Searching for the human genetic factors standing in the way of universally effective vaccines

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Vaccines have revolutionized modern public health. The effectiveness of some vaccines is limited by the variation in response observed between individuals and across populations. There is compelling evidence that a significant proportion of this variability can be attributed to human genetic variation, especially for those vaccines administered in early life. Identifying and understanding the determinants of this variation could have a far-reaching influence upon future methods of vaccine design and deployment. In this review, we summarize the genetic studies that have been undertaken attempting to identify the genetic determinants of response heterogeneity for the vaccines against hepatitis B, measles and rubella. We offer a critical appraisal of these studies and make a series of suggestions about how modern genetic techniques, including genome-wide association studies, could be used to characterize the genetic architecture of vaccine response heterogeneity. We conclude by suggesting how the findings from such studies could be translated to improve vaccine effectiveness and target vaccination in a more cost-effective manner.

1. Host genetics contributes to variability in response to all vaccines

Vaccination has been one of the most successful public health interventions in modern history and continues to save millions of lives every year [1,2]. The aim of any vaccination strategy is to achieve universal protection either directly or indirectly against any vaccine-targeted pathogen in all susceptible individuals in the population [3]. There is, however, a marked variation in how individuals respond and maintain immunity to every existing vaccine that can in turn contribute directly to the risk of primary vaccine failure [4].

The theme of this issue aims to explore the factors contributing to biological variation in vaccine responses and how identifying these factors may contribute to improvements in vaccination strategies. This observed variation in vaccine response is a complex trait [5] resulting from a combination of environmental and genetic factors. Large epidemiological studies and clinical trials have identified a range of environmental factors which are contributors to this variation including: age [6], sex [7], ethnicity [8], size (body-mass index) [9] and health, including smoking status [10], of individuals as well as the dose [11], route of administration and quality of storage of the vaccine [12]. Twin studies have proposed that genetic variation contributes as much as 70% to the total observed variability for the hepatitis B [9,13] and measles vaccines [14], which are also the vaccines with comparatively high rates of primary failure. It is now becoming increasingly possible to characterize the genetic variants contributing to complex traits in the new era of ‘-omics’ technologies. Characterizing and understanding the genetic determinants of vaccine response heterogeneity is, therefore, theoretically possible and could offer a cost-effective and feasible method to significantly reduce the incidence of vaccine failure [9,15].

In this review, we discuss the principles and practicalities of using these genomic technologies to identify the genetic factors contributing to vaccine response
Table 1. Terms used in defining response to vaccines [16,17].

<table>
<thead>
<tr>
<th>term</th>
<th>definition</th>
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<tbody>
<tr>
<td>vaccine efficacy</td>
<td>the reduction of incidence of vaccine-targeted disease in a vaccinated group compared with a non-vaccinated group under ‘ideal’ conditions—usually measured using double blind randomized controlled trials</td>
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<tr>
<td>vaccine effectiveness</td>
<td>the reduction of incidence of disease at the population level—usually determined following introduction of a vaccine on a national scale with subsequent analysis of disease reporting alongside adverse incidence data</td>
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<tr>
<td>protection against infection</td>
<td>successful stimulation of a long-lived immune response that interferes with colonization or replication of the targeted pathogen in the vaccinated individual</td>
</tr>
<tr>
<td>protection against disease</td>
<td>successful stimulation of a long-lived immune response that prevents the development of disease from the targeted pathogen when colonization or replication of the pathogen may still occur</td>
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<td>primary vaccine failure</td>
<td>development of disease in a vaccinated individual due to failure to mount an appropriate immune response against the vaccine-targeted pathogen</td>
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<tr>
<td>secondary vaccine failure</td>
<td>development of disease in a vaccinated individual despite a documented appropriate immune response against the vaccine-targeted pathogen</td>
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<tr>
<td>correlate of protection</td>
<td>an immunological measurement that is responsible for and statistically interrelated with protection; can be against either infection or disease</td>
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<tr>
<td>surrogate of protection</td>
<td>an immunological measurement that substitutes for the true immunologic correlate of protection which may be unknown or not easily measurable</td>
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variability with specific reference to work that has already been published focusing on hepatitis B (HBV), measles (MV) and rubella (RV) vaccines. We conclude by discussing how we anticipate the field to develop based on advances in genomics and how findings from such studies may be used to benefit vaccine development and deployment at a practical level. Throughout this review, we use antibody response as an immunological measure of vaccine response but it is important to note that although this is one of the most commonly used measures, there are alternatives, particularly measures of cellular immunity, that may be more appropriate dependent upon the particular vaccine in question.

2. The practical considerations of undertaking genetic studies of vaccine response

(a) What is a ‘vaccine response’

In order to identify and understand the factors responsible for the variability in vaccine response, it is first necessary to define the vaccine response itself. Terminologies can be confusing and so definitions of terms used in this review can be found in table 1. Ultimately, a vaccine can only be defined as ‘effective’ if it is observed to reduce the prevalence of the infection it is targeting in populations [18]. Given the costs and risks associated with a large-scale introduction of a vaccine, especially if the prevalence of the targeted disease is low, it is necessary to first undertake randomized clinical trials of vaccines that can produce a measure of ‘efficacy’. However, vaccine developers will try to predict whether or not a vaccine will be efficacious based on immunogenicity data before embarking on expensive clinical trials. This requires a detailed appreciation of the biological mechanisms involved in protection of an individual against the vaccine-targeted pathogen and ultimately knowing which immunological measurements constitute suitable correlates or surrogates of protection. It is important to distinguish between protection against disease and infection which may have consequences in terms of chronic carriage and infectivity. Formally correlating immune markers with protection against either infection or disease is challenging although suitable correlates do exist for hepatitis B, measles and rubella [16]. A serum antibody concentration of more than 10 mIU ml$^{-1}$ measured against the hepatitis B surface antigen correlates well with protection against both disease and infection. An antibody titre of more than 120 mIU ml$^{-1}$ against measles measured by the microneutralization assay correlates with protection against disease, whereas levels more than 1000 mIU ml$^{-1}$ are required to protect against infection. Similarly, a titre of more than 10 IU ml$^{-1}$ of antibody against rubella determined using the microneutralization assay can be considered to be protective against disease. It is important to note that studies investigating measles will often report levels of total antibody measured using an enzyme-linked immuno assay rather than measuring the more functional antibody levels using the microneutralization assay. Similar thresholds are defined for other diseases caused by toxin producing bacteria (diphtheria and tetanus) and encapsulated bacteria (Haemophilus influenzae, pneumococcal and meningococcal species), but equivalent correlates of protection are not so well characterized for other important infections, including HIV, malaria and tuberculosis. The absence of simple correlates partly explains why the development of vaccines against these pathogens is particularly challenging [19,20].

Tables 2 and 3 list the studies that have attempted to identify the genetic factors responsible for vaccine response heterogeneity. The majority of these studies have already been reviewed comprehensively by various groups [5,49–51], but new studies continue to emerge every year. The lists are by no means exhaustive and some studies have been specifically excluded, such as those including individuals with recognized immunodeficiency states (including patients receiving dialysis for example [52]) and those studies reporting haplotype associations which are inherently
Table 2. Studies investigating the genetic contribution to variability in the antibody response to hepatitis B vaccine (surface antigen—HBsAg). Some smaller candidate gene studies have not been included in the table [21–23]. HLA, human leucocyte antigen; LOD, logarithm of the odds; SNP, single nucleotide polymorphism; GWAS, genome-wide association study.

<table>
<thead>
<tr>
<th>vaccination schedule</th>
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<th>method used</th>
<th>population sample</th>
<th>top associations</th>
<th>refs</th>
</tr>
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<tbody>
<tr>
<td>HBsAg at 0 and 2 months</td>
<td>dichotomous responder case versus non-responder comparison at 6 months after start of vaccination for cases, unknown timing for controls</td>
<td>HLA candidate gene association using Chi-squared test</td>
<td>84 non-responders (&lt;10 IU ml(^{-1}) after at least four doses) and two cohorts of responders (&gt;1000 IU ml(^{-1}) after two doses) with 117 and 131 individuals — all Caucasian</td>
<td>DQB1*0602 ( (p &lt; 0.001) ) associated with increased antibody</td>
<td>[24]</td>
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<tr>
<td>HBsAg at 0, 1, 2 and 12 months</td>
<td>dichotomous responder case versus non-responder comparison at 3 months after start of vaccination</td>
<td>HLA candidate gene association using Chi-squared test</td>
<td>46 poor responders (&lt;10 IU l(^{-1}) at 3 months) and 100 good responders (&gt;1000 IU l(^{-1})) all Caucasians 20–26 years old</td>
<td>DRB1<em>01</em>, DR5, DQB1<em>0301, DQB1</em>0501, DPB1<em>040</em> each associated with increased antibody (all (p &lt; 0.001))</td>
<td>[25]</td>
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<tr>
<td>HBsAg at 0, 1 and 6 months (adult) or 0, 1 and 2 months (child)</td>
<td>dichotomous responder case versus non-responder comparison 1 month after final vaccination</td>
<td>HLA candidate gene association using Chi-squared test</td>
<td>73 non-responders (&lt;10 IU l(^{-1})) and 53 responders (&gt;10 IU l(^{-1})) from a cohort of 4269 Caucasian adults of variable age and 62 non-responders and 56 responders from a cohort of 1122 Caucasian children 3 months of age</td>
<td>DRB1*13 ( (p &lt; 0.001) in adults and (p = 0.0375) in children) associated with increased antibody response</td>
<td>[26]</td>
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<tr>
<td>HBsAg at 0, 1 and 6 months and booster at 6–12 months if low antibody</td>
<td>dichotomous responder case versus non-responder comparison 1 month after final vaccine</td>
<td>transmission disequilibrium test</td>
<td>10 families (40 subjects) with true non-responder probands recruited and vaccinated at 4 days of life (antibody &lt;10 mIU ml(^{-1}) after booster) and 17 families (51 subjects) with slow responder probands (&lt;10 mIU ml(^{-1}) pre-booster and &gt;100 mIU ml(^{-1}) post-boosted) recruited in Italy</td>
<td>C4A deficiency allele (C4AQ0) always transmitted to non- or slow-responding probands with a LOD score of 1.58 (not statistically significant)</td>
<td>[27]</td>
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Table 2. (Continued)

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<tr>
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<tbody>
<tr>
<td>HBsAg at 0, 1 and 6 months</td>
<td>dichotomous responder case versus non-responder comparison 1 month after final vaccine</td>
<td>HLA and other candidate gene association study using Fisher’s exact test and logistic regression</td>
<td>53 responders (&gt;10 IU l⁻¹) and 73 non-responders (&lt;10 IU l⁻¹) from a cohort of 4269 German individuals</td>
<td>DRB1<em>1301 (p &lt; 0.0005) associated with increased response C44 deficiency alleles (p = 0.001) and deletion alleles (p = 0.006) were associated with non-response. This was independent of DRB1</em>0301 carriage. DRB1*0701 (p &lt; 0.00052) also associated with non-response</td>
<td>[28]</td>
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<tr>
<td>HBsAg varying doses at various times</td>
<td>log-transformed peak anti-HBsAg antibody response 1 – 12 months after last vaccination</td>
<td>candidate gene association study using linear regression</td>
<td>662 infant vaccines from The Gambia as a discovery cohort and 393 similar infants as a replication cohort</td>
<td>ITGAL R719V polymorphism associated with increased peak anti-HBsAg response in both cohorts (p = 0.011) out of 715 SNPs across 133 genes</td>
<td>[13]</td>
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<tr>
<td>HBsAg at 0 and either 6 or 12 months</td>
<td>dichotomous responder case versus non-responder comparison at 18 months following first vaccination</td>
<td>candidate gene association study using Cochran–Armitage, Breslow–Day, Cochran–Mantel–Haenszel tests</td>
<td>405 cases (undetectable anti-HB) and 530 controls (100–1000 IU l⁻¹) for discovery and 304 cases and 538 controls in replication from Indonesian adults over 5 years of age</td>
<td>SNPs in FOXP1 (p = 9.2 × 10⁻⁶), BTLN2 (p = 8.5 × 10⁻⁷), and HLA-DQB1 (p = 6.7 × 10⁻⁶) associated with increased response HLA-DRA (p = 5.6 × 10⁻⁶) associated with non-response</td>
<td>[29]</td>
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<tr>
<td>HBsAg at 0 and either 6 or 12 months</td>
<td>categorical comparison 6 months after final dose</td>
<td>genome-wide association study using ordinal logistic regression</td>
<td>631 low (0–99.9 IU l⁻¹), 597 medium (100–999.9 IU l⁻¹) and 829 high (&gt;1000 IU l⁻¹) responders as discovery and 667 low, 653 medium and 836 high responders as a replication cohort from 5100 Indonesian adults</td>
<td>SNP in HLA-DP (p = 2.91 × 10⁻¹⁴) associated with increased antibody responses SNPs in HLA-DR (p = 6.53 × 10⁻²²), and class III HLA tagging SNP (p = 1.24 × 10⁻¹⁷) both found to be independently associated with lower antibody responses</td>
<td>[30]</td>
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<tr>
<td>HBsAg booster</td>
<td>dichotomous responder case versus non-responder comparison 4–6 weeks after booster</td>
<td>HLA candidate gene association study using Chi-squared test</td>
<td>510 responders (&gt;1 mIU ml⁻¹) and 171 non-responders (&lt;1 mIU ml⁻¹) from a cohort of 2057 neonatally vaccinated Taiwanese students</td>
<td>DPB1 alleles 02:02 (p = 9.1 × 10⁻⁴), 03:0101 (p = 0.0041) and 04:0101 (p = 0.017) were significantly associated with response DPB1 alleles 05:01 (p = 1.9 × 10⁻⁸) and 09:01 (p = 0.017) were associated with non-response</td>
<td>[31]</td>
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(Continued.)
complex to interpret, albeit relevant [24,53,54]. All of the studies listed in tables 2 and 3 tested for association between genetic polymorphisms and antibody correlates of protection. Most of the studies defined participants either as dichotomous ‘responders’ (those likely to be protected against disease in the case of HBV) and ‘non-responders’ using the cut-offs defined above. Other studies, particularly those looking at MV and RV, investigated the antibody response as a continuous quantitative variable. Analysing vaccine responses as a quantitative variable makes no assumptions about defined cut-offs. The approach may also provide significant benefits in terms of study design, because it allows study investigators to include every individual with a measured outcome rather than having to select a number of defined ‘non-responders’ and it avoids any potential selection bias that may result from the more familiar case–control approach. Defining a trait as continuous may also provide increased statistical power to find novel associations [55–57], and there are a range of methodologies available to analyse such traits in genetic studies that have proved to be very successful in looking at other traits, including height [58] and lipid levels [59].

(b) Defining vaccine failure

Defining individuals who have failed vaccination (table 1) rather than depending upon immune correlates of protection may be useful because vaccine failure has more clinical relevance and provides the opportunity to consider the immune system as a whole rather than focusing on one or two elements of humoral or cellular immunity. However, the numbers of individuals who experience vaccine failure are often small in number, difficult to trace and may have other clinically undiagnosed causes for their immunodeficiency which are obviously important to define prior to their inclusion in the study. One HBV candidate gene study listed in table 2 attempted to look at hepatitis B core antibody levels in vaccinated individuals as a marker of failure of the vaccine to prevent hepatitis B infection, but final numbers were too small to make any definitive conclusions [13]. A more recent study that is not highlighted in table 3 suggested that variants in the measles receptor CD46 and the pathogen nucleic acid sensing receptor TLR8 were associated with failure of MV [60]. The results from this study should be interpreted with caution because the study participants defined as vaccine failures did not always have a laboratory confirmed diagnosis of measles. The selection of control individuals deemed ‘vaccine responders’ in such analyses is also of fundamental importance. Any controls should have been exposed to the pathogen of interest. This could be made more reliable by using family or other household members as controls or limiting analyses to diseases where near ubiquitous exposure in the population is likely. One study that demonstrates such careful control selection looked at *Haemophilus influenzae* vaccine failures compared with adult population controls and found that mutations in the MAL/TIRAP gene, which is critical in pathogen sensing, and the anti-inflammatory IL-10 cytokine gene were associated with substantially increased risks of non-meningitis and epiglottitis disease, respectively [61]. These are, unfortunately, the only published examples of studies that have, to the best of our knowledge, aimed to identify genetic variants associated with vaccine failure, highlighting the need for studies investigating these issues in the future.
Table 3. Studies investigating the genetic contribution to variability in the antibody response to rubella and measles vaccines. Many studies have been published investigating similar gene associations and phenotypes in the same cohorts so only the most recent studies are discussed for each cohort in question. Significance values are shown for the top SNP in each gene or locus only.

<table>
<thead>
<tr>
<th>Vaccination Schedule</th>
<th>Phenotype</th>
<th>Method Used</th>
<th>Population Sample</th>
<th>Top Associations</th>
<th>Refs</th>
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<tbody>
<tr>
<td>Live attenuated rubella vaccine with second dose received at median of 12 years of age</td>
<td>Log-transformed rubella neutralizing antibody at median of 5.2 years since last vaccination</td>
<td>Candidate gene association study using linear regression</td>
<td>887 children and young adults with interquartile range 13–17 years of age (Rochester cohort) and 542 adult Army personnel with range 22–27 years of age (San Diego cohort)</td>
<td>Multiple SNPs associated (meta-analysis ( p &lt; 0.05 )) with variable effect on antibody including SNPs in PVR ((p = 0.008)), PVRL2 ((p = 0.025)), IL6 ((0.027)) and retinoic acid receptor beta ((0.037)) all associated with reduced antibody levels</td>
<td>[33–35]</td>
</tr>
<tr>
<td>Live attenuated rubella vaccine with second dose received at median of 12 years of age</td>
<td>Log-transformed rubella neutralizing antibody at median of 5.2 years since last vaccination</td>
<td>Candidate HLA gene association study</td>
<td>706 children and young adults with interquartile range 13–17 years of age (Rochester cohort) and 1012 adult Army personnel with range of 22–27 years of age (San Diego cohort)</td>
<td>HLA-DPB1<em>04:01 ((p &lt; 0.001) in Rochester and ( p = 0.08 ) in San Diego) associated with increased antibody responses HLA-B</em>27:05 ((p = 0.067) in Rochester and ( p = 0.047 ) in San Diego) associated with reduced antibody responses</td>
<td>[36,37]</td>
</tr>
<tr>
<td>Live attenuated rubella vaccine at median of 15 months and 7 years of age</td>
<td>Log-transformed neutralizing antibody at median of 6.4 years since last vaccination</td>
<td>Genome-wide association study using linear regression</td>
<td>897 healthy Caucasian children and younger adults from USA as discovery cohort with no replication</td>
<td>SNPs in HLA-DPB1 associated with reduced antibody levels (top SNP ( p = 8.62 \times 10^{-6} ))</td>
<td>[38]</td>
</tr>
<tr>
<td>Live attenuated measles vaccine at median of 15 months and 5 years of age</td>
<td>Log-transformed measles neutralization antibody levels at median of 7.5 years since last vaccination</td>
<td>Candidate gene association study using linear regression</td>
<td>764 individuals from two independent age-stratified random cohorts of healthy schoolchildren and young adults from USA</td>
<td>SNPs in retinoic acid receptor beta ((p = 0.001)), RIG1 ((p = 0.01)) and IL12B ((p = 0.037)) consistent with findings from earlier study [39] associated with increased antibody response SNPs in CD46 ((p = 0.0007)) consistent with [39] associated with reduced antibody response Some evidence of variants in toll-like receptors 2 and 4 associated with variable effects on antibody levels ((p = 0.0012–0.0071)) although not replicating gene-specific results from earlier smaller study [40]</td>
<td>[41–45]</td>
</tr>
<tr>
<td>Live attenuated measles vaccine at median of 15 months and between 4 and 12 years of age</td>
<td>Log-transformed measles specific IgG (cohort 1) and neutralization antibody (cohort 2) between 2 and 6 years since last vaccination</td>
<td>Candidate HLA gene association study using mixed effects linear models</td>
<td>346 children between 12 and 18 years old from the USA (cohort 1) ( p = 0.03) and 388 children between 11 and 19 years old from USA differing significantly in terms of age of enrolment, age of first and second vaccinations and ethnicity</td>
<td>Increased antibody responses seen with HLA-DQA1<em>0201 ((p = 0.03)) and HLA-DQA1</em>0101 ((p = 0.004)) Other reported replicated associations with antibody response not observed below consistent ( p = 0.05 ) significance level (see main text)</td>
<td>[46–48]</td>
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(c) Selecting the study design

As can be appreciated from the studies listed in tables 2 and 3, several study designs have been used to investigate the contribution of host genetics to vaccine response, consistent with efforts employed to dissect the contribution of genetics to other complex traits. The approaches used can be categorized into twin, family association, candidate gene and genome-wide association (GWAS) studies. The practicalities, benefits and challenges associated with each of these different study types have been extensively reviewed elsewhere [50,62,63]. Twin studies have traditionally been used to estimate the differential contribution of genetic and environmental effects to biological complex traits, whereas candidate gene and family association studies are used to test for the association of plausible genes or loci with such traits. Candidate gene studies are commonly plagued by a failure to include sufficient numbers of cases and controls which limits the statistical power of the study. They are also susceptible to bias attributable to cryptic population stratification and there is an inherent risk of false-positive association especially in the absence of formal replication using other cohorts analysed independently by, preferably, separate investigators.

GWAS has recently become a hugely popular and successful study design dependent upon the nascent understanding of common genetic variation within the human genome and the ability to ‘tag’ this variation by typing a large number (commonly 500 000 to 2 million) of single-nucleotide polymorphisms (SNPs) across every chromosome using high-throughput technology. This concept relies upon a comprehensive understanding of the extent of linkage disequilibrium between the typed SNPs, and other variation which is starkly population-dependent [64]. GWAS are expensive to undertake because of the requirement for large numbers (thousands if not tens of thousands) of individuals and they do come with a risk of missed true associations owing to their stringent thresholds for significance (currently statistical test p-values of less than 5 × 10⁻⁸). These challenges can be overcome with careful study design, and the approach provides significant benefits over other methods such as candidate gene studies in terms of providing a reliable and accurate picture of population structure in the studied cohorts. The comprehensive genotyping employed in GWAS also enables the fine mapping of associated variants to be undertaken using advanced statistical approaches such as imputation, which can help increase our understanding of associations in a biological context [65].

The majority of the studies listed in tables 2 and 3 follow the candidate gene format with the exception of two GWAS which have been published for HBV and the one for RV. This apparent bias towards candidate studies is not entirely surprising given the relatively recent introduction of GWAS and the popularity of candidate gene studies owing to the costs and availability of the technology over the past 20 years. Some of the published candidate gene studies included large numbers of individuals, attempted replication and corrected for multiple testing [9,13], but the vast majority of published studies were not so carefully designed. Likewise, the majority of the associations reported in the candidate studies have not yet been replicated in the GWAS. This absence of replication may either suggest that the original candidate gene findings were false-positives, or that the stringent threshold required for the GWAS resulted in the associations being missed. These inconsistencies are not unique to vaccine response as a phenotype and can, indeed, be observed for any complex trait which has undergone genetic analysis over the past few decades.

(d) Origins of study heterogeneity

When comparing the findings of the studies listed in tables 2 and 3 and attempting to rectify the inconsistencies such as those highlighted between the candidate gene and GWAS findings above, it is important to identify any potential sources of differences between the studies. Nearly all of the studies listed in table 2 were superimposed upon clinical trials testing immunogenicity or efficacy of HBV or population studies investigating baseline seropositivity and response to booster vaccination. It can be appreciated that the time points between receipt of the vaccine and measurement of response varied quite substantially between the studies. This was also a particular problem in the studies listed in table 3 where the median time between the final vaccine and immunological measurement could vary substantially (often more than 5 years). Choosing the appropriate time to measure the vaccine response requires an appreciation of the kinetics of antibody generation. Antibody concentrations rise in the days to months following vaccine administration (with the rate of rise dependent upon a number of key non-genetic factors, including the number of prior doses of vaccine received and age of the individual) with a subsequent peak and decay of antibody levels which are again dependent on a variety of factors. Unfortunately, the decay is not always predictably linear, and indeed, the different elements of the response are dependent on different cellular mechanisms [6]. It would therefore be preferable to measure the response at the peak, but because the time of peak response can vary substantially, it is often necessary to try to model these responses mathematically [13,66].

There are other factors that are important when comparing existing or planning future studies. Careful note should be made of any other drugs delivered at the same time as the studied vaccines, because some vaccines are well recognized to interact with the immunogenicity of others [67]. Furthermore, population differences may be important to consider. The proportion of non-responders following the routine number of doses of HBV, for example, is noted to vary significantly between different ethnic groups [8,68,69]. This may relate to differing environmental surroundings or their differing genetic background, most notably at key polymorphic loci such as the human leucocyte antigen (HLA) region. Fortunately, there are now several ways to account for this diversity in GWAS approaches, including random effect mixed models [70,71] or inclusion of summaries of the genetic variance between individuals using principal component analysis [72]. Differences in sex composition between studies may also play an important role in differential study findings, because sex is recognized to be an important predictor of immune response to some vaccines [7]. Sex is of course dependent upon genetics, and many immunological genes are present on the X chromosome, so accounting for gender differences may help identify important contributors to vaccine response heterogeneity.
(e) The genetic determinants of vaccine response heterogeneity identified to date

Despite the multiple limitations highlighted for the studies in tables 1 and 2, there are some clear reproducible associations. Various HLA-DRB1 alleles are differentially associated with both increased and reduced or non-response to the HBV. DRB1*01, 13 and 15 are relatively consistently associated with an increased response, whereas 03 and 07 are consistently associated with lower antibody responses. These differences have been explored experimentally in detail. Multiple groups have found that antigen-presenting cells (APCs) from peripheral blood mononuclear cells of non-responders are able to stimulate DR-matched helper T cells from responders as effectively as APCs from high responding individuals making a deficit in antigen processing and presentation less likely [25,73–75]. Conversely, these studies found that the T-cells from non-responders could not be activated by either their non-responding APC counterpart or DR-matched APCs from responders. The currently favoured theory is, therefore, that there is a ‘hole’ in the T-cell repertoire associated with these particular HLA alleles resulting in reduced antibody levels. The mechanism for increased responses observed with some HLA alleles is proposed to be a consequence of improved antigenic epitope binding [54]. These theories are yet to be proven correct.

Other interesting consistent observations from the HBV studies are the findings of associations between vaccine response variation and HLA-DP alleles and complement factor C4A gene which resides in the so-called class III region of the HLA complex. These associations appear to be independent of the other class II associations in the HLA region. Identifying the precise variant associated with the HBV response variation in the HLA locus is inherently difficult because of the complex and often long-range linkage disequilibrium that exists across the region. The HLA-DP findings are particularly interesting given the concurrent associations of SNPs in the gene with viral clearance in chronic hepatitis B infection in Asian populations [76], and the replicated C4A findings may go some way towards explaining the recently reported class III lagging SNP found associated with HBV response in the largest GWAS performed to date [30]. This complement association may have significant biological relevance given the reported importance of complement to enhancing B-cell activation, survival and class-switching in the presence of T-cell-dependent antigens such as hepatitis B surface antigen [28,77].

MV and RV are often compared concurrently because they are commonly administered simultaneously and are both of the same class of live, attenuated virus vaccine. The majority of the studies that have been published looking at these vaccines have been undertaken by a single group and have been reviewed extensively elsewhere [78,79], but there has been a series of more recent studies looking at these vaccines which have resulted in some interesting new observations. A large discovery GWAS has been completed looking at RV demonstrating again that the HLA-DRB1 locus is likely to be associated with rubella neutralization antibody response [38]. The top SNP ($p = 5.62 \times 10^{-8}$) did not surpass the classical level of GWAS significance and will, therefore, require independent replication especially because another association with HLA-DRB1*04:01 allele reported by the same group in an independently recruited cohort was in the opposite direction [36]. This latter candidate gene study also proposed an association between the class I HLA-B*27:05 allele and rubella antibody levels, although no independent class I associations were observed in the GWAS. No GWAS studies have yet been published involving MV, but a series of candidate gene studies provide some evidence of HLA associations. Most recently, using two cohorts consisting of over 300 adolescents and adults each, an association was observed between the class II DQA1*0201 allele and increased antibody response to the attenuated vaccine [46]. Other class I (B*3503) and class II (DRB1*0701) associations were reported although these did not pass the conservative significance threshold ($p = 0.05$) in either cohort. The class II associations for both MV and RV are highly relevant given the discussion relating to HBV response above, and it is tempting to speculate that similar biological mechanisms may be responsible for the observed variation, although this has yet to be formally tested. The role of the relatively less studied DP alleles rather than DR is certainly interesting. The class I associations are novel, but because the measles and rubella vaccines are live attenuated viruses, class I HLA alleles are very likely to be relevant in mounting an effective CD8T cell immune response and the associations merit further investigation.

HLA is estimated to contribute only to a minor proportion of the variation in HBV, MV and RV post-vaccination antibody titres with variants in other non-HLA genes estimated to contribute to the majority of the observed variation [37,80]. Notably, and consistent with the HBV GWAS, the RV GWAS did not identify any such variants. Although there have been various plausible extra-HLA variants that have been proposed to be associated with variation in response to all of the vaccines discussed herein, none of these findings have been independently replicated and should therefore be considered with caution.

The associations reported above for HBV, MV and RV are both interesting and potentially informative. However, when considered altogether, the studies highlight the fact that there are still substantial gaps in our knowledge and understanding of vaccine response. It is therefore essential to perform more studies to independently replicate existing putative associations, exclude false-positive associations, and identify variants that explain the remainder of the variation in vaccine response and appreciate how other non-genetic factors interrelate with such observations. Large-scale GWAS offer an attractive method to help dissect the variable contributions of genetic and environmental factors but it will be vital to learn from existing studies to maximize the chances of making novel discoveries.

3. Making the most of genetic association findings

The findings of large-scale GWAS looking at multiple vaccines will be available in the near future. These studies should focus on multiple populations, using large numbers of individuals, paying careful attention to the measured phenotype and analytical strategy employed aiming to maximize the changes of both validating putative associations from existing studies and uncovering novel associations. Combining the findings of such studies, including work from our laboratories involving various initiatives such as the VaccGene
consequently help reduce worldwide outbreaks of pertussis. Similarly, although MV generates a protective immune response in over 90% of vaccinated individuals, failure to elicit a protective immune response in 10% of vaccinated individuals may have contributed to the outbreaks of measles observed in the USA in the 1990s [1]. Consequently, a twodose vaccination schedule has been introduced in many developed countries that reportedly increases the proportion of vaccinated individuals mounting a protective immune response to 98% [89,90] while also boosting immunity to measles in older children. If it does become possible to characterize the genetic factors contributing to both primary vaccination failure and immunogenicity, it may become cost-effective to identify and offer a second dose of vaccine only to those individuals who are identified, based on their genetics, to be at high risk of failure and/or poor maintenance of immunity to measles following a single dose of MV.

4. Future perspectives and conclusions

Our understanding of common genetic variation in humans is now at a point where we are becoming intent on studying other elements of variation including analysing rare (less than 1% minor allele frequency in the population) variants and epistatic modifications [91]. The field of rare variants is a particularly exciting field because it is suspected that individuals at the extremes of vaccine response may harbour large-effect low-frequency variants giving rise to the so-called extreme phenotypes that may represent forms of ‘primary immunodeficiencies’ when at the lower end of the response spectrum [92]. Identifying these variants requires new strategies to look at the human genome largely centred upon exome or whole-genome sequencing. Although such technologies are at an early stage of development they are also beginning to shed light on areas of the genome that have been under natural selection pressure throughout our evolution [93,94]. Techniques are now available that can begin to integrate the findings of GWAS studies with these discoveries to help verify plausible associations [95] and, because vaccine responses are signatures of how our immune system has been shaped by pathogen-driven selection, these studies may help shed light on this area which has long fascinated scientists and the general public alike [96].

This review summarizes some of the discussions at a meeting where it was made clear that the new ‘-omics’ era may hold substantial promise in terms of understanding the various elements contributing to biological variation in response to vaccination. Human genetics undoubtedly plays a major role in this variation but it is essential that any studies investigating the contribution of genetics to vaccine response are carefully planned and should account for known environmental variables. The results of these studies should help us to understand how we can either design better vaccines that protect nearly 100% of those vaccinated and how we can deploy vaccines in a more cost-effective way.

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