A genome-wide association study of host genetic determinants of the antibody response to Anthrax Vaccine Adsorbed

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ABSTRACT

Several lines of evidence have supported a host genetic contribution to vaccine response, but genome-wide assessments for specific determinants have been sparse. Here we describe a genome-wide association study (GWAS) of protective antigen-specific antibody (AbPA) responses among 726 European-Americans who received Anthrax Vaccine Adsorbed (AVA) as part of a clinical trial. After quality control, 736,996 SNPs were tested for association with the AbPA response to 3 or 4 AVA vaccinations given over a 6-month period. No SNP achieved the threshold of genome-wide significance ($p = 5 \times 10^{-8}$), but suggestive associations ($p < 1 \times 10^{-5}$) were observed for SNPs in or near the class II region of the major histocompatibility complex (MHC), in the promoter region of SPSB1, and adjacent to MEX3C. Multivariable regression modeling suggested that much of the association signal within the MHC corresponded to previously identified HLA DR-DQ haplotypes involving component HLA-DRB1 alleles of *15:01, *01:01, or *01:02. We estimated the proportion of additive genetic variance explained by common SNP variation for the AbPA response after the 6 month vaccination. This analysis indicated a significant, albeit imprecisely estimated, contribution of variation tagged by common polymorphisms ($p = 0.032$). Future studies will be required to replicate these findings in European Americans and to further elucidate the host genetic factors underlying variable immune response to AVA.

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1. Introduction

The Anthrax Vaccine Research Program (AVRP) human clinical trial (clinicaltrials.gov identifier NCT00119067, hereafter referred to as AVA000) was a phase 4 study of the safety and immunogenicity of Anthrax Vaccine Adsorbed (AVA). Before 2008, the licensed AVA regimen consisted of subcutaneous (SQ) vaccine administration in 0.5 ml doses at weeks 0, 2, and 4 and months 6, 12, and 18 with yearly booster doses thereafter. AVA000 was designed to assess the impact on serological responses to AVA of switching from SQ to intramuscular (IM) administration and of omitting one or more doses from the licensed schedule. AVA000 was a multicenter, randomized, double-blind, placebo-controlled, noninferiority trial that enrolled 1563 healthy adults (18–61 years of age at baseline) between 2002 and 2008. A planned interim analysis of the first 1005 enrolled subjects demonstrated that a 3-dose IM regimen provided noninferior immunological priming by 7 months while producing fewer injection site adverse events [1]. The Food and Drug Administration has since modified their recommendations for the administration of the vaccine accordingly [2].

Anthrax produces cytotoxic effects through the interaction of three proteins: lethal factor, edema factor, and a binding component protective antigen (PA). No firm serologic correlate of protection in humans has been established. However, as PA is known to be an important component of an effective anthrax vaccine, antibodies to PA (AbPA) are commonly used as the primary

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measure of AVA immunogenicity [3]. While AVA has been judged safe and effective according to the Institute of Medicine, data from AVA000 and smaller previous studies have indicated significant inter-individual variability in the AbPA immune response [4,5]. This variability suggests potential host genetic influences, which are supported by observed differences in the AbPA response between European and African-Americans [1,6].

Twin studies have estimated that genetic effects on vaccine responses are strong, with estimated heritabilities for antibody levels ranging from 38.8% for mumps to as high as 88.5% in the case of measles [7]. The human leukocyte antigen (HLA) system has been the primary focus of research into specific genetic variants, including previous work by our group on response to AVA within the AVA000 trial population [8]. In that study, three HLA class II haplotypes with component *HLA-DRB1* alleles of *01:01,*01:02, or *15:01 were associated with a decreased AbPA response among European-American participants. Candidate gene studies of other vaccines have also implicated variation outside of the major histocompatibility complex (MHC), but genome-wide assessments of host genetic variation and vaccine response are scarce. We are only aware of published studies on antibody response to hepatitis B vaccine and T cell response to the MRKAd5 HIV-1 gag/pol/nef vaccine [9,10].

Much current research is directed at the development of next-generation anthrax vaccines, including recombinant vaccines and monoclonal antibodies designed to block the anthrax toxin [11]. Understanding the host contribution to the observed variability in the AbPA response will continue to be important as PA will likely remain a major component of future candidate vaccines. In addition, the National Biodefense Science Board recently endorsed conducting a clinical trial to study the anthrax vaccine response in children [12]. Immunogenetic characterizations may also serve to inform computational models aimed at "predictive vaccinology" [13]. One such mathematical model is that proposed by Kumar et al. [14] for the inflammatory response to natural anthrax infection. Another involves ongoing AVRP efforts using aerosolized *Bacillus anthracis* to infer a correlate of human immune protection by extrapolating results from rhesus macaques [15]. In that model one particular component aims at predicting future post-vaccination AbPA levels in order to estimate probabilities of subsequent protection. These predictions might be improved by the identification of suitable genetic correlates to explain portions of the population variability in AbPA response. With these potential applications in mind, here we report the results of a genome-wide association study (GWAS) conducted in the AVA000 trial population.

2. Materials and methods

2.1. Population Genetics Analysis Program (PopGen) study of the AVA000 trial population

The design and participant characteristics of the AVA000 trial population have been described previously [1,8]. Briefly, 1563 subjects were randomized to seven study arms: group 1 received the licensed regimen (8 doses, SQ), while group 2 also received 8 doses but with IM administration. Groups 3 through 5 received between 4 and 7 doses via IM administration, while groups 6a (SQ) and 6b (IM) received saline placebo.

2.2. Measurement of IgG antibody to protective antigen

Methods for the measurement of AbPA using a quantitative enzyme-linked immunosorbent assay (ELISA) are described in Semenova et al. [16]. As in our previous analysis of HLA polymorphisms [8], we focus here on responses during the initial 7 months

of the AVA000 trial, with AbPA measured at 4 time points (4, 8, 26, and 30 weeks post-baseline) in response to either 3 or 4 doses of AVA. A full discussion of the impact of route of administration and number of vaccinations on the AbPA response has been described elsewhere [1]. We excluded AbPA measurements taken after missed vaccinations or at times that deviated significantly from those specified in the study protocol (Supplementary Table 1).

2.3. Genotyping and quality control

Of the 1303 AVA000 participants randomized to AVA-containing regimens, 1070 individuals were genotyped (sample call rate > 95%, 1 sample excluded) using the Affymetrix Genome-Wide 6.0 array (Affymetrix Inc., Santa Clara, CA, USA). Due to the multi-ethnic composition of the trial population, we inferred familial relationships (kinship coefficients) using the algorithm implemented in *KING*, which is designed to be robust against population structure [17]. We examined sample pairs with kinship coefficients greater than or equal to $2^{-4.5} = 0.04419$ (threshold for third-degree relatives suggested by the *KING* developers), removing both samples for pairs estimated to be monozygotic twins (8 pairs) and randomly retaining one individual from any other type of pair-wise familial relationship (73 samples excluded in total). On the basis of a multidimensional scaling (MDS) analysis of the remaining 997 samples (see Supplementary Fig. 1), we retained individuals in the present analysis that clustered into the majority racial/ethnic group representing European-Americans ($n = 726$). The numbers of participants in other racial/ethnic population groups available from AVA000 (including African-Americans and Hispanic-Americans) precluded a meaningful analysis of these subgroups. Using *PLINK* [18], we excluded SNPs with individual genotyping call rates < 99%, minor allele frequencies (MAF) < 5%, or evidence of deviation from Hardy–Weinberg Equilibrium (HWE; *p*-value < 0.0001).

2.4. Supplementary genotyping with the ImmunoChip

Of the 726 European-Americans included in the GWAS analysis, 671 (due to sample availability) were also genotyped on the ImmunoChip, a custom Illumina Infinium array (196,524 SNPs) designed for replication and fine mapping of established GWAS loci and strong candidate genes for autoimmune and inflammatory diseases [19]. We applied the same quality control criteria as with the Affymetrix 6.0 array data. In total, we evaluated a total of 736,996 autosomal SNPs (651,201 from the Affymetrix array and 85,795 non-overlapping SNPs from the ImmunoChip) for association with the AbPA response to AVA.

2.5. Statistical modeling of AbPA response

As in our previous analysis of HLA polymorphisms, we modeled the association of each SNP (assuming an additive mode of inheritance) with the longitudinal profile of $\log_{10}$ transformed AbPA measured at 4, 8, 26, and 30 weeks from baseline [8]. Briefly, parametric regression models were implemented using the *survival* package in the R Statistical Computing Environment [20] to account for left-censoring induced by the detection limit of the ELISA assay (3.7 μg/ml). Generalized Estimating Equations (GEE) were used to account for multiple AbPA measurements on the same individual. All models included age at enrollment (<30, 30–39, 40–49, and 50–61 years of age), sex, study site, cumulative number of AVA doses (1, 2, 3, or 4), route of administration (SQ vs IM), time since last AVA vaccination, and the top four principal components (computed on the entire multi-ethnic sample)

Table 1
Characteristics of European-American (EA) Anthrax Vaccine Research Program human clinical trial participants included in GWAS of the immune response to AVA.

Variable	EA's included in GWAS N = 726	Remaining participants[a] N = 577	
	Frequency (%)[b]	Frequency (%)[b]	p-Value
Sex			0.850
Male	356 (0.49)	279 (0.48)	
Age at enrollment			5.29×10^{-9}
<30 years	160 (0.22)	204 (0.35)	
30–39 years	160 (0.22)	141 (0.24)	
40–49 years	234 (0.32)	153 (0.27)	
50–61 years	172 (0.24)	79 (0.14)	
Clinical site			$<2.2 \times 10^{-16}$
Rochester, MN	204 (0.28)	47 (0.08)	
Atlanta, GA	122 (0.17)	60 (0.10)	
Washington, DC	106 (0.15)	106 (0.18)	
Houston, TX	135 (0.19)	135 (0.23)	
Birmingham, AL	159 (0.22)	154 (0.27)	
AVA vaccination regimen			0.829
Group 1 (4-SQ)	145 (0.20)	114 (0.20)	
Group 2 (4-IM)	144 (0.20)	118 (0.20)	
Group 3 (3-IM)	142 (0.20)	114 (0.20)	
Group 4 (3-IM)	138 (0.19)	120 (0.21)	
Group 5 (3-IM)	157 (0.20)	111 (0.19)	

GWAS, genome-wide association study; AVA, Anthrax Vaccine Adsorbed; 4-SQ, subcutaneous administration at 0, 2, 4, and 26 weeks; 4-IM, intramuscular administration at 0, 2, 4, and 26 weeks; 3-IM, intramuscular administration at 0, 4, and 26 weeks.

[a] Of the 1303 participants randomized to AVA (non-placebo) regimens (see Section 2).

[b] Percentages may not sum to one due to rounding.

as covariates. SNP annotation was performed using *SNPDOC* (https://wakegen.phs.wfubmc.edu/public/snpdoc/index.cfm).

2.6. Genome-wide estimation of explained variance for week 30 AbPA response

We also estimated the cumulative variance explained by common SNP variation through linkage disequilibrium (LD) with causative variation using a variance components approach [21,22]. We used the AbPA response measured at week 30 as the primary quantitative phenotype so as to avoid left-censoring issues with the detection limit of the ELISA assay; virtually all individuals displayed AbPA levels above the detection limit at that measurement time point. The variance components model was estimated

using restricted maximum likelihood as implemented in the *GCTA* software package [23]. We applied the definition of unrelated individuals used in Yang et al. [21], removing individuals with estimated relationships of greater than 0.025.

3. Results

Characteristics of the European-American AVA000 participants included in the GWAS are displayed in Table 1. Compared with individuals randomized to an AVA-containing regimen excluded from genetic analyses, included participants were slightly older (mean age of 41.0 ± 11.1 (SD) years compared to 36.6 ± 11.0). The percentage of male participants and the distribution across each of the vaccination regimens are generally similar. The top SNP associations ($p \leq 1 \times 10^{-5}$) from the GWAS analysis are displayed in Table 2. Supplementary Table 2 presents a complete listing of SNPs displaying evidence of association with $p < 1 \times 10^{-4}$. No single SNP achieved the commonly utilized threshold for genome-wide significance ($p \approx 5 \times 10^{-8}$) [24], although the genome-wide distribution of p-values did exhibit some evidence of deviation from the null expectation of no association (Supplementary Fig. 2).

Among the strongest associations observed were those of three SNPs located in or near *HLA-DRB1* – rs9269821 ($p = 7.06 \times 10^{-6}$), rs482044 ($p = 9.80 \times 10^{-6}$), and rs3104402 ($p = 5.83 \times 10^{-6}$) (Fig. 2 and Table 2). As shown in Fig. 1, these SNPs are in moderate LD with the common HLA DR-DQ haplotypes identified in our previous analysis, HLA-DRB1-DQA1-DQB1 *01:01–*01:01–*05:01 ($r^2 = 0.359$ with rs9269821) and *15:01–*01:02–*06:02 ($r^2 = 0.456$ with rs3104402) [8]. We subsequently performed additional regression analyses including these haplotypes (along with *01:02–*01:01–*05:01) as additional covariates (Fig. 2). While most of the observed association localizing to the class II region was attenuated by adjusting for the effect of these haplotypes, we did observe a modest associations independent of variation linked to *HLA-DRB1*. For example, the A allele at rs2301226, which is in LD with the *HLA-DPB1* *02:01 allele ($r^2 = 0.622$), was associated with a higher AbPA response (conditional $\beta = 0.099$, SE = 0.031, $p = 0.001$). We observed similar associations localizing to the class I region, with the T allele at rs2735035 (conditional $\beta = -0.063$, SE = 0.021, $p = 0.003$, $r^2 = 0.814$ with *HLA-A* *02) and the G allele at rs4947306 (conditional $\beta = 0.084$, SE = 0.028, $p = 0.003$, $r^2 = 0.216$ and 0.133 with HLA-C *06 HLA-B *57 and HLA-Cw *06-HLA-B *13 haplotypes, respectively).

Table 2
Top associations ($p \leq 1 \times 10^{-5}$) with the immunoglobulin G antibody to protective antigen (AbPA) response to AVA.

SNP	Chr	Position	Array	Gene	Allele	MAF	Beta (SE)	p
rs11121382	1	9,334,056	Affy 6.0	SPSB1	C	0.309	−0.099 (0.022)	4.08×10^{-6}
rs2647264	4	106,488,043	Affy 6.0	TET2|PPA2	G	0.081	0.165 (0.037)	8.67×10^{-6}
rs634308	5	134,591,056	Affy 6.0	PITX1|H2AFY	G	0.349	−0.100 (0.022)	3.51×10^{-6}
rs9269821	6	32,657,786	ImmunoChip	HLA-DRB1	G	0.280	−0.109 (0.024)	7.06×10^{-6}
rs482044	6	32,684,042	ImmunoChip	HLA-DRB1|HLA-DQA1	G	0.388	0.091 (0.021)	9.80×10^{-6}
rs3104402	6	32,789,654	ImmunoChip	HLA-DRB1|HLA-DQA1	A	0.073	−0.165 (0.036)	5.83×10^{-6}
rs10758161	9	32,745,028	Affy 6.0	TAF1L|TMEM215	G	0.106	−0.159 (0.036)	8.23×10^{-6}
rs6478282	9	118,964,916	Affy 6.0	ASTN2	A	0.262	0.101 (0.022)	5.59×10^{-6}
rs12683718	9	118,982,293	Affy 6.0	ASTN2	G	0.262	0.099 (0.022)	7.25×10^{-6}
rs732949	13	52,415,885	ImmunoChip	PCDH8|OLFM4	C	0.066	0.166 (0.037)	7.73×10^{-6}
rs7230711	18	46,944,617	Affy 6.0	−|−	C	0.075	−0.162 (0.033)	1.22×10^{-6}
rs1539857	18	46,951,934	Affy 6.0	SMAD4|MEX3C	C	0.076	−0.158 (0.033)	2.49×10^{-6}
rs16952919	18	46,989,825	Affy 6.0	MEX3C|−	T	0.071	−0.140 (0.031)	7.28×10^{-6}
rs16952953	18	46,999,850	Affy 6.0	MEX3C|−	G	0.074	−0.162 (0.034)	1.93×10^{-6}
rs7238856	18	47,000,228	Affy 6.0	MEX3C|−	A	0.077	−0.156 (0.033)	2.38×10^{-6}
rs7241194	18	47,004,131	Affy 6.0	MEX3C|−	C	0.079	−0.151 (0.033)	4.51×10^{-6}

AVA, Anthrax Vaccine Adsorbed. Beta denotes the estimated regression coefficient corresponding to the additive mean change in $\log_{10}$[AbPA] for the displayed allele based on a longitudinal model fit to measurements at 4, 8, 26, and 30 weeks. All models were adjusted for age, sex, study site, route of administration (SQ vs IM), cumulative number of AVA doses, time since last vaccination, and the top 4 principal components (see Section 2). For SNPs in intergenic regions, Gene lists the nearest gene upstream and downstream within ±500 kb.
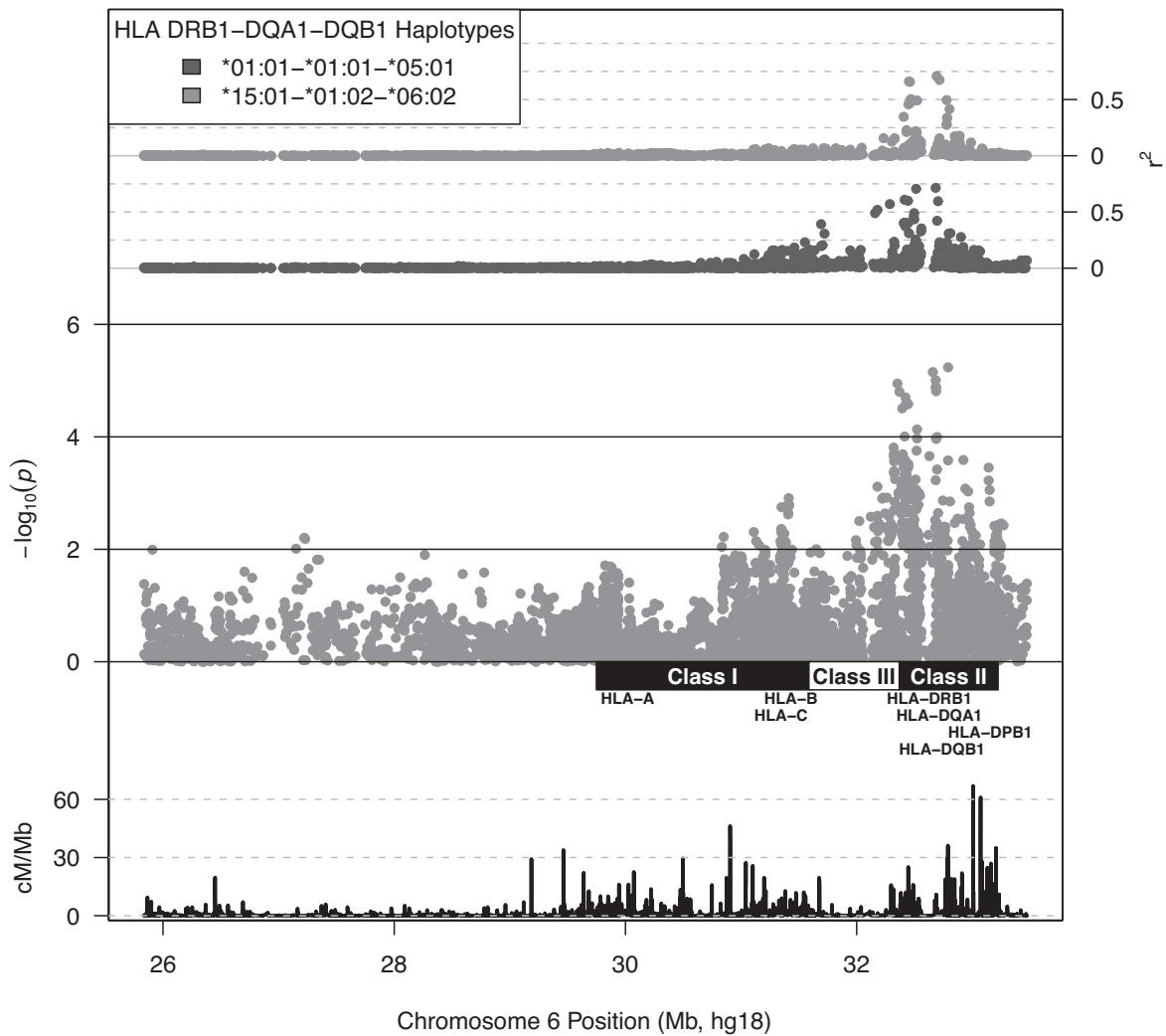
Fig. 1. SNP associations with the immunoglobulin G antibody to protective antigen (AbPA) response to AVA within the extended major histocompatibility complex. AVA, Anthrax Vaccine Adsorbed. Middle axis displays SNP associations with longitudinal AbPA response measured at 4, 8, 26, and 30 weeks adjusted for age, sex, study site, route of administration (SQ vs IM), cumulative number of AVA doses, time since last vaccination, and the top 4 principal components (see Section 2). Bottom axis displays local recombination rate estimates from the International HapMap Project [44]. Top axis displays linkage disequilibrium (r^2) between each SNP and selected HLA DRB1-DQA1-DQB1 haplotypes.

Beyond the human MHC, we also observed comparable associations for a cluster of intergenic SNPs on chromosome 18q21.2 near the mex-3 homolog C (*MEX3C*) gene. The strongest association was observed for rs16952953 ($p = 1.93 \times 10^{-6}$), with the G allele associated with a lower AbPA response ($\beta = -0.162$, SE = 0.034). We also note the association of rs11121382 ($p = 4.08 \times 10^{-6}$), which lies in the promoter/regulatory region of the splA/ryanodine receptor domain and suppressor of cytokine signaling (SOCS) box containing 1 (*SPSB1*) gene on chromosome 1p36.22, where the C allele was similarly associated with a lower AbPA response ($\beta = -0.099$, SE = 0.022). Finally, using a variance components modeling approach, we estimated that common SNP variation explained 90.12% (SE = 0.4864, $p = 0.032$) of the AbPA response at 30 weeks (following 3 or 4 AVA doses). Although this analysis suggested a statistically significant genetic contribution to variability in 30-week AbPA response, the large standard error clearly indicates an imprecise estimate. Thus, we would caution against the interpretation that common SNP variation can account for the majority of the residual variability in the AbPA response (after accounting for age, number of vaccinations, etc.), as predictive accuracy based on a variance components model would be expected to be substantially lower [25].

4. Discussion

GWAS have been a popular tool for investigating human genetic correlates of various disease outcomes. In our evaluation of variable AbPA responses induced by AVA vaccination, no SNP displayed an association surpassing the generally accepted, but conservative, threshold of genome-wide significance. Some of the strongest associations mapped to the MHC class II region near the adjacent *HLA-DRB1* and *HLA-DQA1* loci – those previously associated with lower AbPA [8]. While many of the SNP signals in this region appeared to tag previously identified HLA DR-DQ haplotypes, some evidence remains for modest residual association within the MHC (Fig. 2). The small size of the AVA000 trial and the lack of an immediate replication cohort prevented us from teasing out independent MHC association signals. A recent GWAS of response to hepatitis B vaccine indicated likely independent association signals corresponding to *HLA-DR*, *HLA-DP*, and the class III region [10]. The conditional analyses performed here similarly suggested a possible association with SNPs linked to *HLA-DPB1* independent of the effect of HLA DR-DQ. As with the other associations reported here, this result naturally requires replication.
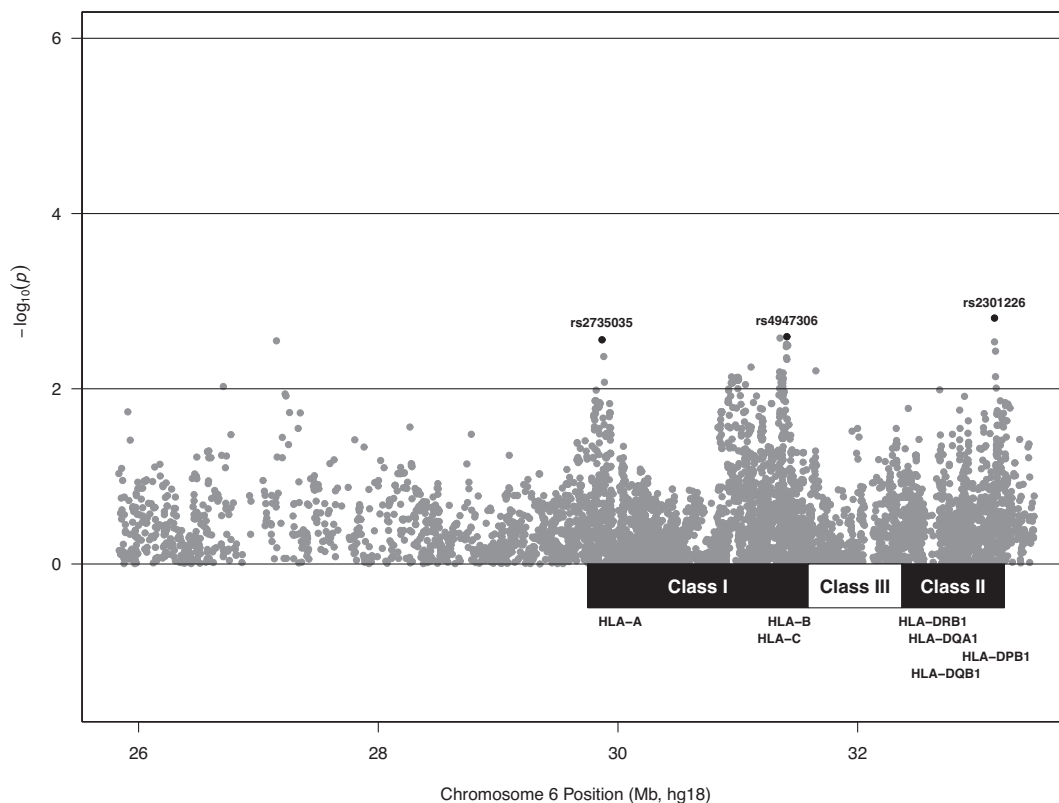
Fig. 2. SNP associations with the immunoglobulin G antibody to protective antigen (AbPA) response to AVA within the extended major histocompatibility complex conditional on previously identified HLA DR-DQ haplotypes. AVA, Anthrax Vaccine Adsorbed. Values denote the association with the longitudinal AbPA response measured at 4, 8, 26, and 30 weeks adjusted for age, sex, study site, route of administration (SQ vs IM), cumulative number of AVA doses, time since last vaccination, top 4 principal components, and for the effect of HLA DR-DQ haplotypes *01:01–*01:01–*05:01, *01:02–*01:01–*05:01, and *15:01–*01:02–*06:02 (see Section 2). (rs2301226) $r^2 = 0.622$ with HLA-DPB1 *02:01. (rs2735035) $r^2 = 0.814$ with HLA-A *02 (rs4947306) $r^2 = 0.216$ and 0.133 with HLA-C *06 HLA-B*57 and HLA-C *06–HLA-B *13 haplotypes, respectively.

None of the other polymorphisms with the strongest associations detected (Table 2) occur at loci for which a clear role in vaccine immune response is currently known. For example, there is little previous work implicating variation in or near *MEX3C* (also designated as the RING finger and KH domain-containing protein 2, *RKHD2*) with any trait; no associations have been reported in the NHGRI GWAS catalog [26]. Guzmán et al. [27] reported the lone genetic association of two SNPs in *RKHD2* with essential hypertension in a case–control study from Spain. Two recent systems biology studies have both reported significant changes in *MEX3C* expression in peripheral blood mononuclear cells following influenza vaccination [28,29], however the paucity of experimental data on *MEX3C/RKHD2* function makes any suggestion of a mechanistic role in vaccine response highly speculative.

Perhaps more plausible was the association of rs11121382, which lies in the promoter/regulatory region of *SPSB1*. The SPSB1 protein interacts with MET, a receptor protein-kinase for hepatocyte growth factor (HGF) [30]. The SPSB1 protein also regulates inducible nitric-oxide synthase levels (iNOS), which in turn regulates the production of nitric oxide (NO) in activated macrophages [31]. Lewis et al. recently demonstrated that the induction of iNOS by SPSB1 is controlled by a negative feedback loop involving SPSB2, downstream of regulation by TLR3 and TLR4 [32]. The role of *SPSB1* in the production of NO suggests a potential pathway through which genetic variation might influence the host immune response. No other SNP in this region that passed quality control appeared associated with AbPA variation, though none was even in moderate LD with rs11121382 (maximum $r^2 = 0.18$ within a 50 kb window). Visual inspection of the intensity data for this SNP did not suggest any technical problem with genotyping. As with the SNPs near

MEX3C, interpretation of the association with rs11121382 must await further research on vaccine response.

The current investigation only had adequate power (>80%) to detect very large effects (~5.3% of the variance for a continuous trait) at the genome-wide significance level of 5×10^{-8}. While a suitable replication cohort is not immediately available, an effort to replicate our findings here is underway, including a component of functional/systems biology research. Furthermore, the design of the AVA000 trial and the sensitivity of the ELISA assay used for AbPA could have obscured associations of host genetic variants contributing to the innate immune response to anthrax vaccine. The first available AbPA measurement occurred at 4 weeks, at which time all participants randomized to 4 dose regimens had already received 2 AVA doses; and at that point a large majority (~75%) of individuals randomized to the 3 dose regimen had AbPA levels below the assay detection limit. More informative research on host genetic factors that influence variability in the innate immune response to AVA may therefore require carefully timed observations soon after the first dose.

In view of the high estimated heritabilities for vaccine-induced antibody levels mentioned earlier, our failure to discern effects attributable to individual SNPs here raises the "missing heritability" question facing the genetics community at large [33,34]. The absence of large effects and our results using variance-components modeling are both consistent with a highly polygenic genetic architecture underlying the response to AVA. This architecture resembles the infinitesimal model of quantitative genetics proposed by Fisher (summarized in Refs. [35,36]). This model envisioned a large number of subtle additive effects on trait variation, whereas large deleterious effects on the immune response (or other

traits that bear on reproductive fitness) would be eliminated from the population through natural selection.

Although response to as "simple" an agent as a vaccine might be more homogeneous than would be expected for a naturally occurring infection, as a quantitative trait the response is nevertheless relatively complex. Both theoretical and empirical evidence [37] have supported a highly polygenic, largely additive genetic basis for complex traits in humans, including evidence from recent large-scale studies for schizophrenia/bipolar disorder [38], height [39], body mass index [40], and blood lipid levels [41]. Of course, a polygenic host contribution would complicate the search for individual causative polymorphisms, but analyses using variance component models have suggested that the aggregate contribution of multiple SNPs can be accurately estimated with sufficient sample sizes [21,22]. The implication for predictive vaccinology is that it may be possible to construct predictive models of vaccine response without directly mapping all associated SNPs. This modeling approach would require both substantial sample sizes to achieve a useful degree of predictive accuracy [25,42] and longitudinal measurements of outcomes less sensitive to assay detection limits. New approaches including "systems vaccinology" could perhaps provide mechanistic and predictive insight into the immune response to AVA, while alleviating some (though not all) of the requirement for large sample size [43]. Whether these approaches can identify specific genetic characteristics that can guide the design of future candidate anthrax vaccines remains to be seen.

Acknowledgements

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2012.05.032.

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