Retrospectively Estimating Prevalence of Peanut Allergy Genetic Markers in an Air Force Population

Technical Support
Katherine Kohnen
Summer Hughes, MFS
John Trombley
Amy Walters
Shana Huntsberger

Technical Director
Richard R. Chapleau, Ph.D.

January 2018

Air Force Research Laboratory
711th Human Performance Wing
U.S. Air Force School of Aerospace Medicine
Aeromedical Research Department
Applied Technology and Genomics Division
2510 Fifth St., Bldg. 840
Wright-Patterson AFB, OH 45433-7913
Using Government drawings, specifications, or other data included in this document for any purpose other than Government procurement does not in any way obligate the U.S. Government. The fact that the Government formulated or supplied the drawings, specifications, or other data does not license the holder or any other person or corporation or convey any rights or permission to manufacture, use, or sell any patented invention that may relate to them.

Qualified requestors may obtain copies of this report from the Defense Technical Information Center (DTIC) (http://www.dtic.mil).

AFRL-SA-WP-SR-2018-0002 HAS BEEN REVIEWED AND IS APPROVED FOR PUBLICATION IN ACCORDANCE WITH ASSIGNED DISTRIBUTION STATEMENT.

//SIGNATURE//       //SIGNATURE//

COL ALBERT BONNEMA  DR. RICHARD A. HERSACK
Chief, Applied Technology & Genomics Div  Chair, Aeromedical Research Department

This report is published in the interest of scientific and technical information exchange, and its publication does not constitute the Government’s approval or disapproval of its ideas or findings.
Food allergies are complex, multifactorial traits that pose great risk to health and operational success. Latent food allergies could manifest catastrophic outcomes during missions, resulting in potential failure and loss of life. Understanding an individual’s risk of developing severe allergies is one of many components that can make up a high-quality surveillance and public health/preventive medicine program. We therefore assessed whether two specific genetic markers identified as related to peanut allergies in a civilian population of children were over- or under-represented in a U.S. Air Force population. Our results showed that for a single polymorphism, there was a significant difference between the U.S. Air Force population and open-source literature genomes. Although not strictly a validation of externally identified markers, our pilot study suggests that genetic markers derived from civilian populations may not always correlate with military cohorts, and internally validating genetic tools prior to developing screens is of the utmost importance for providing Trusted Care, Anywhere.
This page intentionally left blank.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF FIGURES</td>
<td>ii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>ii</td>
</tr>
<tr>
<td>1.0 SUMMARY</td>
<td>1</td>
</tr>
<tr>
<td>2.0 INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>3.0 METHODS</td>
<td>2</td>
</tr>
<tr>
<td>3.1 Sample Collection</td>
<td>2</td>
</tr>
<tr>
<td>3.2 SNP Genotyping</td>
<td>2</td>
</tr>
<tr>
<td>4.0 RESULTS</td>
<td>3</td>
</tr>
<tr>
<td>4.1 Genotyping a Collection of USAF Nasal Wash Samples</td>
<td>3</td>
</tr>
<tr>
<td>4.2 Comparing Risk Groups Between a USAF Population and the U.S. and Global Populations</td>
<td>3</td>
</tr>
<tr>
<td>4.3 Allele Frequencies for Polymorphisms Associated with Peanut Allergy Risk</td>
<td>4</td>
</tr>
<tr>
<td>4.4 Combined Risk Group Distributions for USAF Population</td>
<td>4</td>
</tr>
<tr>
<td>5.0 DISCUSSION</td>
<td>4</td>
</tr>
<tr>
<td>6.0 CONCLUSION</td>
<td>5</td>
</tr>
<tr>
<td>7.0 REFERENCES</td>
<td>6</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS AND ACRONYMS</td>
<td>7</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

Page

Figure 1. Genotype frequencies for USAF School of Aerospace Medicine nasal wash archive samples ................................................................. 3

LIST OF TABLES

Page

Table 1. Frequency of Overall Risk Group Combinations ................................................................. 4
1.0 SUMMARY

Food allergies are complex, multifactorial traits that pose great risk to health and operational success. Latent food allergies could manifest catastrophic outcomes during missions, resulting in potential failure and loss of life. Understanding an individual’s risk of developing severe allergies is one of many components that can make up a high-quality surveillance and public health/preventive medicine program. We therefore assessed whether two specific genetic markers identified as related to peanut allergies in a civilian population of children were over- or under-represented in a U.S. Air Force population. Our results showed that for a single polymorphism, there was a significant difference between the U.S. Air Force population and open-source literature genomes. Although not strictly a validation of externally identified markers, our pilot study suggests that genetic markers derived from civilian populations may not always correlate with military cohorts, and internally validating genetic tools prior to developing screens is of the utmost importance for providing Trusted Care, Anywhere.

2.0 INTRODUCTION

The Department of Defense considers peanut allergy an unwaiverable medical condition [1], partially because much of the food provided to military personnel may be prepared and manufactured in a few facilities. Additionally, downrange operations often require logistics footprints that would be cost-prohibitive for accommodating food allergies in a small proportion of active duty (AD) service members. For example, food allergies affect 2-10% of U.S. children in the general population [2], and although such allergies are increasing in prevalence, they account for a negligible fraction of potential AD warfighters. The scientific community currently has little consensus about how and why food allergies develop, and there has been interest in studying the possible genetic markers causing an increased risk of developing food allergies in individuals.

A person can develop a food allergy at any age, with peaks in childhood and early adulthood [3], and having knowledge about who is at risk of developing a food allergy may be valuable to the U.S. Air Force (USAF), as Airmen potentially can develop allergies while they are AD. Mild allergic reactions may include nasal drip, itchy skin and eyes, digestive problems, and even respiratory distress [4]. Anaphylaxis is a rare, systemic reaction to an allergen that may cause death in some severe cases and may be very difficult to treat in the field. In fiscal year 2009, the Department of Defense launched a research program funding four laboratories to investigate the genetics of food allergies. A study from that program identified two single-nucleotide polymorphisms (SNPs) as being associated with the development of peanut allergies [5]. An individual homozygous at either SNP for the risk allele resulted in a threefold higher risk of developing a peanut allergy, while a heterozygous (one risk allele) individual had an increased risk of 1.7-fold.

The overall goal of this pilot study was to determine if there are any differences between allele and genotype frequencies in these two SNPs between a USAF population and the U.S. and global populations. Here, we extracted DNA from a USAF nasal wash repository and genotyped by polymerase chain reaction (PCR) to compare allele frequencies available from the 1000 Genomes Project [6]. Our results suggested a statistically significant increase in risk allele frequency for one of the two SNPs and an overall increase in risk genotype frequency in the USAF samples. Further research efforts are necessary to confirm these results and determine if
the genetic predispositions are consistent across a current AD population and if they are associated with childhood or adult-onset allergies. Ultimately, we hope that by identifying an increased risk of developing food allergies, we may provide medical personnel with improved surveillance on elevated risk Airmen for annual screening to prevent surprise allergic reactions while on duty. In essence, our results in this pilot study may serve as support for additional resources to investigate the genetic associations of these SNPs to peanut allergy risk and develop screening tools.

3.0 METHODS

The Air Force Research Laboratory Institutional Review Board reviewed the research protocol and classified the research as non-human subjects research (Protocol Number: FWR20170097N).

3.1 Sample Collection

A de-identified nasal wash archive located at the USAF School of Aerospace Medicine contains more than 2100 samples diagnosed with coronavirus collected from military medical treatment facilities between 2013 and 2014. Of these, we extracted DNA from 465 samples using the Maxwell 16 Cell DNA Purification Kit on a Maxwell 16 DNA extractor (Promega, Madison, WI). Extractions were quantified for concentration of DNA and A260/A280 ratio using the NanoDrop 1000 Spectrophotometer (ThermoFisher Scientific, Waltham, MA) with 2 μL of sample. A quality control filter eliminated samples with a DNA concentration less than 1 ng/μL or an A260/A280 ratio outside 0.8-3. After quality filtering, 247 samples remained available for further analyses, putting the extraction efficiency at 53%.

3.2 SNP Genotyping

We used the BioMek FX\textsuperscript{3} (Beckman Coulter Life Sciences, Indianapolis, IN) automated liquid handler to prepare samples in 96-well plates for genotyping by PCR. Each plate consisted of 46 samples assayed for both SNP assays (rs9275596 and rs7192) and four no template controls. We performed PCR genotyping using the 7500 Fast Real-Time thermocycler (Applied Biosystems, Foster City, CA) according to the manufacturer’s instructions for TaqMan-based assays. We repeated samples once with insufficient amplification in either one of the two assays. Automated genotype calling was supplemented with manual annotation, when necessary, using the 7500 Fast software. Of the 247 samples, 204 had successful amplification in both SNPs after the reruns, making the final PCR success rate 83%.

We assigned risk groups for each sample based on genotype per SNP. Samples homozygous for the risk allele were “high risk,” heterozygous samples were “medium risk,” and homozygous samples for the normal allele were “low risk.” The allele and risk group (genotype) frequencies were then calculated and compared to global and U.S. frequencies obtained from the 1000 Genomes Project online database \cite{6}. The risk alleles for rs9275596 and rs7192 are C and T, respectively \cite{5}.
4.0 RESULTS

4.1 Genotyping a Collection of USAF Nasal Wash Samples

For the SNP rs9275596, the low risk group was most frequent in the USAF samples followed by the medium risk group. The highest risk group, associated with a threefold increase of risk of peanut allergy development, was least frequent (Figure 1A). As peanut allergies are disqualifying for military service, we expected to see a low frequency of elevated risk groups. In contrast, the medium and low risk groups for rs7192 were nearly identical in frequency, while the highest risk group had a higher frequency than in rs9275596 (Figure 1B).

![Diagram of genotype frequencies for USAF School of Aerospace Medicine nasal wash archive samples.]

Figure 1. Genotype frequencies for USAF School of Aerospace Medicine nasal wash archive samples.

4.2 Comparing Risk Groups Between a USAF Population and the U.S. and Global Populations

Our analysis of the 1000 Genomes Project’s online database found the global risk group frequencies for rs7192 are 12.1%, 46.7%, and 36.6%, for high, medium, and low risk groups, respectively (Figure 1A). For the U.S. population, we calculated risk group frequencies of 13.7%, 39.3%, and 47.0%, respectively. Neither comparison between our sample population and the global or the U.S. population showed significant difference ($P=0.39$ and 0.23, respectively). The $P$-values were calculated using chi-square.
In contrast, for rs9275596 we found significant deviation within the USAF population compared to the global and U.S. populations. The global population distributions are 6.7%, 33.1%, and 60.2%, for high, medium, and low risk, while the U.S. distributions are 7.7%, 32.8%, and 59.58%, respectively. There were significant differences in the risk group frequencies across both populations. Our population’s distributions of 8.9%, 43.6%, and 47.4% (Figure 1B) were dramatic deviations from the global population \(P=0.001\) and the U.S. population \(P=0.002\).

4.3 Allele Frequencies for Polymorphisms Associated with Peanut Allergy Risk

Comparing risk allele frequencies between our USAF population and either the global or U.S. population resulted in finding significant differences for rs7192 (35.3%, 34.0%, and 32.1%, respectively; \(P=0.7\) and 0.3, respectively). In contrast, the risk allele frequency difference for rs9275596 for our population and the global population was significant (68.9% vs. 76.7%, \(P=0.008\)), while the difference from the U.S. population was not significant (73.4%, \(P=0.1\)).

4.4 Combined Risk Group Distributions for USAF Population

We calculated an overall risk classification based upon the genotypes of each SNP to identify the frequency of overall risk group combinations between the two SNPs for our population sampling. The most frequent risk group observed was low-low (LL, 35.78%), indicating homozygous for the non-risk allele for both SNPs. A combined 16% of the USAF population has at least one genotype suggesting heightened risk of developing peanut allergies (Table 1). Overall, the difference between the risk group frequencies and allele frequencies for both SNPs together was significant (USAF vs. global: \(P=0.006\), USAF vs. U.S.: \(P=0.03\)).

<table>
<thead>
<tr>
<th>Overall Risk Group</th>
<th>USAF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-Low</td>
<td>35.78</td>
</tr>
<tr>
<td>Low-Medium</td>
<td>15.20</td>
</tr>
<tr>
<td>Low-High</td>
<td>2.45</td>
</tr>
<tr>
<td>Medium-Medium</td>
<td>32.84</td>
</tr>
<tr>
<td>Medium-High</td>
<td>7.84</td>
</tr>
<tr>
<td>High-High</td>
<td>5.88</td>
</tr>
</tbody>
</table>

5.0 DISCUSSION

Despite decades of active research, the process by which food allergies develop remains a complex issue. Evidence suggests that genetic predisposition makes up some portion of risk [5]; however, it does not explain all facets of food allergies. In particular, why some adults develop allergies to foods they once were able to consume without a reaction is a puzzle open to interpretation. Using genetic indicators to help predict risk of developing an allergy may be advisable for the USAF as a precision-based care and risk management strategy.

Our work highlighted a specific polymorphism in the histocompatibility complex, hypervariable region as having a reduced risk allele frequency compared to civilian populations. However, we still found that approximately 16% of the sampled population had at least one
“high risk” genotype, indicating at least a threefold increase in chance of peanut allergy development. It is important to remember that the higher risk genotypes do not guarantee peanut allergies, but merely suggest and increased risk of allergy development. While having a peanut allergy is disqualifying and an unwaiverable medical condition, being able to survey for risk of peanut allergy development could allow an Airman to be more aware of reactions to food sources and possibly self-limit intake of trigger foods, strategies that may reduce the chance of severe allergic reaction [7].

Our original hypothesis was that the risk alleles and high-risk genotypes should be present less frequently in USAF populations. However, the data suggest that for one polymorphism (rs9275596), our population has an increased frequency of elevated risk and there is no decrease in risk group frequency for the other polymorphism (rs7192). As the nasal washes used in our study came from military medical treatment facilities that treat both military personnel and their families, a portion of our population is likely to have been non-AD. The number of diagnosed peanut allergies should be limited to the family members treated at the facility, which may account for a portion of the genetic risk alleles. However, as discussed above, merely having an elevated risk genetic profile does not guarantee an individual will develop an allergy. We also expect, therefore, that a systematic investigation of diagnoses vs. genotype of only AD Airmen would still find a portion of the population with risk genotypes, albeit we expect a lower value than for the civilian population. We suggest thoroughly examining this assertion in a formal manner before any effort begins to develop administrative, policy, or medical decisions.

6.0 CONCLUSION

Food allergies are complex, multifactorial traits that pose great risk to health and operational success. Latent food allergies could manifest catastrophic outcomes during missions, resulting in potential failure and loss of life. Understanding an individual’s risk of developing severe allergies is one of many components that can make up a high-quality surveillance and public health/preventive medicine program. We therefore assessed whether two specific genetic markers identified as related to peanut allergies in a civilian population of children were over- or under-represented in a USAF population. Our results showed that for a single polymorphism, there was a significant difference between the USAF population and open-source literature genomes. Although not strictly a validation of externally identified markers, our pilot study suggests that genetic markers derived from civilian populations may not always correlate with military cohorts, and internally validating genetic tools prior to developing screens is of the utmost importance for providing Trusted Care, Anywhere.
7.0 REFERENCES


**LIST OF ABBREVIATIONS AND ACRONYMS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD</td>
<td>active duty</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>SNP</td>
<td>single nucleotide polymorphism</td>
</tr>
<tr>
<td>USAF</td>
<td>U.S. Air Force</td>
</tr>
</tbody>
</table>