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جامعة الملك سعود
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Asthma Research Chair

Screening for Genetic Variants of IL-4 and ADAM33 Genes in a Sample from Asthmatic Saudi Children in Riyadh

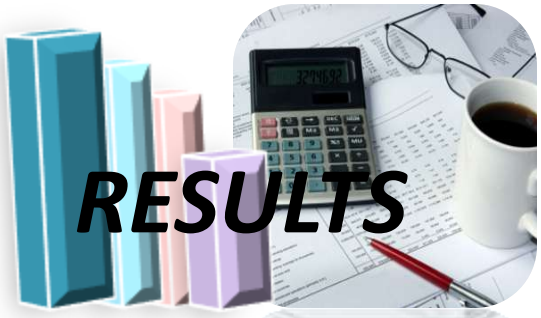
Arwa Ishaq AL-Khayyat

INTRODUCTION

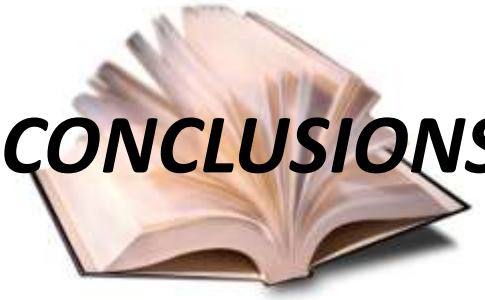
ASTHMA

GENETIC OF ASTHMA

EPIDEMIOLOGY STUDY



RESULTS



CONCLUSIONS

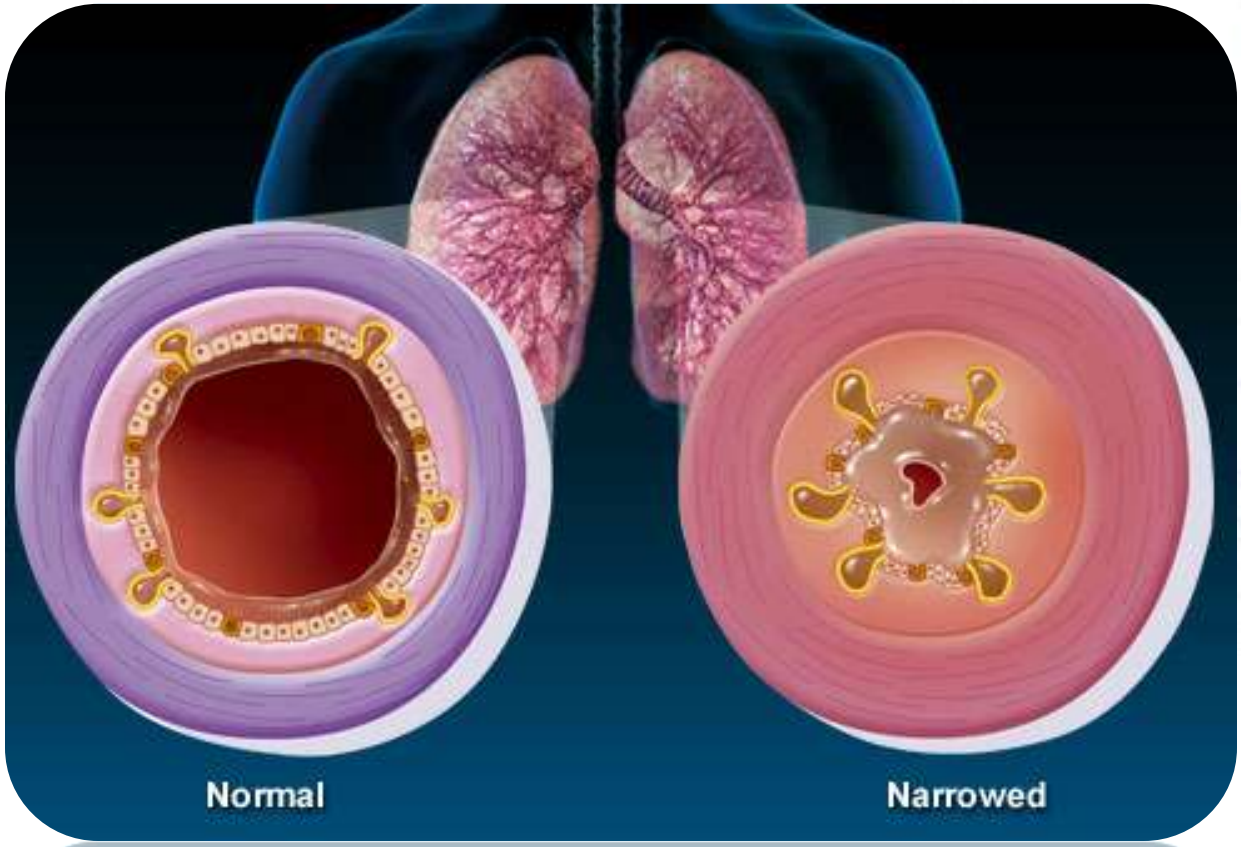
OBJECTIVES

RESEARCH PROTOCOL

INTRODUCTION

Asthma is considered as one of the most prevalent pulmonary diseases in the world.

intermittent narrowing of the small airways of the lung with subsequent reversible airflow obstruction



bronchial hyperresponsiveness (BHR)

airway inflammation

EPIDEMIOLOGY STUDY

•The epidemology studies indicate that there was a significant increase in the prevalence of asthma in the last 30 years worldwide; approximately 300 million individuals affected.

•11% (2,200,000) of Saudi Arabia population suffers from Asthma.

GENETIC OF ASTHMA

Genetic play a critical role in the development of asthma, which is considered to be a complex disease

Multifactorial

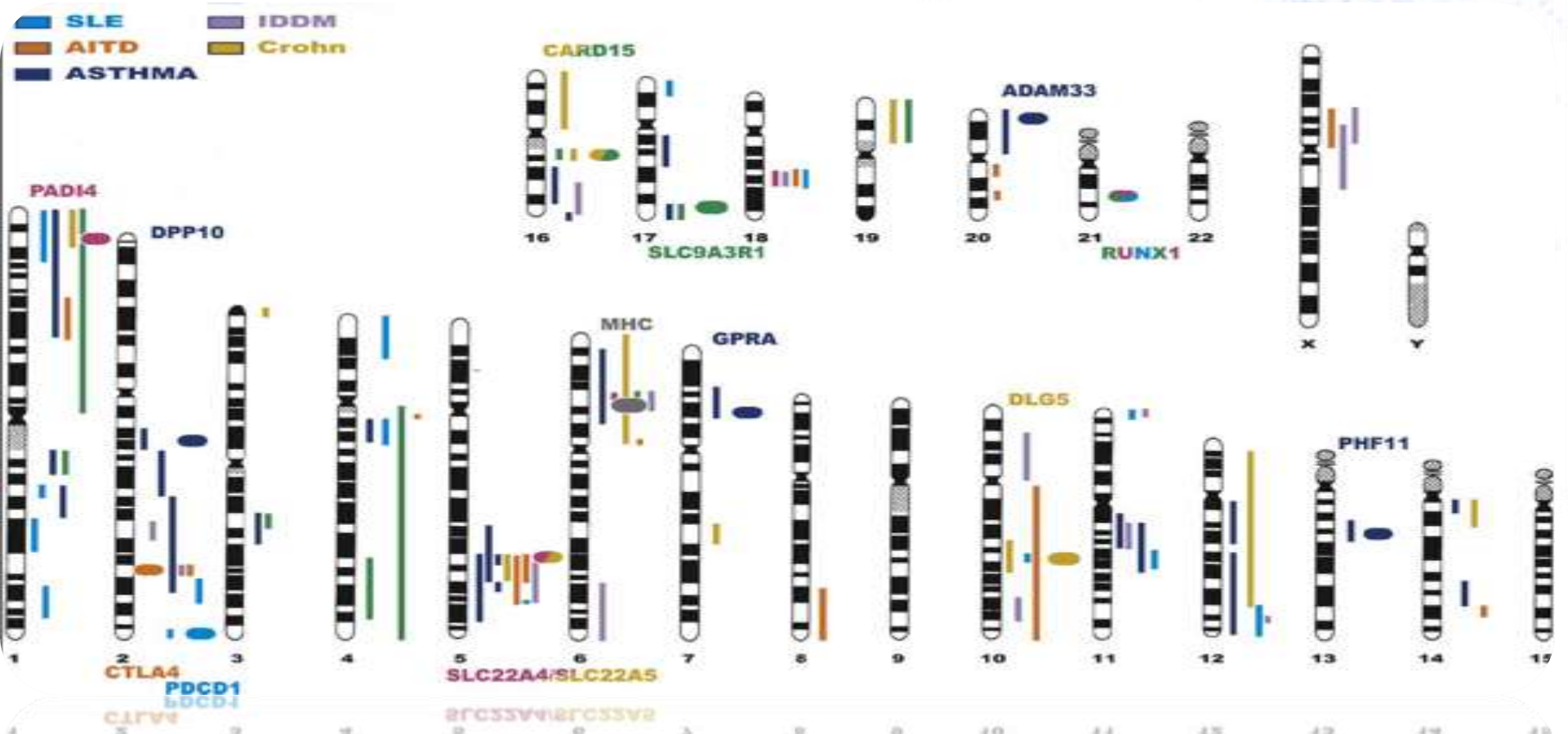
(genetics and environment)

Polygenic

GENETIC OF ASTHMA

•The genes possibly involved with asthma are many and diverse.

• Linkage studies indicated many chromosomal region that have strong relation with asthma: 2q, 5q, 6p,12q, 16q, 17q and 20p.



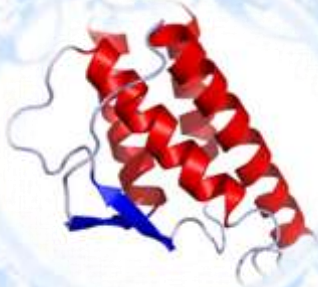
GENETIC OF ASTHMA

- Associations studies have demonstrated that over 100 variants in candidate genes are associated with asthma in different ethnic group. These include: ADAM33, IL-5, ORMDL3, IL-4 ,TNF, NOS1 and ARG2

COMMON GENES ASSOCIATION WITH ASTHMA AND ASTHMA PHENOTYPE

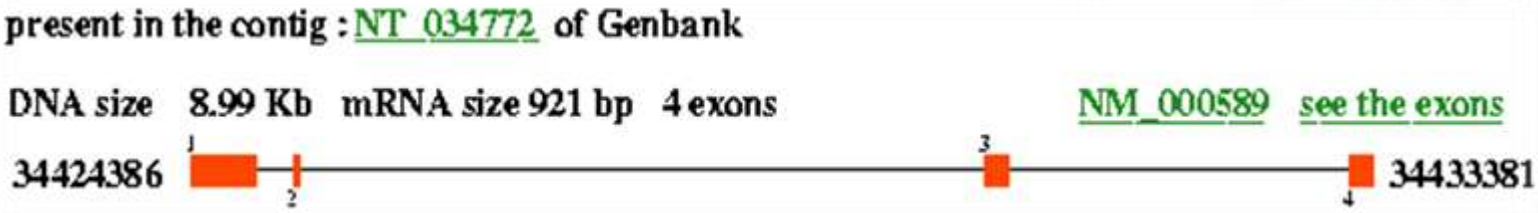
Genes	Chromosomal region	Phenotype
IL-10	1q31-q32	Asthma
CTLA4	2q33	Asthma, high IgE
IL-1 gene family	2q14	Asthma, atopy
IL-13	5q31	Asthma , high IgE
1L-4	5q31.1	High IgE,
CD14	5q31.1	High IgE, atopy
STAT6	12q13	Asthma, High IgE
LTC4s	5q35	Asthma
TNF	6p21.3	Asthma
NOD1	7p14	Asthma
ORMDL3	17	Asthma
ADAM33	20q13	Asthma

Interleukin -4 (IL-4)

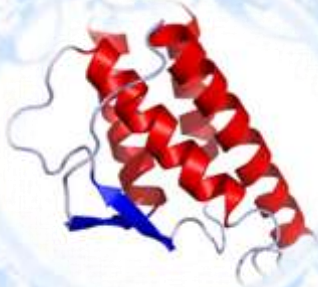


- IL4 gene has been mapped to chromosome 5q31 .1 where asthma and atopy have also been linked.

- The human IL-4 gene contains four exons and has a length of approximately 8.99 k b.



Interleukin -4 (IL-4)



- IL-4 gene encode IL-4 cytokines.

- This cytokine produced by activated T cells and mast cells which plays crucial role in the development of of allergic inflammation in asthma

Critical Role in Asthma……

1. It is associated with secretion IgE by B lymphocytes, which has a pivotal role in the development of immediate allergic reactions.

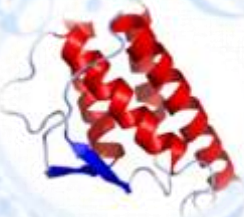
2. IL-4 contributes to airway obstruction in asthma is through the induction of mucin gene expression and the hypersecretion of mucus.

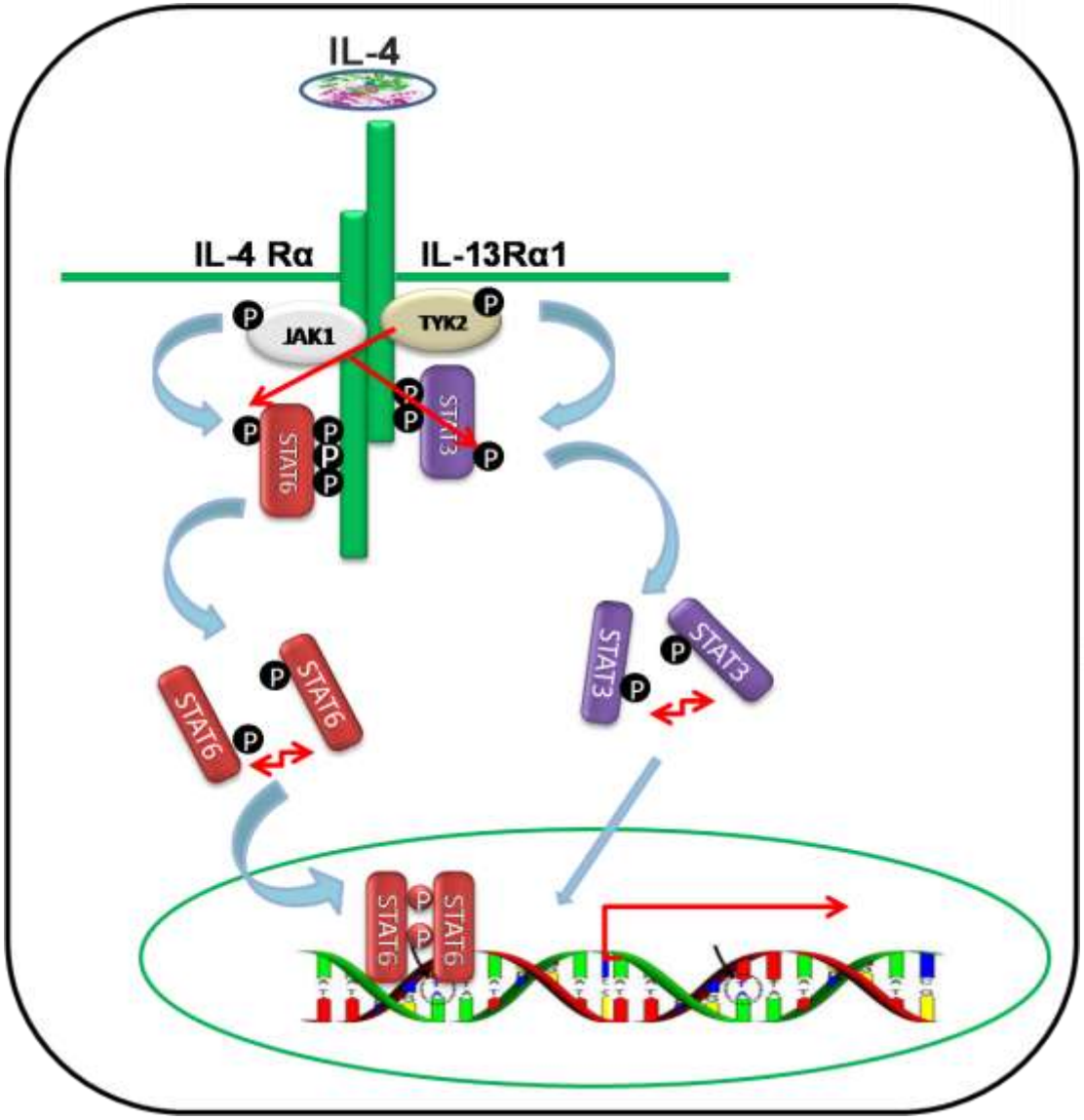
3. IL-4 increases the expression of eotaxin and other inflammatory cytokines from fibroblasts that might contribute to inflammation and lung remodeling in chronic asthma.

4. An important activity of IL-4 in promoting cellular inflammation in the asthmatic lung is the induction of vascular cell adhesion molecule (VCAM)-1 on vascular endothelium

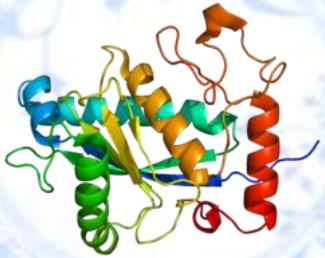
5. IL-4 inhibits eosinophil apoptosis and promotes eosinophilic inflammation by inducing eosinophil chemotaxis and activation through the increased expression of eotaxin

Interleukin -4 signaling pathway





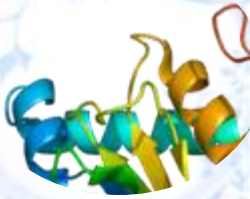
ADAM33



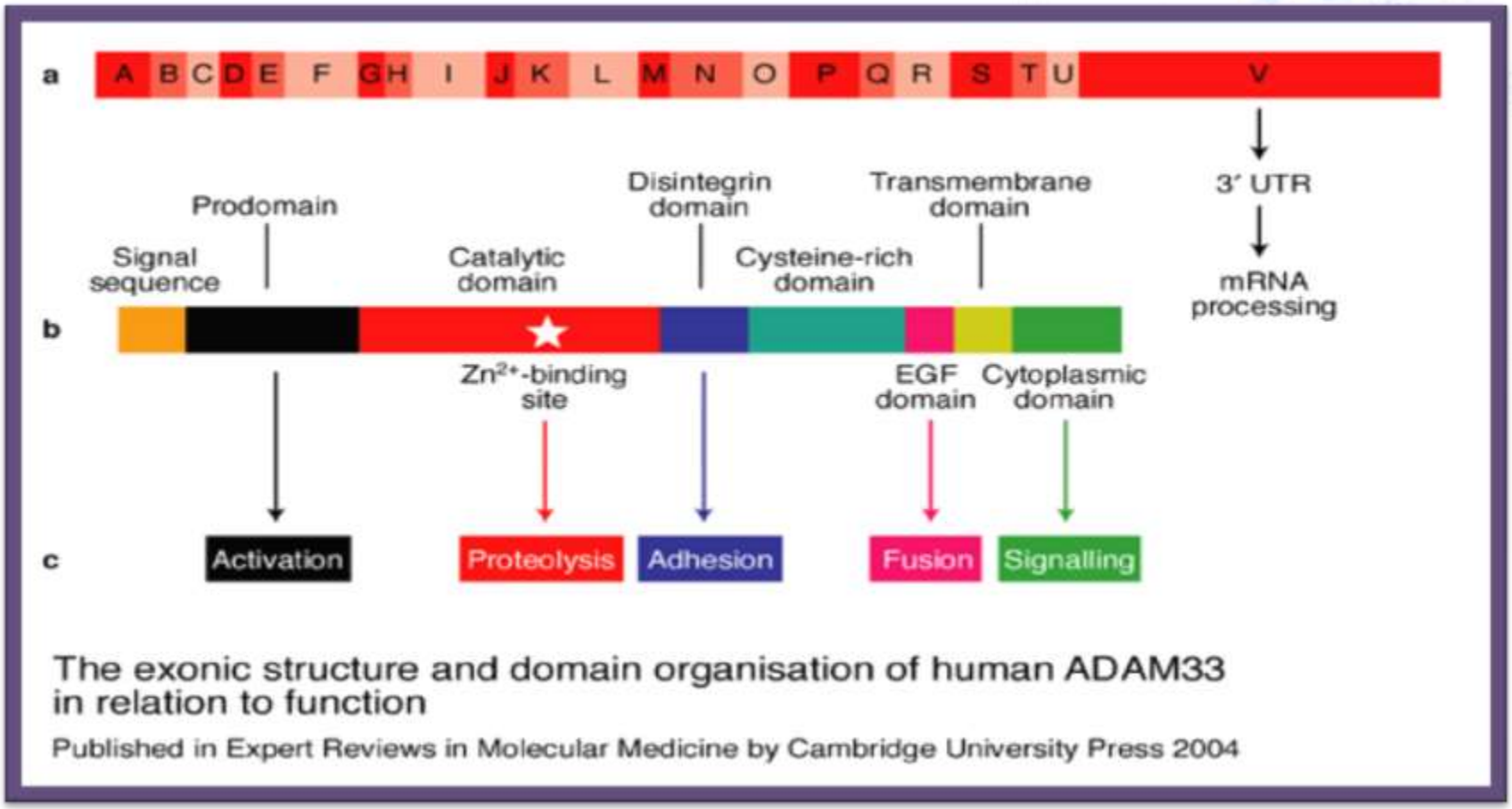
- ADAM33 gene located on chromosome 20p13.

- ADAM33 gene identify by the positional cloning as the first candidate gene for the development of asthma and bronchial hyperresponsiveness.

ADAM33



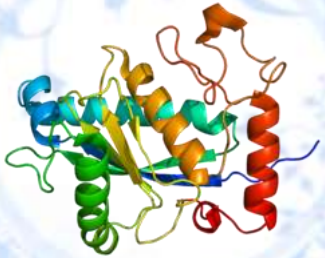
ADAM33 gene has 22 exons (A-V) that encode respectively different domains



The exonic structure and domain organisation of human ADAM33 in relation to function

Published in Expert Reviews in Molecular Medicine by Cambridge University Press 2004

ADAM33



- ADAM33 gene encodes A disintegrin and metalloproteinase 33 protein (ADAM33)

- ADAM33 may play a role in airway remodeling in asthma .

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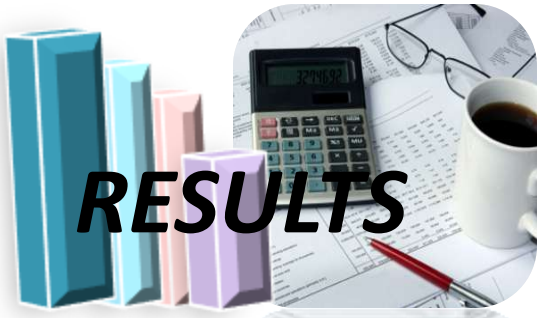

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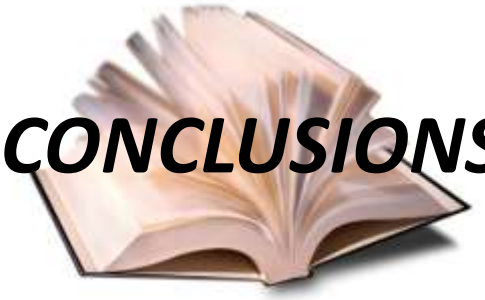

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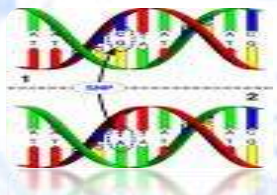
- Determine the common variants in IL-4 and ADAM33 genes Saudi asthmatic children.

- Determine the SNP frequency of IL-4 and ADAM 33 variants in asthmatic and normal Saudi children.

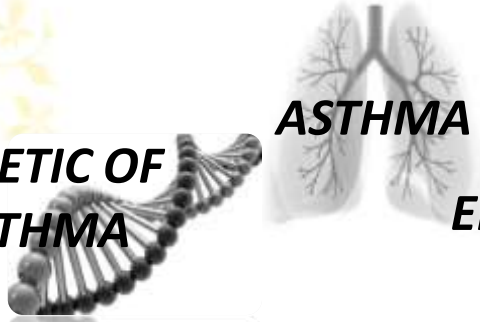
- Compare the genetic variation in Saudi asthmatic children with results from other population

SNPs in our project



Gene	SNPs	Location	Variation
ADAM33	T1	Exon 20	A/G
	T2	Exon 20	G/A
	ST+4	Intron 19	A/C
	S1	Exon 19	C/T
IL-4	RS#2243250	Promoter region	C/T
	RS#4986963	Promoter region	C/T

INTRODUCTION



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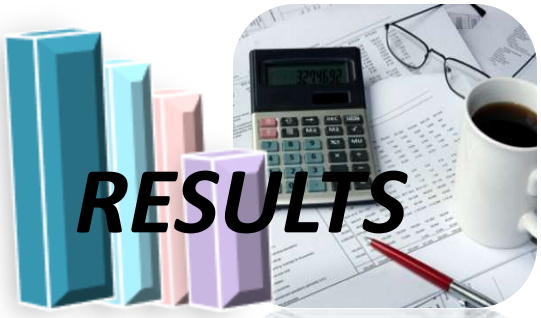

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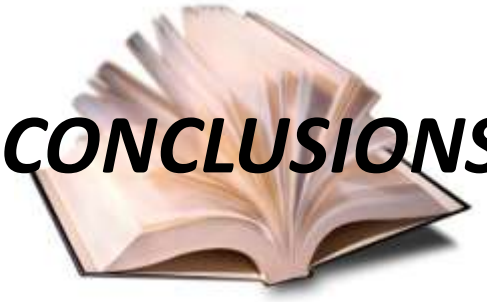

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Blood sample collection from KKUH
107 asthmatic patients, 87 normal control

DNA extraction

RT-PCR

(*Allele specific discrimination*)

- ADAM33 RS#2280090 RS#2280091
- IL-4 RS#2243250

PCR-RFLP

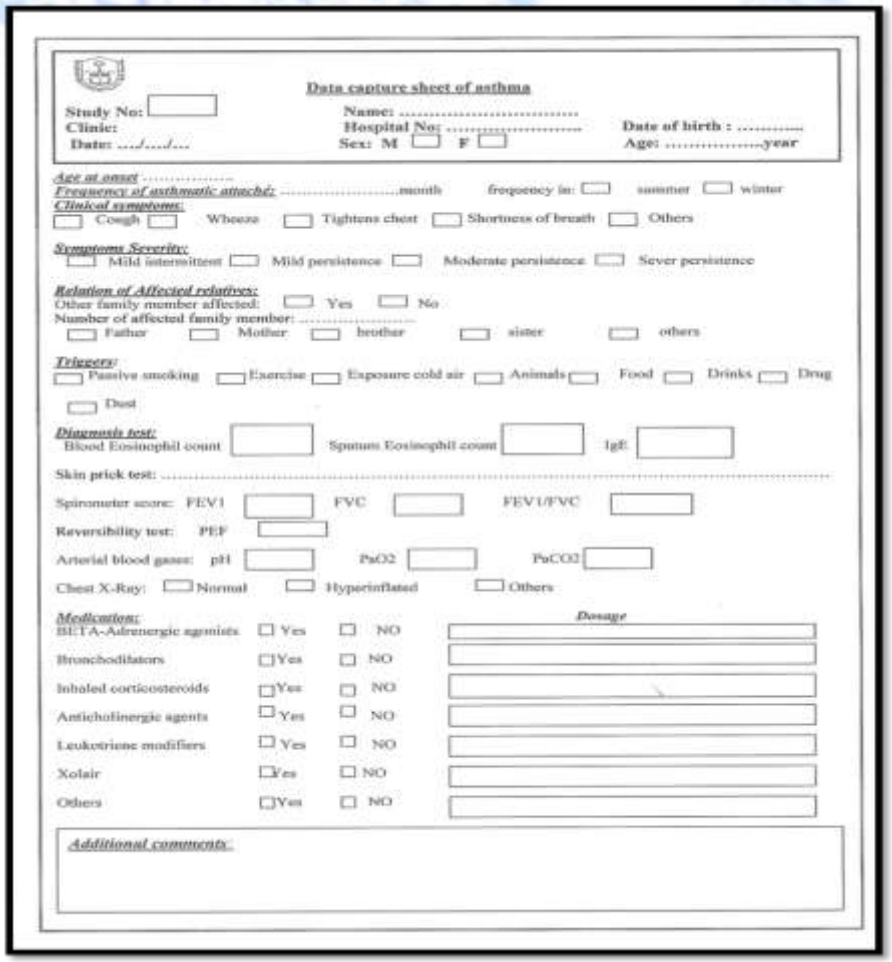
- ADAM33 Rs2280090
- IL-4 RS#4986963

Multiplex PCR-ARMS

- ADAM33 RS#44707
- IL-4 RS#2234250

Asthma Data Sheet

Consent Form



Data capture sheet of asthma

Study No: ____
Clinic:
Date: ..../..../....

Name: .....
Hospital No: .....
Sex: M ☐ F ☐

Date of birth : .....
Age: .....year

Age at onset .....
Frequency of asthmatic attacks: .....month frequency in: ☐ summer ☐ winter

Clinical symptoms:
☐ Cough ☐ Wheeze ☐ Tightens chest ☐ Shortness of breath ☐ Others

Symptoms Severity:
☐ Mild intermittent ☐ Mild persistence ☐ Moderate persistence ☐ Sever persistence

Relation of Affected relatives:
Other family member affected: ☐ Yes ☐ No
Number of affected family member: .....
☐ Father ☐ Mother ☐ brother ☐ sister ☐ others

Triggers:
☐ Passive smoking ☐ Exercise ☐ Exposure cold air ☐ Animals ☐ Food ☐ Drinks ☐ Drug ☐ Dust

Diagnosis test:
Blood Eosinophil count ____ Sputum Eosinophil count ____ IgE: ____

Skin prick test: .....

Spirometer score: FEV1 ____ FVC ____ FEV1/FVC ____
Reversibility test: PEF ____
Arterial blood gases: pH ____ PaO2 ____ PaCO2 ____
Chest X-Ray: ☐ Normal ☐ Hyperinflated ☐ Others

Medications:

			Dosage
BETA-Adrenergic agonists	☐ Yes	☐ NO	
Bronchodilators	☐ Yes	☐ NO	
Inhaled corticosteroids	☐ Yes	☐ NO	
Antichofinergic agents	☐ Yes	☐ NO	
Leukotriene modifiers	☐ Yes	☐ NO	
Xolair	☐ Yes	☐ NO	
Others	☐ Yes	☐ NO	

Additional comments:

جامعة الملك سعود
أقسام العلوم والدراسات الطبية
عمادة الدراسات العليا

إقرار بالموافقة على المشاركة في بحث علمي

عنوان البحث : البحث عن المتغيرات الجينية في انترلوكين-4 (IL-4) و ادم33 (ADAM33)33 في عينة من الأطفال السعوديين المصابين بمرض الربو في الرياض.
اسم الباحث: أروى بنت إسحاق بن عبد الله خياط
اسم المشارك:
عينة دم حجم 5 ملليلتر (لاستخلاص المادة الوراثية ومحاولة البحث عن صفات المرض الوراثي)

أوافق المشاركة في هذا البحث العلمي بعد أن شرح لي القائم بالبحث تفاصيل البحث وأهميته ونوعية مشاركتي وأجاب على كل تساؤلاتي. وبناء على ذلك فأنا لا مانع لدي من إعطاء عينة دم لاستخلاص المادة الوراثية واستخدامها في هذا البحث حسب التعليمات المقررة في المملكة العربية السعودية. وبناء على ذلك فأني اصرح بالتالي

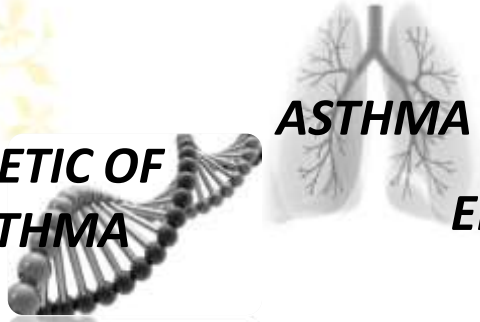
1- استخدام عينتي في هذا البحث
نعم ☐ لا ☐

2- استخدام المتبقي من عينتي في أبحاث أخرى ذات صلة بهذا البحث
نعم ☐ لا ☐

إقر بأنني قد درست النموذج أعلاه وأوقع على سبيل البيانات المطابقة للمتطوع أو ولي الأمر :
الاسم: .....
التوقيع: .....
التاريخ: .....
صلة القرابة (إذا التوقيع غير المريض المشارك): .....

الباحث قد من ينوب عنه:
إقر بأنني شرحت للمتطوع / أو ولي أمرة تفاصيل البحث وأهدافه :
الاسم: .....
التاريخ: ..... التوقيع: .....

INTRODUCTION



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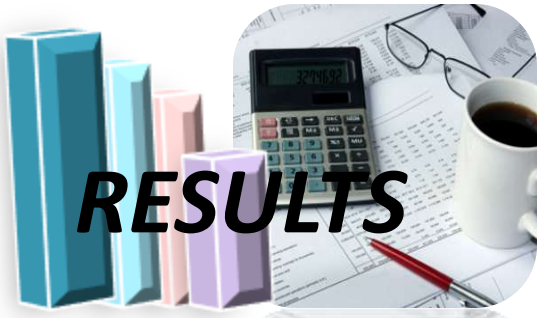

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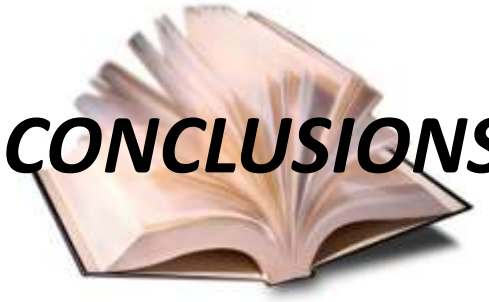

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RESEARCH PROTOCOL

RESULTS

ADAM33

Genotype frequency and P-value for ADAM33 SNPs and asthma susceptibility in different ethnic groups.

SNPs	Saudi		Dutch		African American		US Caucasian		US Hispanic	
	P	CA	P	CA	P	CA	P	CA	P	CA
T2										
AA	0.074	0.083	0.02	0.03	0.04	0.03	0.02	0.01	0.01	0.01
GA	0.489	0.297	0.24	0.23	0.29	0.26	0.21	0.32	0.19	0.09
GG	0.436	0.619	0.74	0.74	0.66	0.71	0.77	0.67	0.80	0.90
P-value	0.03		NS		NS		0.02		0.04	
T1										
AA	0.391	0.617	0.74	0.74	0.67	0.75	0.77	0.68	0.64	0.66
AG	0.494	0.329	0.24	0.23	0.29	0.22	0.21	0.31	0.34	0.32
GG	0.113	0.058	0.02	0.03	0.04	0.03	0.02	0.01	0.03	0.03
P-value	0.014		NS		NS		0.03		0.06	
ST+4										
AA	0.489	0.533	0.20	0.19	0.42	0.33	0.36	0.33	0.34	0.37
AC	0.459	0.466	0.51	0.44	0.45	0.47	0.46	0.50	0.50	0.53
CC	0.051	-	0.29	0.37	0.13	0.20	0.18	0.17	0.16	0.10
P-value	0.52		NS		NS		NS		NS	
S1										
CC	0.937	0.975	0.86	0.83	0.96	0.97	0.82	0.81	0.91	0.92
CT	0.062	0.024	0.14	0.15	0.04	0.03	0.17	0.19	0.09	0.08
TT	-	-	-	0.02	-	-	0.01	-	-	-
P-value	0.22		NS		NS		NS		NS	

	Haplotypes					Haplotype frequencies				
no.	T1	T2	ST+4	S1		Total	Healthy	Asthmatic	OR (95% CI)	P-value
H1	A	G	A	C		0.42	0.52	0.35	1	---
H2	G	A	A	C		0.26	0.21	0.31	2.4 (1.2-4.6) *	0.009 *
H3	A	G	C	C		0.25	0.23	0.27	1.9 (0.8-4.8)	0.13
H4	G	G	A	C		0.02	0.01	0.02	3.4 (0.5-21.4)	0.19
H5	A	G	A	T		0.02	0.01	0.20	2.4 (0.4-14.8)	0.33
	Global haplotype association p-value: 0.03 *									

Table 1. Analysis of haplotype associations with response.
The asterisk (*) indicates significance (P<0.05) of odds ratios (OR) and confidence intervals (95% CI) for the haplotype in reference to the most frequent homozygous haplotype (no. 1).

SNP	Genetic Model	Genotype frequency, no. (%)			OR (95% CI)	P-value	AIC (BIC)
		Genotype	Healthy	Asthmatics			
T1	Dominant	A/A	53 (61.6)	38 (39.6)	1.00	0.0013 *	241 (251)
Rs2280091		A/G-G/G	33 (38.4)	58 (60.4)	2.70 (1.46-5.01) *		
T2	Dominant	G/G	52 (61.9)	41 (43.6)	1.00	0.0083 *	238 (247)
Rs2280090		A/G-A/A	32 (38.1)	53 (56.4)	2.28 (1.22-4.23) *		
ST+4	Codominant	A/A	32 (53.3)	48 (48.5)	1.00	0.064	208 (220)
rs44707		A/C	28 (46.7)	46 (46.5)	1.1 (0.58-2.18)		
		C/C	0 (0)	5 (5)	NA (0.0-NA)		
S1	-----	C/C	80 (97.6)	90 (93.8)	1.00	0.28	244 (254)
Rs3918396		C/T	2 (2.4)	6 (6.2)	2.38 (0.46-12.37)		
		T/T	0 (0)	0 (0)	NA (0.0-NA)		

Table 2. Genotype frequency distribution of ADAM33 T2, T1, ST+4 and S1 polymorphisms and its association with asthma (n=182; OR values were adjusted by gender). P-values with asterisk (*) indicate significance for the best fit model of association of SNP with asthma, adjusted by the variable Gender. For each SNP,only the best fit genetic model is shown, selected by their lowest index criteria values (AIC and BIC). The most frequent homozygous genotype was used as reference.

•There was strong linkage disequilibrium between T1 (rs2280091) and T2 (rs2280090) (r^2=0.83; D'=0.95; P<0.001).

•The haplotype [A-G-A-C] was more frequent in the healthy group,
while the [G-A-A-C] haplotype was significantly more frequent in asthmatics.

• Thus supporting the association of T1 G-allele and T2 A-allele with increased predisposition to asthma (P=0.007).

IL-4

Genotype frequency of IL-4 SNPs and asthma susceptibility in different ethnic groups from NCBI populations.

	Genotype frequency			Allele frequency		P-value
RS#2243250	CC	CT	TT	C	T	
SAUDI Patients	0.5614	0.368	0.070	0.745	0.254	0.64
Controls	0.648	0.296	0.055	0.796	0.203	
EUROPEN	0.707	0.259	0.034	0.836	0.164	
JAPANES	0.045	0.455	0.500	0.273	0.727	
AFRICAN AMRICAN	0.125	0.333	0.542	0.292	0.708	
CAUCASIN	0.800	0.200	-	0.271	0.729	
HISPANIC	0.545	0.409	0.046	0.750	0.250	
RS#4986963	CC	CT	TT	C	T	
SAUDI Patients	0.112	0.884	-	0.556	0.443	0.02
Controls	0.269	0.730	-	0.634	0.365	
EUROPEN	1.000	-	-	1	-	
JAPANES	1.000	-	-	1	-	
CAUCASIN	1.000	-	-	1	-	
HISPANIC	1.000	-	-	1	-	

Comparing between asthmatic patients and control within males and females groups

Gene	SNPs	SEX	P/C	Genotype Frequency			Allele Frequency		p-value	
ADAM33	T2			GG	GA	AA	G	A	M	F
		M	P	48.38	46.77	4.83	0.717	0.282	0.036	0.193
			C	65.62	18.75	15.62	0.75	0.25		
		F	P	36.66	50	13.33	0.616	0.383		
			C	55.88	38.23	5.88	0.75	0.25		
				AA	AG	GG	A	G		
	T1	M	P	42.85	49.20	12.12	0.674	0.325	0.032	0.184
			C	69.69	18.18	12.12	0.787	0.212		
		F	P	33.33	48.48	18.18	0.575	0.424		
			C	50	47	2.94	0.735	0.264		
				CC	CT	TT	C	T		
	S1	M	P	91.93	8.06	0	0.959	0.040	0.476	0.85
			C	96.77	3.22	0	0.983	0.016		
		F	P	96.96	3.03	0	0.984	0.015		
			C	96.96	3.03	0	0.984	0.015		
				AA	AC	CC	A	C		
	ST+4	M	P	46.87	50	3.12	0.718	0.281	0.0001	0.019
			C	65.21	34.78	0	0.826	0.173		
		F	P	51.51	39.39	9.09	0.712	0.28		
			C	50	50	0	0.75	0.25		
IL-4				CC	CT	TT	C	T		
	RS#2243250	M	P	59.45	32.43	8.10	0.756	0.243	0.56	0.15
			C	72.72	18.18	9.09	0.818	0.181		
		F	P	50	45	5	0.725	0.275		
			C	58.62	37.93	3.44	0.775	0.224		
				CC	CT	TT	C	T		
	RS#4986963	M	P	11.62	88.37	0	0.558	0.441	0.96	0.110
			C	13.63	86.36	0	0.568	0.431		
		F	P	10.714	89.285	0	0.553	0.446		
			C	33.33	66.66	0	0.666	0.333		

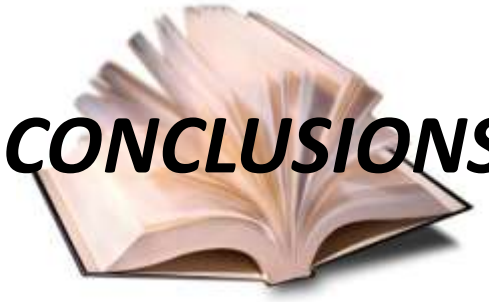
INTRODUCTION

ASTHMA

GENETIC OF ASTHMA

EPIDEMIOLOGY STUDY

RESULTS



CONCLUSIONS

OBJECTIVES

RESEARCH PROTOCOL

CONCLUSIONS:

•This preliminary data indicate significant statistical association in asthmatic children in the following SNPs:

1. RS#2280090 in *ADAM33*
2. RS#2280091 in *ADAM33*
3. RS# 4986963in *IL-4*.

•Similar association was found in other population like Caucasian and Hispanic. However, no associations were found in Dutch and Afro –Americans populations

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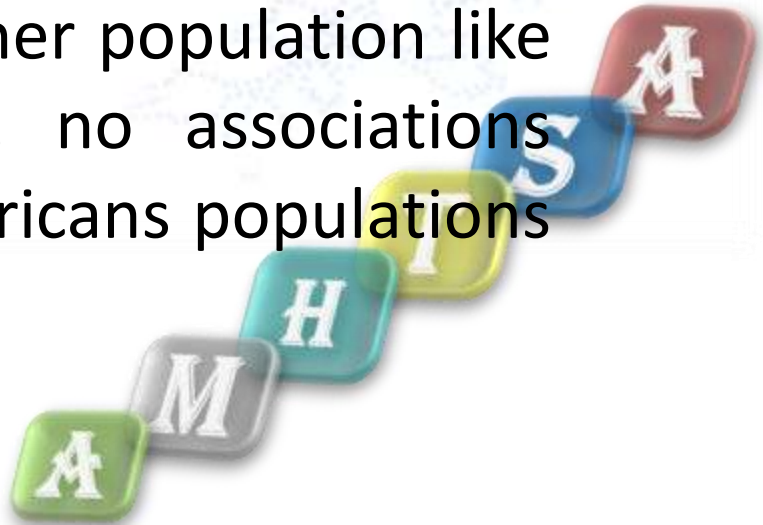


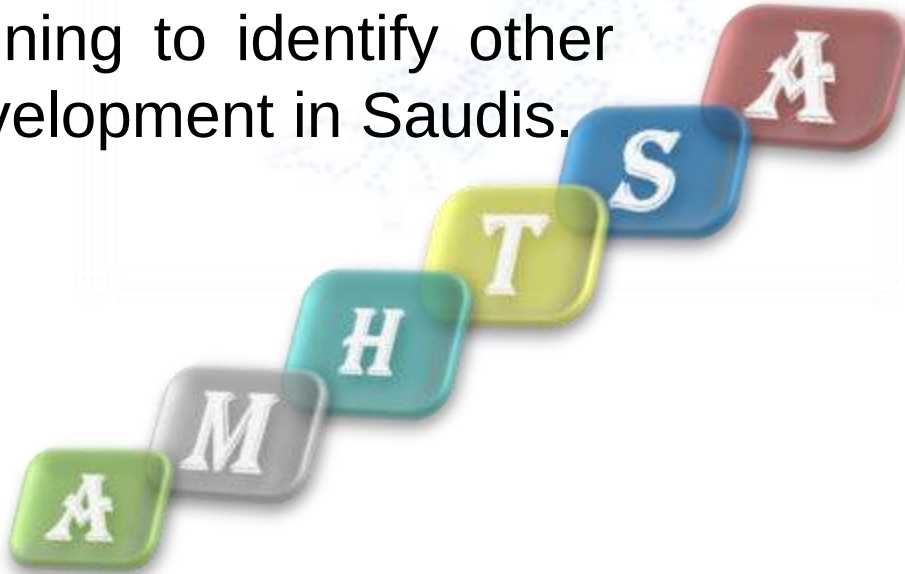
Further Studies

The results of this investigation have highlighted areas that need further detailed investigations. We propose the following future studies:

Further Studies

i. Screening of the entire ADAM33 and IL-4 genes in large population groups of Saudi Arabia from different geographic region in Saudi Arabia, to identify markers specific for the Saudi population.

ii. Conduct whole genome scanning to identify other genes contributing to asthma development in Saudis.



Further Studies

iii. Determine the relationship between genetic variations and drug response, i.e. to conduct pharmacogenetic study of asthma.

iv. Study the relationship between environmental factors and asthma development.

Acknowledgement

- Dr. Mohammed Al-Anazi, PhD
- Prof. Arjmuand Warsy, PhD
- Dr. Saleh Al-Muhsen,MD