mucus hypersecretion in non-eosinophilic asthma are not regularly assessed in asthma control questionnaires. This may explain why successful macrolide therapy improves asthma-related quality of life yet does not impact on asthma control score.

Will this approach be useful? Probably yes. Studies in respiratory epidemiology and other disciplines have found important relationships between circulating white cell count and mortality.^{14 15} Parenthetically, this cautions the need carefully to examine the specificity of the results, particularly in populations where other influences may alter blood cell numbers, for example ageing or unrelated exposures. Several other studies have related white cell count to respiratory outcomes such as atopy¹⁶ and, importantly, the effect of exposures.^{17 18} To date, the characterisation of the clinical phenotype of subjects in genetic studies has been limited, and this may partly explain the inconsistency between studies and the small size of the genetic effects that have been identified. I have long puzzled over how scientists can define a person's genotype to the level of a single nucleotide yet accept a phenotype characterisation in the same individual that is as (im)precise as the answer to the question "Do you wheeze?". The solution to this problem does not lie in bigger and better gene machines that are sold with

the promise of "personalised medicine"; it lies in better characterisation of the phenotype that can then be linked to genetic characterisation.

The use of circulating granulocyte counts is a simple yet effective way to give scientists access to better phenotypic classification in large-scale studies. It is one of those observations that prompts responses like, "anyone can do that", or "I wish I'd thought of that!". To which the reply might be, "Yes. So now let's make use of it".

Competing interests: None.

Thorax 2009;**64**:369–370. doi:10.1136/thx.2008.109710

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The art of replication

Michael Kabesch

Hot topics in science, like fashion, come and go. Asthma genetics is no exception to this rule. Asthma candidate genes come and very few stay for good, changing from "candidate" gene to "disease" gene. Only an exclusive few survive a thorough replication process.

The asthma candidate genes studied in the replication approach by Blakey and colleagues¹ (pages 381–7) were originally discovered by positional cloning, a technique based on linkage studies using microsatellite markers and subsequent fine mapping of these linkage signal loci in families. Until recently, when genome-wide association studies (GWAS) entered the field, this was

the method of choice to detect novel asthma candidate genes. In contrast to candidate gene studies, which are hypothesis driven and always only as good as the underlying hypothesis, positionally cloned genes had the aura of being the better asthma candidate genes, as they were detected by a systematic genomic approach not potentially biased by the researcher's own belief in a hypothesis. Seven years after the first positionally cloned asthma candidate genes were published in 2002/2003, positional cloning has lost its nimbus. It became clear that these genes are no better candidates than those detected by hypothesis-driven approaches. Bias is not excluded by positional cloning. What counts at the end of the day in complex disease studies is replication and verification.

Interestingly, the interpretation of replication has become a matter of debate itself. Some argue that larger populations

may not necessarily be better for replication of genetic signals in complex diseases. Disease definitions may be broader in large studies as phenotype assessment may be limited in larger studies. Environmental conditions in the replication study may be very different from the population where the initial result was acquired. As gene–environment interactions are an important factor in complex diseases and as asthma could be a syndrome with different underlying mechanisms, this diversity may influence the prospect for replication.

Another important issue in replication is that different but related phenotypes are sometimes used synonymously to argue for positive replication. This approach has been called "loose replication". For example, instead of replicating the association of single nucleotide polymorphism (SNP) A in gene 1 with asthma, an association of SNP B in gene 1 with elevated immunoglobulin E (IgE) in the replication study is observed. As elevated IgE may be related to asthma, this is counted as a loose replication of the initial finding. However, this seems rather like

Correspondence to: Professor Michael Kabesch, Hannover Medical School, Center for Pediatrics, Clinic for Pneumology and Neonatology, Carl-Neuberg Strasse 1, D-30625 Hannover, Germany; Kabesch.Michael@mh-hannover.de

an extension of the original finding, which needs further replication itself to increase the likelihood that the reported association is real. Nevertheless, these studies, as well as failures to replicate, should be published to evolve a full picture of a gene's possible effects. Much can be learned from these findings and, in the end, they may be even more important for our understanding of genetics and disease mechanisms in complex diseases than straightforward replication.

Blakey *et al* studied five positionally cloned genes, initially found in linkage and association studies in children. They now aimed to replicate associations with asthma, wheezing and IgE in their large British population sample. They found small but significant effects for polymorphisms in *DPP10* and *ADAM33* (and

borderline for *GPR154*), with odds ratios of around 1.1 per allele.

Is this the final verdict on the role of these genes in asthma? Rather not. Although this is a large and well phenotyped cross-sectional and probably unbiased population sample, it is a snapshot in time. The individuals studied were born in 1 week of 1958 in Britain. It may be assumed that environmental factors were very different at the time these individuals developed their asthma compared with children growing up nowadays. Looking back on the second half of the 20th century we have witnessed rising prevalence rates for asthma, and fundamental changes in lifestyle, diet, and personal and meta-environments. It could very well be that asthma of today's children and youth is the result of

mechanisms different from those relevant for asthma development 50 years ago. However, when associations are found consistently across time, this could point to very basic and timeless asthma mechanisms. This is nothing but a hypothesis—but one to consider when indulging in replication studies such as this that look simple at first glance but may hold more food for thought than expected.

Competing interests: None.

Thorax 2009;64:370–371. doi:10.1136/thx.2008.105197

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Change in smoking status after low-dose spiral chest CT screening for lung cancer: opportunity for smoking intervention

Matthew M Clark,¹ James R Jett^{2,3}

Several studies have examined the effectiveness of low-dose spiral chest CT scan screening for early detection of lung cancer. Currently, most patients with lung cancer are diagnosed at an advanced stage. Early detection of lung cancer could help reduce the high mortality rate associated with lung cancer. It is hoped that the advanced technology of this new screening procedure may prove to be comparable to the reduction in mortality associated with having the recommended mammographies for early detection of breast cancer.

However, unlike most cancers, lung cancer is associated with a specific behaviour—namely, smoking cigarettes. It has been proposed that informing cigarette smokers of negative lung cancer screening

results could give them permission, licence or a “green light” to continue smoking. If a screening procedure leads to increased smoking rates, it is possible that any health benefits associated with early detection of lung cancer would be offset by an increase in the prevalence of lung cancer due to increased smoking rates.

Fortunately, several non-randomised screening programmes have examined this concern and, so far, negative screening results do not appear to increase smoking rates. For example, we found that CT screening results did not affect the smoking rates of 1475 adults with a smoking history of at least 20 pack-years at 1-year follow-up.¹ Interestingly, in this same sample we found that three abnormal screens over a 3-year period promoted smoking abstinence (41.9%). Those with three negative screens still had a higher than expected quit rate (19.8%) over the 3 years.²

The study by Ashraf and colleagues in this issue of *Thorax* (see page 388) is unique in that participants were randomised to receive either CT screening or to not have any

screening.³ A randomised study design helps to further understand the possible effects that lung cancer screening may have on smoking rates. The fact that the authors also found that the screening and control subjects both demonstrated a quit rate of almost 12% is further support of previous research findings from single-arm studies that negative screening results do not promote or encourage continued cigarette smoking. The study also demonstrates the strength of a randomised controlled trial, and showed that smoking cessation was equal in the two arms regardless of whether or not the subjects received CT screening. Without the control arm it would be possible mistakenly to attribute the successful smoking cessation to having CT screening.

What is striking is that the quit rate found in participants in CT screening—either in randomised trials or single-arm studies—is higher than expected. Participants usually have a 20 pack-year history of smoking so they are long-term addicted smokers. That almost 12% quit at 1 year in this study and that 14% quit at 1 year in our study shows that this is a population of smokers with a high motivation to quit smoking. Our attempt to take advantage of this teachable moment by enhancing smoking abstinence with either internet resources or written materials proved ineffective.⁴ It is our current opinion that more intensive, truly multi-disciplinary approaches are needed for smoking cessation. We therefore suggest that smoking cessation interventions that combine pharmacotherapy with nicotine counselling and use screening results to enhance motivation for quitting smoking may prove to be efficacious for this

¹ Department of Psychiatry and Psychology, Mayo Clinic, Rochester, Minnesota, USA; ² Division of Pulmonary and Critical Care Medicine, Mayo Clinic, Rochester, Minnesota, USA; ³ Division of Medical Oncology, Mayo Clinic, Rochester, Minnesota, USA

Correspondence to: Dr J R Jett, Division of Pulmonary and Critical Care Medicine, Mayo Clinic, Rochester, MN55905, USA; jett.james@mayo.edu