



## REVIEW

# Genetic testing for asthma

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**ABSTRACT:** Asthma is a genetically complex disease caused by multiple genetic and environmental factors. An increasing number of asthma susceptibility genes are currently being identified. The present study addresses the question as to whether this genetic information can be used to predict asthma, particularly in pre-school children.

The predictive value of a single gene test in a complex disease is very limited for diagnostic or preventive purposes and thus cannot be recommended. Based on data of simulation studies and other complex diseases, the use of genetic profiling that incorporates multiple genetic risk factors holds promise for clinical application. The results of genome-wide association studies will be crucial in establishing this genetic risk profile for asthma.

In the future, asthma prediction may be possible, based on a prediction model that incorporates genes, personal factors and environmental risk factors. Studies in general and at-risk populations are needed to investigate and validate this approach.

**KEYWORDS:** Asthma, gene, genome-wide association, prediction, testing

Asthma is a chronic inflammatory disease of the airways, characterised by respiratory symptoms such as wheezing, breathlessness, intermittent airway obstruction and airway hyperresponsiveness. Asthma is the result of the interaction of multiple genetic and environmental factors [1].

Over the last 15 yrs, significant progress has been made in genetic studies of asthma using candidate gene and positional cloning studies and, most recently, genome-wide association (GWA) studies. In  $\geq 10$  populations, 10 candidate genes for asthma susceptibility or severity have been replicated. These genes encode *interleukin (IL)4*, *IL4 receptor- $\alpha$  chain (IL4RA)*, *IL13*,  *$\beta 2$  adrenergic receptor*, *human leukocyte antigen (HLA)-DRB1* and *DQB1*, *tumour necrosis factor- $\alpha$* , *lymphotoxin- $\alpha$* , the  *$\beta$ -chain of the high-affinity immunoglobulin E receptor*, *CD14*, and *A disintegrin and matrix metalloprotease-33 (ADAM33)* [2].

Seven asthma genes have been discovered through positional cloning, a technique based on the identification of chromosomal regions that are linked with asthma in families. Subsequent detailed analyses of genes within this chromosomal region can reveal potential novel genes for asthma. The first of these genes, *ADAM33* [3], has been shown to be important in susceptibility to asthma and bronchial hyperresponsiveness in multiple populations [2]. Other novel identified

genes, such as those encoding *dipeptidylpeptidase 10* [4], *PHD finger protein 11* [5], *SFRS8* [6], *CYFIP2* [7] and *HLA-G* [8], are currently subject to replication studies. If these genes are confirmed in other populations, as recently shown for the gene encoding G-protein coupled receptor A (*Neuropeptide S receptor-1*) [7, 9–11], new insights into the pathogenesis of asthma will be obtained in the future.

One novel asthma gene has recently been identified through GWA studies. In GWA studies, the complete genome is scanned using a chip with 317,000–1,000,000 single nucleotide polymorphisms (SNPs) and significant allele frequency differences between asthma cases and controls are investigated. MOFFATT *et al.* [12] have identified a sequence variant on chromosome 17q that is associated with *ORMDL3* expression to be significantly associated with childhood asthma. This finding has now been replicated in two populations [13, 14].

In addition to genetic factors, personal factors, such as sex and age, and environmental factors are important in asthma development (table 1). The increase in the prevalence of asthma over the last 20 yrs can't be explained by the small changes in the genetic constitution of the Western population that occur over time, but by the changing environment. Different environmental factors, such as indoor and outdoor

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pollution, allergen exposure, tobacco smoke and other factors related to the "Western lifestyle" like contact with microbial products, may be implicated in asthma pathogenesis [15, 16].

It is unknown if these genetic, environmental or personal risk factors act independently or in interaction with each other in the development of asthma. More studies are needed to characterise the interaction of multiple genes and multiple environmental factors. These investigations are complicated by the fact that large sample sizes are needed [17].

The identification of the genetic basis of asthma opens up avenues for the clinical application of this knowledge. The present review will discuss the possibilities of applying genetic information to the prediction of asthma, with a specific focus on early childhood asthma. The latter focus is based on the assumption that it is likely that gene–environment interactions in early life contribute to the development of asthma and that primary preventive approaches will be specifically targeted in this age group. Moreover, up to 40% of children in early childhood have chronic respiratory symptoms. It is a real challenge for clinicians to identify children who will develop chronic asthma. Currently, use of easy applicable, noninvasive tests that are not time consuming to study bronchial hyperresponsiveness or lung function, phenotypes crucial to an objective diagnosis of asthma, are lacking in clinical practice for preschool children. Thus, genetic profiling may open new areas of investigation to identify children at risk.

Before discussing the application of genetic tests in asthma, the current status of genetic research in asthma and the prediction of asthma in childhood will be examined.

### GENETICS OF ASTHMA: CURRENT STATUS

Asthma is a genetically complex disease. In contrast to monogenic Mendelian diseases, no one-to-one relationship between the gene and the disease exists, which may be due to incomplete penetrance (*i.e.* subjects with the disease gene variants but without the disease), phenocopies (subjects with asthma but without a genetic cause) and genetic heterogeneity (subjects with asthma caused by different genes).

TABLE 1 Risk factors for asthma			
Candidate genes	Positional cloned genes (chromosome)	Environment	Personal factors
<i>IL4</i>	<i>ADAM33</i> (20p)	Tobacco smoke exposure	Sex
<i>II4RA</i>	<i>GPRA1</i> (7p)	Diet	Age
<i>IL13</i>	<i>DPP10</i> (2q)	Pollutants	
<i>TNF</i>	<i>PHD11</i> (13q)	Microbial products	
<i>LTA</i>	<i>HLA-G</i> (6p)		
<i>B2AR</i>	<i>SFRS8</i> (12q)		
<i>HLA-DR and DQ</i>	<i>CYF1P2</i> (5q)		
<i>CD14</i>			
<i>FCER1B</i>	Whole genome association <i>ORMDL3</i> (17q)		

Twin studies suggest that 40–60% of asthma is caused by genetic factors. The number of genes contributing to asthma remains a matter of speculation, but based on the current evidence from linkage and association studies, it is likely that  $\geq 30$ –100 genes will be important in different populations. Several studies have been published that provide a comprehensive review of asthma genetics [2, 18].

It is most likely that asthma genes do not confer risk for asthma autonomously. The effects of a specific gene will rather depend on other genes and environmental exposures. The first example of a gene–gene interaction in asthma was shown in a Dutch study of asthmatic adult patients and their spouses, which investigated gene variants (alleles) in *IL13* and *IL4RA*. Carriers of risk alleles in either *IL13* or *IL4RA* had a modestly increased risk of asthma (odds ratio (OR) 1.85 and 1.83, respectively), whereas subjects with risk alleles in both genes had an increased OR of 4.83 (95 % confidence interval (CI) 2.0–11.9) of developing asthma [19]. In that study, *IL4RA* and *IL13* had an additive effect.

Gene by environmental interaction in asthma is a likely interpretation of the results from a study in farmers' children living in Germany, Austria and Switzerland. Prevalence rates of asthma and allergies were reported to be lower in children living on farms than in children from rural populations not living on farms. It has been hypothesised that contact with microbial products, such as endotoxin, modifies the immune response and asthma development [20]. Important receptors for microbial products are Toll-like receptors (TLR), in particular TLR2 and TLR4. EDER *et al.* [21] showed that farmers' children carrying a T-allele in *TLR2-16934* were significantly less likely to have a diagnosis of asthma than farmers' children with genotype AA (3 versus 13%;  $p=0.012$ ). This indicates that the environment (*i.e.* living on farms) modifies the association between the *TLR2* gene and asthma. In other words, no association of *TLR2* gene variants and asthma was observed in children not raised on farms [21].

Therefore, asthma is an example of a genetically complex disease, with multiple genes that may act additively and increase each other's effect by gene–gene and gene–environment interactions. Thus, any method to predict asthma should take into account the complexity of these interactions.

### PREDICTING ASTHMA IN CHILDHOOD

Predicting asthma in early childhood remains one of the most difficult challenges in daily clinical practice; this is illustrated by the following case. A 2-yr-old male enters his doctor's practice complaining of intermittent wheezing and cough during the previous 4 months. According to his mother, he continuously suffers from a runny nose with episodes of fever since he started attending day care aged 1 yr. The child did not have eczema at infancy. One of the child's grandmothers has asthma and his mother has hay fever. The father smokes but not, it was assured, in the presence of the child.

Does this child have asthma? Is prescription of inhaled corticosteroids beneficial for this child? One may consider making an assumption based on epidemiological studies that have identified several risk factors for childhood asthma, *i.e.* family history, male sex, maternal smoking during pregnancy,

atopy and the presence of infant eczema [22]. Their predictive value for asthma development will be discussed shortly.

Family history of asthma is an important risk factor for asthma in an individual. However, a familial aggregation of asthma may be due to both effects of shared genes and shared environmental factors. In 10 published studies, family history had positive predictive values (*i.e.* the proportion of patients with positive test results that were correctly diagnosed) ranging 11–37%, whereas the negative predictive values (*i.e.* the proportion of subjects with negative test results that were correctly diagnosed) ranged 86–97% [23]. Thus, a family history of asthma is not a strong predictor of asthma in a child, yet the absence of a family history is a good predictor that the child will not develop asthma.

Clinical scores that use symptoms and clinical tests next to family history seem to provide better prognostic values for asthma than family history alone. One clinical score has been developed based on the data of the Tucson Children's Respiratory Study [22]. This was a prospective study from birth onwards that assessed, at 3 yrs of age, which factors are associated with doctor diagnosed asthma at 6–13 yrs of age. A clinical score for the prediction of asthma includes two major criteria (family history of asthma in one of the parents and doctor-diagnosed eczema in the child) and three minor criteria (doctor-diagnosed allergic rhinitis in the child, wheezing apart from colds, and blood eosinophilia at age 10 yrs). For a "stringent index", children had to have reported early frequent wheezing during the first 3 years of life, in addition to one of the two major criteria and two of three minor criteria. This stringent index had a positive predictive value ranging 42.0–51.5% for asthma at age 6–13 yrs, and a negative predictive value ranging 84.2–91.6% [22]. It appears that this index may improve the ability to predict asthma at school age in this population raised in Tucson (AZ, USA), whereas the negative predictive value is comparable to the presence of a family history of asthma. A drawback of the Tucson Children's Respiratory Study [22] is that the blood eosinophil data were collected at age 10 yrs not at age 3 yrs, thus not allowing an initial set of data for risk assessment. Other studies have proposed similar scoring systems with comparable results for asthma or symptoms associated with asthma, such as persistent wheeze [24, 25]. The question arises as to whether genetic information will further increase the ability to predict asthma.

## GENETIC TESTING

A genetic test has been defined as "the analysis of human DNA, RNA, chromosomes, proteins and certain metabolites in order to detect heritable disease-related genotypes, mutations, phenotypes or karyotypes for clinical purposes" [26]. In the present review, only DNA was analysed.

Two factors determine the predictive value of a genetic test for a disease with a given population prevalence: genotype relative risk (GRR) and allele frequency. GRR is defined as the ratio of disease risks between those with and those without the susceptibility genotype (comparable to an OR). What is the purpose of risk profiling the GRR of asthma genes in an individual? In the literature, results of association studies have not consistently been expressed as GRR. Therefore, the present

authors reviewed published data on two of the best replicated candidate genes for asthma: *IL13* and *IL4RA*. The results of these studies were expressed as GRR, and a strict definition was employed, *i.e.* the same SNP, the same allele, the same genetic model (*i.e.* codominant or recessive) and the same phenotype (asthma) were required for a definition of replication [27]. The current study was limited to investigations of asthma in Western, Caucasian case-control populations (table 2). Table 2 shows that the GRR of SNPs in *IL4RA* and *IL13* is generally found to be 1.0–3.2 with some studies estimating even higher risk estimates (range 0.49–7.27). This risk estimation is in concordance with a meta-analysis of eight populations performed on *ADAM33*, which showed that the best associated SNP (ST+7) had an OR of 1.46 (95% CI 1.21–1.76) for asthma development [39]. Results from GWA studies in asthma and other complex diseases show that the GRR for genes identified through GWA is between 1.05–1.8 [12, 40].

However, the GRRs summarised in table 2 may be overestimated. In complex diseases, estimation of the effect size of the genotype has been shown to differ between small and large studies, with small studies generally reporting a higher genotype effect than large studies [41]. One reason for this may be population selection; if families are selected with multiple affected individuals, the contribution of the genotype to the phenotype may be higher compared with its contribution in the general population. One example is familial breast cancer. When two genes associated with familial breast cancer were discovered, *BRCA1* and -2, it was initially estimated that almost all carriers of the mutations in these genes would develop breast cancer. However, these calculations were based on families where multiple family members had breast cancer and segregating at the *BRCA1* and *BRCA2* locus. Subsequent population-based assessments showed a lower lifetime risk of disease (~60%) in carriers [42]. Therefore, given the fact that risk calculations of asthma genes in selected study groups are possibly biased, some of the GRRs in table 2 may be overestimated.

What is the allele frequency of the risk alleles of asthma genes in the general population? Based on the information obtained to date, the allele frequency of risk alleles varies considerably, *i.e.* between 10–85%. Indeed, in some cases the prevalent allele appears to confer risk for asthma, as shown previously in a meta-analysis for *ADAM33* [39]. In the meta-analysis of eight studies, the risk allele frequency for ST+7 G was 84.9% in the asthma population and 79.1% in the control population. Based on an estimated asthma prevalence of 8%, this SNP can be attributed to ~50,000 asthma cases in the UK population. Thus, asthma appears to be a complex genetic disease associated with SNPs in genes with moderate-to-high allele frequency in the population, low penetrance but substantial population attributable risk.

## A SINGLE GENE TEST FOR ASTHMA?

The positive predictive value of a single gene test for a complex disease, such as asthma, is limited. This has been illustrated by HOLTZMAN and MARTEAU [43] (table 3) who assessed the relationship between GRR and allele frequency in order to predict a disease, as in this example, with a population frequency of 5% (table 3). The positive predictive value of a single gene test appears to be lower with higher risk allele

**TABLE 2** Results from genetic association studies on interleukin (IL)13 and IL4 receptor  $\alpha$ -chain (IL4RA) in asthma

Population	[Ref.]	Phenotype	Risk genotype frequency		Risk genotype		Wild genotype		Genetic risk calculation	
			Cases	Controls	Cases	Controls	Cases	Controls	OR (95% CI)	GRR
<b>IL13 Arg130Gln: risk genotype Gln/Gln</b>										
The Netherlands	[28]	Asthma	11	9	141	111			0.96 (0.39–2.40)	0.96
Canada	[29]	Probable asthma aged 2 yrs	2	12	20	247			2.06 (0.43–9.84)	1.96
Finland	[30]	Physician-diagnosed asthma	17	19	146	113			0.69 (0.34–1.39)	0.72
Germany	[31]	Asthma	16	12	212	258			1.62 (0.75–3.51)	1.58
UK	[32]	Atopic asthma	24	11	126	139			2.41 (1.13–5.11)	2.18
<b>IL13 - 1055 CT: risk genotype TT</b>										
The Netherlands	[33]	Allergic asthma	13	2	88	105			7.76 (1.70–35.30)	6.89
Canada	[29]	Probable asthma aged 2 yrs	1	8	21	253			1.51 (0.18–12.62)	1.48
The Netherlands	[28]	Asthma	9	2	162	117			3.25 (0.69–15.32)	3.13
<b>IL4RA R576Q: risk genotype R576/R576</b>										
Finland	[30]	Physician-diagnosed asthma <sup>#</sup>	121	82	46	48			1.54 (0.94–2.52)	1.15
The Netherlands	[19]	Asthma	8	5	143	109			1.22 (0.39–3.83)	1.21
Italy	[34]	Atopic asthma	8	9	37	43			1.03 (0.36–2.95)	1.03
USA Caucasian	[35]	Asthma	19	1	130	56			8.18 (1.07–62.64)	7.27
UK	[32]	Atopic asthma	19	26	131	124			0.69 (0.36–1.31)	0.73
New Zealand and Australia	[36]	Mild-to-moderate asthma	3	5	83	138			1.00 (0.23–4.28)	1.00
Sweden	[37]	Atopic asthma	10	9	160	189			1.31 (0.52–3.31)	1.29
USA Caucasian	[38]	Asthma (non)-allergic	18	3	175	53			1.82 (0.52–6.41)	1.74
<b>IL4RA Ile50Val: risk genotype Ile/Ile</b>										
UK	[32]	Atopic asthma	52	48	98	102			1.13 (0.70–1.82)	1.08
Sweden	[37]	Atopic asthma	48	83	122	201			0.95 (0.63–1.45)	0.97
USA Caucasian	[38]	Asthma (non)-allergic	65	17	137	39			1.09 (0.57–2.07)	1.06
Finland	[30]	Physician-diagnosed asthma	10	17	140	108			0.45 (0.20–1.03)	0.49
The Netherlands	[19]	Asthma	31	25	78	62			0.99 (0.53–1.84)	0.99

Data are presented as n, unless otherwise stated. OR: odds ratio; 95% CI: 95% confidence interval; GRR: genotype relative risk. <sup>#</sup>: risk genotype is Q576 / Q576.

**TABLE 3** Positive predictive values of tests for susceptibility conferring genotypes for a disease with a lifetime risk in a given population<sup>#</sup>

Risk genotype frequency %	Relative risk				
	1.5	2.0	5.0	10.0	20.0
0.1	7.5	10.0	24.9	49.6	98.1
0.5	7.5	10.0	24.5	47.8	91.3
1.0	7.5	9.9	24.0	45.9	84.0
10.0	7.1	9.1	17.9	26.3	34.5
30.0	6.5	7.7	11.4	13.5	14.9

<sup>#</sup>: assuming a population frequency of 5%. Reproduced from [43] with permission from the publisher.

frequencies and lower GRRs. Even with a GRR of 5.0, the presence of a disease susceptibility allele with an allele frequency of 30% results in a positive predictive value of 11.4% [43].

#### MULTIPLE GENE TESTS FOR ASTHMA: GENETIC PROFILING

Clearly, the complex nature of asthma is best reflected by tests that account for multiple genes. These tests have not been published for asthma to date, but results from simulation studies and other genetically complex diseases may illustrate its use.

Simulation studies express the usefulness of genetic testing as the area under the receiver operating characteristic curve (AUC), which reflects the combination of sensitivity and specificity. An AUC of 0.5 is nondiscriminatory. It is generally accepted that an AUC can be used for screening if it is >0.8, and can be used for pre-symptomatic diagnosis if >0.99. In a simulation study, YANG *et al.* [44] showed that a multiple gene

test consisting of 20 genes with GRRs ranging 1.5–1.7 results in an AUC of 0.77. This figure is comparable to the predictive value of serum cholesterol levels for coronary heart disease. JANSSENS *et al.* [45] argued that lower GRRs and a higher number of genes are more realistic. Thus, they investigated the predictive value of genetic testing in relation to a gene number up to 400 using a multiplicative model, allele frequency, GRR and the heritability. Table 4 demonstrates that with 50 genes that relevant for disease development, an AUC of 0.8 can be reached if GRR is 1.5 and the risk allele frequency is 10%. Moreover, JANSSENS *et al.* [45] demonstrated that excellent discrimination (AUC >0.95) is possible for complex genetic diseases with a prevalence >1% and heritability of 0.23; this is compatible with the current authors' understanding of asthma with regards to heritability and disease prevalence. WRAY *et al.* [46] showed that GWA studies may be used to identify these genetic risk variants. For example, if a disease is controlled by 1,000 gene loci with a mean GRR of only 1.04, a case–control study with 10,000 cases and controls may lead to the selection of ~75 loci that control >50% of the genetic variance of the disease [46]. A genome-wide study for asthma of this sample size is currently ongoing in Europe.

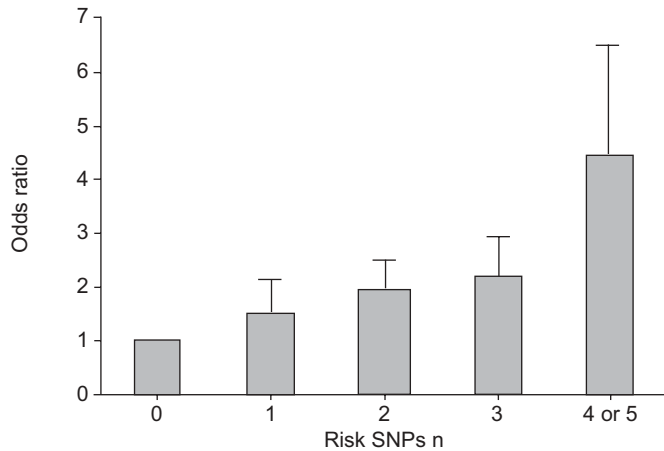
Two empirical studies into other genetically complex diseases may illustrate the predictive power of multiple gene tests.

First, YIANNAKOURIS *et al.* [47] have investigated the genetic contribution of 11 known risk genes to the incidence of coronary infarct in a Greek population. In total, 11 SNPs were investigated in a nested case–control study of 202 cases and 197 controls. A risk score was calculated for each individual by assigning a score of 1 if a subject was homozygous for the risk allele, and 0.5 for heterozygotes. These scores were added together for each gene up to a maximum score of 11. For scores of  $\geq 3$ , the OR for coronary infarction development was 1.55. For scores  $\geq 3.5$ , the OR increased to 2.02. In these cases, 22% of the variance of the disease in the population could be explained by genetic risk factors [47]. The study by YIANNAKOURIS *et al.* [47] marks the first step towards the

**TABLE 4** Multiple gene testing: effect of odds ratio (OR) and risk allele frequency

OR	Risk allele frequency %	AUC of multiple test with 50 genes	Genes needed for AUC of:		
			0.80	0.90	0.95
1.05	5	0.53	>400	>400	>400
	10	0.54	>400	>400	>400
	30	0.56	>400	>400	>400
1.1	5	0.56	>400	>400	>400
	10	0.58	>400	>400	>400
	30	0.62	>400	>400	>400
1.25	5	0.63	320	>400	>400
	10	0.69	170	>400	>400
	30	0.76	80	240	>400
1.5	5	0.74	100	280	>400
	10	0.80	50	150	330
	30	0.88	23	70	160

AUC: area under the curve of the receiver operating characteristic. Reproduced from [45] with permission from the publisher.



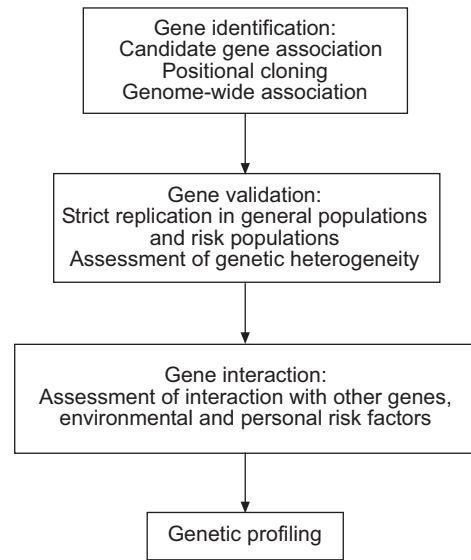
**FIGURE 1.** Odds ratio of the genetic risk for prostate cancer in relation to the number of risk genotype alleles in the Swedish population. SNP: single nucleotide polymorphisms.

analyses of multiple genes as predictive risk factors, even though there were several limitations, including: the fact that each gene had been attributed an equal risk; the fact that gene–gene interactions were not modelled [48]; and that over-fitting may limit the applicability of the results to other samples.

Secondly, ZHENG *et al.* [49] investigated SNPs from five chromosomal regions known to be associated with prostate cancer in a large Swedish case–control cohort of 2,893 patients and 1,781 controls. The five most significant SNPs, together with family history, were used to assess cumulative genetic risk for carriers of one to five risk alleles. When corrected for family history, having an increased number of risk SNPs significantly increased the prostate cancer risk (fig. 1). In a study subject with a positive family history, the prostate cancer risk was even higher and increased for carriers of one, two, three, four and five risk SNPs to 1.62, 2.07, 2.71, 4.76 and 9.46, respectively. There are several interesting aspects of this study. First, the functional role of these SNPs is not known, yet they can be used for risk prediction. Secondly, incorporating family history increased the predictive power of these risk SNPs. Thirdly, although the predictive power was higher in subjects carrying four out of five SNPs, this only accounted for 5.4% of the cases compared with 2.2% of the controls. Finally, these five SNPs, together with a family history, accounted for 46% of all prostate cancer cases in this population. Although these findings need to be assessed in a prospective study, they confirm that relevant risk SNPs can be assessed and used for genetic profiling [49].

#### Combination of genes and personal factors

The example of prostate cancer shows that combining risk SNPs with personal factors, including family history, may increase the predictive power of genetic profiling. Familial breast cancer may serve as an additional example. Two known breast cancer genes (*BRCA1* and *-2*), and a third, hypothetical, gene were statistically modelled to investigate the predictive value of genetic tests. The cumulative risk of breast cancer for *BRCA1* carriers was estimated to be 0.49 at 50 yrs of age and 0.71 at 70 yrs of age, whereas the risk estimates for *BRCA2* at the same



**FIGURE 2.** Flow diagram showing each stage from gene identification through to genetic profiling.

ages were 0.28 and 0.84, respectively. An interesting feature is that additional risk factors, such as parity, age at menarche, age at first child and body mass index, could be identified in a combined model and were found to increase predictive properties of the genetic tests. The present study has demonstrated that it is possible to predict risk of disease using a combination of genetic and personal information [50]. It should be noted that the penetrance of *BRCA1* and *-2* is high compared with asthma genes but that the allele frequency is low.

#### IMPLICATIONS FOR GENETIC TESTING OF ASTHMA

To date, genetic testing for asthma is not possible and genetic tests for asthma that are currently available through the internet cannot be recommended [51]. The positive predictive value of single-gene tests is limited. First, genes for asthma need to be identified and strictly replicated for asthma (fig. 2). Secondly, investigations in other diseases show that disease prediction may be possible based on genetic profiling incorporating genes, personal factors and environmental risk factors. The predictive value of such a system will depend critically on the ability to model all these risk factors and to have large data sets available that are needed to validate these models. Furthermore, difference in the genetic make-up in different populations (genetic heterogeneity; for instance differences between Chinese and Caucasian populations) may seriously affect the ability to predict disease, and this should also be investigated [52]. Follow-up studies of general populations and risk groups, commencing before birth, should assess whether an approach based on multiple genetic tests that model personal and environmental factors has an increased positive predictive value compared with the current approaches.

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