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ORIGINAL ARTICLE

HLA Immune Response Genetics WILEY

Genome-wide association studies of response and side effects to the BNT162b2 vaccine in Italian healthcare workers: Increased antibody levels and side effects in carriers of the *HLA-A*03:01* allele

Chiara Magri[1] | **Eleonora Marchina**[1] | **Emanuele Sansone**[2] | **Adamo Pio D'Adamo**[3,4] | **Stefania Cappellani**[4] | **Carlo Bonfanti**[1,5] | **Luigina Terlenghi**[5] | **Giorgio Biasiotto**[1,6] | **Isabella Zanella**[1,7] | **Emma Sala**[8] | **Arnaldo Caruso**[1,5] | **Massimo Lombardo**[9] | **Paolo Gasparini**[3,4] | **Giuseppe De Palma**[2,8] | **Massimo Gennarelli**[1,10]

[1]Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy

[2]Department of Medical and Surgical Specialties, Radiological Sciences and Public Health, Unit of Occupational Health and Industrial Hygiene, University of Brescia, Brescia, Italy

[3]Department of Medicine, Surgery and Health Sciences, University of Trieste, Trieste, Italy

[4]Institute for Maternal and Child Health-IRCCS "Burlo Garofolo", Trieste, Italy

[5]Laboratory of Microbiology, ASST Spedali Civili, Brescia, Italy

[6]Highly Specialized Laboratory, ASST Spedali Civili di Brescia, Brescia, Italy

[7]Clinical Chemistry Laboratory, Section of Cytogenetics and Molecular Genetics, ASST Spedali Civili di Brescia, Brescia, Italy

[8]Unit of Occupational Health, Hygiene, Toxicology and Prevention, ASST Spedali Civili, Brescia, Italy

[9]Chief Executive Office, ASST Spedali Civili di Brescia, Brescia, Italy

[10]Genetics Unit, IRCCS Istituto Centro San Giovanni Di Dio Fatebenefratelli, Brescia, Italy

Correspondence
Chiara Magri, Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy.
Email: chiara.magri@unibs.it

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The remarkable variability of response to vaccines against SARS-CoV-2 is apparent. The present study aims to estimate the extent to which the host genetic background contributes to this variability in terms of immune response and side effects following the administration of the BNT162b2 vaccine. We carried out a genome wide association study (GWAS) by genotyping 873 Italian healthcare workers who underwent anti-SARS-CoV-2 vaccination with the BNT162b2 vaccine and for whom information about anti-SARS-CoV-2 spike antibodies titers and vaccine side effects were available. The GWAS revealed a significant association between the HLA locus and the anti-SARS-CoV-2 Spike antibodies level at 2 months following the first dose of vaccine (SNP: rs1737060; $p = 9.80 \times 10^{-11}$). In particular, we observed a positive association between the antibody levels and the presence of the *HLA-A*03:01* allele. The same allele was found associated with a 2–2.4-fold increased risk of

experiencing specific side effects such as fever, chills and myalgia and a 1.5–-1.8-fold increased risk of joint pain, nausea, fatigue, headache and asthenia, independently of age and sex. This study confirms that the heterogeneity in the immune response to the BNT162b2 vaccine and in its side effects are at least partially influenced by genetic variants. This information, integrated with individual biological and lifestyle-related correlates, could be of use in the definition of algorithms aimed at the identification of subjects in which the administration of additional vaccine doses would be particularly beneficial to maintain immunity against the virus.

KEYWORDS

BNT162b2, GWAS, *HLA-A*03:01*, SARS-CoV-2, SARS-CoV-2 spike antibodies, side effects, vaccine

1 | INTRODUCTION

A new infectious respiratory disease, caused by a novel coronavirus 2 (SARS-CoV-2) and named coronavirus disease 19 (COVID-19), emerged in China in December 2019 and caused over 600 million cases and more than 6 million deaths worldwide.[1]

The COVID-19 pandemic has strengthened the interest in the biological mechanisms underlying the complex interplay between infectious agents and the human host.[2,3]

Since the launch of the vaccination campaigns, the spectrum of phenotypes associated to the SARS-CoV-2 infection has widened to include serological and clinical response to the vaccines, highlighting the question concerning the extent to which the inter-individual variability is influenced by the hosts' genetic background.

The BNT162b2 vaccine (BNT162b2, Pfizer-BioNTech) was the first RNA-based vaccine to obtain a marketing authorization by the European Medicines Agency for COVID-19 prevention. This vaccine is based on a messenger RNA (mRNA) technology which inoculates the mRNA encoding for the antigen. More specifically, BNT162b2 inoculates the mRNA coding for SARS-CoV-2 spike protein, found on the outer surface of the virus, and used to enter cells and replicate.

BNT162b2 has shown high efficacy during clinical trials and has been administered to millions of people around the world to date.[4] However, vaccine efficacy has changed during time as a result of the circulation of new viral variants. In Italy, the "real-life" efficacy of vaccination is estimated weekly by the Istituto Superiore di Sanità and reported in an epidemiological surveillance bulletin.[5]

Previously published studies generally revealed a large inter-individual variability in terms of immune response, mainly related to age and clinical history concerning previous SARS-CoV-2 infections.[6–9] It is widely acknowledged that the response to vaccines can be influenced by numerous factors including characteristics of the infectious agent, features of the vaccine itself (e.g., type of vaccine, adjuvant, dose, and administration route and schedule), and pertaining to the host (e.g., age, sex, ongoing therapies, and comorbidities), and, including his/her genetic background.[10,11] Twin studies have shown the heritability of the immune response to vaccines to be markedly heterogeneous, with estimates ranging between 39% and 89%.[12] The importance of the host's genetic background in determining the immune response to vaccines is further corroborated by the observation that different ethnic groups, even when exposed to the same environment, show different seroconversion rates, and are characterized by different durations of immunity.[11] Genome-wide association studies (GWASs) and candidate gene analyses have shown that genes involved in the response to vaccines can be generally grouped into those involved in antigen processing and presentation, genes related to the innate immune system and genes implicated in cell signaling pathways. Genetic variants in major histocompatibility complex genes as well as in genes encoding Toll-like receptors (TLR) or RIG-like receptors (RLR), cytokines or cytokine receptors and viral or vitamin receptors have been found associated with both humoral and cell-mediated vaccine responses.[11,13,14]

To our knowledge, genetic studies pertaining to the immune response to the BNT162b2 vaccine are limited to date. A genetic screening of a specific polymorphic region within the 3′ regulatory region 1 (3′RR1) of the human immunoglobulin constant-gene (IgH) locus, allowed the identification of different single-nucleotide polymorphisms (SNPs) associated with either high or low antibody response.[15] Moreover, a very recent GWAS

performed on the UK population show that individuals carrying *HLA-DQB1*06* alleles are characterized by a stronger antibody responses against the SARS-CoV-2 spike protein and the receptor-binding domain (RBD) following vaccination with both ChAdOx1 nCoV-19 (manufactured by AstraZeneca) and BNT162b2 vaccines than non-carriers.[16] In addition, a study performed on the Italian population revealed an association between a low level of antibody titer following BNT162b2 vaccination and specific HLA alleles.[17]

The widespread vaccine administration in the population has also highlighted a remarkable heterogeneity in terms of side effects, with age, personal history of previous SARS-CoV-2 infections and the genetic variability of the host (mainly concerning variants in the HLA locus) identified as the main predictors of the reactogenicity.[18] Of note, a distinct study performed on the Japanese population identified 14 loci associated with adverse events following COVID-19 vaccination.[19]

Taken together, these findings suggest that the host's genetic variability, is an important predictor of both the immune response and potential side effects observed following the administration of a COVID-19 vaccine.

On December 27th, 2020, the anti SARS-CoV-2 vaccination campaign with the BNT162b2 vaccine started on a national scale in Europe. Concurrently, the ASST Spedali Civili in Brescia launched a campaign aiming to study the efficacy and duration of seroconversion of the BNT162b2 vaccine in health care workers (HCWs).[20–22] The present study aims to estimate the extent to which the host genetic background contributes to the variability in terms of immune response and adverse reactions following the administration of the BNT162b2 vaccine in the same cohort of HCWs.

2 | MATERIALS AND METHODS

2.1 | The cohort

The samples analyzed in this study were a subset ($N = 926$) of a previously described cohort.[23] Briefly, the original cohort included HCWs employed in ASST Spedali Civili di Brescia (Brescia, Italy) assembled to study the proportion, level and the determinants of humoral response from 21 days to up to 1 year after the first dose of anti SARS-CoV-2 vaccine. The vast majority of participants received a 2nd dose of BNT162b2 vaccine 21 days after the 1st dose; results collected at 2 (T1) and 4 months (T2) after the 1st dose administration have been included in the present study.

After providing written informed consent, participants underwent blood sampling for genetic analyses and filled an online questionnaire pertaining to their demographic information, life style, and potential side effects experienced following vaccine administration.

A total of 873 individuals remained eligible for the final analysis, satisfying the following inclusion criteria:

i. having received two doses of BNT162b2 anti-SARS-CoV-2 vaccination;
ii. no serological evidence of pre-vaccine SARS-CoV-2 infection;
iii. no serological evidence of post-vaccine SARS-CoV-2 infection up to T1;
iv. being of Caucasian ancestry;
v. having passed all quality controls for the GWAS analysis.

Their demographic characteristics are reported in Table 1. Of Note, five individuals contracted a Covid-19 infection between the first (T1) and the second (T2) serological measurements. These individuals were excluded from the analyses concerning antibody titers at T2 and the potential change of antibody titers from T1 to T2.

The study and the questionnaire were approved by the Ethics Committee of Spedali Civili di Brescia (NP. 4139 of 25 May 2021).

2.2 | Antibody detection

As previously reported,[23] vaccine response (from T1 onwards) was assessed using the Electrochemiluminescence immunoassay (ECLIA) Elecsys® anti SARS CoV2 S for anti-S (IgG/A/M) detection (Roche Diagnostics International Ltd, Rotkreuz, Switzerland). Elecsys® Anti-SARS-CoV-2-S is an immunoassay for the in vitro quantitative determination of antibodies (IgG/A/M) targeting the SARS-CoV-2 Spike (S) protein receptor binding domain (RBD) in human serum and plasma. The assay uses a recombinant protein representing the RBD of the S antigen in a double-antigen sandwich assay format. Results are expressed as U/mL, with a cut-off of 0.8 U/mL, and the upper limit of detection was 250 U/mL. Since the antibody titers elicited in immunized individuals were very high, we tested all serum samples at a dilution rate of 1:20 in accordance with Roche indications. Consequently, the upper limit for detection was raised to 5000 U/mL and the dynamic range was extended.

ECLIA Elecsys® anti SARS-CoV-2 (anti-N) was also used to test participants. In this case, results are

TABLE 1 Demographic and life-style related characteristics of the study cohort.[a]

Demographic information	
Sample size	873
Median age (range)	48 (22–65)
No. of females (%)	722 (82.7%)
Blood groups	
A	314 (36.0%)
B	68 (7.8%)
AB	26 (3.0%)
O	316 (36.2%)
Unknown	149 (17.1%)
Life style	
Median BMI (range)	22.7 (14.5–50.8)
Smoke	
1: No	714 (81.8%)
2: 5–10 cigarettes/day	91 (10.4%)
3: >10 cigarettes/day	68 (7.8%)
Physical activity (%)	
0: No	420 (48.1%)
1: At least once a week	453 (51.9%)
Alcohol consumption	
0: No	251 (28.8%)
1: 0.5–1 glass/day	605 (69.3%)
2: 2–3 glasses/day	16 (1.8%)
3: >3 glasses/day	1 (0.1%)
Antibody titers	
Median T1 level (range)	1167 (36–5000) U/mL
Median T2 level (range)	926 (4–5000) U/mL
Median percentage increase/ decrease from T1 to T2	−23% (−89% to +226%)

[a]All information except for the antibody titers are self-reported.

expressed as cut-off index, with the cut-off being 1. Whenever available, the results of pre-vaccine serological screenings performed during 2020 were considered and cumulated with the baseline (T0) to further minimize the risk of misclassification.

2.3 | DNA extraction

Genomic DNA was extracted from 1.2 mL of venous whole blood using the MagCore® Genomic DNA Whole Blood Kit and the MagCore® automatic nucleic acid extractor according to the manufacturer's operating instructions. Quantity and purity were evaluated with the Nanodrop spectrophotometer.

2.4 | Genotyping

Samples were genotyped using the Illumina Infinium Global Screening Array v3.0. DNA was processed according to the Illumina instruction manuals and genotype data were analyzed using GenomeStudio® Genotyping Module v2.0 software. Quality control (QC) of the data was performed with Plink 1.9 (www.cog-genomics.org/plink/1.9/).[24,25] Single nucleotide polymorphisms (SNPs) with a call rate <0.95, duplicated or non-polymorphic were excluded from the analysis. Individuals were excluded if more than 5% of SNPs were classified as missing, if derived genetic sex did not match reported sex, or showed unusual heterozygosity (<0.20 or >0.40). Cryptic relatedness was calculated for all pairs of individuals and for pairs with a pi_hat ≥20 only one of the two member was retained.

The risk of spurious associations due to population stratification was controlled using the Principal Component Analysis (PCA) implemented in Plink v1.9 software. The PCA was performed on a pruned subset of SNPs obtained after removing SNPs in linkage disequilibrium by means of the plink command "indep-pairwise" and using a window size of 50 SNPs a number of SNPs to shift the window of 5 and a r2 threshold of 0.6. Samples that resulted as being outlier with respect to the first two PCs at a visual inspection were removed from the analysis. The first five PCs were included as covariates in GWAS.

2.5 | Imputation

After initial quality controls, data was prepared for imputation with the toolbox provided by Will Rayner (https://www.well.ox.ac.uk/~wrayner/tools/). Briefly this tool checks and—if needed—updates strand, alleles, position, and Ref/Alt assignment. Furthermore, it removes A/T and G/C SNPs if MAF > 0.4, SNPs with differing alleles, SNPs with >0.2 allele frequency difference and SNPs not in the adopted reference panel. Genotypes were imputed using the European subset of the HRC (Version r1.1 2016—http://www.haplotype-reference-consortium.org) reference panel a with the Minimac 4 software through the Michigan Imputation Server.[26] Plink 2 (www.cog-genomics.org/plink/2.0/)[24] was used to filter only biallelic variants with an imputation quality (r2) > 0.8, and to convert vcf files into plink format. HLA imputation was performed using the Four-digit Multi-ethnic HLA v1 (2021) reference panel[27] available on the Michigan imputation server using recommended settings.

After imputation, only SNPs with a MAF > 0.05 and in HWE ($p > 1 \times 10^{-6}$) were retained for statistical analyses.

2.6 | Statistical analyses and GWAS

Associations between vaccine response and demographic and/or life-style variables were investigated using multivariate linear regressions. Variables associated with response were used as covariates in the GWAS. Three distinct variables were used as response indicators: the log-transformed level of antibodies at T1 and T2, and the percentage of increase/decrease from T1 to T2 (measured as $((T2 - T1)/T1) \times 100$). Antibody levels were log-transformed to take into account the skewness of the distribution.

Associations between SNPs and the log-transformed levels of antibodies at T1 and T2 were explored using linear regression analyses under an additive model, with "age," "smoke" and the first five PCs included as covariates. Potential association between SNPs and percent increase/decrease of antibodies from T1 to T2 was explored using linear regression analyses under an additive model, with "BMI," "alcohol consumption" and the first five PCs included as covariates. All the analyses were performed using Plink 1.9.[24] To identify lead SNPs and to define genomic risk loci significantly associated with vaccine response, we performed variant clumping by means of FUMAGWAS web tool v1.4.1.[28] Gene-based association analyses were performed with MAGMA v1.08[29] implemented in the FUMAGWAS web tool, starting from GWAS summary statistics. The same analyses were performed for the HLA imputation outputs: (1) binary marker for classical HLA alleles; (2) binary marker for the presence/absence of a specific amino acid residue; (3) HLA intragenic SNPs, and (4) binary markers for insertion/deletions.

For multiple testing correction, we considered a threshold of $p = 5 \times 10^{-8}$ to declare significance at the single SNP level. Since input SNPs were mapped to 18,178 protein coding genes, genome wide significance at the gene-level was defined at $p = 0.05/18{,}178 = 2.751 \times 10^{-6}$. For the HLA locus we analyzed 40,599 outputs, and we considered a threshold of $p = 1.23 \times 10^{-6}$ (0.05/40,599).

The genome-wide associations with vaccination side effects were tested individually for each side effect, taking into account only those present in at least 1% of our cohort (Pain at the inject. site, Swelling/Redness, Rush/pethechiae, Fatigues, Asthenia, Joint pain, Myalgia, Headache, Chills, Fever >37.5°C, Nausea, Lymphadenopathy, Diarrhea, and Tachycardia). Associations were explored using logistic regression analyses, with "age," "sex" and the first five PCs included as covariates. Gene-based association analyses were performed with MAGMA v1.08. To declare significance, we used the same multiple testing correction thresholds of above ($p = 5 \times 10^{-8}$ to declare significance at the single SNP level, and of $p = 2.751 \times 10^{-6}$ to declare significance at the gene-level).

Association between side effects and the presence of the *HLA-A*03:01* allele, were explored using logistic regression analyses. Along with the first five PCs, "age" and "sex" were included as covariates in the study since they were predictive of a number of side effects, as shown in Supplementary Table 1.

3 | RESULTS

3.1 | The effect of biological and lifestyle factors

Eight hundred and seventy-three HCWs were involved in the final analysis.

Their median age was of 48 years (range: 22–65), and the majority were females (82.7%) (Table 1). No significant differences were observed in terms of lifestyle indicators between the two sexes except for the declared alcohol consumption (Chi-squared p-value <0.01). The majority of females (74%) declared an alcohol consumption of 0.5–1 glass/day and 24.8% described themselves as teetotaller. For males, these percentages were 45.7% and 47.7%, respectively. As far as the impact of biological and lifestyle factors on antibody levels and adverse reactions to vaccines are concerned, multivariate regression analyses revealed that the main predictors of antibody titers at T1 and T2 were "age," "smoke" and to a minor extent "physical activity" (Table 2, Supplementary Figure 1). The percent change in antibody titers from T1 to T2 emerged instead as being slightly influenced by "BMI" and "alcohol consumption" (Table 2). After stratifying individuals into four classes based on their BMI, we noted that underweight individuals (BMI < 18.5) had a significantly lower decrement of antibody titers from T1 to T2 compared to overweight ($25 \leq$ BMI < 30; Wilcoxon test $p = 0.022$) and obese (BMI ≥ 30; $p = 0.028$) subjects (Supplementary Figure 2).

The vast majority (92.9%) of individuals reported at least one side effect of vaccination. The frequency of the reported reactions is shown in Table 3 together with the frequency reported by the Food & Drug administration (FDA) for the general population aged 18–55.[30] The main biological risk factors for the onset of adverse reactions to the vaccine resulted to be age and sex. In particular, older people had a lower risk of experiencing pain, headache, fatigue, asthenia, fever, or chills after vaccination and a higher risk of rush/petechiae; whereas females had a higher risk than males to experience swelling/redness at the injection site, headache, fatigues, myalgia, asthenia, fever, chills, joint pain, nausea, diarrhea and lymphadenopathy (Supplementary Table 1). Lifestyle-related factors, on the contrary, did not emerge

TABLE 2 Results of multivariate regression analyses on biological and lifestyle-related factors influencing antibody titers.

	Estimate	Std. error	*t* value	Pr(>|*t*|)
Antibody titers T1				
(Intercept)	8.310927	0.232422	35.758	<2e−16
Age	**−0.017003**	**0.002258**	**−7.530**	**1.27e−13**
Sex	0.009549	0.062850	0.152	0.8793
BMI	0.005005	0.005431	0.922	0.3570
Smoke	**−0.287438**	**0.039744**	**−7.232**	**1.05e−12**
Alcohol	−0.067890	0.048045	−1.413	0.1580
Physical activity	**−0.114623**	**0.047530**	**−2.412**	**0.0161**
Antibody titers T2				
(Intercept)	8.363120	0.238581	35.054	<2e−16
Age	**−0.018710**	**0.002317**	**−8.075**	**2.26e−15**
Sex	−0.006798	0.064694	−0.105	0.91634
BMI	−0.004188	0.005580	−0.750	0.45321
Smoke	**−0.310337**	**0.041201**	**−7.532**	**1.26e−13**
Alcohol	−0.024893	0.049700	−0.501	0.61660
Physical activity	**−0.128665**	**0.048820**	**−2.635**	**0.00855**
Percentage of variation in antibody levels from T1 to T2				
(Intercept)	−2.51517	0.1035650	−0.243	0.8082
Age	−0.05861	0.0010057	−0.583	0.5602
Sex	−0.76774	0.0280829	−0.273	0.7846
BMI	**−0.61315**	**0.0024224**	**−2.531**	**0.0115**
Smoke	−1.77897	0.0178849	−0.995	0.3202
Alcohol	**4.58047**	**0.0215743**	**2.123**	**0.0340**
Physical activity	−1.05883	0.0211922	−0.500	0.6175

Note: Significant results are reported in bold.

as vaccination side effect predictors. No association with side effects was found for BMI, smoking, or physical activity, while alcohol consumption was negatively associated with the onset of pain.

3.2 | GWAS results

To test the hypothesis that the genetic background could influence both the variability in terms of antibody response to vaccination and the variability in the manifestation of side effects, we performed a GWAS including the first five PCs as covariates together with the main biological and lifestyle-associated identified as predictors in the analyses described in the previous paragraph.

3.2.1 | Association to antibody levels at T1

As far as the antibody levels at T1 are concerned, the analysis of 4,989,554 SNPs revealed a genome-wide significant association for 114 SNPs located on a locus on chromosome 6 spanning the HLA region (Figure 1A,B, Supplementary Table 2), with the lead SNP being rs1737060 (6:29732969:G:C; $p = 9.80 \times 10^{-11}$). MAGMA gene-wise analysis confirmed the association with the HLA locus, by identifying a positive association just below the significance threshold for the *HLA-F* gene ($p = 3.04 \times 10^{-6}$) (Figure 1C). In addition to the *HLA-F* gene, four other HLA genes were ranked among the top 10 associated genes (Figure 1, Supplementary Table 3).

3.2.2 | Association to antibody levels at T2

The GWAS did not reveal any genome-wide significant association with antibody levels at T2. However, the most significant locus was again the HLA locus (Supplementary Figure 3A,B, and Supplementary Table 2), with the near-significant lead SNP being rs2743941 (6:29755451:T:C; $p = 8.739 \times 10^{-8}$). In addition to this locus on chromosome 6, three other loci among the top 10 in terms of association

TABLE 3 Frequency of side effects to vaccination in our cohort compared to those reported by the FDA in persons aged 18–55 years.[30]

Number of adverse reactions to vaccine	Our cohort	FDA data (% after 1st dose–% after 2nd dose)[a]
Local AEs		
Pain at the inject. site	703 (80.5%)	83.1%–77.8%
Swelling/redness	117 (13.4%)	5.8%–6.3% Swelling
		4.5%–5.9% Redness
Rush/pethechiae	14 (1.6%)	nr
Systemic AEs		
Fatigues	538 (61.6%)	47.4%–59.4%
Asthenia	386 (44.2%)	nr
Joint pain	320 (36.7%)	11%–21.9%
Myalgia	266 (30.5%)	21.3%–37.3%
Headache	241 (27.6%)	41.9%–51.7%
Chills	233 (26.7%)	14%–35.1%
Fever >37.5°C	169 (19.4%)	7.5%–31.4%
Nausea	87 (9.9%)	nr
Lymphadenopathy	61 (7.0%)	64 cases reported
Diarrhea	39 (4.5%)	11.1%–10.4%
Tachycardia	31 (3.6%)	nr
Vomiting	15 (1.7%)	1.2%–1.9%
Facial paralysis	7 (0.8%)	4 cases of Bell's palsy
Bruising	3 (0.3%)	nr
Anaphylaxis	3 (0.3%)	nr
None	62 (7.1%)	

Abbreviation: nr, not reported.
[a]Reactions reported in persons aged 18–55 years derived from tables 15 and 17 of the Vaccines and Related Biological Products Advisory Committee Meeting December 10, 2020.[30]

strength were shared between the two analyses (Supplementary Table 2). MAGMA analysis did not identify any gene-based significance but, of note, among the top 10 associated genes figured *HLA-F* and *HLA-DRB1* (Supplementary Figure 3C, Supplementary Table 3).

3.2.3 | Percentage of variation of antibody levels from T1 to T2

Finally, as described in the previous section, we tested the hypothesis that the genetic background could influence the rate of antibody level change over time by studying the association with the percent variation of antibody levels from T1 to T2. No significant associations emerged either from the single SNP analysis or from the gene-wise analysis (Supplementary Figure 4, Supplementary Tables 2 and 3).

Of note that 16 genes, including *HLA-F* and *HLA-A*, were found to be nominally associated with all three traits (Supplementary Table 4).

3.2.4 | HLA imputation and association with HLA alleles

Since the most significant result involved the HLA locus, we refined our association analysis in order to verify if the association between the level of antibodies at T1 and the HLA locus could be due to the effect of specific HLA alleles. Imputation of the HLA locus as reported in the methods, allowed imputing 40,599 variants/alleles with info >0.80 and MAF < 0.05, of which 115 were HLA alleles and 2269 amino acids (AA) changes. At the SNP level, the association with rs1737060 was confirmed. The summary statistics of all tested HLA alleles are reported in Supplementary Table 5. The HLA allele most strongly associated with T1 antibody levels was *HLA-A*03:01* ($p = 3.089 \times 10^{-8}$, beta = 0.3092, SE = 0.05533), the same result was obtained for the four-digit allele *HLA-A*03:01:01:01*, whereas the most significant associations with an amino acid change were with "AA_A_161_29911255_exon3_D" ($p = 6.0 \times 10^{-8}$, beta = 0.2965, SE = 0.05423). Of note, the presence of aspartic acid at position 161 is a defining mutation of the *HLA-A*03* allele. The second most significant association with antibody levels at T1 was the absence of the "AA_A_70_29910741_exon2_H" allele ($p = 5.809 \times 10^{-7}$, beta = 0.1859, SE = 0.03691), another amino acid change defining the *HLA-A*03* allele. In our cohort 8 individuals were predicted homozygous for the *HLA-A*03:01* allele, 148 were heterozygous, and 717 had others alleles.

In order to evaluate if there were any correlations between the association with rs1737060 and the *HLA-A*03* allele, we performed a regression analysis conditioned for the SNPs rs1737060 allele. In the conditional analysis, the *HLA-A*03* allele was not significant, suggesting the associations with rs1737060 and with the *HLA-A*03* allele to be overlapping signals. Unfortunately, the gene-based association detected by MAGMA between antibody levels at T1 and *HLA-F* could not be investigated further as no *HLA-F* alleles were imputed.

No significant associations of HLA alleles with antibody levels at T2 or with the percent change in antibody levels from T1 to T2 were observed after multiple testing correction. However, the *HLA-A*03:01* allele was a nominal predictor of antibody levels at T2 ($p = 5.86 \times 10^{-4}$, beta = 0.1983, SE = 0.05746) (Supplementary Table 6).

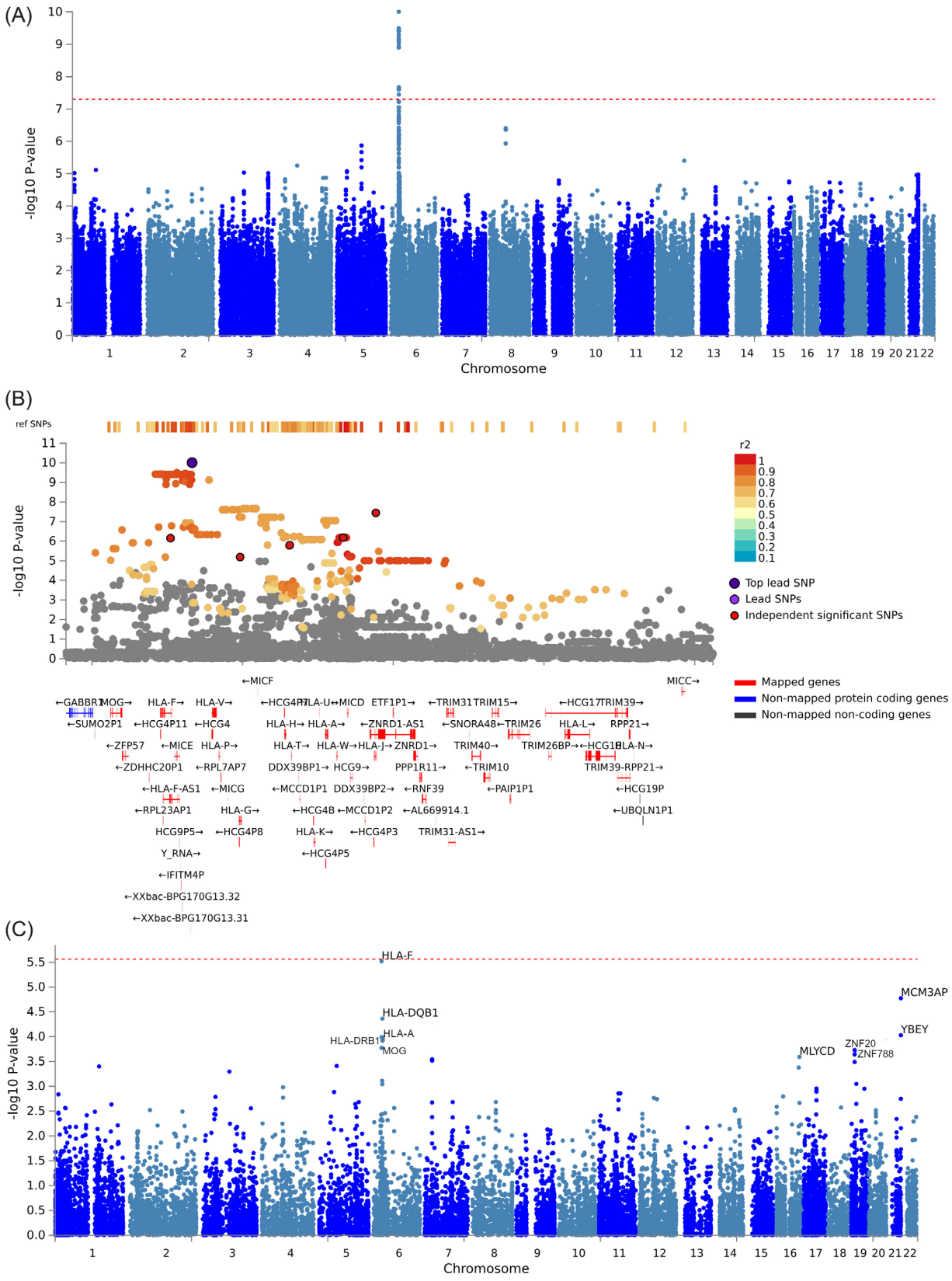

FIGURE 1 Manhattan plots for the association with antibody levels at T1. (A) Single SNP Manhattan plot. (B) Regional Manhattan plot of the locus on chromosome 6 associated with T1 antibody levels. (C) Manhattan plot of the gene-based test as computed by MAGMA based on the GWAS summary statistics for the association with T1 antibody levels. Significance threshold was set at $p = 2.751 \times 10^{-6}$.

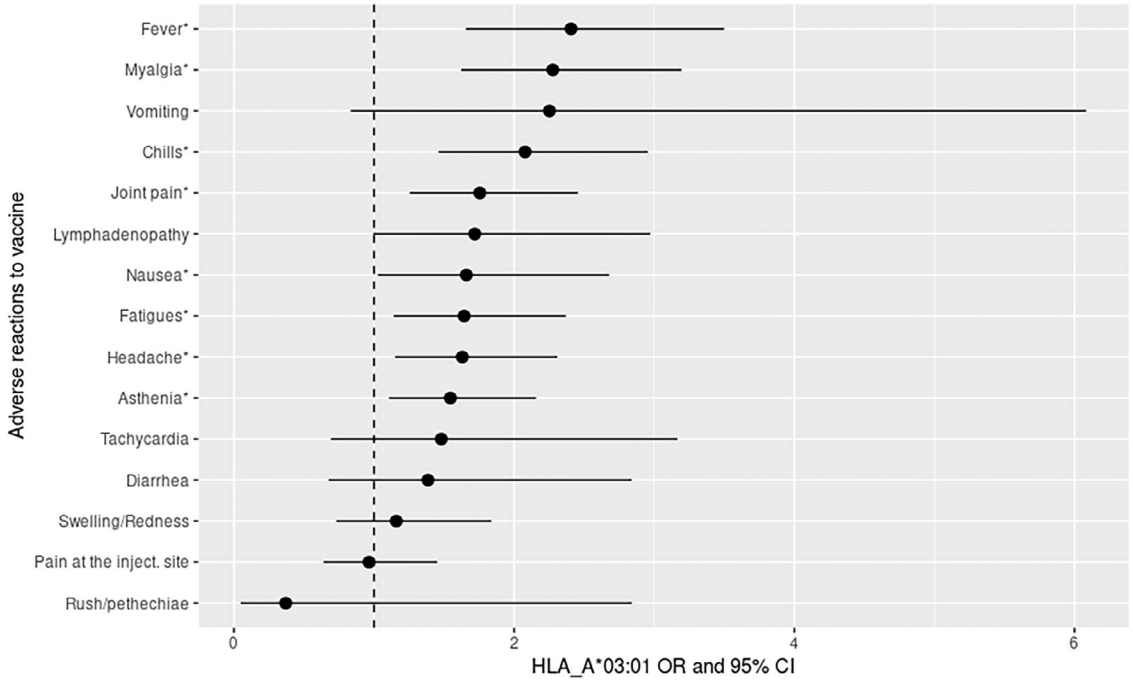

FIGURE 2 Forest plot of the odd ratios and 95% confidence intervals (CI) for specific vaccine responses associated with the *HLA-A*03:01* allele. Asterisk indicates side effects for which the association is statistical significant ($p < 0.05$).

3.2.5 | Association with vaccination side effects

We surveyed genetic associations with all side effects reported in >1% cases (Table 2). The logistic regressions revealed only a genome-wide significant association between the presence of lymphadenopathy and two SNPs that map in a locus on chromosome 20 (chr20: 57,982,201–58,009,888), spanning a gene desert. The lead SNP was rs271982 (20:58002985:A:G; $p = 1.791 \times 10^{-9}$, OR = 4.205, SE = 0.2283), mapping in an intergenic region between the *EDN3* and the *PHACTR3* genes (Supplementary Figure 5A). MAGMA gene-wise analysis did not reveal any significant gene-wise association (Supplementary Figure 5B).

In light of recent reports on the *HLA-A*03:01* allele being associated with an increased risk of fever, chills, and stronger side effects from Pfizer-BioNTech COVID-19 vaccination,[18] we also tested if the *HLA-A*03:01* allele was a predictor of side effects in our cohort. The results confirmed this association with comparable effects (Figure 2). As in the paper of Bolze and collegues,[18] *HLA_A*03:01* allele was a particularly strong predictor of fever and chills. In particular, carriers of *HLA-A*03:01* had a 2–2.4-fold increased risk of fever, chills and myalgia, and a 1.5–1.8-fold increased risk of joint pain, nausea, fatigue, headache and asthenia, independently of age and sex (Figure 2).

4 | DISCUSSION

In this study, we investigated biological, lifestyle-associated and genetic factors that could contribute to explain the heterogeneity in terms of immune response and side effects following vaccination with the BNT162b2 vaccine in a cohort of HCWs who had not previously come into contact with the SARS-CoV-2 virus.

The analysis revealed that age and smoking habits are the main predictors of antibody response: older people and smokers have on average lower antibody titers than young people and non-smokers. The correlation with age had already emerged in previously published studies performed on a cohort comprising the one described here[22,31] and it is in line with what has been reported in several other analyses.[32–34] The correlation with smoking instead had not been previously investigated in the same cohort but has been reported in another sample of Italian HCWs.[35] In particular, Ferrara and colleagues observed a difference in vaccine-induced IgG titer between current smokers and non-smokers 60 days after the completion of the vaccination cycle.[35] In the past, other study has shown that smokers have a generally reduced production of IgA, IgG, and IgM.[36] The correlation between smoking and antibody titers has also been suggested for other vaccines, although the underlying causes of these correlations remain, at least in part, unknown.[37] Cigarette smoke may act as a double-edged sword by stimulating

autoimmunity on the one hand, while attenuating the normal defensive functions of the immune system on the other.[37] Moreover, obese and overweight subjects showed a faster decrease in the antibody levels. Obesity has been already related with poor vaccine-induced immune responses in humans[38] and it worsens outcomes from COVID-19[39] and like other states of malnutrition, it is known to impair the immune response, altering leucocyte counts as well as cell-mediated immune responses. Various plausible explanations have been proposed for the phenomenon, such as changes in adipokine secretion, fatty acid-induced inflammation, oxidative stress, ER stress, or adipose tissue hypoxia, and they are likely to act in a synergistic manner.[40] Our study also revealed that variability in antibody response is strongly influenced by genetic variants. In particular, we observed a positive association with polymorphisms at the HLA locus: the *HLA-A*03:01* allele was associated with an increased antibody titer 39 days after the completion of the vaccination cycle (T1), regardless of age and smoking habits. Factors that appear to influence antibody titers at T1 also influence antibody levels 2 months later (T2). However, although the effect of age and smoking habits remains unchanged, the effect of the *HLA-03*01* allele on the log-transformed antibody levels appears to lose strength over time (T1: beta = 0.31, SE = 0.05; T2: beta = 0.18, SE = 0.06). This could be explained by the different range of antibody levels themselves, or may be due to real reduction of the effect of genetic variation over time. However, we did not observe an effect of the genetic background on the rate of decrease/increase in antibody titers from T1 to T2.

An association between polymorphisms at the HLA locus and the immune response to SARS-CoV-2 vaccines was recently reported in a multicenter GWAS conducted on the UK population.[16] Unlike our study, however, the identified signal was associated with the *DQB1* gene, and in particular with the *DQb1*06* allele, which has been reported to increase antibody titers and reduce the risk of breakthrough infection. Currently, we do not have an explanation for this apparent discrepancy between the two studies. Certainly, the HLA is a complex locus, difficult to study using the GWAS approach; indeed, the HLA locus is one of the loci with the highest gene density in our genome: more than 200 genes in less than 3600 kb.[41] It is an extremely polymorphic region and the variants show a high linkage disequilibrium with each other, this complicates the association of specific genes or alleles with observed traits. Furthermore, the genetic diversity of the HLA locus is extremely population-specific and reflects the evolutionary history of the different populations and the different selective pressures that human populations have faced over millennia.[42] In light of this, the different results obtained in the two analyses could be also explained by the ancestral diversity of the population studied by Mentzer and colleagues[16] (largely from the United Kingdom) compared with ours (almost entirely of Italian origin). In support of this hypothesis, another study conducted on an Italian HCW cohort highlighted a significantly reduced frequency of the *HLA-A*03:01* allele in subjects with a weak antibody response to BNT162b2 vaccine.[17] These data, therefore, suggest also a population-specific effect of the *HLA-A*03:01* allele on the antibody response to BNT162b2 vaccine.

The two Italian cohorts differ from the UK one not only for a different geographical origin but also for other characteristics that could account for the different results, namely: (a) differences in vaccine type; (b) differences in the timing of antibody titer measurements and the number of doses administered; (c) the single-center nature of one study versus the multicentric approach of the other, potentially introducing center-specific confounding variables; and (d) the notable age difference between cohorts, although the inclusion of age as a covariate may minimize its impact.

While our manuscript was under review, another study was published online, which confirmed our association between the *HLA-A*03:01* allele and high antibody concentrations in the Italian population, using a different approach.[43]

Another interesting result that emerged from our study is the association of the *HLA-A*03:01* allele with an increased risk of experiencing specific side effects such as fever, chills and myalgia after vaccination with BNT162b2. In fact, the analysis showed side effects associated with the BNT162b2 vaccine to be more frequent in young and female subjects, but also in *HLA-A*03:01* carriers. While sex and age had been previously showed to correlate with side effects,[44–46] the detected association with *HLA-A*03:01* is remarkable and, to our knowledge, is the first confirmation of the data reported in the 23andme's blog (https://blog.23andme.com/articles/reaction-to-covid-vaccine) and of the result obtained by Bolze and colleagues,[18] who demonstrated that the *HLA-A*03:01* allele was associated with a 2-fold increase in the risk of self-reported severe difficulties with daily routine following vaccination. Notably, the computed ORs are very similar across the two studies.

It is difficult to speculate on the potential molecular mechanism underlying the associations found in this study with the HLA locus in absence of specific functional validation results. However, a possible explanation of the association between *HLA-A*03:01* and high antibody titers could be sought in the CD8+ T cells SARS-CoV-2-HCoV cross-reactivity conditioned by this allele. In fact, in a recent study, Buckley and colleagues

propose that subjects possessing some HLA alleles, including the *HLA-A*03:01* allele, are more likely to have SARS-CoV-2-HCoV cross-reactive CD8+ T cells if they have previously been exposed to endemic coronaviruses.[47] The subjects analyzed in this study are all healthcare workers and, according to the World Health Organization, respiratory infections are among the most common occupational infections in this category of workers. Therefore, we hypothesize that the association between the *HLA-A*03:01* allele and high antibody levels observed in our study may be attributed to the fact that the examined individuals are more likely to have been exposed to endemic coronaviruses in the past. This exposure may have triggered cross-reactive immune responses in individuals with the *HLA-A*03:01* allele, resulting in an enhanced response to the vaccine. Whether this is the real explanation is unknown, but it is certainly a hypothesis worthy of further investigations in future studies.

Finally, from the GWAS emerged also a significant association between a locus on chromosome 20 (lead SNP was rs271982) and the presence of lymphadenopathy. This locus overlaps a gene-desert region that is predicted as a weak transcription region in the stomach mucosa by ChromHMM,[48] and includes the pseudogene *PIEZO1P1* (Piezo Type Mechanosensitive Ion Channel Component 1 Pseudogene 1). It is certainly noteworthy that the functional PIEZO1 gene is involved in the lymphatic system, since it encodes a mechanically activated cation channel required for lymphatic valve formation.[49]

The study is characterized by some limitations that need to be taken into consideration. The vast majority of analyzed samples are of Italian origin, therefore we cannot assume these results to hold in the contexts of other Caucasian and non-Caucasian populations. In addition, the study was conducted on a cohort of HCWs aged between 18 and 65 years, who had not come into contact with the SARS-CoV-2 virus and received two doses of the BNT162b2 vaccine. While this contributes to the homogeneity of the analyzed population, it implicates that results cannot be necessarily extended to individuals infected with the virus prior to vaccination, individuals who have followed a different vaccination plan, or older/younger people. Moreover, the HLA alleles analyzed in this study have not been genotyped, but imputed from SNP-array genotypes. Although we cannot rule out some imputation bias, the imputation accuracy at G-group resolution has been estimated to be 97.8% for the European population.[27] As far as the correlation with side effects is concerned, it must be taken into account that data on the participants' socio-demographic and health characteristics were collected with an online questionnaire, and the possibility of self-reporting bias should also be considered. Furthermore, the questionnaires were filled, by participants, months after the administration of the 2nd dose of the vaccine, therefore it is not possible to discriminate between side effects caused by the first and by the second vaccine doses. The study, however, has the strength of including medical professionals that can accurately identify and interpret signs and symptoms, potentially reducing under- or over-reporting of specific adverse events increasing the reliability of the findings.

In conclusion, this study confirms that the heterogeneity in the immune response to the BNT162b2 vaccine and in its side effects are at least partially influenced by individual genetic variability. This information, integrated with individual biological and lifestyle-related correlates, could be of use for example in the definition of algorithms aimed at the identification of subjects in which the administration of additional vaccine doses would be particularly beneficial to maintain immunity against the virus.

AUTHOR CONTRIBUTIONS

Chiara Magri contributed to conceptualization, study design, data analysis and manuscript writing; Eleonora Marchina contributed to funding acquisition, drafting the first version of the manuscript and blood samples extraction; Emanuele Sansone and Emma Sala contributed to blood samples collection; Adamo Pio D'Adamo and Stefania Cappellani performed genotyping. Carlo Bonfanti, Arnaldo Caruso, and Luigina Terlenghi performed immunoassays and antibody measures; Giorgio Biasiotto and Isabella Zanella contributed to study design and data interpretation. Massimo Lombardo contributed to funding acquisition and blood samples collection and genotyping. Paolo Gasparini supervised genotyping; Giuseppe De Palma coordinated blood collection and storage and contribute to conceptualization. Massimo Gennarelli contributed to conceptualization, study design, funding acquisition and supervised the project. All authors revised the manuscript critically for important intellectual content, read and approved the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors confirm that there are no conflicts of interest.

DATA AVAILABILITY STATEMENT

GWAS summary statistics are available on the NHGRI-EBI Catalog of human genome-wide association studies

at https://www.ebi.ac.uk/gwas/deposition, reference numbers from GCST90256681 to GCST90256698.

ORCID

Chiara Magri https://orcid.org/0000-0001-9933-8666

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SUPPORTING INFORMATION

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