Genetic Basis for Adverse Events after Smallpox Vaccination

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(See the editorial commentary by Relman, on pages 4–5.)

Identifying genetic factors associated with the development of adverse events might allow screening before vaccine administration. Two independent clinical trials of the smallpox vaccine (Aventis Pasteur) were conducted in healthy, vaccinia virus–naive adult volunteers. Volunteers were assessed repeatedly for local and systemic adverse events (AEs) associated with the receipt of vaccine and underwent genotyping for 1442 single-nucleotide polymorphisms (SNPs). In the first study, 36 SNPs in 26 genes were associated with systemic AEs (P \leq .05); these 26 genes were tested in the second study. In the final analysis, 3 SNPs were consistently associated with AEs in both studies. The presence of a nonsynonymous SNP in the methylenetetrahydrofolate reductase (MTHFR) gene was associated with the risk of AE in both trials (odds ratio [OR], 2.3 [95% confidence interval {CI}, 1.1–5.2] [P = .04] and OR, 4.1 [95% CI, 1.4–11.4] [P < .01]). Two SNPs in the interferon regulatory factor–1 (IRF1) gene were associated with the risk of AE in both sample sets (OR, 3.2 [95% CI, 1.1–9.8] [P = .03] and OR, 3.0 [95% CI, 1.1–8.3] [P = .03]). Genetic polymorphisms in genes expressing an enzyme previously associated with adverse reactions to a variety of pharmacologic agents (MTHFR) and an immunological transcription factor (IRF1) were associated with AEs after smallpox vaccination in 2 independent study samples.

Although reactions occurring after inoculation of vaccinia virus were commonly observed in recent population-wide vaccination programs [1], the biological basis for these adverse events (AEs) is not well understood. Performance of 2 independent clinical studies of a single vaccinia virus vaccine at our study site afforded us the unique opportunity to assess genetic factors that might predict systemic AEs. All of the vaccinia virus–naive subjects who were enrolled in the study developed pock formation at the vaccination site, and a subset experienced systemic reactions that included fever, rash, or regional lymphadenopathy. Because poxviruses have developed multiple mechanisms by which to evade host immune responses, such as targeting of primary innate immunity and manipulation of intracellular signal transduction pathways [2], we questioned whether subjects experiencing AEs exhibited unique genetic polymorphisms in these pathways that made them more susceptible to these reactions.

The funding organizations played no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; or in the preparation, review, or approval of the manuscript.

# Potential conflicts of interest: J.E.C. received research funding from Sanofi-Aventis and Vaxgen and a joint Small Business Technology Transfer award (with Mapp Pharmaceuticals); He has consulted for MedImmune, Vaxin, Evogenix, Symphogen, and Syngenta. K.M.E. received research funding from Sanofi-Aventis, MedImmune, Vaxgen, Merck, and Wyeth. She has also consulted for MedImmune and Wyeth. All other authors: no conflicts.

# Financial support: Vaccine Trials and Evaluation Unit, National Institutes of Health (NIH)/National Institute of Allergy and Infectious Diseases (NIAID) (contract N01-AI-25462 for Division of Microbiology and Infectious Diseases (OMID) studies 02–054 and 03–044); NIH/NIAID (grants K25-AI-64625, R21-AI-59295, and R01-AI-59994); and NIH/National Institute of General Medical Sciences (grant R01 GM-62758). The clinical study was supported in part by the National Center for Research Resources, NIH (grant M01 RR-00095).

The Journal of Infectious Diseases 2008;198:16–22
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0022-1899/2008/19801-0006$15.00
DOI: 10.1086/588670

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In earlier studies, we characterized humoral and cellular immune responses and outlined patterns of systemic cytokine expression after smallpox vaccination [3–8]. In the present study, we utilized data collected during 2 independent studies to identify stable genetic factors associated with AEs. Because there is failure to replicate the results of many genetic association studies during subsequent studies, we sought to repeat the assessment in an additional study group [9, 10]. The fact that the results of our first study were independently replicated in the second study strengthens the plausibility of these genetic associations. An identical panel of candidate single-nucleotide polymorphisms (SNPs) was evaluated in each of the studies. Subjects with systemic AEs, including fever, lymphadenopathy, or generalized aciform rash, were compared with subjects who did not experience these reactions. The data used in both studies were the genotypes at 1442 SNPs across at least 386 candidate genes. The present investigation provides, for 2 independent data sets, important preliminary findings addressing the contribution of common genetic variants to a complex clinical phenotype, which also is of substantial importance with respect to public health.

SUBJECTS AND METHODS

Study subjects. The vaccines, study subjects, and study design used in both of the clinical trials have been previously described in detail. Both trials were conducted at the National Institutes of Health (NIH)–funded Vaccine and Treatment Evaluation Unit at Vanderbilt University (Nashville, Tennessee) [4, 8, 11]. The first study [7] enrolled 85 healthy, vaccinia virus–naive adults in genotyping studies, and the second study [11] enrolled 46 healthy, vaccinia virus–naive adults. In both studies, individuals were asked to self-identify their ethnic background. Both studies complied with the policies of the internal review boards of Vanderbilt University and the NIH, and written informed consent was obtained from all individuals.

Clinical assessments. For both studies, the same team of trained physicians and nurses used the same forms to obtain a medical history and to record local and systemic AEs occurring after vaccination. Subjects were examined at regular intervals (on days 3–5, 6–8, 9–11, 12–15, and 26–30 after vaccination). Local and systemic AEs were recorded. Subjects who had an oral temperature >38.3°C at any time during the study, generalized skin eruptions on areas noncontiguous to the site of vaccination [11], or enlarged or tender regional lymph nodes associated with vaccination were defined as subjects experiencing systemic AEs.

Identification of genetic polymorphisms. We used a previously described custom SNP panel based on the National Cancer Institute (NCI) SNP500 Cancer Project [12]; this panel targets investigation of soluble-factor mediators and signaling pathways, many of which have known immunological significance [13]. In this panel, there is heavy weighting toward nonsynonymous SNPs (i.e., SNPs that result in an amino acid substitution). Genotyping for SNPs was performed using DNA directly amplified from Epstein Barr virus (EBV)–transformed B cells generated from peripheral blood samples collected from each subject. Genotyping was performed at the Core Genotyping Facility of the NCI in Gaithersburg, Maryland. Genotypes were generated using Illumina GoldenGate assay technology. Of the 1536 SNPs assayed, a total of 1442 genotypes passed quality control filters for both the first and second sample sets.

Statistical analysis. The clinical and demographic characteristics, including age, sex, and race, noted in the first and second studies were compared using Student’s t test (for age) and 2-sample tests of proportions (for AE status for and for sex and race). Allele frequencies were estimated by dividing the total number of copies of individual alleles by the total number of alleles in the sample, and the frequencies noted in the 2 studies were compared using a 2-sample test of proportions. Deviations in the fitness for Hardy-Weinberg proportion were evaluated using the exact test as described by Wigginton et al. [14].

We chose a 2-stage design for identifying and replicating genetic associations in the independent clinical trials. This study design was selected with the goal of minimizing type I errors (false-positive results). For comparison, we also performed genetic association analysis in a single pooled sample. In the first study, we tested for potential associations between each of the 1442 SNPs passing quality control filters, as well as for the occurrence of AEs, by use of logistic regression. For each SNP in the first sample set, we recorded the odds ratio (OR) estimate and P value of the likelihood ratio test for a univariate logistic model. No correction for multiple comparisons was made in the first sample set, because we reserved the second study sample set for determination of probable true-positive results. In the second sample set, we tested only those SNPs that had an AE-associated P value of ≤.05 in the first study. A significant SNP association in the first study was considered to have been replicated if it met the following criteria in the second study: (1) an OR that consistently associated the risk of AE with the same genotypes and (2) a P value ≤.05. To obtain an empirical probability of meeting our replication criteria purely by chance, we generated 1000 simulated data sets from both study sample sets by permuting case-control labels. An additional association for which P = .06 is discussed below because of its high biological plausibility.

Patterns of linkage disequilibrium (LD) between replicated SNPs on the same chromosome were assessed using Haploview software (version 3.32; Broad Institute) [15]. Haplotypes were inferred for SNPs in high LD, by use of the iterative approach described by Lake et al. [16]. The resulting haplotypes were tested for association with AEs by use of univariate logistic models. Statistical analyses and simulations were performed using software that we wrote using the R language (version 2.5.1; R
Table 1. Summary of data on adverse event (AE) status and subject age, sex, and race in both studies.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>First study (n = 85)</th>
<th>Second study (n = 46)</th>
<th>P&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>AE status&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16/69</td>
<td>24/22</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Age, mean ± SD, years</td>
<td>23.2 ± 3.9</td>
<td>24.2 ± 3.8</td>
<td>.15</td>
</tr>
<tr>
<td>Sex&lt;sup&gt;c&lt;/sup&gt;</td>
<td>40/45</td>
<td>27/19</td>
<td>.20</td>
</tr>
<tr>
<td>Race&lt;sup&gt;d&lt;/sup&gt;</td>
<td>84/0/1</td>
<td>44/1/1</td>
<td>.25</td>
</tr>
</tbody>
</table>

<sup>a</sup> For the difference between the studies. Two-sided P value for Student’s t test (for age) or a 2-sample test of proportions (for AE status and for sex and race).

<sup>b</sup> No. of subjects with AEs after vaccination/no. of subjects without AEs after vaccination.

<sup>c</sup> No. of males/no. of females.

<sup>d</sup> No. of whites/no. of blacks/no. of Asians.

RESULTS

Clinical and demographic characteristics of subjects included in genetic analysis. In both studies, all participants were invited to donate genetic samples. In the first study, of the 148 vaccinia virus–naive participants enrolled in the clinical trial, a total of 96 individuals provided written informed consent for the genetic sub-study. Of those 96 subjects with genetic data, 16 experienced systemic AEs after immunization. An additional 11 subjects who had genotyping performed and who reported only a localized rash near the inoculation site were removed from the analysis to focus only on systemic AEs. The other 69 subjects who reported no AEs were used as controls. Thus, the first study analyzed 85 subjects. In the second study, which originally included 48 vaccinia virus–naive healthy adults, 46 adults gave consent for genotyping and were enrolled. Of these 46 individuals, 24 experienced systemic AEs.

Table 1 summarizes the age, race, sex, and AE status decompositions noted in both studies. Table 1 also describes the results of comparisons of the clinical and demographic characteristics of subjects in the first and second studies. As table 1 indicates, there was no statistical difference in age, sex, or race between the 2 study populations. In the first study, 40 individuals (47%) were men, 84 (99%) were white, and 1 (1%) was Asian. In the second study, 27 individuals (59%) were men, 44 (96%) were white, 1 (2%) was black, and 1 (2%) was Asian.

Genetic associations with AEs. A total of 36 SNPs (within 26 genes) that showed significant associations in the first study were tested for potential associations in the second study. Three variant genotypes were confirmed to be associated with AEs in the second study. These included 1 SNP in the methylenetetrahydrofolate reductase gene (MTHFR) (P < .01) and 2 SNPs in the interferon regulatory factor–1 gene (IRF1) (P = .03). The strong significance of the association in the replication study suggested a high level of plausibility that the gene products were involved in the pathogenesis of the AEs. The results of our simulation study indicated that the probability of meeting our replication criteria (i.e., an OR that consistently associated the risk of AE with the same genotypes and a P value ≤ .05) entirely by chance was P < .001. It is important to note that we also reanalyzed the data as a single pooled sample and found the same pattern of statistically significant associations. Table 2 shows the statistical results that were replicated in the second study alongside the results from the first study.

Three SNPs in a third gene (IL4) had P values of .06 in the second study. Although this finding was not significant when a strict requirement that P ≤ .05 was used, we thought that this association was of great interest because of prior biological studies showing a central role for this cytokine in the biological profile of poxvirus [19–21]. Considering the reduced size of the second sample and the fact that the risk of AE associated with variant genotypes was consistent across studies, these SNPs in IL4 warrant further study, because additional variants in linkage disequilibrium could also be associated with the outcomes of AE (table 3).

The SNPs located in IRF1 and IL4 are located in the same chromosomal region (5q31.1), suggesting an indirect associa-

Table 2. Genetic polymorphisms associated with adverse events in both studies.

<table>
<thead>
<tr>
<th>Gene, SNP&lt;sup&gt;a&lt;/sup&gt;</th>
<th>SNP location,&lt;sup&gt;b&lt;/sup&gt; bp</th>
<th>Chromosomal location</th>
<th>First study OR&lt;sup&gt;c&lt;/sup&gt; (95% CI)</th>
<th>P&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Second study OR&lt;sup&gt;c&lt;/sup&gt; (95% CI)</th>
<th>P&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTHFR</td>
<td>1801133</td>
<td>6393745</td>
<td>1p36.3</td>
<td>2.3 (1.1–5.2)</td>
<td>.04</td>
<td>4.1 (1.4–11.4)</td>
</tr>
<tr>
<td>IRF1</td>
<td>9282763</td>
<td>34237146</td>
<td>5q31.1</td>
<td>3.2 (1.1–9.8)</td>
<td>.03</td>
<td>3.0 (1.1–8.3)</td>
</tr>
<tr>
<td>839</td>
<td>34234139</td>
<td>5q31.1</td>
<td>3.2 (1.1–9.8)</td>
<td>.03</td>
<td>3.0 (1.1–8.3)</td>
<td>.03</td>
</tr>
</tbody>
</table>

NOTE. CI, confidence interval; OR, odds ratio; SNP, single-nucleotide polymorphism.

<sup>a</sup> By rs number.

<sup>b</sup> As determined according to dbSNP (Human Genome Build 36.1; National Center for Biotechnology Information).

<sup>c</sup> Estimated.

<sup>d</sup> By likelihood ratio χ<sup>2</sup> test with 1 df.
Table 3. Distribution of genotypes at single-nucleotide polymorphisms (SNPs) in MTHFR, IRF1, and IL4.

<table>
<thead>
<tr>
<th>Gene, SNPa</th>
<th>SNP location,b bp</th>
<th>Genotype</th>
<th>First (n = 85)</th>
<th>Second (n = 46)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTHFR, 1801133</td>
<td>6393745</td>
<td>CC</td>
<td>36(42)</td>
<td>18(39)</td>
</tr>
<tr>
<td>IRF1 9282763</td>
<td>34237146</td>
<td>AA</td>
<td>39(46)</td>
<td>17(37)</td>
</tr>
<tr>
<td>IL4 2070874</td>
<td>34424723</td>
<td>CC</td>
<td>52(62)</td>
<td>34(74)</td>
</tr>
<tr>
<td>IRF1 839</td>
<td>34234139</td>
<td>GG</td>
<td>3(4)</td>
<td>5(11)</td>
</tr>
<tr>
<td>IL4 2243268</td>
<td>34428976</td>
<td>AA</td>
<td>52(62)</td>
<td>34(74)</td>
</tr>
<tr>
<td>IL4 2243290</td>
<td>34433182</td>
<td>CC</td>
<td>53(62)</td>
<td>34(74)</td>
</tr>
<tr>
<td>IL4</td>
<td></td>
<td>AA</td>
<td>26(31)</td>
<td>12(26)</td>
</tr>
<tr>
<td>IL4</td>
<td></td>
<td>AC</td>
<td>6(7)</td>
<td>0(0)</td>
</tr>
</tbody>
</table>

a By rs number.
b As determined according to dbSNP (Human Genome Build 36.1; National Center for Biotechnology Information).

This region of chromosome 5q31 contains discrete haplotype blocks [22]. Accordingly, haplotypes were inferred for AE-associated SNPs in IRF1 (rs839 and rs9282763) and IL4 (rs2070874, rs2243268, and rs2243290). In both studies, 2 IRF1 haplotypes accounted for all subjects. The common IRF1 haplotype listed in table 4 was found in 71% of the first sample set and 63% of the second sample set. The rare IRF1 haplotype was significantly associated with AE in both studies (P = .03). Across both studies, 2 different 3-SNP haplotypes in IL4 were found among 99% of subjects. The common IL4 haplotype shown in table 4 was found in 78% of the first sample set and 87% of the second sample set. The rare IL4 haplotype was significantly associated with the risk of AE in the first study (P = .05); the association was similar in the second study (P = .06).

**DISCUSSION**

**MTHFR and IRF1.** The candidate genes noted to have the strongest association with AE in both studies include a metabolism gene previously associated with adverse reactions to a variety of pharmacologic agents (MTHFR) and an immunological transcription factor (IRF1) gene. The statistical results from these studies have strong biological plausibility and are in agreement with previous work on the immune response to poxviruses.

An SNP in the 5,10-methylenetetrahydrofolate reductase gene (MTHFR; rs1801133) was associated strongly with the risk of AE in both studies. This nonsynonymous SNP in exon 5 causes an amino acid change from alanine to valine, and functional characterization of this SNP demonstrated that it is thermostable and affects both the quantity and activity of the MTHFR enzyme [23]. The enzyme catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which is a cosubstrate for homocysteine remethylation to methionine. MTHFR function provides pools of methyl groups that are crucial for the control of DNA synthesis and repair mechanisms [24]. MTHFR is a key enzyme in homocysteine metabolism, which plays a major role in regulating endothelial function. In the future, it may be of interest to examine the association of genetic variation in this gene with the rare cardiac events that occur after vaccination.

Genetic variation of MTHFR has been associated with a range of clinical outcomes, including altered cardiovascular function, organ transplantation, toxicity of immunosuppressive drugs, and systemic inflammation [25–28]. Elevated plasma levels of homocysteine stimulate endothelial inflammatory responses, which could contribute to the development of systemic AEs. Alternatively, because vaccination elicits immune responses involving the rapid proliferation of cells, demand for DNA synthesis metabolites would be elevated, and alterations in the level or activity of the MTHFR enzyme may exert significant influence over this process.

**Interferon (IFN) regulatory factor–1 (IRF-1).** The IRF1 gene is part of the immunological gene cluster on chromosome 5q31. We found 2 SNPs in IRF1 that were significantly associated with AE in both study samples. The IRF1 gene encodes an important member of the IFN regulatory transcription factor (IRF) family. The IRF family regulates IFNs and IFN-inducible genes. IRF1 activates transcription of type 1 IFN-α and IFN-β, as well as genes induced by type 2 IFN-γ [29]. Many viruses target IRFs to evade host immune responses by binding to cellular IRFs and blocking transcriptional activation of IRF targets [30].

Polymorphisms in the gene coding for a transcription factor with such far-reaching effects as IRF1 could have pro-
found effects on the proper immune response to and clearance of vaccinia virus. Mice deficient in IFN receptors are especially susceptible to vaccinia virus infection, suggesting an important role for these molecules in controlling vaccinia virus infection [31]. Vaccinia virus dedicates several host-modifying genes to counteracting IFNs. For example, the viral gene B18R encodes a protein that serves as a viral IFN-α/β-binding protein that binds IFNs from several species [32]. This protein also can bind to the cell surface after secretion, thus preventing host IFN from binding to cellular IFN receptors [33]. Although the SNPs identified in IRF1 and IL4 do not change amino acids in the encoded proteins, recent evidence suggests that synonymous SNPs, such as rs839, can alter regulation of mRNA or splice junctions [34, 35]. It is also plausible that one or both SNPs are in LD with the causal variant not tested in this study.

Interleukin-4. Genetic polymorphisms in this major cytokine gene involved in adaptive immunity to viruses also may be associated with AEs, albeit with a $P$ value of .06 in our relatively small replication study. We found 3 SNPs in IL4 that may be associated with AEs in both studies. There was high intragenic LD ($r^2 > 0.9$) between the tested SNPs within each gene (IRF1 and IL4) and haplotypes inferred separately for each of these genes mirrored the significant risk patterns of the SNPs observed individually. Thus, the fact that multiple SNPs that were in high LD were identified in regions of IRF1 and IL4 strongly suggests that there are additional markers in LD, several of which could functionally contribute to the risk of AEs.

Table 4. Haplotypes inferred for adverse event (AE)–associated single-nucleotide polymorphisms (SNPs) in IRF1 (rs839 and rs9282763) and IL4 (rs2070874, rs2243268, and rs2243290).

<table>
<thead>
<tr>
<th>Gene, SNP</th>
<th>Haplotype at baseline</th>
<th>Risk haplotype</th>
<th>First study</th>
<th>Second study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>OR (95% CI)</td>
<td>$P$</td>
</tr>
<tr>
<td>IRF1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs839</td>
<td>A</td>
<td>G</td>
<td>3.2 (1.0–10.2)</td>
<td>.03</td>
</tr>
<tr>
<td>rs9282763</td>
<td>G</td>
<td>A</td>
<td>3.0 (1.0–9.0)</td>
<td>.03</td>
</tr>
<tr>
<td>IL4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs2070874</td>
<td>C</td>
<td>T</td>
<td>2.4 (1.0–5.7)</td>
<td>.05</td>
</tr>
<tr>
<td>rs2243268</td>
<td>A</td>
<td>C</td>
<td>3.8 (1.0–14.4)</td>
<td>.06</td>
</tr>
<tr>
<td>rs2243290</td>
<td>C</td>
<td>A</td>
<td>3.0 (1.0–9.0)</td>
<td>.03</td>
</tr>
</tbody>
</table>

NOTE. CI, confidence interval; OR, odds ratio.

* By rs number.

* Most common haplotype, considering 2 SNPs in IRF1 or 3 SNPs in IL4.

* Rare (variant) haplotype, considering 2 SNPs in IRF1 or 3 SNPs in IL4.

* Estimated OR comparing the risk haplotype with the haplotype at baseline (95% CI).

* By likelihood ratio $\chi^2$ test with 1 df.
The IL4 gene encodes a pleiotropic cytokine produced by a variety of immune cells, especially activated T cells. IL4 controls humoral immune responses, isotype switching, and suppression of cytotoxic T cell function and expansion. Thus, genetic polymorphisms related to inappropriate regulation of IL4 expression and/or activity of IL-4 cytokine could be associated with overstimulated inflammatory responses leading to the development of clinical AEs. Previous studies of the role of IL4 in the pathogenesis of poxvirus have shown that IL4 has a central role in altering the adaptive immune response. Overexpression of IL4 during infection with recombinant poxviruses encoding IL4 suppresses the induction of cytotoxic T cell activity by inhibiting CD8+ T cell proliferation, which increased the pathogenicity of such recombinant viruses even in previously immunized animals [36]. IL4 also plays a role in preventing optimal innate immune responses to poxviruses. Secretion of IL-4 during vaccinia virus infection in individuals with atopic dermatitis alters the cytokine milieu, resulting in blocking of production of the antimicrobial peptide LL-37; this accounts, in part, for the increased risk of vaccinia virus infection in subjects with atopic dermatitis [37].

**Model of pathogenesis.** Because the outcome of interest here was the aggregation of specific AEs, it is logical that >1 gene may be involved. The genes with variants for which we discovered an association with AEs are all potentially involved in pathways that are in line with our previously hypothesized mechanism of AEs involving excess stimulation of inflammatory pathways and the imbalance of tissue damage repair pathways. This model was developed from studies of circulating cytokines and relevant immunological effector cells [3–5]. For subjects experiencing AEs, vaccination appears to trigger an acute inflammatory response that is excessive. Antigen presentation to T cells in the dermis leads to the release of T cell cytokines that trigger a cascade of cytokines and chemokines whose release enhances the inflammatory response by (1) promoting the migration of monocytes into the lesion and their maturation into macrophages and (2) further attracting T cells [38, 39]. Taken together, these findings suggest that systemic AEs occurring after smallpox vaccination may be consistent with low-grade macrophage activation syndrome caused by virus replication and vigorous tissue injury and repair.

There are limitations to the present study. The numbers of subjects are too small for a genetic association study of low-penetrance, high-frequency alleles. The association between IL4 variations and AEs was weaker than that between variations in other genes and AEs. Nevertheless, the observation of the same variants in 2 independent clinical trials, the high biological plausibility of these associations in light of what is known about the biological profile of poxvirus, and the potential public health significance suggest that the findings are of interest.

**Conclusions and future directions.** These data provide the rare opportunity to (1) study 2 independent cohorts of smallpox vaccine recipients and (2) attempt to identify associations between common genetic variations and the occurrence of AEs after vaccination. Statistical analysis of the results of the first study revealed potentially significant associations between SNPs in biologically interesting candidate genes. Of the AE-associated genes identified in the first study, 2 were replicated in an independent study, with one additional candidate gene having results just beyond the cut-off value used to denote statistical significance but nevertheless having a high level of biological plausibility. It is possible that our findings could be due to chance, but we avoided multiple testing issues by testing only the most promising results in the validation sample. Although all SNPs were tested in the first study, only those SNPs that were significantly associated with AEs were tested in the second study, and our empirically derived probability of replication by chance alone was <0.1%. The association of SNPs in 3 genes across both studies and the biologically plausibility that these SNPs were associated with the development of AEs lend credence to the reproducibility of these associations.

As with any statistical association, follow-up studies in additional populations are needed to identify the particular genetic susceptibility variants and examine the functional consequences of polymorphisms in the AE-associated genes. The polymorphisms that were identified show consistently high heterozygosity across Hispanic, African, African-American, Asian, and white population samples [40]. Therefore, although future population samples may reveal population-specific differences in allele frequencies that require analytical consideration, the variability in these SNPs makes them reasonable candidates for association studies in more racially diverse populations. Because we found multiple AE-associated SNPs in regions of IRF1 and IL4, focused studies should be undertaken to characterize the genetic variability in these candidate regions. Indeed, haplotypes in IRF and IL4 displayed altered susceptibility to a specific systemic AE (i.e., fever) after smallpox vaccination [41]. Although the association between AEs and a nonsynonymous polymorphism in the gene for MTHFR points toward functional significance of this SNP, fine mapping of this locus should determine whether this is the case. For all 3 candidate genes, both follow-up replication and functional studies are needed to establish the plausibility of the association of common genetic polymorphisms with the hypothesized etiological pathways.

**Acknowledgments**

We thank Jennifer Hicks, Karen Adkins (Vanderbilt Pediatric Clinical Research Office, Vanderbilt University Medical Center, Nashville, Ten-
nessee), and the staff at the Vanderbilt General Clinical Research Center (Vanderbilt University Medical Center), for nursing support.

References


