HLA-A*03:01 is associated with increased risk of fever, chills, and more severe reaction to Pfizer-BioNTech COVID-19 vaccination

Alexandre Bolze¹, Iva Neveux², Kelly M. Schiabor Barrett¹, Simon White¹, Magnus Isaksson¹, Shaun Dabe³, William Lee¹, Joseph J. Grzymski², Nicole L. Washington¹, Elizabeth T. Cirulli¹

¹Helix, 101 S Ellsworth Ave Suite 350, San Mateo, California 94401
²Center for Genomic Medicine, Desert Research Institute, 2215 Raggio Pkwy, Reno, Nevada 89512
³Renown Health, 1155 Mill St., Reno, Nevada 89502

*Corresponding Author:
Elizabeth T. Cirulli, liz.cirulli@helix.com

NOTE: This preprint reports new research that has not been certified by peer review and should not be used to guide clinical practice.
Abstract

COVID-19 vaccines are safe and highly effective, but some individuals experience unpleasant reactions to vaccination. As the majority of adults in the US have received a COVID-19 vaccine this year, there is an unprecedented opportunity to study the genetics of reactions to vaccination via surveys of individuals who are already part of genetic research studies. Here, we have queried 17,440 participants in the Helix DNA Discovery Project and Healthy Nevada Project about their reactions to COVID-19 vaccination. Our GWAS identifies an association between severe vaccine side effects and HLA-A*03:01. This association was statistically significant only for those who received the Pfizer-BioNTech vaccine (BNT162b2; \( p=4.70\times10^{-11} \)), but showed a trending association in those who received the Moderna vaccine (mRNA-1273; \( p=0.005 \)) despite similar sample sizes for study. In Pfizer-BioNTech recipients, HLA-A*03:01 was associated with a two-fold increase in risk of severe vaccine side effects. The effect was consistent across ages, sexes, and whether the person had previously had a COVID-19 infection. The reactions experienced by HLA-A*03:01 carriers were driven by associations with chills, fever, fatigue, and in general feeling unwell.
Introduction

Less than one year after the first publication of a SARS-CoV-2 sequence, COVID-19 vaccines were developed, clinically tested and authorized to be administered in the general population. Within months, hundreds of millions of adults worldwide were vaccinated, and rates of hospitalization among vaccinated individuals dropped precipitously; potential side effects were mild\(^1\). During the clinical trials of mRNA vaccines (Pfizer-BioNTech BNT162b2 and Moderna mRNA-1273), both local reactions, such as pain at the injection site, and systemic symptoms such as fatigue, fever, chills, and myalgia were observed in some participants\(^2,3\). Both clinical trials showed that only a small fraction of these reactions could be categorized as severe, and most of the severe reactions followed the second dose. These studies raise the question of what factors explain the interindividual variability of reactions following COVID-19 vaccination.

Younger age and a personal history of SARS-CoV-2 infection prior to vaccination are two factors leading to increased reactogenicity to the vaccine\(^4\). However, these factors alone do not explain the large interindividual variability in the degree of severity of the reaction following COVID-19 vaccination. The aim of this study is therefore to identify factors associated with severe reactions following COVID-19 vaccination. Similarly to our hypothesis that genetic factors play a role in the severity of COVID-19 disease\(^5,6\), we hypothesized that genetic factors help explain differences in reactions following COVID-19 vaccination. To test our hypothesis, we administered online surveys to 17,440 participants from the Helix DNA Discovery project and the Healthy Nevada Project asking about their vaccination status and their reactions following the vaccine. These surveys included questions about 4 local and 20 systemic symptoms, as well as the overall severity of the reaction and its impact on daily activities in the days following vaccination. These participants were previously sequenced with Helix Exome+ assay, which allowed us to perform rare and common variant genetic associations.
Methods

Cohort and survey

We administered an online survey in June and September of 2021 and received responses from 8,125 Helix DNA Discovery Project participants and 9,315 Healthy Nevada Project participants (Table 1)\textsuperscript{7,8}. These are unselected Helix customers and patients in the Renown Health System who chose to consent to participate in research projects and respond to our survey. The survey takes approximately 10 minutes to complete and can be found in the supplement. The participants in this cohort are aged 18 to 89+, 65% are female, and 85% are of European genetic ancestry (Table 1).

Table 1. Study and cohort information.

| Sample size | 17,440 |
|-------------|--------|
| Median age (range) | 58 (18-89+) |
| N female (%*) | 10,402 (64.9%) |
| Genetic ancestry N (%*) | \begin{tabular}{l|c}
African & 264 (1.6%) \\
East Asian & 301 (1.9%) \\
European & 13,643 (84.9%) \\
Hispanic & 1,391 (8.7%) \\
South Asian & 60 (0.4%) \\
Other / mixed ancestry & 403 (2.5%) \\
\end{tabular} |
| N with COVID-19 vaccination (%) | \begin{tabular}{l|c}
Pfizer & 8,041 (46.1%) \\
Moderna & 7,086 (40.6%) \\
J&J & 790 (4.5%) \\
Other/Unsure & 227 (1.3%) \\
\end{tabular} |
| N with positive COVID-19 test before vaccination (%) | 1,280 (7.3%) |
| N reporting vaccine reactions (%) | \begin{tabular}{l|c}
4- Extreme difficulties / unable to perform daily routine & 1,029 (8.0%) \\
3- Severe difficulties with daily routine & 1,237 (9.6%) \\
2- Moderate difficulties with daily routine & 2,597 (20.2%) \\
1- Mild difficulties with daily routine & 3,502 (27.2%) \\
0- No difficulties with daily routine & 4,500 (35.0%) \\
\end{tabular} |

*The total here is adjusted to remove individuals who do not have their sex and ethnicity available: this demographic information was collected separately and was not yet available for some individuals for this round of analysis.

Respondents indicated whether they had been vaccinated and which vaccine type they had received. They rated the severity of their vaccine response as indicated in Table 1. They also answered questions about 24 specific side-effects that can occur after COVID-19 vaccination (see Supplemental survey). There were 3,323 individuals who took the survey in both June and
September, updating their COVID-19 vaccination status and infection status. For these individuals, the highest severity score and all reactions were used as a phenotype, regardless of in which survey either were reported.

**Genotyping**

DNA samples were sequenced and analyzed at Helix using the Exome+® assay as previously described\(^9\). Imputation of common variants was performed by pre-phasing samples and then imputing. Pre-phasing was performed using reference databases, which include the 1000 Genomes Phase 3 data. This was followed by genotype imputation for all 1000 Genomes Phase 3 sites with MAF\(\geq 1\%) that have genotype quality (GQ) values less than 20. Imputation results were then filtered for quality (GP\(\geq 0.95\)) so that only high precision imputed variant calls were reported\(^10\).

HLA genotypes for A, B, C, DPB1, DQA1, DQB1, and DRB1 were imputed using HIBAG with the default recommendations\(^11\). Individual genotypes were imputed using the model for the appropriate genetic ancestry for each individual\(^11\). Probabilities higher than 0.5 were used as genotype calls.

**Genetic analysis**

We used Regenie for the genetic analysis\(^12\). Briefly, this method builds a whole genome regression model using common variants to account for the effects of relatedness and population stratification, and it accounts for situations where there is an extreme case-control imbalance, which can lead to test statistic inflation with other analysis methods. The covariates we included were age, sex, age*sex, age*age, sex*age*age, 10 PCs, type of COVID-19 vaccine, whether the individual had COVID-19 prior to getting vaccinated, and bioinformatics pipeline version. We only utilized phenotypes with at least 50 cases, with a minimum minor allele count (MAC) cutoff of 5.

As previously described, a representative set of 184,445 coding and noncoding LD-pruned, high-quality common variants were identified for building PCs and the whole genome regression model\(^9\). Each genetic ancestry group was analyzed separately: African, East Asian, European, Hispanic, South Asian.
Results

A minority of individuals have a severe reaction following COVID-19 vaccination

We received survey responses from 8,125 Helix participants and 9,315 HNP participants in June and September 2021. As a control, we first checked whether we observed the same trends as reported in the clinical trials of the Pfizer-BioNTech, Moderna and J&J COVID-19 vaccines. The large majority of participants in these clinical trials did not have a personal history of SARS-CoV-2 infection. To assess the frequency of reactions in our cohort, we therefore restricted our initial analysis to participants who reported no previous infection by SARS-CoV-2. Our results were in agreement with what was previously reported\(^2,3,13\). For the Pfizer-BioNTech vaccine, we observed that only 2.7% of individuals (152 of 5,590) had a severe or extreme reaction after the first dose, and 10.2% (569 of 5,583) had a severe or extreme reaction after the second dose (Figure S1). For the Moderna vaccine, we observed that 4.4% of individuals (243 of 5,575) had a severe or extreme reaction after the first dose, and this number grew to 19.5% (1,083 of 5,558) after the second dose (Figure S1). For the J&J vaccine, we observed that 17.7% (80 of 452) had a severe reaction after the single dose (Figure S1). Moreover, we observed a higher number of severe reactions in respondents 18-55 years old compared to those over 55 (Figure S1). These analyses supported the observation that there are large differences in reactions to COVID-19 vaccination between individuals, and validated our self-reported survey as a reliable tool to investigate the genetic basis of these differences.

GWAS identifies HLA region associated with severe reaction to COVID-19 vaccination

To test our hypothesis that genetic variation drives some of the differences seen in vaccine reaction, we performed a genome-wide association study (GWAS) using a phenotype of extreme or severe reaction (1,709 cases) compared to no or mild reaction (6,203 controls). Individuals reporting moderate reaction were excluded from the GWAS (Table 1). Our analysis of 12,602,619 SNPs identified 188 genome-wide significant variants on chromosome 6 (\(p<5\times10^{-8}\); Figure 1). The lead SNP was rs144943243 / chr6:29820015:AAAAT:A, \(p=3.51\times10^{-11}\). This variant is in a region of AAAT repeats found upstream of \textit{HLA-G}, with a MAF of 24% in the European genetic ancestry subset of our cohort.

We next analyzed imputed HLA types against the phenotype to identify whether a specific HLA type was associated with the GWAS signal. We identified a significant association with HLA-A\(^*\)03:01 (\(p=5.00\times10^{-11}\)), which had a MAF of 15% in the European genetic ancestry subset of our cohort. The distribution of HLA-A\(^*\)03:01 across the globe is shown in Figure S2\(^14\). A regression including both this HLA type and rs144943243 identified similar signals for each variant, with rs144943243 retaining a better \(p\)-value in a joint analysis. The subsequent analyses assessing the impact of the genetic variant by phenotype lead to similar results for rs144943243 and HLA-A\(^*\)03:01. In this manuscript, we decided to show the results for HLA-A\(^*\)03:01, a common allele for the well-studied \textit{HLA-A} gene.
Figure 1. Manhattan plot for main phenotype of severe / extreme vaccine reaction against mild or no reaction to any vaccination event with Pfizer-BioNTech, Moderna, J&J, or other COVID-19 vaccines. The lambda GC was 1.07.

**HLA-A*03:01 association is specific to vaccine type**

HLA-A*03:01 had an odds ratio (OR) of 1.5 for individuals to experience a severe / extreme vaccine reaction as opposed to a mild or no reaction. We found that this effect was additive, with individuals with 1 copy of HLA-A*03:01 having a phenotype intermediate to those with 0 or 2 copies (Figure 2). We found the effect of this variant to track similarly across age, sex, and whether the person had a history of SARS-CoV-2 infection prior to vaccination (Figure 2, S3). However, our analyses beyond European (p=5.00E-11, OR=1.6) and Hispanic (p=2.93E-04, OR=2.7) genetic ancestries were underpowered to identify associations (Table S1). We also observed that most of the effect seems to occur at the second dose for two-dose vaccines (Figure S4), and that severe reactions subsided within 2 days for 66% of those who experienced severe reactions (Figure S5).

Importantly, we identified that the association signal came almost entirely from reactions to the Pfizer-BioNTech vaccine (p=4.70E-11, OR=2.07), with very little impact on Moderna vaccine response (p=0.005, OR=1.32) despite similar sample sizes for analysis (Pfizer 552 cases vs. 3,142 controls; Moderna 1,018 cases vs. 2,592 controls). While the main phenotype of severe / extreme reaction was more common in Moderna recipients compared to Pfizer-BioNTech recipients, even restricting to a more stringent case definition did not result in statistical significance for the Moderna subset (p=0.008, OR=1.5; 473 extreme reaction cases vs. 1,425 no reaction controls). The sample sizes for those who received J&J or other vaccinations were
too low to clearly assess an association. Detailed counts of reaction severity split by genetic ancestry, HLA-A*03:01 status, and vaccine type are reported in Table S1.
Figure 2. Risk of vaccine side effects by HLA-A*03:01 genotype and vaccine type. A and D) Broken down by whether they had COVID-19 prior to vaccination. B and E) Broken down by sex (COVID-19 prior to vaccine excluded). C and F) By age (COVID-19 prior to vaccine excluded). Top row (A-C): Fraction with severe or extreme reaction; Bottom row (D-F): Number of vaccine reaction symptoms per person. European genetic ancestry with Pfizer-BioNTech or Moderna only shown.
Specific vaccine reaction phenotypes are associated with HLA-A*03:01

To understand the severity scores more deeply, we next analyzed the individual symptoms that participants reported as occurring after receiving the vaccine, split into Pfizer-BioNTech and Moderna subsets. We identified that in Pfizer-BioNTech recipients, HLA-A*03:01 was most strongly associated with increased risk of fever (p=1.76E-14), chills (p=6.15E-13), feeling unwell (p=5.73E-10) and fatigue (p=5.60E-07) after receiving the vaccine (Figure 3). The ORs for these reactions ranged from 1.44-2.09, with 41% of those with 1 HLA-A*03:01 copy who received a Pfizer vaccine having at least two of these four symptoms, 27% of those with 2 copies, and 19% of those with 0 copies. Associations with joint pain, headache, swollen lymph nodes, headache and nausea were less predictive but still statistically significant (p<0.001). In contrast, none of these symptoms were significantly associated with HLA-A*03:01 in Moderna recipients (Figure 3).
Figure 3. Odds ratios and 95% Confidence Interval (CI) for specific vaccine responses in an additive genetic analysis (regenie) of HLA-A*03:01 in European ancestry individuals (n=9,636). *p<0.001 in Pfizer-BioNTech recipients (A); no associations were significant in Moderna recipients (B).
Discussion

Here, we identified an HLA type, HLA-A*03:01, with a strong association with reactions to COVID-19 vaccines. We find that, all else being equal, individuals with this HLA type who received the Pfizer-BioNTech vaccine are approximately twice as likely to have a severe or extreme reaction following COVID-19 vaccination. Chills and fever were the two specific side effects that were most enriched in individuals carrying 1 or 2 copies of HLA-A*03:01 compared to individuals with two other HLA-A alleles. This association was present in the Helix DNA Discovery cohort, as well as the Healthy Nevada Project cohort. We find this association to trend across age groups, sex, and whether the person had a personal history of COVID-19 prior to vaccination, all of which are known to be associated with severity of vaccine reaction. We find that the effect of this variant was almost entirely driven by participants who had received the Pfizer-BioNTech vaccine. The signal for the association was very weak in those receiving the Moderna vaccine and did not approach genome-wide significance, despite a sample size roughly equal to that of the Pfizer vaccine recipients. This difference is surprising given the similarities between the two mRNA vaccines, but it is possible that the increased prevalence of vaccine reactions in Moderna recipients may make the signal harder to identify in that subgroup (Figure 2, S1, S4). Our sample size for J&J and other vaccines were too low for adequate analysis power.

Replication of the result in other cohorts who were mostly vaccinated with non-mRNA COVID-19 vaccines will be informative to hypothesize about potential mechanisms leading to these severe reactions. An effect could be linked to a common peptide derived from all vaccines showing an association. The alternative where the effect is only seen robustly for the Pfizer-BioNTech mRNA vaccine could lead to the hypothesis that the reaction is driven by a peptide unique to the Pfizer-BioNTech vaccine. Importantly, as we were preparing this manuscript, 23andMe published on their blog (https://blog.23andme.com/23andme-research/reaction-to-covid-vaccine/, accessed 14 November 2021) showing that HLA-A*03:01 was also the strongest genetic association with COVID-19 vaccine response in their cohort with a p-value p=1.5E-130. No additional details regarding the effect by vaccine type, dose, or symptoms were available yet.

An association of HLA-A*03:01 with reactogenicity to other non-COVID-19 vaccines could also be informative. However, to our knowledge, very few studies have been published reporting genetic associations with reactogenicity after non live-attenuated vaccines. In the past, a few studies have investigated the genetics of response to vaccines by looking at the levels of antibodies after a certain time period in vaccine recipients, often identifying associations with HLA genes\textsuperscript{15,16}. Other studies have elucidated why some patients presented with a rare life-threatening disease following vaccinations with live-attenuated vaccines\textsuperscript{17}. For example, genetic defects in the IL-12 dependent IFN-gamma pathway cause BCGitis after BCG vaccination\textsuperscript{18}. The difficulty of collecting appropriate phenotype information on the reaction to a
vaccine with standard medical data or electronic health records could be one reason to explain the small number of these studies. Another reason may be the difficulty to justify sequencing individuals to investigate a transient phenotype. The ability for us to ask new questions and gain new biological insights highlight the importance of continuing to survey and engage participants who are enrolled in ongoing genetic research projects.

This study raises many new questions about the potential role of HLA-A*03:01 in severe reactions following vaccination. Lastly, it is important to emphasize that our study defined a severe reaction to the vaccine as a reaction that would interfere with daily routine shortly after receiving the vaccine. These reactions were symptoms such as chills or fever, which cannot be compared with the severity of COVID-19 disease experienced by many individuals. COVID-19 vaccines have consistently been shown to be safe and very effective to prevent hospitalizations and life-threatening disease following SARS-CoV-2 infections\textsuperscript{2,3,13}. 

\textsuperscript{2} CC-BY-NC 4.0 International license
It is made available under a CC-BY-NC 4.0 International license.
Acknowledgements

Funding was provided to DRI by the Nevada Governor’s Office of Economic Development. Funding was provided to the Renown Institute for Health Innovation by Renown Health and the Renown Health Foundation. We acknowledge the entire Helix Bioinformatics team for their contributions to the production exome sequencing pipeline. We thank C. Clinton, KT Farley and E. Levin for their contribution running the Helix DNA Discovery Project and the work with the IRB. We thank all of the genomic representatives of the Healthy Nevada Project (HNP). We thank Renown Health and DRI marketing for helping to launch the HNP project. We thank the participants in the Helix DNA Discovery Project, and the Healthy Nevada Project.

References

1. Chapin-Bardales, J., Gee, J. & Myers, T. Reactogenicity Following Receipt of mRNA-Based COVID-19 Vaccines. JAMA 325, 2201–2202 (2021).

2. Polack, F. P. et al. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. N. Engl. J. Med. 383, 2603–2615 (2020).

3. Baden, L. R. et al. Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. N. Engl. J. Med. 384, 403–416 (2021).

4. Menni, C. et al. Vaccine side-effects and SARS-CoV-2 infection after vaccination in users of the COVID Symptom Study app in the UK: a prospective observational study. Lancet Infect. Dis. 21, 939–949 (2021).

5. COVID-19 Host Genetics Initiative. Mapping the human genetic architecture of COVID-19. Nature (2021) doi:10.1038/s41586-021-03767-x.

6. Casanova, J.-L., Su, H. C. & COVID Human Genetic Effort. A Global Effort to Define the Human Genetics of Protective Immunity to SARS-CoV-2 Infection. Cell 181, 1194–1199 (2020).

7. Introducing the Helix DNA Discovery Project.
   https://blog.helix.com/helix-dna-discovery-project/ (2018).

8. Grzymski, J. J. et al. Population Health Genetic Screening for Tier 1 Inherited Diseases in Northern Nevada: 90% of At-Risk Carriers are Missed. bioRxiv 650549 (2019)
doi:10.1101/650549.

9. Cirulli, E. T. et al. Genome-wide rare variant analysis for thousands of phenotypes in over 70,000 exomes from two cohorts. *Nat. Commun.* **11**, 542 (2020).

10. Helix’s Exome+ Performance White Paper. (2019).

11. Zheng, X. et al. HIBAG--HLA genotype imputation with attribute bagging. *Pharmacogenomics J.* **14**, 192–200 (2014).

12. Mbatchou, J. et al. Computationally efficient whole genome regression for quantitative and binary traits. *Cold Spring Harbor Laboratory* 2020.06.19.162354 (2020) doi:10.1101/2020.06.19.162354.

13. Sadoff, J. et al. Safety and Efficacy of Single-Dose Ad26.COV2.S Vaccine against Covid-19. *N. Engl. J. Med.* **384**, 2187–2201 (2021).

14. Gonzalez-Galarza, F. F. et al. Allele frequency net database (AFND) 2020 update: gold-standard data classification, open access genotype data and new query tools. *Nucleic Acids Res.* **48**, D783–D788 (2020).

15. Chung, S. et al. GWAS identifying HLA-DPB1 gene variants associated with responsiveness to hepatitis B virus vaccination in Koreans: Independent association of HLA-DPB1*04:02 possessing rs1042169 G - rs9277355 C - rs9277356 A. *J. Viral Hepat.* **26**, 1318–1329 (2019).

16. Png, E. et al. A genome-wide association study of hepatitis B vaccine response in an Indonesian population reveals multiple independent risk variants in the HLA region. *Hum. Mol. Genet.* **20**, 3893–3898 (2011).

17. Pöyhönen, L., Bustamante, J., Casanova, J.-L., Jouanguy, E. & Zhang, Q. Life-Threatening Infections Due to Live-Attenuated Vaccines: Early Manifestations of Inborn Errors of Immunity. *J. Clin. Immunol.* **39**, 376–390 (2019).

18. Lichtenauer-Kaligis, E. G. R. et al. Severe Mycobacterium bovis BCG infections in a large series of novel IL–12 receptor β1 deficient patients and evidence for the existence of partial
IL–12 receptor β1 deficiency. *European Journal of Immunology* vol. 33 59–69 (2003).