Location	SNP	RefSNP
5q31	<i>IL13</i>	Exon 4	Arg110Gln	rs20541
		Promoter	C-1111T	rs1800925
11q13	<i>MS4A2</i>	Exon 7	Glu237Gly	rs569108
16p12.1-p11.2	<i>IL4Rα</i>	Exon 11	Gln551Arg	rs1801275
		Exon 5	Ile50Val	rs1805010

Pathogenesis of asthma





Date: 17.01.1429
26.01.2008 التاريخ: No: 08/1934/R الرقم:

PROF. MOHAMED S. AL-HAJJAJ
Head, Division of Pulmonology
Department of Medicine

Subject: Project No. 07-589

"Case control study of asthmatic patients for IL13, FceRB and IL4Ra polymorphisms in the Saudi population"

Dear Prof. Al-Hajjaj,

Your response to the ethical comments on the above-mentioned research project was reviewed in the Ethics Committee Meeting no: 4 (1428/1429) held on 14.01.1429 (23 January 2008). The Committee found your answers to be satisfactory; therefore, the study is approved from the ethical point of view.

I wish you success in your research.

Sincerely yours,


Professor Jamal S. Jarallah
Chairman, Ethics Committee

Arg144Gln
G>A and
C1111T C>T
affecting IL-13
plasma level in
the presence
of homozygous
mutant allele

Research Methodology

Blood samples
collection



- KKHU
- 100 asthmatics (50 adult and 50 pediatric)
- 100 control (50 adult and 50 pediatric)

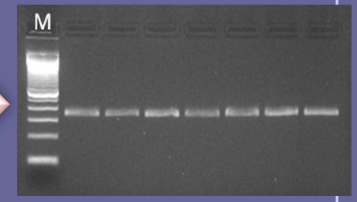
ELISA



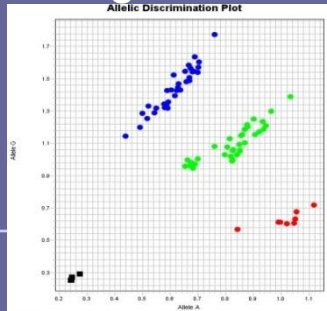
DNA Extraction



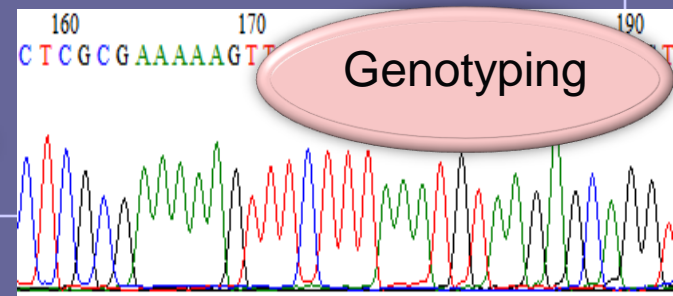
Standard PCR



Allelic Discrimination
through RT-PCR



Automated sequence



إقرار بالموافقة على المشاركة في بحث علمي:

عنوان البحث:

اسم الباحث:

اسم المشارك:

عينة دم حجم ٣ مليلتر (لاستخلاص المادة الوراثية ومحاولة البحث عن مسببات المرض الوراثي)

أوافق على المشاركة في هذا البحث العلمي بعد أن شرح لي الباحث/ الباحثة تفاصيل البحث وأهميته ونوعية مشاركتي وأجابت/ أجابت على كل تساؤلاتي. وبناء على ذلك فإنه لا مانع لدي من إعطاء عينة دم لاستخلاص المادة الوراثية واستخدامها في هذا البحث حسب النظم والقوانين في المملكة العربية السعودية. وبناء على ذلك فأبني أسمح التالي:

١ - استخدام عيناتي في هذا البحث فقط.

نعم لا

٢ - استخدام المتبقي في عيناتي في أبحاث أخرى ذات صلة في هذا البحث.

نعم لا

أقر بأنني قد درست النموذج أعلاه وأوقع على صحة البيانات السابقة.

المتطوع أو ولي الأمر:

الاسم:

التوقيع:

التاريخ:

صلة القرابة (إذا الموقع غير المريض المشارك):

الباحث أو من ينوب عنه:

أقر بأنني شرحت للمتطوع/ لقريبه/ أو ولي أمره تفاصيل البحث وأهدافه:

الاسم:

التاريخ:

التوقيع:



ASTHMA CASE REPORT FORM

STUDY NO.

HOSP. NO.

AGE SEX M F

AGE AT DIAGNOSIS

FAMILY HISTORY

1 ASTHMA YES NO NO. OF FAMILY MEMBERS AFFECTED

2 ALLERGY YES NO NO. OF FAMILY MEMBERS AFFECTED

RELATIONSHIP TO AFFECTED RELATIVE

BROTHER MATERNAL AUNT/UNCLE MATERNAL 1ST COUSIN

SISTER PATERNAL 1ST COUSIN MATERNAL 2ND COUSIN

PATERNAL AUNT/UNCLE PATERNAL 2ND COUSIN OTHERS

CLINICAL FEATURES

WHEEZE SHORTNESS OF BREATH COUGH

INVESTIGATION FINDINGS

1 BLOOD EOSINOPHILS cells/uL

2 ARTERIAL BLOOD GASES pH paO2 paCO2

3 CHEST X-RAY NORMAL HYPERINFLATED

4 PULMONARY FUNCTION TEST

VC TLC MVV

FEV1 RV DLCO

PEF SPIROMETER SCORE

MEDICATIONS

		YES	NO	DOSAGE	DURATION OF TREATMENT
1 BETA-ADRENERGIC AGONISTS	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		
1 BRONCHODILATORS	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		
1 INHALED CORTICOSTEROIDS	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		
1 ANTICHOLINERGIC AGENTS	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		
1 LEUKOTRIENE MODIFIERS	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		
1 OTHERS (pls. specify)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		

ADDITIONAL COMMENTS HERE:

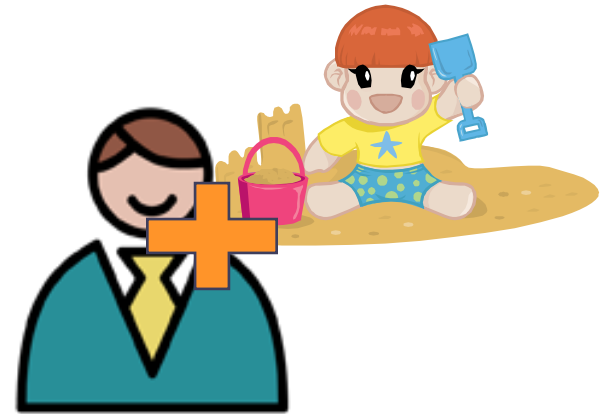
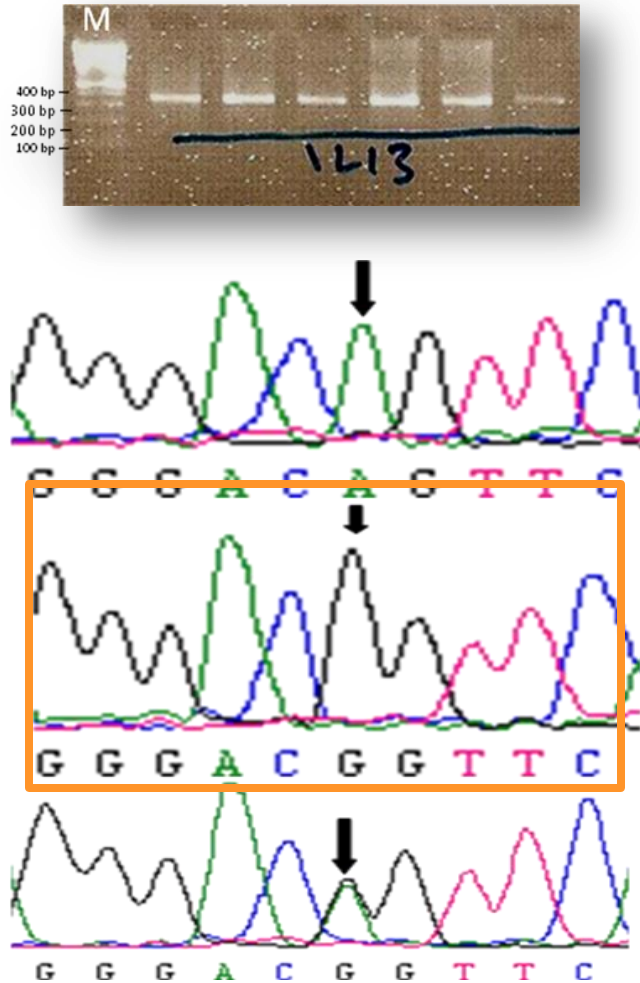


Statistical analysis

- Allele frequencies were obtained by allele counting and errors were assigned. For allele analysis, 2 x 2 tables were generated between asthmatics and non-asthmatics and between other populations using chi-square test or Fischer's exact test, 95 % CI and odds ratio.
- For ELISA calculations student un-paired *t*-test was used.
- All analysis were calculated by computerized methods using excel sheet and statistical software GraphPad InStat version 2.04 (Ralf Stahlman, Purdue Univ. 931897s). a *P*-value of <0.05 was considered statistically significant for all analysis.

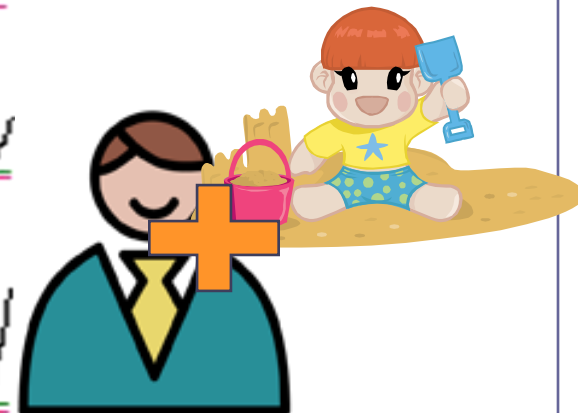
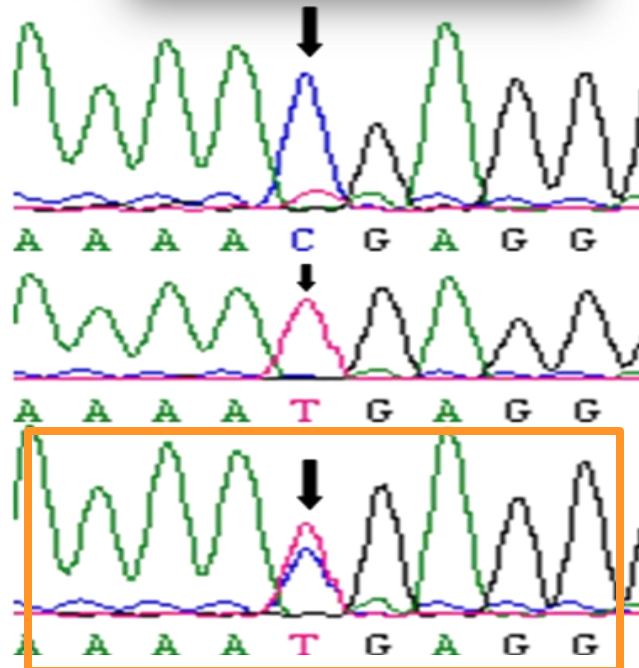
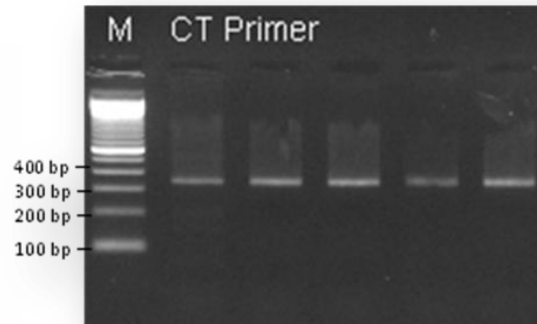
Results

Detection of the *IL13* Arg144Gln G>A variant



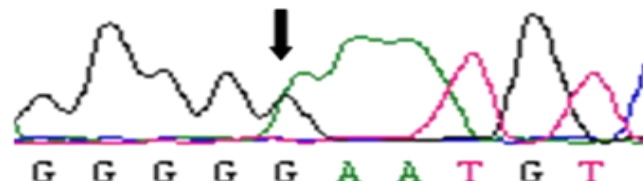
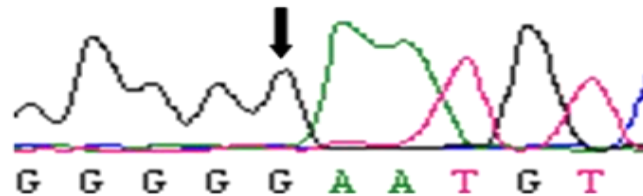
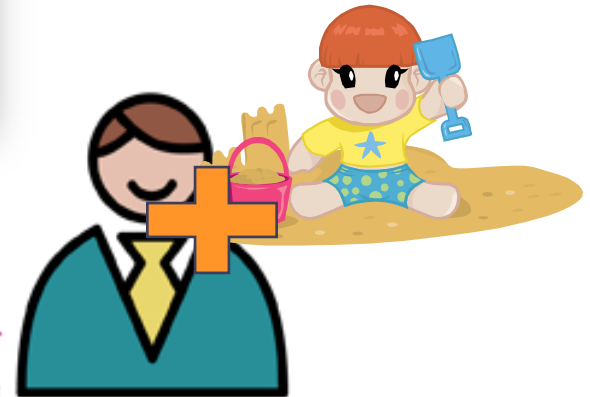
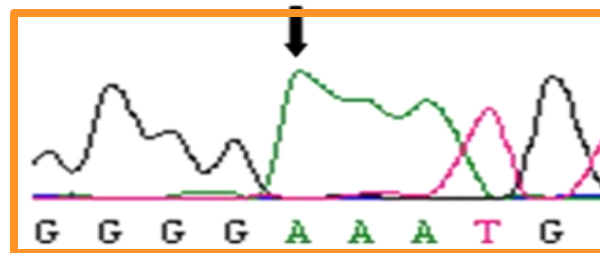
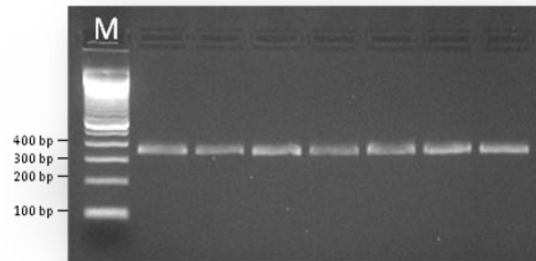
Results *Cont.*

Detection of the *IL13* C1111T C>T variant



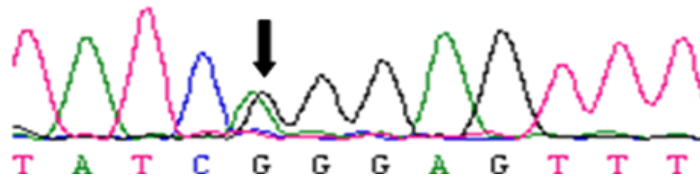
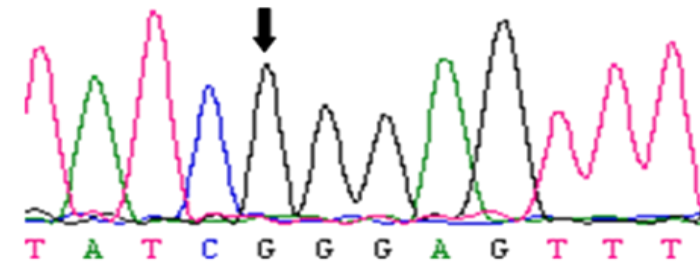
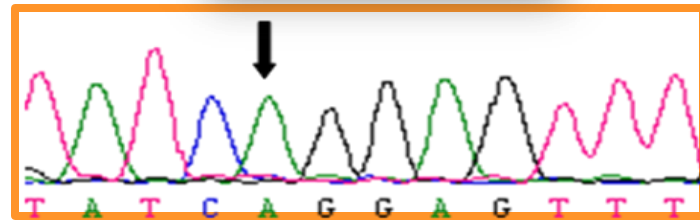
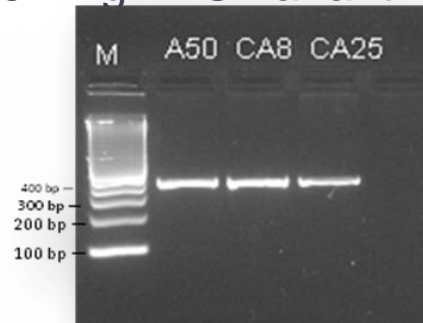
Results *Cont.*

Detection of the *MS4A2* Glu237Gly A>G variant



Results *Cont.*

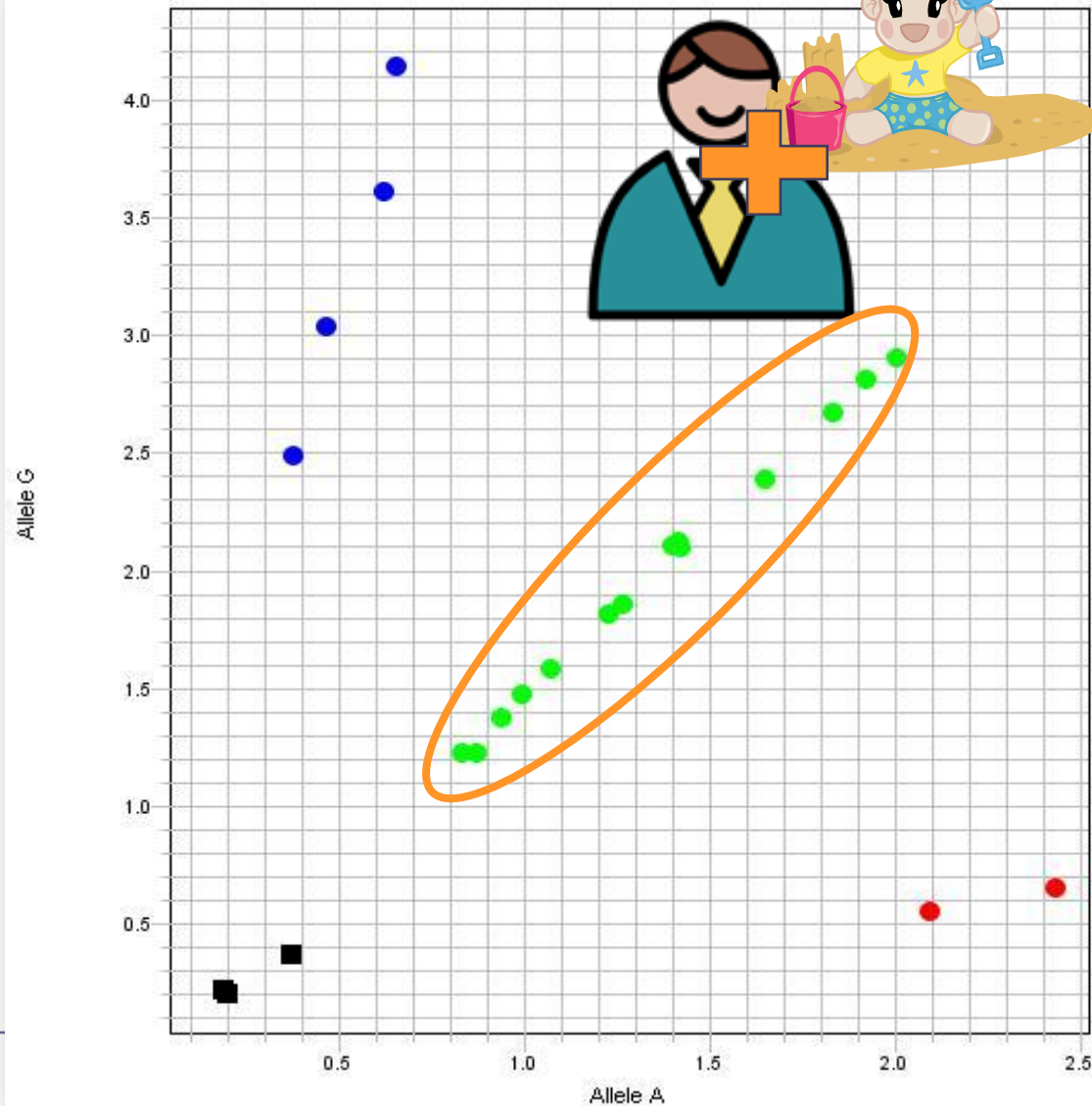
Detection of the *IL4Rα* Gln551Arg A>G variant



Results *Cont.*

Detection of the *IL4Rα* Ile50Val
A>G variant

Allelic Discrimination Plot



Results *Cont.*

Allele associations

SNP	Adult <i>Case Control</i>	Pediatric <i>Case Control</i>
<i>IL13</i> Arg110Gln	<i>p</i> value= 1	0.5530
C-1111T	<i>p</i> value= 1.1405	0.6375
<i>MS4A2</i> Glu237Gly	<i>p</i> value= 0.8056	0.5870
<i>IL4Rα</i> Gln551Arg	<i>p</i> value= 0.1239	0.0537
Ile50Val	<i>p</i> value= 0.3943	0.8857

Results *Cont.*

IL-13 Plasma Level

IL-13 pg/ml	Adult		Pediatric	
	<i>Case</i>	<i>Control</i>	<i>Case</i>	<i>Control</i>
N	50	50	49	48
Mean	3.985	6.157	12.244	10.901
<i>p</i> value	0.5869		0.7503	

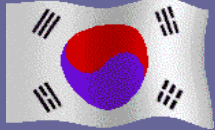
Results *Cont.*

IL-13 genotype	Adult		Pediatric	
	Case	Control	Case	Control
Arg110Gln A>G				
A/A	0		0	
G/G	0.2223		0.6005	
A/G	0.9631		0.6619	
C-1111T C>T				
C/C	0.4773		0.4162	
T/T	0		0	
C/T	0.6375		0.9987	

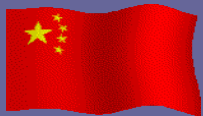
Results *Cont.*

Population analysis of study SNPs

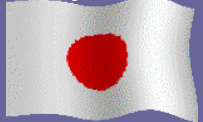
Korea



China



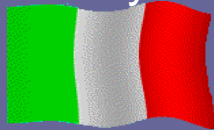
Japan



Finland



Italy



Sweden



Netherland



UK



US



Australia



Demark



Germany





Conclusion

- The five polymorphic sites investigated during this study in the Saudi adult and pediatric groups did not reveal any association with asthma development in the Saudis. Furthermore, more detailed investigation is essential.



Acknowledgments

- This study was funded by King Saud University and King Abdulaziz City for Science and Technology.
- We thank: the study subjects for their contributions in making this study possible, Prof. Al-Hajjaj, Dr. Al-Muhsen, nurses for their contribution to the recruitment of asthmatic patients, KKUH, genetic unit members at King Faisal Specialist Hospital and research center and central lab members at King Saud University for providing us with required facilities.



*Thank you for
your attention*