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CICLO XXXV

Coordinatore Prof. Di Virgilio Francesco

Exploiting Host Genetics and Sex differences to predict immune response after anti-SARS-CoV-2 vaccination

Settore Scientifico Disciplinare MED/03

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SUMMARY

1. INTRODUCTION

In December 2019, in the city of Wuhan, Hubei Province of China, a new virus emerged, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). This virus caused an outbreak in China and a pandemic that affected the entire world. The disease was designated COVID-19 (Coronavirus Disease 2019) by the World Health Organization (WHO) in February 2020 [1].

To combat this invisible enemy, the world's scientific society has focused studies on this new disease to better understand and treat its effects. Given the importance and urgency of obtaining this knowledge, the COVID-19 Host Genetics Initiative (HGI) was born in 2020. The human genetics community came together in this initiative to produce, share and analyze data to understand the genetic causes of COVID-19 susceptibility, severity and outcomes. These results have increased understanding of the biology of infection and disease. This has made it possible to identify individuals at high or low risk and has helped generate hypotheses for the formulation of new drugs [2].

As described on the website, HGI has set three main objectives [3]:

- Create a resource sharing system to facilitate and optimize the COVID-19 host genetics research (e.g., protocols, questionnaires);
- Coordinate the analytical activities of the different studies to identify genetic determinants that influence the susceptibility and severity of COVID-19;
- Create a platform for sharing results that is available to the entire scientific community.

This initiative was also adhered to by our research group coordinated by Prof. Donato Gemmati, with a study titled "*Extreme-genotype-comparison and extreme-clinical-phenotype-comparison in CoV-2 patients: direct candidate genes-pathways and GWAS*". This study aimed to investigate individual genetic susceptibility to COVID-19 disease infection, its progression, and sex disparity in prognosis by identified candidate genes and by doing Pharmacogenetics/Pharmacogenomics studies and epigenetic investigations [4].

Due to HGI's findings regarding how interindividual genetics affects disease outcome, it made us hypothesized that this individual genetic variability could also somehow affect the outcome of the immune response in terms of antibodies production after SARS-CoV-2 vaccination. This hypothesis led us to conduct a study in which the possible influence of specific genetic variants on the dynamics of immunoglobulin levels produced by anti-SARS-CoV-2 vaccination over time was investigated, all of which led to a publication titled "*Host genetics impact on SARS-CoV-2 vaccine-induced immunoglobulin levels and dynamics: The role of TP53, ABO, APOE, ACE2, HLA-A, and CRP genes"* [5].

The interesting results obtained from this study provided the starting point for this thesis, in which the research was continued by abandoning the time variable and testing the dynamicity of antibody levels over the entire time period between the second dose of

vaccine and the following six months. In addition, the number of subjects enrolled, candidate genes were increased and the focus on sex disparity was maintained.

1.1. SARS-CoV-2 MAIN CONSIDERATIONS: EPIDEMIOLOGY

The origin of SARS-CoV-2 is still unclear. It was first supposed to be a zoonotic disease, with origin in Wuhan's "wet" marketplaces for animal-human transmission. However, the samples collected from the market did not allow for the establishment of the precise zoonotic precursor strain. Therefore, additional research is required to determine the precise origin as well as any potential intermediate hosts [6] [7].

The SARS-CoV-2 belongs to the genus Betacoronavirus (family Coronaviridae) and is an enveloped, positive-sense, single-stranded RNA virus. The genus also comprises six human well-known coronaviruses, like Severe Acute Respiratory Syndrome (SARS-CoV), and Middle East Respiratory Syndrome (MERS-CoV), which lead to a significant number of deaths in the outbreaks of 2002 and 2012, respectively [8]. However, the SARS-CoV-2 genome was considered novel because it did not entirely match any other sequenced Coronaviridae viral genome [9].

SARS-CoV-2 spread quickly and it was responsible for a significantly larger number of deaths when compared to SARS-CoV and MERS-CoV. Until the end of 30 September 2022, a total of 614 385 693 reported SARS-CoV-2 infections and 6 522 600 related deaths in over 100 countries around the world. In particular, Italy registered a total of 22 432 803 infections and 177 054 deaths [10]. The fatality rate of COVID-19 was 1%, and between 3 to 20% of the cases required hospitalization, out of this percentual 10 to 30% were admitted to intensive care [11].

Epidemiologic studies highlighted that male sex with older age (>60 years old), host genetic variants and specific comorbidities (i.e., diabetes, hypertension, chronic lung disease, cardiovascular disease, obesity and cancer) were associated with severe cases of SARS-CoV-2 infection [12]. Specifically, the male sex experienced the worst outcomes with mortality rates above 50% than the female.

According to estimation, 30% of the infections were asymptomatic, and more than 50% of transmission happened during the pre-symptomatic stage. This made the disease very challenging to detect and contain [13]. SARS-CoV-2 induces severe respiratory illness, and the pathogenesis is characterized by diffuse alveolar damage, occasionally together with microthrombi, vascular damage, and immunological depletion. SARS-CoV-2 can spread by a variety of routes, including aerosol, surface contamination, and faecal-oral route, which can cause severe flu-like symptoms such as fever, coughing, and dyspnea [14] [8]. It was also observed that respiratory failure in severe SARS-CoV-2 sickness is linked to

hyper-inflammation, which may result from a cytokine storm syndrome [8]. According to a global meta-analysis of more than 3 million cases, males are nearly three times as likely to require intensive treatment unit (ITU) admission than females (Odd Ratio - OR, 2.84; 95% CI, 2.06-3.92) and higher odds of death (OR, 1.39; 95% CI, 1.31-1.47) [15].

SARS-CoV-2 changed over time, just like other viruses. The majority of SARS-CoV-2 genomic alterations did not affect viral function. Due to their fast appearance in populations and evidence of transmission or clinical consequences, several variations had drawn considerable attention and were regarded as variants of concern. The WHO assigned labels for significant variations based on the Greek alphabet. Each variant had many names based on the nomenclature used by various evolutionary categorization schemes [10].

In response to COVID-19's disastrous effects during the last two years, global efforts were/are being made to create herd and community immunity, which starts at the individual level and eventually scales up to the population level [8].

1.2. SARS-COV-2 MOLECULAR STRUCTURE AND PATHOGENESIS

SARS-CoV-2 is a positive-sense, single-stranded RNA (+ssRNA) enveloped virus. The genomic virus structure contains cis-acting secondary RNA structures essential for RNA synthesis, located in 5′ and 3′ untranslated regions (UTRs). The RNA virus has two large open reading frames (ORF1a and ORF1b), that occupy two-thirds of the genome. They are located at the 5′ ends that make up the replicase complex's 16 non-structural proteins (Nsps 1–16) [16].

In the SARS-CoV-2 structure, Spike (S), Membrane (M), Envelope (E), and Nucleocapsid (N), are the four primary glycoproteins. In particular, the N glycoprotein is the only structural protein inside the virion and contains the RNA genome. The primary function is to modulate RNA unwinding upon entrance into cell [17]. The S glycoprotein is exposed at the membrane, giving the virus its corona-like appearance, and plays an important role in binding to the host cell's membrane. The M and E glycoproteins, are the main structural glycoproteins in the lipid viral envelope and are responsible for the assembly, replication and release of the virus particle [18] (Figure 1A).

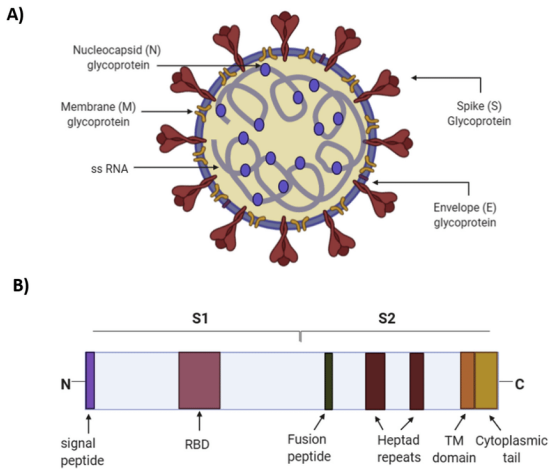


Figure 1. Structure of SARS-CoV-2 and Schematic representation of Spike (S) glycoprotein. A) The main structural glycoproteins in SARS- CoV- 2 virion. **B)** The N-termius S1 units contains the receptor binding domain (RBD) which is necessary for the virus to bind to ACE2. The functional components necessary for fusion are located in the C-terminus S2. Additionally, it also contains a transmembrane domain (TM) for membrane anchoring and a cytoplasmatic tail for proper intracellular trafficking [14].

The viral life cycle in the host cells involves several steps and starts with the entrance of the SARS-CoV-2 in human host cells using the membrane receptor Angiotensin-converting Enzyme 2 (ACE2), followed by important conformational changes required to achieve infection. Most studies showed that SARS-CoV-2 S glycoprotein binding mechanism to ACE2 is not sufficient to infect host cells; rather, it appears that the S glycoprotein has to be further processed and cleaved by the host cell's proteases at specific locations, to fuse the virus with the target cell membrane [19,20]. This is mediated by the members of type II transmembrane serine proteases (TTSP), in particular, the transmembrane protease serine 2 (TMPRSS2). Further, the S glycoprotein is cleaved in S1/S2 sites by TMPRSS2 resulting in unlocked, fusion-catalyzing forms at the cell surface that promote the faster early entrance of the virus into host cells. For instance, it was demonstrated that the kidney epithelial cell line (VeroE6) that expresses TMPRSS2 was extremely sensitive to SARS-CoV-2 infection, indicating that TMPRSS2 protease is important for viral entry into cells [18]. The S1 subunit is composed of the receptor binding domain (RBD), which is essential for attachment to host cell receptors, and it is located at the N-terminus of the S glycoprotein. While additional domains are located at the C-terminus of the S2 glycoprotein, and they are important for fusion and intracellular trafficking inside the cell (Figure 1B) [18]. Due to its importance for the virus survival and infectivity in the host cells, the S protein serves as the primary target for the creation of vaccinations, entrance inhibitors, and neutralizing antibodies [18 21].

Once inside the cytoplasm of the cell, the virus releases its RNA genome for translation to produce the viral replicase polyproteins pp1a and pp1b, which are further divided into smaller components by proteases that are also encoded by the virus. Several

sub-genomic mRNAs are continuously transcribed by the viral polymerase and translated into the structural proteins of the virus. While the S, E, and M proteins are inserted into the viral envelope at the ER and Golgi intermediate compartments, the N protein forms a complex with the genomic RNA. Exocytosis is then used to expel the freshly constructed virus particles from the infected cells [21] (Figure 2).

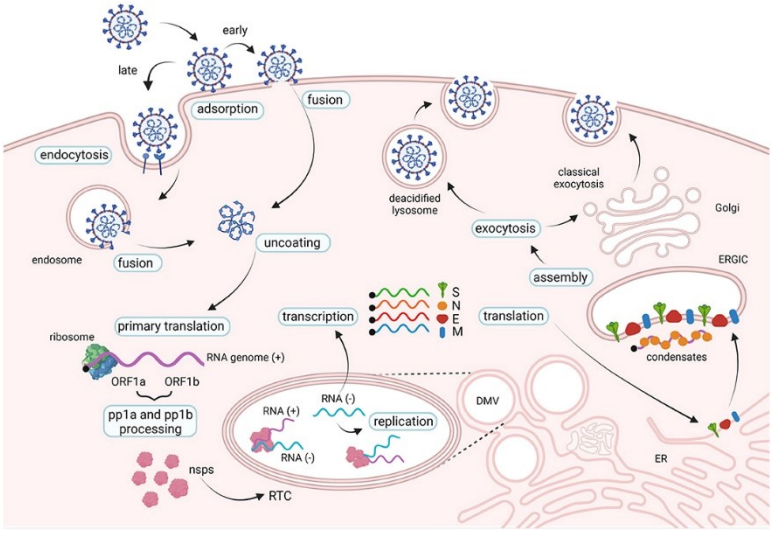


Figure 2. The SARS-CoV-2 life cycle. Angiotensin-converting enzyme 2 (ACE2) and host proteases are two cellular receptors with which SARS-CoV-2 binds to activate the spike proteins. There are two possible pathways for viral entry into the cell cytoplasm: either the viral membrane fuses with the cell membrane at the cell surface (late pathway) or the viral particle is endocytosed before fusing with the endosomal membrane (early pathway). Two large open reading frames, (ORF1a and ORF1b) are rapidly translated in polyproteins, pp1a and pp1b. These polyproteins are then processed into the distinct non-structural proteins (nsps), which make up the viral replication and transcription complex (RTC). In defense-like double-membrane vesicles, viral genomic RNA replication takes place (DMVs). The endoplasmic reticulum (ER) membrane receives structural proteins created during transcription and translation of the negative template, and transit through the ER-to-Golgi intermediate compartment (ERGIC). Here, newly synthesised genomic RNA condensates and N proteins interact with E and M proteins to form viral particles that bud into the lumen of secretory vesicular compartments. Both the traditional exocytosis pathway through the Golgi compartment and the incorporation in deacidified lysosomes that merge with the cellular surface membrane are used to release virions from the infected cell [12].

1.3. ACE2: BIOLOGICAL ROLE

ACE2 is a type I transmembrane metallocarboxypeptidase that exists both as a membrane-associated form and as a secreted form. ACE2 has an important homeostatic function regulating Renin-Angiotensin System (RAS) along with Angiotensin Converting Enzyme (ACE), which is pivotal for both the cardiovascular and immune systems [22].

ACE2 is associated with the vasoconstrictor/proliferative axis and differs from ACE, which is correlated to the vasodilator/anti-proliferative axis, in its physiological role, and is considered to be a negative regulator of RAS [23].

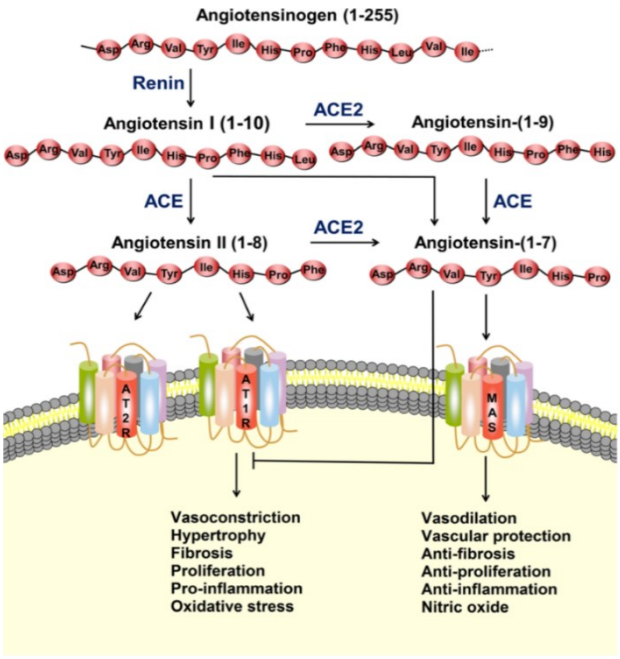


Figure 3. The interaction between the Renin-Angiotensin System (RAS) and ACE2/angiotensin-1 (1-7)/MAS axis [22].

Indeed, the RAS system affects the function of the heart, blood vessels, and kidneys, by playing an important role in controlling blood pressure as well as electrolyte and fluid balance [24]. The most typical bioactive peptide in the RAS, Angiotensin-II (Ang-II), has a significant function in the development of cardiovascular disorders such as hypertension, myocardial infarction, and heart failure because is a major vasoactive peptide in the RAS system, and acts as a potent vasoconstrictor through its receptor Angiotensin Type 1 (AT1R) [25]. In the classic RAS system, Renin cleaves the angiotensinogen substrate to produce the decapeptide Angiotensin-I (Ang-I), subsequently ACE modifies Ang-I by removing two amino acids from its carboxyl terminus to produce Ang-II [26]. Ang-II binds to AT1R promoting vasoconstriction, cell proliferation, inflammatory responses and blood coagulation, whereas Angiotensin Type 2 Receptor (AT2R) counteracts the effects of AT1R [22 27]. Conversely, ACE2 can behave in two different ways. Specifically, it produces Angiotensin (Ang)-(1-7) following Ang-II cleavage. A second traditional method involves the hydrolysis of Ang-I by ACE2 to produce Angiotensin (Ang)-(1-9), and the subsequent hydrolysis of Ang-(1-9) by ACE to produce Ang-(1-7) [24 28]. After, Ang-(1-7) interacts with the G protein-coupled receptor MAS, which has the opposite effect of Ang-II, causing vasodilation and the synthesis of anti-inflammatory chemicals. Because ACE and Ang-I have a higher affinity, the classical pathway of Ang-II to Ang-(1-7) is more common [24] (Figure 3).

1.4. ACE2 EXPRESSION

The SARS-CoV-2 infection first affects airway epithelial cells [29]. According to animal models and human transcriptome databases, ACE2 is moderately expressed in type II alveolar cells (AT2) in the lower lung, but is highly expressed in the upper bronchial epithelia, and is significantly expressed on the surface of epithelial cells, particularly ciliated cells. The difference observed in the expression of ACE2 is directly associated with the

SARS-CoV-2 infection gradient, in which nasal ciliated cells are the primary target for the virus replication in the early stage of infection [8] [30] (Figure 4).

Aside from the lungs, ACE2 is also found in cardiac cells, proximal tubule cells of the kidney, and bladder urothelial cells, and is highly expressed in the enterocytes of the small intestine, particularly in the ileum [26].

Since ACE2 is the target receptor of SARS-CoV-2, its expression is linked to the virus's organic susceptibility. There is a link between the tissue expression of ACE2 and the locations of SARS-CoV-2 infection. For instance, the lung and heart are more susceptible organs to SARS-CoV-2 infection, due to the high amounts of expression of ACE2 [31] [23].

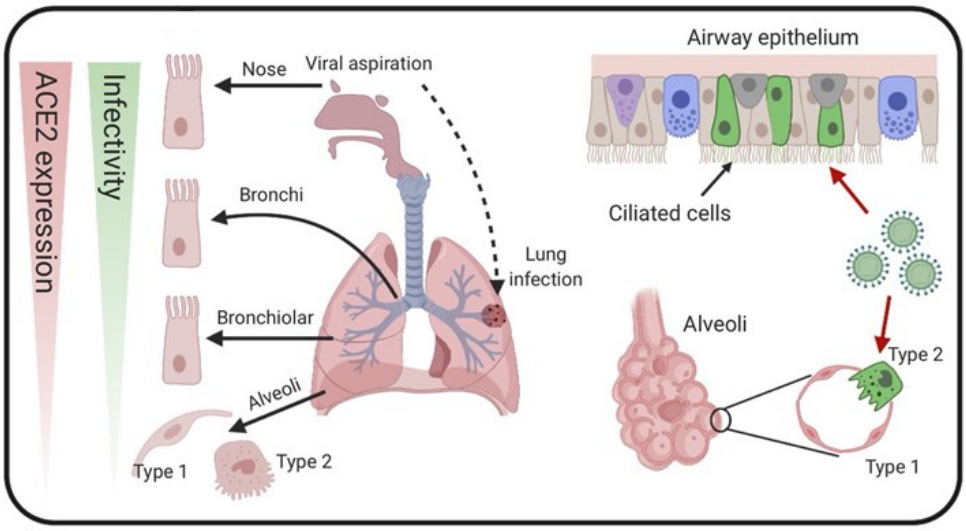


Figure 4. ACE2 expression and SARS-CoV-2 infection association [26].

Following viral entry, ACE2 is down-regulated, leading to high ACE/Ang-II activity, increased lung vascular permeability, and subsequent lung injury [32].

Since ACE2 acts as a counter-regulator of RAS, a decreased expression of ACE2 results in a weaker ACE2-Ang (1e7)-MAS axis, this is a result of an increase in Ang-II and a decrease in the amount of the vasodilator Ang-(1e7). This altered ACE/AC2 expression contributes to severe outcomes in COVID-19 patients, as it does for cardiovascular diseases.

A cohort study showed that SARS-CoV-2 infected patients had plasma levels of Ang-II that were considerably greater than those of uninfected people. In addition, in individuals with SARS-CoV-2 infection, the level of Ang II was linked with viral titre and lung injury [29]. After SARS-CoV-2 infection, the impaired ACE2-Ang (1e7)-MAS axis was associated with damages, including myocardial fibrosis, increase in ROS production, inflammation, and cardiovascular disease [23]. Due to Ang-II's activity on its AT1 receptors promotes NAD(P)H oxidase upregulation, oxidative stress, and cytokine production.

The combination of cytokine storm, ROS, and inflammation leads to vascular permeability, diffuse alveolar damage, pulmonary oedema, and, eventually, Acute Respiratory Distress Syndrome (ARDS), which can results in death [33].

1.5. IMMUNE RESPONSE

The immune system is divided into two types, innate and adaptative, they are responsible to respond to diverse diseases, including viral infections. A range of different cells is part of the innate immune system, such as Dendritic cells (DCs), monocytes, macrophages, neutrophils, and Natural Killers (NKs). Those cells express pathogen-recognition receptors (PRRs) that can detect pathogen-associated molecular patterns (PAMP), including C-type lectin receptors, NOD-like receptors (NLRs), RIG-I-like receptors (RLRs) and Toll-like receptors (TLRs). The coronaviruses are recognised by cytosolic and endosomal RNA receptors, such as RIG-I and TLRs (TLR2, TLR3 and TLR7). Downstream activation by TLRs and RIG-1 leads to stimulation of the nuclear factor (NF-kB) interferon regulatory factor 3 (IRF3), resulting in the expression of pro-inflammatory cytokines and chemokines [34].

Meanwhile, the adaptative immune response is mediated by T cells ($CD4^+$ and $CD8^+$) and B cells. T cells are associated with cellular immunity upon activation by antigen-presenting cells (APCs), differentiation, and proliferation. In general, there are three types of T cells: cytotoxic ($CD8^+$), helper ($CD4^+$), and regulatory (T reg). The $CD8^+$ T cells interact with the Major Histocompatibility Complex I (MHC I) producing cytotoxic granules rich in granzyme B into the target cell, leading to cell death. While the $CD4^+$ T cells are subdivided into the Th1, Th2, Th17, and TfH; they interact with the Major Histocompatibility Complex II (MHC II) and are well known for several cytokines production, such as IL(Interleukin)-2, IL-6, IL-8, IL-12, Tumor Necrosis Factor- Alpha (TNF-α), and Interferon-gamma (IFN-γ); They have different roles, including as APCs, activation of B and multiple innate cells. The B cells are part of humoral immunity, producing immunoglobulin (Ig) and neutralizing antibodies (NAbs) [34] [35]. The B cells produce 5 Igs: IgG, IgA, IgE, IgM and IgD; IgG is the principal isotype in the blood and extracellular fluid and efficiently opsonizes pathogens for engulfment by phagocytes and activates the complement system. An essential and specific defence against viral invaders is provided by NAbs. In general, the NAbs might block the interaction between virus particles and receptors or bind to a viral capsid to inhibit the spread of the virus. After infection, it can take some time for the host to produce highly effective NAbs but these persist to protect against future encounters with the agent. After a first encounter with the antigen by vaccination or natural infection, immunological memory

allows for more rapid production of neutralizing antibodies following the next exposure to the virus [36] [37].

1.6. CLINICAL MANIFESTATIONS

SARS-CoV-2 is transmitted through respiratory droplets and aerosols and was demonstrated that it can survive for up to 3 hours in aerosolized form, around 24 hours on cardboard, and for 3 days or more on plastic or stainless steel. Most individuals may exhibit symptoms within 11.5 days of exposure, and the median incubation time is 4-5 days.

The infection of SARS-CoV-2 results in severe flu-like symptoms. The majority of patients present with mild to moderate respiratory illness, including coughing, fever, headache, myalgia, and diarrhoea. Approximately one week following the onset of symptoms, severe sickness generally starts [38] (Figure 5).

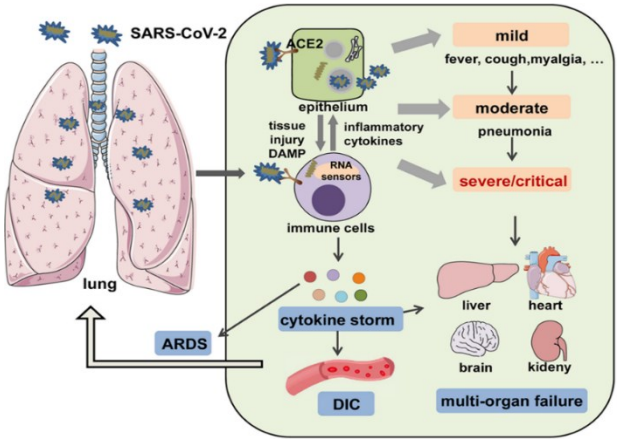


Figure 5. COVID-19 systemic clinical manifestations. By binding to ACE2 receptors, SARS-CoV-2 infects airway epithelial cells or immune cells, resulting in tissue damage, the release of Damage-Associated Molecular Patterns (DAMPs), and the production of inflammatory cytokines.
Then, the crosstalk between immune and epithelial cells results in a variety of clinical manifestations, ranging from mild forms (such as fever, cough, and myalgia) to moderate forms (such as pneumonia and localized inflammation) requiring hospitalization, to severe/critical forms with a fatal outcome [40].

The most common symptom of severe disease is dyspnoea (shortness of breath), which is a result of hypoxaemia. In individuals with severe COVID-19, dyspnoea and hypoxia might appear quickly followed by ARDS, pneumonia, disseminated intravascular coagulation (DIC), renal failure, low blood pressure, and multiorgan failure with consequent death. Specifically, ARDS is a form of lung injury that is characterized by an uncontrolled

immune response, pulmonary vascular leakage and consequently a loss of aerated lung tissue [31 39 40 41].

Several studies have shown that COVID-19 severe symptoms are due to a strong immune response. In general, following virus infection, the innate immune response will produce high levels of type I IFN. The type I IFN will induce an anti-viral state in the cell and the adjacent cells, stimulates the antigen-presenting and also the high-affinity antigen-specific T and B cell responses [42 43]. However, when the viral load stimulates a greater amount of ROS from infected epithelial cells, a hyperinflammatory immune response takes place. Once, ROS stimulates the synthesis of NLRP3 and nuclear factor (NF-κB), will contribute to the development of the cytokine storm.

In COVID-19 patients extremely high levels of pro-inflammatory cytokines like IL-1, IL-6, IL-8, IL-12, and Tumor Necrosis Factor- Alpha (TNF-α), are produced uncontrollably. This happens during the crosstalk between epithelial cells and immune cells, as well as elevated levels of inflammatory markers like D-dimer, ferritin, and C- reactive protein (CRP) [44]**.** Additionally, it was observed that some chemokines were highly expressed in COVID-19, such as CCL2, CCL3, CCL5, and IP-143-10, being crucial in determining host mortality during SARS-CoV-2 infection [45].

This initial activation of inflammatory cytokines and chemokines with subsequent recruitment of monocytes, macrophages, neutrophils, DCs, NK cells and activation of T and B cells from peripheral tissues results in the production of sustained inflammatory cytokines like IL-2, IFN-γ, and TNF- α, generating a positive feedback loop that drives the elevation and continuation of the pathological inflammation, observed in COVID-19 severe cases. Thus causing myelopoiesis and emergency granulopoiesis and worsening lung and epithelial damage. Moreover, excessive levels of systemic cytokines such as IL-2, IFN-γ, GM-CSF, and TNF- α, lead to macrophage activation and erythro-phagocytosis, which eventually causes anaemia that will affect the coagulation and vascular haemostasis, generating capillary leak syndrome, thrombosis and DIC. Combined, these incidents cause ARDS, multiorgan failure, and death [44].

1.7. SARS-COV-2 VACCINES

The virus increased infectivity ratio and dissemination mobilized researchers all around the world in the search for effective protection against the virus. In December 2020 the Food and Drug Administration (FDA) authorized the first vaccines as prophylactic measures for the SARS-CoV-2 virus and consequently COVID-19 [42]. In general, vaccines mainly act by simulating a natural infection, thereby promoting the development of a humoral and cellular immune response aimed at defending the host against a specific

pathogen. Compared to previous vaccinations, the anti-SARS-CoV-2 vaccines were produced in a much less time [46].

Most of the vaccines developed focused on the S glycoprotein due to its greater number of neutralizing epitopes in the RBD domain. Mainly because is the only surface protein readily accessible by antibodies and immunological cells in the body [47].

Before administration in Europe, all vaccines were previously authorised by the European Medicines Agency (EMA), and in Italy also for the Italian Medicines Agency (AIFA). Until October 2022, about 6 billion of the worldwide population received at least one dose of the vaccine, from this 75.2% were located in Europe, and more specifically in Italy, a total of 86% of the population were vaccinated (Figure 6) [48]. It has been established that the global COVID-19 vaccination programs reduced the risk of developing symptomatic and/or severe illness in the short-term [49].

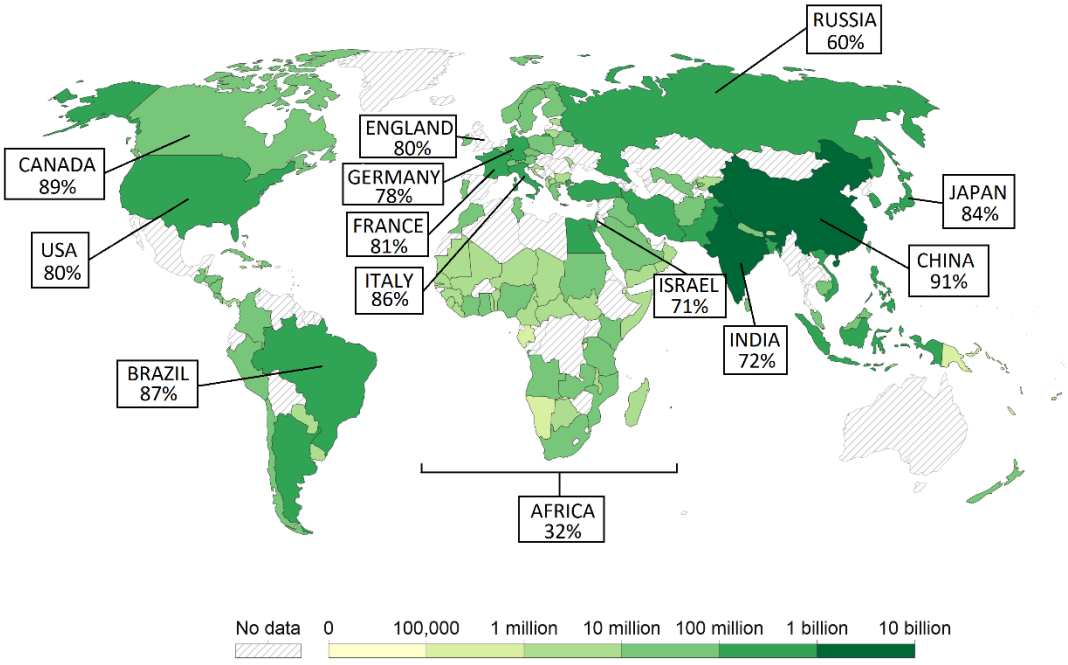


Figure 6. Global coverage of COVID-19 vaccination campaigns [44].

Different technologies were applied in the development of the SARS-CoV-2 vaccines, such as:

- Messenger RNA (mRNA) vaccine
- Adenoviral vector (AdV) vaccine
- Inactivated virus vaccine
- Protein subunits vaccine

Of these vaccine types, the first ones produced and approved by regulations were the mRNA and the Viral Vector Vaccines. After, other vaccine technologies were applied and distributed most recently around the world. Vaccine types, doses, advantages, and disadvantages are summarized in Table 1 [50] [51].

Like the clinical reaction to SARS-CoV-2, the immunological response following vaccination appears to be characterized by high inter-individual variation, it is responsible for the different antibody responses to vaccination. Recent studies have confirmed that there are host-specific factors associated with the efficacy of the anti-SARS-CoV-2 vaccines but are not yet clearly outlined. They may include the factors that affect immune system functionality, such as host genetics, age, sex, psychosocial factors, lifestyles, drugs, and clinical history, as well as a basal inflammatory state [52] [53] [54].

Table 1. General information about the different vaccine types [46] [47].

Vaccine techonologies	Type	Protection	Approval	Age groups	Dosage	Advantages	Disadvantages
mRNA	Pfizer/BioNTech	95.6% (3 doses)	WHO, FDA, EMA	5 years and up	2 doses (21 days apat)	- High adaptibility to control immunogenicity - Immunogenicity is high - Long-term immunity	- In-vivo stability
	Moderna	93%	WHO, FDA, EMA	Adults (FDA) 6 years and up (EMA)	2 doses (28 days apart)		
Vector	Oxford/Astrazeneca	76%	WHO, EMA	Adults	2 doses (8- 12 weeks apart)	- Unwanted antigens can be removed - No adiuvant are required - High efficiency in stimulating immune response	- The existance of pre-existing host immunity reduces efficacy - Expensive
	Johson & Johson	66%	WHO, FDA, EMA	Adults	1 dose		
Inactivated	Coronavac/ Sinovac	51%	WHO	Adults (WHO) 3 years and up (other)	2 doses (14 days apart)	- Good safety profile - Better transportation and storage stability - Long shelf life	- Immunogenicity deficit - Protection lasts less time - Adjuvants are required - A higher dose is required
	Covaxin	77.8%	WHO	Adults	2 doses (28 days apart)		
	Sinopharm	79%	WHO	Adults (WHO) 3 years and up (oyher)	2 doses (21 days apart)		
Subunit	Novavax	90.4%	WHO, EMA	Adults	2 doses (3-4 weeks apart)	- Fewer adverse effects	- Immunogenicity is limited - Adjuvants are required

1.7.1. MESSENGER RNA (mRNA) VACCINE

The mRNA-based vaccines, developed by Pfizer-BioNTech (BNT162b2) and Moderna (mRNA-1273) were the most innovative manufacturing method. This technology uses a single-stranded RNA molecule that codifies for a small part of the S glycoprotein, encapsulated within a lipid nanoparticle (LNP). This nanoparticle is injected intramuscularly into the human body and once inside the cell, the ribosomes translate the information and synthesise it, to then deliver it to the cell's surface where the immune cells can recognise the antigen. This will trigger the immune response, especially the adaptative immune cells, such as T and further B cells for the production of antibodies, such as IgG and Nabs that can recognize the antigen and this will induce further immunological memory [55].

The Pfizer-BioNTech and Moderna mRNA vaccination series is comprised of two doses given at intervals of three weeks for Pfizer-BioNTech and one month for Moderna.

A robust humoral response to mRNA vaccines has been described in several types of research. These vaccinations have demonstrated effectiveness against the virus variants [56] [55].

Two doses of these vaccines administrate in individuals 16 years old or more, were demonstrated to provide more than 94.1% of protection against COVID-19. Aside from brief topical and systemic reactions, no safety problems were noticed [57] [58].

1.7.2. ADENOVIRAL VECTOR VACCINE

Current Adenoviral vector (AdV)-based vaccines against SARS-CoV-2 are AZD1222 (ChAdOx1 nCoV-19) by Oxford-Astrazeneca, and Ad.26.COV2.S/JNJ- 78436725 by Janssen Pharmaceuticals (Johnson & Johnson, USA). The two vaccines' mechanisms of action are comparable. Specifically, genetic material from the SARS-CoV-2 is inserted into a modified strain of a different virus (usually an adenovirus that is unable to replicate) to stimulate an immunological response only aimed at the viral S glycoprotein encoded in the host DNA and not at the adenovirus itself. When the viral vector vaccine is injected into a host it enters the cells, and the DNA is released to the cytoplasm and then migrates to the nucleus. The host enzymes convert the DNA into mRNA, which migrates back into the cytoplasm where it interacts with the ribosomes to translate viral proteins.

The viral proteins start to be expressed on the surface of the cell, allowing the immune cells to identify it. The cells undergo differentiation and activation, and eventually activate B cells, producing IgG and NAbs to counteract the virus in case of infection[46].

These vaccines are administrated to people aged 18 years old or older. The Ad.26.COV2.S vaccination just needs one dose, but the AZD1222 vaccine requires two doses given 28 days apart. The AZD1222 was shown to be 59.5% effective in the prevention of the symptoms of the disease. While the effectiveness of AZD1222 in avoiding symptomatic illness was 58.3%, quite comparable with Ad.26.COV2.S. In participants who received the second dose of AZD1222 12 weeks after the first, the efficacy 14 days after the second dose was higher, coming to 82.4%. While for Ad.26.COV2.S 66.1% of the effectiveness was shown 28 days after immunisation [46] [58]. In general, both vaccinations showed a significant decrease in COVID-19 cases with time [58].

1.8. COVID-19 RISK FACTORS

It is still unclear why some people develop severe COVID-19 symptoms and some do not. Like for other diseases is believed that age, underlying medical conditions, and environmental factors including socio-economic determinants of health are risk factors that contribute to this observed difference in the symptomology [59].

In general, the disease affects people over 60 years old and those with comorbidity conditions. According to the systemic analysis of COVID-19 effects, individuals with a history of high blood pressure (17%), hyperglycemia (8%), and heart disease (5%) are more prone to develop the severe illness than those patients without any history of chronic medical conditions; hence have serious/life-threatening COVID-19[60] [61].

Ages 50 to 64 have a 4-fold rise in mortality risk compared to those under 40, while those 85 or more a tenfold increase. Similar to this, individuals with one comorbidity or more than 10 comorbidities had a 1.5 and 3.8 times greater chance of dying than those without any underlying diseases, respectively [61].

The systemic inflammation caused by SARS-CoV-2 infection leads to an increase in blood viscosity, endothelial dysfunction, activation of the coagulation cascade and atherosclerotic plaque rupture [62].

1.9. GENETIC VARIANTS

Several studies demonstrated that human genome variants can affect the disease outcome.

A group of 427 members of the COVID-19 Host Genetics Initiative, after a large human genetic study of SARAS-CoV-2 infection, identified 13 locations (or loci) in the human genome that affect COVID-19 susceptibility and severity [63]. The genetic risk factors vary from rare to high-impact mutations, which can contribute to the differences seen between mild symptoms and life-threatening illness, to more-common genetic variants that only moderately affect symptom severity [59] [2].

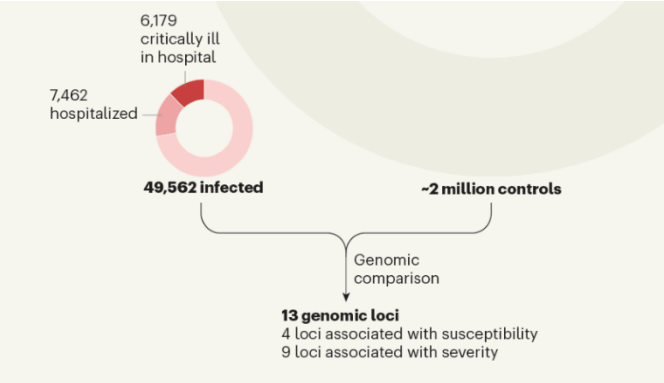


Figure 7. Identifying regions of the human genome associated with COVID-19 susceptibility and severity. The COVID-19 Host Genetic Initiative looked for genetic variations that could explain the variation in each individuals susceptibility to COVID-19 as well as the severity of the illness [59].

Additionally, in early 2020 in Spain and Italy, a Genome Wild Association Study (GWAS) was performed to determine whether common variants drive susceptibility to severe COVID-19. Both studies identified two genomic regions associated with respiratory failure in the disease: one region is located on chromosome 3 (3p21.31) which arises from Neanderthal DNA, and the other one is on chromosome 9 (9q34.2) coincident with the ABO blood group [64].

Alongside these genomic regions, other genes and single nucleotide polymorphisms (SNPs) not located in these chromosomes were ascribed to affect the outcome of the disease and also the immune response, such as *ACE* (rs1799752), *ACE2* (rs2285666), *APOE* (rs7412/rs429358), *CFH* (rs1061170), *CRP* (rs876538/rs2808635), *HLA-A* (rs2571381/rs2499), *OAS1/OAS2/OAS3* (rs10735079), and *TP53* (rs1042522).

1.9.1. *ACE* GENE

The *ACE* gene is located on chromosome 17 (locus 17q23.3). It is 21 kb full-length, consists of 26 exons and 25 introns, and codes for a gene product with 1306 amino acids. This gene includes numerous intragenic variants: most of them are SNPs, a small number of which are found in the coding region, and a few of them are missense mutations [12]. Among the several gene variants, the most investigated is an insertion/deletion (I/D) of 287 bp in the Alu-elements of intron 16, represented by four individual SNPs (rs4646994, rs1799752, rs4340 and rs13447447) that modulate *ACE* expression. Alu elements are DNA repeat sequences known as Short Interspersed elements (SINE). *ACE* with Alu- elements insertion (I) is believed to cause alternative splicing responsible for protein shortening and the loss of one of the two enzyme active sites. While the counterpart whit Alu- elements deletion (D), still maintains the two active sites favouring Ang-I to Ang-II formation [65]. Fuch et al. and Agerholm et al., demonstrated that *ACE* I activity is lower than *ACE* D [66] [67].

It is reported that the D/D genotype occurs in 55% of the population and is associated with the highest serum/tissue ACE activity, the I/D genotype shows intermediate, and the I/I genotype has the lowest ACE levels [12] [68]. The presence of the D allele was linked with disease pathologies associated with RAS activity, such as hypertension, coronary artery disease, stroke, and nephropathies [69] [68].

There is a correlation between *ACE* I/D genotype in SARS-CoV-2. A poor clinical outcome of ARDS is addressed to the D/D genotype, the worst prognosis, with higher mortality rates than the I/I genotype [70]. *ACE* I/D genotype distribution in the worldwide population is different. It is observed that the *ACE* D/D rate reduces as one moves geographically from Europe to Asia, with the European population having a higher *ACE* D/D genotype [12].

1.9.2. *ACE2* GENE

In combination with *ACE*, functional variations in the *ACE2* gene were extensively researched during the COVID-19 period. Several studies reported that functional *ACE/ACE2* gene polymorphisms have been associated with the risk of cardiovascular and pulmonary diseases, but also with acute lung injury, and infectivity making COVID-19 patients substantially more likely to develop ARDS [71] [72]. Moreover, ACE2 has been associated with hypertension, heart failure, and diabetes.

The *ACE2* gene is located on chromosome X (locus Xp22.22) and is recognized as an escape X inactivation gene. Contains 18 exons, accounting for a 41.04 kb full-length, and codes for a gene product of 805 amino acids. Among several SNPs that influence *ACE2* activity levels, the transition G8790A (c.439+4 G>A; rs2285666) at nucleotide +4 of intron 3 of the *ACE2* gene, received special attention, which is regarded as a risk factor for hypertension and heart failure [70] [12].

Different reports showed that this SNP is related to acute lung injury, making COVID-19 patients significantly prone to develop severe and critical outcomes. In particular, the G/- (male hemizygote) or G/G (female homozygote) genotype is responsible for a lower expression of the ACE2 receptor, almost 50% lower compared to the A/- or A/A genotype, while heterozygous G/A individuals had an intermediate expression [12] [73]. Martínez Gómez L. E. et al, attributed to A-allele to a risk factor for severe and critical outcomes (oxygen requirement) in COVID-19, especially in men [71]. Concerning the allele frequency, Cao and colleagues, was observed that the frequency of rare A-allele beings 0.2 in Italians and Europeans, and 0.55 in East Asians (uncorrected $p=2.2*10^{-16}$ for difference in Italians vs East Asians; corrected $p=7.9*10^{-15}$). In addition, the homozygous polymorphic rate in males (0.550) was much higher than females (0.310) in the Chinese population. Taken together, the differences in the frequency of ACE2 variants among different populations suggested that the diverse genetic basis might affect ACE2 functions among populations [74].

Regarding the risk in the sex differences, Gemmati et al. reported that an unbalanced ratio of ACE/ACE2 might show significant differences in the outcomes of COVID-19 in both sexes. Indeed, this unbalance could induce a higher inflammatory mediators/ receptors expression; hence, men might show a worse clinical scenario than women, as women could activate a mosaic advantage due to their X-heterozygosity [75].

1.9.3. NEANDERTHAL SNPs

H. Zeberg and S. Pääbo in 2020 identified a genomic segment of around 50 kb in size located in chromosome 3p.21.31, inherited from Neanderthals. More specifically, the

Neanderthal haplotype (chr3: 45,859,651 - 45,909,024 (hg19)) contains several genetic variants located on Leucine Zipper Transcription Factor Like 1 (LZTFL1) gene.

These genetic variants are all high in linkage disequilibrium (LD) and constitute a specific haplotype. In general, this region is present in 16% of the European population and 65% of the South Asia population, whereas it is almost absent in East Asia. Mostly importantly, it is strongly associated with severe COVID-19 infection and hospitalization [64] [76] .

The *LZTFL1* gene is located on chromosome 3 (locus 3p21.31) and consists of 16 exons. This gene encodes for a protein that is highly expressed in lung cells. It is responsible for regulating airway cilia and the epithelial-mesenchymal transition, a developmental process critical for the innate immune and inflammatory response [77].

The gene variants associated with disease severity, conferring risk for severe/ critical clinical outcomes are rs35044562 and rs11385942. They are located in the intron region and were described previously to impair the physiological immune process, therefore affecting the host's ability to respond properly to the infection.

In patients with severe COVID-19, the rs35044562 genetic variant (A>G), the presence of G-allele, is related to an approximately 70% increased risk of hospitalisation.

Meanwhile, the insertion/deletion of a single nucleotide G>GA (rs11385942) is the second genetic variation linked to COVID-19 severity and was associated with a higher risk of developing COVID-19 with respiratory failure. The patients that have the insertion of A nucleotide (-/A), are characterized by an increased risk of developed respiratory failure of 1.7 greater risks, compared to those who are A/A that have the risk of 3 times [64].

Additionally, Pairo-Castiniera E. et al, were performed a Genetics of Mortality in Critical Care (GenOMICC) study including 2,244 critical patients whit COVID-19 illness. They identified on chromosome 12 (12q24.13) a genetic variant (G>A; rs10735079), associating to A- allele with the major risk for developing the severe form of COVID-19 (OR 1.3; 95% confidence interval 1.18- 1.42) [78].

Using this data Zeberg H. and Paabo S., showed that a haplotype of around 75 kb on chromosome 12 (chr12: 113,350,796 – 113,425,679 (hg19)) which includes the rs10735079, was inherited from Neanderthal. The frequency of this haplotype is between 25 and 30% in the majority of populations in Eurasia, while it is virtually entirely absent in the African population. In the Americas, in particular in some population of African ancestry, this haplotype occurs in lower frequency due to gene flow from populations of European or Native American ancestry. When exposed to SARS-CoV-2, this haplotype was linked to a 22% relative risk decrease for COVID-19-related severe illness [79].

The variant rs10735079 is located in the intron 2 of the OAS3 gene in the interferon-inducible oligoadenylate synthetase gene cluster (OAS1, OAS2 and OAS3), and is commonly widespread outside of Africa. These genes encode enzymes that after

stimulation by interferons, produce a host antiviral mediator (2',5'- oligoadenylate (2- 5A)). This last one, subsequently, activates ribonuclease L (RNase L) degrades intracellular double-stranded RNA and activates other antiviral mechanisms in cells infected by viruses [79].

1.9.4. *ABO* LOCUS

The gene responsible for determining the ABO blood group is located on chromosome 9 (locus 9q34.1), contains 7 exons, and is associated with the synthesis of specific ABO glycosyltransferase. This gene contains more SNPs, including a one nucleotide deletion in exon 6 (codon 87) which determines the O allele (rs8176719), and four common SNPs responsible to switch enzyme function from A transferase to B transferase activity [80].

Based on GWAS, the authors found that a specific genetic variant (G> T; rs657152) located in the ABO's intron region, is in LD with rs8176719 (the main determinant of group O), but also established that T-allele is strongly associated with COVID-19 severity [64].

Before being discovered to be a risk factor for COVID-19 disease, the T-allele was associated with hypercoagulability, arterial embolism/thrombosis, and other circulatory system issues [64 81]. There is a link between the ABO locus and the gene expression of the clotting von Willebrand factor (vWF), involved in thrombosis and coagulation abnormalities. VWF function in haemostasis is twofold: VWF regulates not only the platelet adherence to the subendothelium of blood vessels but also binds plasma coagulation factor VIII (FVIII), shielding FVIII from proteolytic degradation. High levels of the vWF-FVIII complex are well-known prothrombotic risk factors because have an intrinsic procoagulant property, and because influenced the natural anticoagulant effects [12 82]. It was discovered that pulmonary endothelial cells of non-O blood type individuals have higher VWF plasma levels compared to O-group, with an increased risk for venous thromboembolism, and this is why GWAS has ascribed at ABO-locus the role in COVID-19 [12].

Several studies and meta-analyses confirmed that among individuals with non O-group, COVID-19 is more likely to affect people with blood group A than other blood groups [83 84]. Moreover, the protection of the O-group is may be attributed to the presence of anti-A antibodies that could interfere with the interaction between SARS-CoV-2 and its receptor [85].

The findings are controversial when the research is expanded to include the connections between the ABO blood group and anti-SARS-CoV-2 antibodies produced after infection and/or vaccination. For example, Dutra F. et al. found lower antibodies levels among O- and B- blood groups compared to A- and AB- blood groups. Conversely, Bloch

E.M. et al. found higher NAbs levels in B blood group subjects when compared to the remaining blood groups [86] [87].

1.9.5. *TP53* GENE

The *TP53* gene maps on chromosome 17 (locus 17p13.1), count 11 exons, and is codified for a tumour suppressor protein p53, which is among the most studied of molecules. When DNA damage or other cellular insults are present, the transcription factor p53, also known as the "guardian of the genome," activates the apoptosis pathway and arrests the cell cycle, maintaining cellular integrity [88].

Moreover, several studies showed that in addition to "classic" p53 inducers, its function can be also promoted by hypoxia, nutrient deprivation and viral infection. In fact, p53 is also a direct transcriptional target of type I interferons (IFNs) upon viral infection. Resulting in the activation of virus-induced apoptosis, therefore dampening the ability of a wide range of viruses to replicate and spread. These findings indicate that p53 may play a key role in antiviral innate immunity [89].

In general, the expression of type I- IFN and other proinflammatory cytokines is regulated by the transcription factor NF-kb (nuclear factor- kb) which is considered as a major factor in the proinflammatory signalling pathway [90].

In fact, was demonstrated that coronaviruses, such as SARS-CoV-2, can inhibit the type I- IFN response, and at the same time alternate the signal pathways of NF-kB, activating the expression of inflammatory genes, after virus infection [90] [91]. This condition promotes the inflammatory comorbidity of COVID-19. Therefore, it is possible to hypothesize that NF-kB is a key component of the stress response to SARS-CoV-2 replication in the host cell [84] [91].

One of the most studied polymorphisms in the exon 4 of the TP53 gene, the transition Pro72Arg (P72R; CCC> CGC; C>G, rs1042522) alters the biological functions of the p53 [75]. Interesting, the R72variant and P72 variant allele frequencies differ between ethnic cultures and geographical regions of earth. Indeed, whereas the P72 allele is more frequent in the population near the equator, the frequency of R72 allele increase linearly with latitude.

According to several studies, probably this difference is due to the P72 variant ha better response to innate immunity. This may be explained why it is more common in regions near the equator where infections (e.g. malaria) are more widespread [88].

For instance, Azzam, G.A. et al., found in a study of knock-in humanized p53 mice, encoding either the P72 or R72 variants, showed a significant alteration of the NFkB-

dependent apoptosis levels in the tissue of P71 or R72 mice, with a significantly enhanced response to inflammation in P72 mice than R72 mice [90].

In other studies, the P72 and R72 alleles also show a dramatically different innate immune response against SARS-CoV-2 infection. Interestingly, the P72 variant is less susceptible to viral infection and responsible for a strong pro-inflammatory immune response compared to the R72 variant. As a result, the mortality rate varies, rising at higher latitudes and falling towards or close to the equator. There are these differences because NF-kb target genes are better activated by the P72 variant [92 88 90].

1.9.6. *CFH* GENE

The complement factor H gene (CFH) is a member of the Regulator of Complement Activation (RCA) gene cluster, is located on chromosome 1 (locus 1q31.3) and consists of 23 exons. This gene codified for a protein that represented a main inhibitor of the alternative complement cascade on host cells, and its efficacy depends on its capacity to bind to cell surfaces [93].

The CFH protein is mainly synthesized by the liver, and secreted into the bloodstream in extremely varied quantities, from 116 to 711g/ml. These variable concentrations appear to be the result of both genetic and environmental influences. Indeed, plasma CFH levels rise with ageing while falling in smokers. It has been calculated that genetic differences account for about 60% of the variation in CFH plasma [93 94].

Its principal genetic polymorphism, the transition Tyr402His (Y402H; TAT> CAT; T>C; rs1061170) causes the hyperactivation of the alternative complement pathway. This SNP, as a result, leads to the reduction or complete impairment of the protective function of factor H, which then suppresses the activation of the alternative complement pathway both in plasma and the inflamed tissue, resulting in cell damage.

Moreover, immunohistochemical studies showed that homozygous polymorphic CC-genotype have a 2.5-fold higher immunological response to C-reactive protein (CRP) [95].

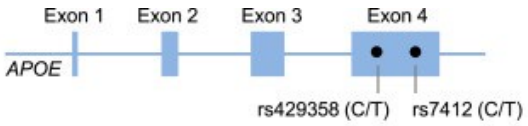
Several studies demonstrated the implication of this SNP in age-related macular degeneration (AMD). The C-allele in AMD patients reduces the activity of CFH, thus promoting its uncontrolled activation and leading to the development of chronic local inflammation with consequent macular lesions [95 96 97] .

1.9.7. *APOE* HAPLOTYPE

The Apolipoprotein E (APOE) gene is located on chromosome (locus 19q13.32), counts 4 exons, and is the major recognised gene associated with AD. It is responsible to codified for the Apo-lipoprotein E which is a multifunctional protein with plays a vital role in cholesterol transport in the brain, and it is responsible for the principal regulator of plasma lipid levels [98] [99].

There are two common SNPs located on exon 4 of the APOE gene: rs429358 (c. Cys (C)112Arg (A); n. 388 T> C) and rs7412 (c. Arg (R)158Cys (C); n. 562 C>T). Three haplotypes emerge (ε2(388 T-526 T), ε3(388 T-526C), ε4(388C-526C)) translating to three protein isoforms E2, E3 and E4, and six combination variants (ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε3, ε3/ε4, ε4/ε4) can be found (Figure 8) [98] [100]. In particular, whilst ε4 is associated with increased risk for AD, ε2 is associated with reduced risk for AD (protective role) and ε3 can be considered neutral [99].

The most prevalent APOE haplotype is ε3 which has a global average frequency of ~ 78%; the average frequency of APOE ε4 is ~ 14%, and the frequency of APOE ε2 is the lowest in all APOE-allele (worldwide average frequency is ~ 8%) [101].



Allele	ε2	ε3	ε4
Haplotype	rs429358 (T) rs7412 (T)	rs429358 (T) rs7412 (C)	rs429358 (C) rs7412 (C)
Aminoacid combination	Cys 112 Cys 158	Cys 112 Arg 158	Arg 112 Arg 158

Figure 8. Schematic representation of the human APOE gene whit the positions of SNPs rs7412 and rs429358 in exon 4. Three haplotypes (ε2, ε3, and ε4) arise for each allele resulting in different amino acid combinations at residues 112 and 158 [96].

In particular, ε4/ε4 has received the most attention due to indisputable epidemiological and experimental evidence that links it to several human pathologies [100]. The APOE ε4 variant is a genetic risk factor not only for Alzheimer's disease but also for cardiovascular disease type 2 diabetes and brain vascular pathologies, which are comorbidities related to SARS-CoV-2 severity [99] [102] [103]. It has also been found an association of APOE to infection response whit the APOE ε4/ε4 genotype that increases the susceptibility to HIV-1 infection and aggravated disease course of AIDS [104].

In the age of COVID-19, there are conducted several studies found a positive association between APOE ε4 and COVID-19 to predict severe disease [105] [106]. Interestingly, a recent study by Lord J. S. et al., demonstrated that the ε4-allele and ε4 haplotype increase the risk of severe COVID-19 by 5 and 17 times, respectively [98]. In addition, APOE was shown to modulate important innate immune responses key to inflammation. It has also been demonstrated that APOE modulates important innate immune responses that are crucial to inflammation. For example, pro-inflammatory cytokines levels including IL-6, IL-1b, IL-3, IFN-g and TNFa are higher in the plasma of human patients carrying the APOE ε4 haplotype. Another in vitro study showed that neurons and astrocytes expressing the ε4-allele were more susceptible to SARS-CoV-2 infection than those expressing the ε3-allele [105]. These results indicate that APOE modulates COVID-19 disease severity and that COVID-19 patients with the ε4 allele exhibit more inflammation associated with severe outcomes [101] [107].

1.9.8. *CRP* HAPLOTYPE

The C- Reactive Protein (CRP) gene lies on chromosome 1 (locus 1q23.2) and counts 2 exons and 1 intron. It is an acute inflammatory protein marker that is expresses at higher levels in infection and inflammatory conditions such as cardiovascular disease [108]. During inflammatory disorders, CRP plasma concentrations dramatically increase up to 1,000-fold [109]. It is synthesized by the liver, and also to a lesser degree by smooth muscle cells, macrophages, endothelial cells, lymphocytes, and adipocytes in response to IL-6. In fact, during the acute phase of inflammation, IL-6 is the only known cytokine to induce the hepatic synthesis of CRP [110].

CRP plasma levels are also correlated with COVID-19 progression [111]. Several studies discovered that the progression of non-severe to severe disease displayed an elevated level of CRP. The CRP levels are different between patients who progressed to severe infection and those who remained on a non-severe form were 43.8 mg/mL and 12.1 mg/mL, respectively [112]. Another study revealed that CRP levels in mildly and severely ill patients were 33.27mg/mL and 57.90 mg/mL, respectively [113]. The level of CRP was seen in about 85% of severe COVID-19 patients before death. A high level of CRP is related both to the severity of the infection and a worse progression of outcome [114].

Also, in the SARS-CoV-2 vaccination context have been found an association between CRP levels and antibody production. Two different studies reveal that after two doses of the BNT162b2 vaccine, the humoral response is significantly associated with pre-booster serum CRP levels. Specifically, the authors discovered that subjects with higher

basal CRP serum levels displayed a faster increase in anti-SARS-CoV-2 IgG levels after receiving two primary doses of BNT162b2 [53] [115].

The two most important genetic variants of CRP (rs876538; C>T and rs2808635; T>G) have been shown to determine serum CRP levels, particularly in response to inflammatory stimuli. Feng X., et al found that the rs2808635 variant was associated with decreased CRP concentration [108]. Parmeggiani F. et al, found that the rs876538 variant was associated with a developing risk of cardiovascular disease [116].

1.9.9. *HLA-A* HAPLOTYPE

Human Leukocyte Antigen (HLA) system contains several genes located on chromosome 6 (locus 6p21.3) [117]. The HLA system contains nearly 27,000 alleles in three distinct classes of genes (Class I, II and III). Of the three classes of genes HLA class, I (including HLA-A, HLA-B, and HLA-C) and class II (including HLA-DR, HLA-DP, and HLA-DQ) play a crucial role in various human immunological functions [117]. These genes encode surface heterodimeric proteins, which are anchored to the plasma membrane and are responsible for antigen presentation to T cells, followed by the development of an adaptive immune response [118].

Different genetic polymorphisms across the HLA-A, HLA-B, HLA-C, HLA-DR, HLA-DP, and HLA-DQ genes have been associated with the predisposition and/or outcome of different infectious diseases such as hepatitis B virus (HBV), hepatitis C virus (HCV), Chikungunya, Chagas, dengue, influenza A(H1N1) and tuberculosis [117] [119] [120]. In addition, several studies found that a large variety of alleles and/or haplotypes are associated with an increased risk for the more severe clinical manifestations of SARS-CoV-2 or capable of exerting a protective effect against the disease [121]. For example, according to Shkurnikov M. et al., and Tomita Y. et al., patients homozygous by HLA-A*02:01 had a severe course of COVID-19 and were admitted to the intensive care unit. Most likely these patients had a relatively lower capacity to present SARS-CoV-2 antigens compared with other frequent HLA class I molecules, HLA-A*11:01 or HLA-A*24:02 [120] [122]. Furthermore, Pisanti S. et al., suggested that B*18:01 alone and within the HLA-A*02.01g-B*18.01g-C*07.01g-DRB1*11.04g haplotype may protect against both incidence and mortality from COVID-19 [123].

Additionally, recent studies demonstrated that two common polymorphisms in the HLA-A*03:01 (rs2571381/rs2499), maybe had a potential role in the development of SARS-CoV-2 immunity also after vaccination. For istance, Crocchiolo R. et al., and Bolze A. et al., identified this locus (HLA-A*03:01, rs2571381/rs2499) to be associated with a weaker

antibody production after vaccination as well as 2-fold increase in the risk of daily management routine post-vaccination, to fever, fatigue, and generally feeling unwell [124] [125].

1.9.10. SEX DIFFERENCES

Since the onset of the COVID-19 pandemic, it has become clear that there are sex differences in SARS-CoV-2 infections with a higher rate of morbidity and mortality in men than in females. Males are more prone to severe COVID-19 disease and post-infectious/vaccine myocarditis, whereas females are more likely to develop long COVID syndrome. Probably, these differences may be due to biological, genetic and lifestyle between the sexes, making men more vulnerable to both infections [126]. Sex differences result in differential regulation of innate and adaptive immune response which in turn regulates sex-biased pathogenesis and mortality towards various pathogens [127]. Some of these differences have been highlighted as important risk factors in sex-related differences in COVID-19; including immunological defence and responses, genetics, and circulating hormones.

Males and females have different incidences and severity among the most common major diseases that affect the population worldwide, such as cardiovascular, musculoskeletal and immune disorders. These differences can be attributed to the diversity of the physiological processes in females and males. For instance, 80% of patients with osteoporosis or autoimmune diseases are female, while cardiovascular diseases affect men at least a decade earlier than they do females [128] [129].

In general female immune system is more active and efficient to clear viral infections than the one from males [130]. Women produce more antibodies than men, and those antibodies remain longer. Regarding the vaccine response, has been observed a higher humoral response after vaccination in females than in men in a variety of vaccines, including those against influenza, hepatitis A and B, herpes simplex 2 and smallpox [131]. These differences observed, are more than only related to hormones sex-related, since it was demonstrated that immune response differences can be seen in children of different sexes after vaccination, leading to conclude that genetic and epigenetics may also influence the outcomes of vaccination [132].

Furthermore, men have a greater death rate from viral infections than females, and this difference can be attributed to differences in the innate and adaptive immune response [133] [134]. It is well known that interferon-α (IFNα) is necessary for the immune system's defence against viral infections. IFNα production has a sex-dependent pathway, which is attributed to females having a higher production of IFNα than males [135]. Probably, this is the reason why females have an advantage in COVID-19 progression over males.

Additionally, compared to men, females have more CD4+ T cells, stronger CD8+ T cell cytotoxic activity, and more B cells that produce immunoglobulin, which results in a stronger humoral response to viral infections [127]. Mostly the activation of the immune cells during viral response is initiated by the mediation of TLR7, which is responsible for IFN-γ production, and it is found in higher levels in females than in males. Also in the COVID-19 context, females with the severe disease showed a stronger antibody response than male patients, and antibodies also showed up in the early stages of the disease [136] [137]. The same results were observed in subjects after vaccination, in which females generally produce higher antibody levels, that have a longer life than men [138]. However, these findings are contradictory. In fact, Klein S. L. et al. found a greater antibody response in the plasma of COVID-19 convalescent males than females; Padoan A et.al observed no sex-related difference in antibody levels both natural infection and after vaccination [139] [140].

Concerning genetic involvement, genetic differences during COVID-19 were also reported, most of which were associated with the X chromosome. One explanation for this finding is the fact that multiple genes that are crucial for innate and adaptive immune response are located in the X chromosome [133].

Moreover, during the early stages of female development, one of the two X chromosomes goes through a normal process of epigenetic silencing. However, X-chromosome inactivation (XCI) is incomplete in humans so many genes (15- 20%) located in these chromosome portions escape inactivation, resulting in overexpression in females. Among those genes, ACE2 is another gene that sits on chromosome X and is one of the genes escaping X inactivation, and that can be overexpressed in females [73] [133]. Therefore ACE2 would be more highly expressed in the female body. The expression of the ACE2 in the lug epithelial cells, decreases with age, especially in the male sex [133]. This aspect may explain why older males have higher COVID-19 mortality than females, who may also have a double-expressed ACE2 gene. Additionally, cellular expression of ACE2 depends also on particular SNPs (ie., rs2285666), in which the G- allele decreases the ACE2 expression, and increases the susceptibility to develop severe COVID-19 outcomes [71].

Additionally, differences in sex hormone milieus play a role to determine the different development of viral infections between the sexes. Circulating hormones are thought to be important in immunity. While women have higher levels of estrogen and progesterone, men have greater levels of testosterone [141]. Several studies reported that estrogen has immunoenhancing effects, while testosterone induces immunosuppressive effects [142]. More studies suggested that 17β-estradiol regulates many aspects of the innate and adaptive immune systems, including stimulation of pro-inflammatory cytokines [143] [144]. Contextually, it has been shown that androgens increase IL-10 levels, which expresses anti-inflammatory properties, therefore limiting the host's immune response to pathogens. In addition, it has been noted that testosterone lowers the quantity of CD8+ T lymphocytes in the blood [126].

On one hand, increased circulating testosterone is associated with reduced neutralizing antibody responses following influenza vaccination [145]. On another, an in vitro study further showed that estrogen enhanced antibody production and increased the survival rate of B-cells [146]. These findings show that sex differences in hormone levels may lead to different immune reactions, which may also explain sex differences in COVID-19 susceptibility, disease severity, and clinical outcomes, but also in the humoral response after COVID-19 vaccination.

2. AIM OF THE STUDY

Individual population characteristics, appear to have played an important role in the progression of COVID-19. The study of genetic variables, seems to have been crucial in determining which individuals would experience more or less severe forms of the disease also due to a strong or weak antibody response against the virus. Similarly, vaccination response in terms of antibody titre might be affected by the same inter-individual variability.

The present study is part of an ongoing project belonging to the COVID-19 Host Genetic Initiative (HGI) titled "*Extreme genotype comparison and extreme clinical phenotype comparison in CoV-2 patients*: *direct candidate genes-pathways and GWAS*" and aims to identify whether gene variants identified to predict the course of disease can also be informative and useful to predict the antibody dynamics after a complete course (two doses) of anti-SARS-CoV-2 vaccination.

Finding out which genotypes/haplotypes and what role they will play in determining who will have a strong or weak and/or long/short vaccine immune response will be our ultimate objective, as it could be the start for future studies aimed at improving the efficacy not only for anti-SARS-CoV-2 vaccine but also for any other future vaccines or campaign of vaccination booster timing included. Moreover, translating this knowledge to the clinical practise by applying sex data dissection could also improve the overall outcome of the vaccine-induced immune responses.

3. MATERIALS AND METHODS

3.1. STUDY DESIGN

This retrospective study evaluated the immune response in healthy adult volunteers (n= 250) vaccinated with two doses of anti- SARS-CoV-2 vaccine: mRNA-based vaccine (Pfizer–BioNTech/ BNT162b2) and adenoviral vector (AdV)-based vaccine (ChAdOx1/ AstraZeneca), belonging to the staff of the University-Hospital of Ferrara. The study starting from January 2021 and involving human participants was reviewed and approved by the local regional ethical committee (CE-AVEC; 405/2020/Oss/UniFe). The study was divided in two parts: (i) an immunological analysis aimed at evaluating the anti- SARS-CoV-2 circulating antibodies (IgG and neutralizing antibodies, Nabs); and (ii) a genotyping profile investigation of a group of selected common gene variants to identify candidate genetic modifiers of the vaccine-induced immune response.

3.2. INCLUSION/ EXCLUSION CRITERIA

The inclusion criteria for participation in this study were:

- Age above 18 years;
- The ability to sign the informed consent form;
- Two doses of vaccines (non-hybrid vaccination, i.e., the first dose of mRNA and the second adenoviral vector);
- Eligibility for vaccination, according to the national (Italian) program for COVID-19 vaccination (i.e., individuals who had no serious allergy problem and especially have not been hospitalized due to a serious allergic reaction (anaphylaxis);
- Negative SARS-CoV-2 real-time polymerase chain reaction (qPCR) result for a nasopharyngeal swab.

While the exclusion criteria for participation in this study included the presence of:

- A history of chronic disorders;
- Active malignant disease under chemotherapy;
- Autoimmune disorder under immunosuppressive therapy.

Among of total 250 individuals asked for study participation 17 were excluded for the reasons listed above.

3.3. BLOOD SAMPLES

Whole blood samples were collected in EDTA vacutainers a two different time points, T1 at least 15 days after the second dose, and T2 at least 90 days after the second dose. Plasma samples from whole blood were obtained after centrifugation (2,500 g x 10 minutes) within 1 h, and they were immediately frozen at -80°C in aliquots and blind tested.

3.4. ANTIBODY ASSAYS

Anti-SARS-CoV-2 immunoglobulin G (IgG) levels were assessed in the plasma samples using Human SARS-CoV-2 Spike (Trimer) IgG ELISA kit (Invitrogen, Thermo Fisher Scientific, USA). It is a no-competitive ELISA array in which a trimerized Spike antigen is pre-coated in the wells of the supplied microplate. Samples and controls are added into the wells and bind to the immobilized Spike antigen. After the addition of HRP-conjugated detector antibody and substrate, is generated a measurable signal. The intensity of this signal is directly proportional to the IgG levels (Figure 9A). The IgG levels were reported as units (U)/mL, and were obtained using a calibration curve.

The SARS-CoV-2 neutralizing antibodies (NAbs) levels were assessed in the plasma samples using SARS-CoV-2 neutralizing antibody ELISA Kit (Invitrogen, Thermo Fisher Scientific, USA). It is a competitive ELISA array in which the wells of the supplied microplate are pre-coated with a SARS-CoV-2 Receptor Binding Domain (RBD) antigen. Samples and controls are added in the wells, and NAbs compete with excess amounts of biotinylated ACE2 to binds to the RBD antigen. After the addition of Streptavidin HRP-conjugated and substrate, is produced a signal (Figure 9B). Signal is inversely proportional to the NAbs levels. The results were obtained by calculating neutralization (%) for samples using the following equation:

Neutralization (%) = [1 – (O.D. of sample/O.D. of negative control)] x 100.

NAbs levels >20% were considered to be a positive result.

Both tests were performed in duplicate using previously frozen plasma samples, following the manufacturers' instructions.

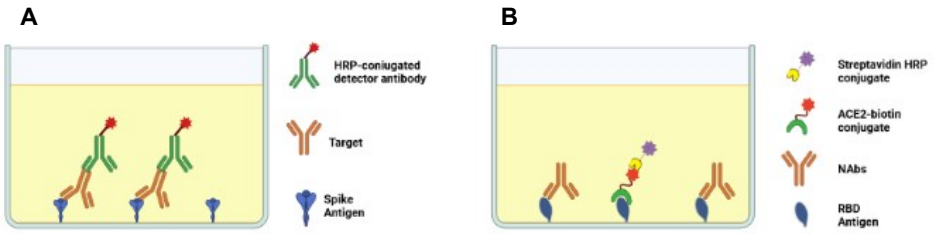


Figure 9. ELISA methods. A) No-competitive ELISA array for the IgG detection**. B)** Competitive ELISA array for NAbs detection. This image is was generated using Biorender.website.

3.5. DNA EXTRACTION AND GENOTYPE ANALYSIS

Genomic DNA was isolated from whole blood by automated DNA extraction and purification robot (BioRobot EZ1 system Qiagen; Hilden, Germany), following the manufacturer's instructions. All the samples of DNA extracted were measured at the Cary60 spectrophotometer (Agilent Techonologies, Mulgrave, Australia), diluted at the concentration of 2.5 ng/µL, and stored at -20°C.

Eleven different gene variants were investigated. The PCR of *ACE* (rs1799752; I/D), *ABO* (rs657152; G>T), *APOE* (R158C, rs7412; C>T, and C112R, rs429358; T>C), *CFH* (Y402H, rs1061170; T>C), *CRP* (rs2808635; G>T, and rs876538; T>C), and *LZTFL1* (rs11385942; -/T) were performed using the thermocycler Agilent SureCycler 8800 (Agilent Technologies, Santa Clara, CA, United States) followed by pyrosequencing (Pyromark ID System, Biotage, AB, Uppsala, Sweden) by using customized specific primers, according to the supplier's instructions [147]. Only for the *ACE* insertion/deletion, the fragments were identified by agarose gel electrophoresis (I/D allele: 490 bp/ 190 bp). In general, PCR amplification reactions contained, 100ng of genomic DNA, 1x buffer (Roche, Mannheim, Germany), 0.2 mM of each dNTP (dATP, dGTP, dTTP, dCTP) (Promega, Madison USA), 0.05 pmol/µl of primers (IDT, Integrated DNA Technologies, Coralville, IA, United States), and 0.05U/µl of Taq DNA Polymerase (Roche, Mannheim, Germany), in a reaction volume of 40 µl. Each PCR cycle was optimised for the different SNPs.

Meanwhile, the PCR of *ACE2* (rs2285666; G>A), *HLA-A* (rs2571381; T>C, and rs2499; T>G), *LZTFL1* (rs35044562; A>G), *OAS3* (rs10735079; A>G) and *TP53* (P72R, rs1042522; C>G) were performed by rhAmp SNP genotyping technology (IDT, Integrated DNA Technologies, Coralville, IA, United States) on the QuantStudio3 Real-Time PCR System (ThermoFisher Scientific, Waltham, USA) according to the supplier's instructions. For these Real-Time PCRs we used 1x rhAmp Genotyping master mix (IDT, Integrated DNA Technologies, Coralville, IA, United States), 1x rhAmp Reporter mix (IDT, Integrated DNA Technologies, Coralville, IA, United States), 1x of SNP assay (IDT, Integrated DNA Technologies, Coralville, IA, United States), distillate water and 5 ng of genomic DNA in a

reaction volume of 10 µl [148]. The analyses of the genotyping were performed in the Thermo Fisher Connect™ software (ThermoFisher Scientific, Waltham, USA).

DNA samples with known genotypes were used as internal control references for all the sequencing, and a random number of samples (15% for each genotype) were reanalysed as the internal quality control procedure as previously described.

Table 2. Gene and variants investigated.

Gene symbol	Chr	Nucleotide and aminoacidic change	db SNP	Method
ACE	17	ins/del 300bp	1799752	Agarose gel electrophoresis
ABO	9	G>T	657152	Pyrosequencing
APOE	19	C>T; R158C	7412	
		T>C; C112R	429358	
CFH	1	T>C; H402Y	1061170	
CRP	1	G>T	2808635	
		T>C	876538	
LZTFL1	3	del/T	11385942	
ACE2	X	G>A	2285666	Real-Time PCR
HLA-A	6	T>G	2499	
		T>C	2571381	
LZTFL1	3	A>G	35044562	
OAS3	12	A>G	10735079	
TP53	17	C>G; P72R	1042522	

3.6. STATISTICAL ANALYSIS

Statistical analyses were performed using GraphPad Prism version 9 (GraphPad Software, Inc.) and MedCalc version 20.113 (MedCalc Software Ltd.). Regression analyses and scatter diagrams accounted for antibody levels and genetic stratification analysis, respectively; *p*-values for intercept accounted for the comparison differences. Moreover, Welch's *t*-test for antibody levels comparison, Chi-square test for genotype distributions or allele frequency, and Hardy- Weinberg equilibrium to check possible deviation of genotype/allele distribution.

The Mann- Whitney non-parametric paired test was performed in case of multiple blood samples from the same donors over time, to evaluate the changes in antibody levels. Spearman's test was used to assess correlation analyses. *p*-values were one-sided with a threshold for statistical significance fixed to $p \leq 0.05$.

4. RESULTS

4.1. CHARACTERISTICS OF SUBJECTS

From January 2021 we progressively enrolled 233 healthy subjects among the worker staff at the University- Hospital of Ferrara at least 15 days after the second dose (15- 199 days) and before the booster dose (third dose) of anti- SARS- CoV-2 vaccine. Table 3 shows the epidemiological characteristics of the vaccinated subjects such as the sex, the percentile of age with mean ± SD, in the whole cohort and stratified by the two main vaccine formulations received: mRNA-based vaccine, n= 166 (Pfizer- BioNtech) and AdV-based vaccine n= 67 (AstraZeneca). Despite the significance in the difference between the number among the two sexes, we continued the study as sex balance was not considered as an exclusion variable. In addition, there were not significant differences between males' and females' mean age in both groups of vaccines.

Table 3. Baseline characteristics of the subjects involved in the study.

		mRNA	AdV	Total	*P*
Subjects – n (%)		166 (71.2)	67 (28.8)	233	
Sex - n (%)					
	Females	104 (62.7)	32 (47.8)	136 (58.4)	**0.04**
	Males	62 (37.3)	35 (52.2)	97 (41.6)	
Age - range					
	25th percentile	38.25	42	39	
	50th percentile	50	50	50	
	75th percentile	58	59	58	
Mean ± SD		47.92± 13.81	50.78± 11.85	48.74± 13.31	ns

In bold significant *p*-value ($p< 0.05$). ns indicates not significant.

4.2. ANTIBODY LEVELS (IgG AND NAbs)

4.2.1. LEVELS DISTRIBUTION AND DYNAMIC OF IgG AND NAbs

Figure 10 shows the distribution and dynamics of anti-SARS-CoV-2 IgG and NAbs levels in the whole cohort, and subsequently stratified by vaccine type and sex, from 15 to 199 days after the second dose of vaccine. Overall, we observed a significant fall in IgG and NAbs levels over time.

As first analysis, we divided the subjects into those whose blood was collected within 90 days (T1, n= 127) and after 90 days (T2, n= 106) after the second dose of vaccine (Figures 10A, B). In detail, we observed a significant fall in antibody levels especially 3 months after vaccination. During the first 90 days (T1, green dots) the reduction was negligible compared with that observed after 90 days (T2, blue dots). These evidences were found for both IgG and NAbs levels (IgG: T1, r^2= 0.004; *p*= 0.43 and T2, r^2= 0.245; *p*< 0.0001; NAbs: T1, r^2= 0.002; *p*= 0.64 and T2, r^2= 0.154; *p*< 0.0001).

Leaving aside the distinction between time points, overall, a significant decrease was observed for both IgG (Figure 10C) and Nabs (Figure 10D) levels over the entire period. (IgG, r^2 = 0.185; p < 0.0001 and NAbs, r^2 = 0.222; p < 0.0001).

Comparing the two vaccine formulations showed different distributions for both IgG and NAbs (Figures 10E, F) levels. The specific kinetics and dynamic distributions for IgG levels were characterized by r^2= 0.320; *p*< 0.0001 and r^2= 0.152; *p*= 0.001, while for NAbs levels by r^2= 0.342; *p*< 0.0001 and r^2= 0.180; *p*= 0.0003, respectively, for mRNA-based vaccine and AdV-based vaccine. Also comparing the intercepts of two vaccine formulations, we observed a significant difference in IgG and NAbs levels distribution (*p*< 0.0001).

By considering a sex stratification (Figure 10G, H), the same analysis showed slightly higher antibody distributions in females than in males for the whole cohort (IgG, r^2 = 0.185; *p*< 0.0001 and NAbs, r^2= 0.228; *p*< 0.0001 for females and IgG, r^2= 0.189; *p*< 0.0001 and NAbs, r^2= 0.222; *p*< 0.0001 for males). However, comparing the intercepts of IgG levels distribution not statistical difference was observed, a borderline result was obtained instead for NAbs levels (IgG: *p*= 0.14; NAbs: *p*= 0.06).

The statistical parameters of regression analyses were reported in Table 4.

To verify that the above results were not due to, or biased by, any imbalance in the number of subjects with different sex or vaccine types, or in the mean age of the sub-groups we counted the relative numbers of these variables in the whole recruitment period stratified by 30 days. Accordingly, not significant differences were documented.

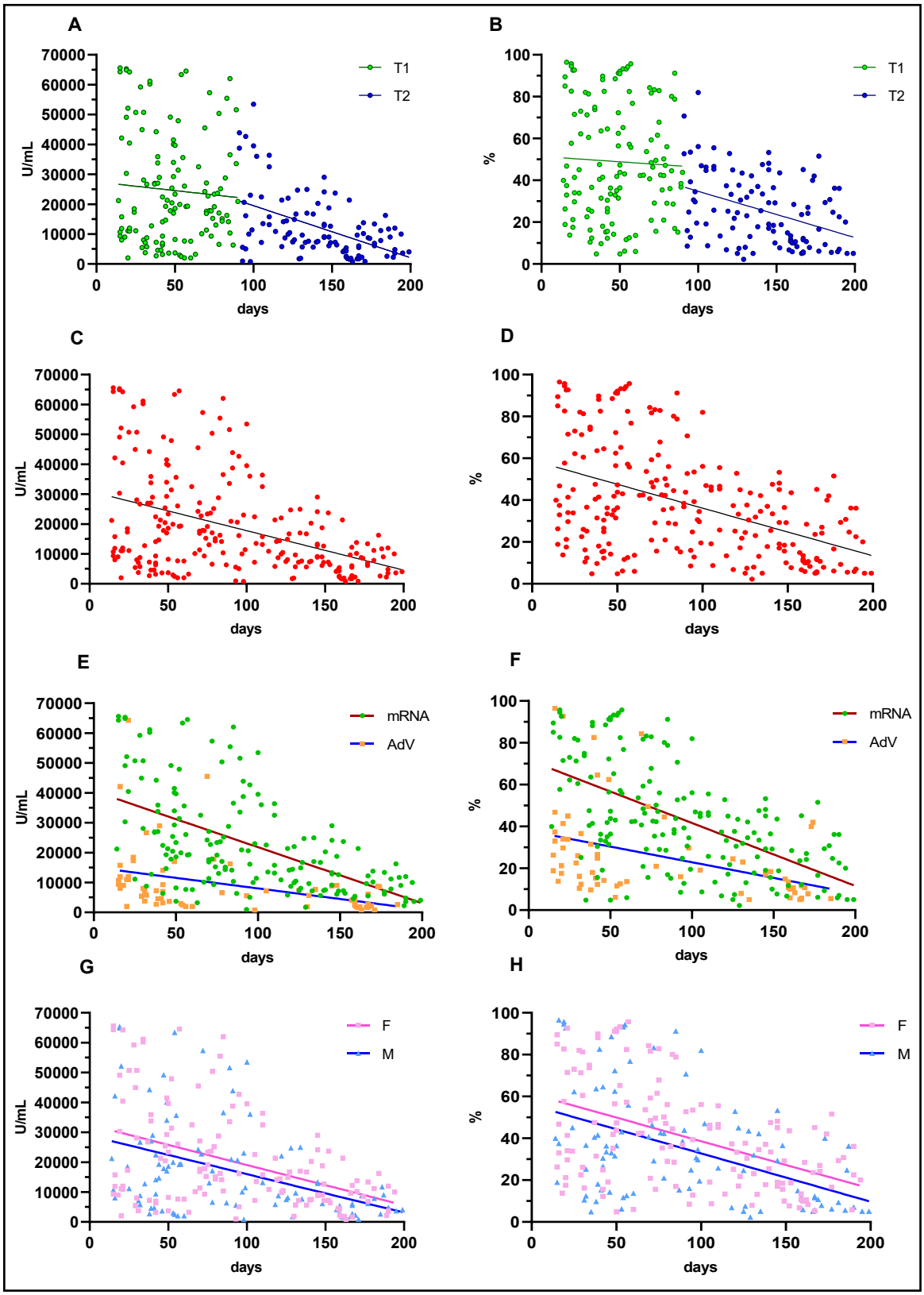


Figure 10. Scatter plots of the distribution of antibody levels after second dose of vaccine. (A, B) IgG and NAbs distribution, respectively, stratified by T1 and T2 recruitment time. **(C, D)** IgG and NAbs kinetic distributions in the whole cohort of subjects. **(E, F)** IgG and NAbs distribution stratified by the vaccine formulation (mRNA-based vaccine and AdV-based vaccine). **(G, H)** IgG and NAbs distribution stratified by sex in the whole cohort.

Table 4. Main statistical findings.

	mRNA	AdV	Females	Males	Total
Subjects – n (%)	166 (71.2)	67 (28.8)	136 (58.4)	97 (41.6)	233
IgG – U/mL					
Mean ± SD	22919.32± 16645.15	9243.48± 10972.06	20274.69± 16563.28	17181.0± 16140.67	18986.78± 16424.87
r^2	0.3201	0.1520	0.1850	0.1889	0.1848
Slope	-178.65	-70.73	-134.83	-128.56	-132.09
Intercept	40602.77	15095.30	32535.76	28855.88	30992.13
***P* comparison**	**< 0.0001**			0.14	
NAbs - %					
Mean ± SD	43.41± 25.80	25.47± 21.31	40.67± 25.05	34.89± 26.73	38.25± 25.86
r^2	0.3421	0.1804	0.2277	0.2222	0.2224
Slope	-0.30	-0.15	-0.23	-0.23	-0.23
Intercept	71.74	37.85	61.21	55.86	58.99
***P* comparison**	**< 0.0001**			0.06	

In bold significant *p*-values ($p < 0.05$).

4.2.2. CORRELATION ANALYSIS

Additionally, we conducted a correlation analysis, in which we observed a strong positive correlation between IgG and NAbs levels in the whole cohort of subjects during the whole frame of time considered (Figure 11A; $r^2 = 0.809$, $p < 0.0001$). Finally, even though the strong difference in the antibody levels distribution detected between the two vaccine types, both vaccines maintained a strong IgG/NAbs correlation when separately assessed (Figure 11B; $r^2 = 0.769$, $p < 0.0001$ for mRNA-based vaccine, and $r^2 = 0.880$, $p < 0.0001$ for AdV-based vaccine). Also, when we divided the whole cohort by sex, we found a strong IgG/NAbs correlation (Figure 11C; $r^2 = 0.779$, $p < 0.0001$ and $r^2 = 0.847$, $p < 0.0001$ for females and males, respectively).

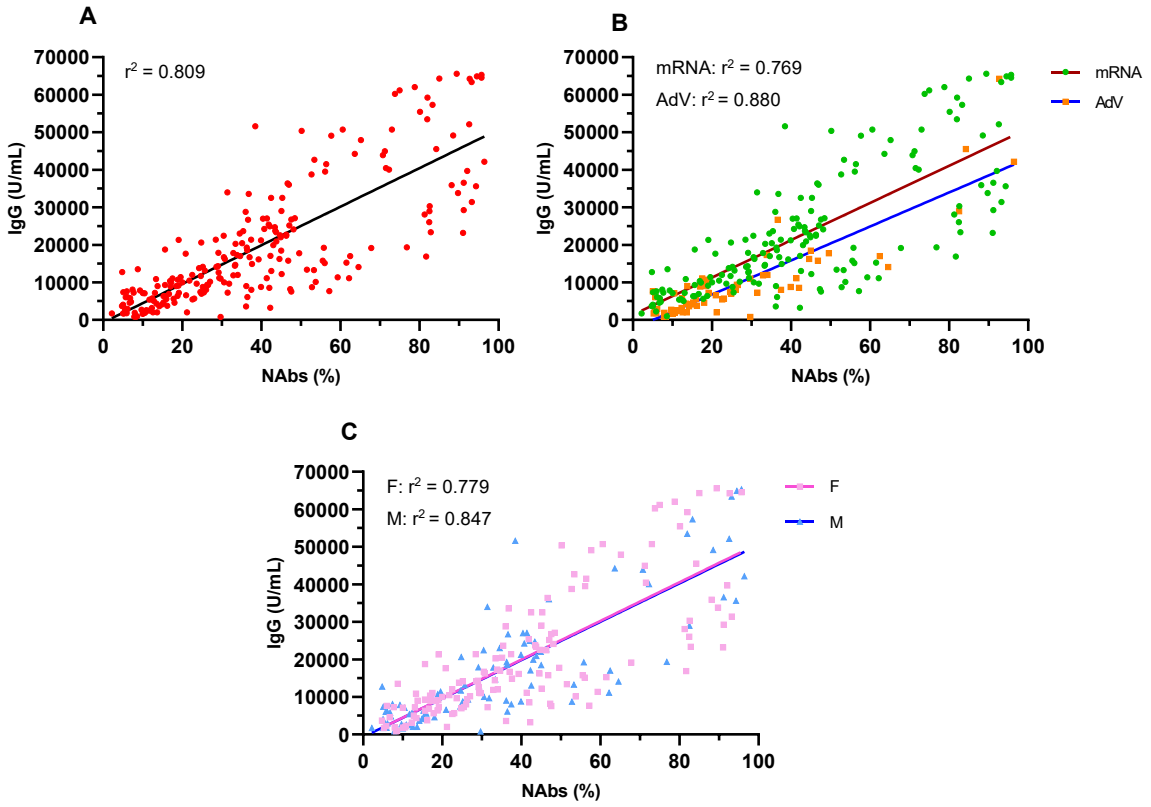


Figure 11. IgG and NAbs correlation analysis. (A) Correlation between IgG and NAbs level distributions in the whole cohort of subjects. **(B)** Correlation between IgG and NAbs stratified by vaccine type. **C)** Correlation between IgG and NAbs stratified by sex. Each panel shows the specific regression line and r-coefficient.

4.2.3. MEAN OF IgG AND NAbs LEVELS

In this analysis we calculated mean of IgG and NAbs levels in the whole cohort of subjects, successively stratifying by two vaccine formulations and sex, without considering the different time points (T1 and T2).

Figure 12 shows the mean of IgG levels. We observed a significant difference in IgG levels mean between mRNA-based vaccine and AdV-based vaccine (Figure 12A, $p< 0.0001$). Considering a sex stratification, the same analysis showed higher of IgG levels in females than in males in the whole cohort of vaccinated subjects, but the differences were borderline (Figure 12B, $p= 0.07$). Table 5 shows in details the main statistical findings of analysed groups.

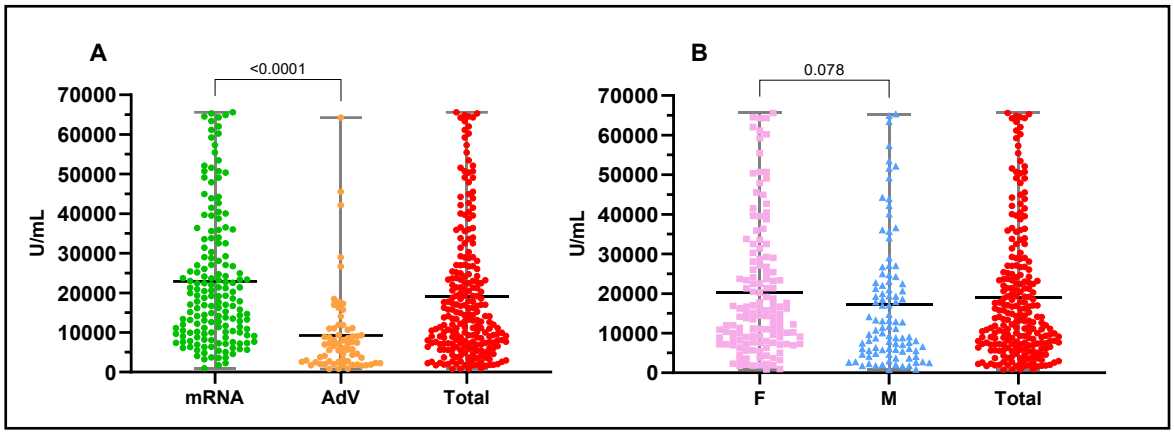


Figure 12. IgG levels distribution. (A) IgG levels distribution stratified by vaccine formulation (mRNA- and AdV-based vaccine). **(B)** IgG levels distribution stratified by sex. Significant differences $p < 0.05$.

Table 5. Main statistical findings.

	IgG – U/mL							
	mRNA	**AdV**	**Total**	***P***	**Females**	**Males**	**Total**	***P***
Subjects - n (%)	166 (71.2)	67 (28.8)	233		136 (58.4)	97 (41.6)	233	
25th percentile	10024.31	2587.29	7194.14		8138.61	5590.00	7194.14	
50th percentile	18644.97	6589.15	13499.29		15028.53	11430.41	13499.29	
75th percentile	31153.88	10831.01	25168.98		27348.80	22735.33	25168.98	
Mean ± SD	22919.32± 16645.15	9243.48± 10972.06	18986.78± 16424.87	**<0.0001**	20274.69± 16563.28	17181.0± 16140.67	18986.78± 16424.87	0.07

In bold significant *p*-value ($p < 0.05$).

Subsequently, we conducted a sub-analysis stratified by sex the IgG levels for each vaccine formulation. Figure 13A shows the mean of IgG levels stratified by sex in subjects vaccinated with mRNA-based vaccine. There were not differences between the two sexes. We observed a slightly difference of IgG levels mean in AdV-based vaccine subjects in which females showed more IgG levels compared with males, but the differences were not significant (Figure 13B, $p = 0.15$). The main statistical findings about the differences between the sexes, stratified by vaccine formulation, are shown in Table 6.

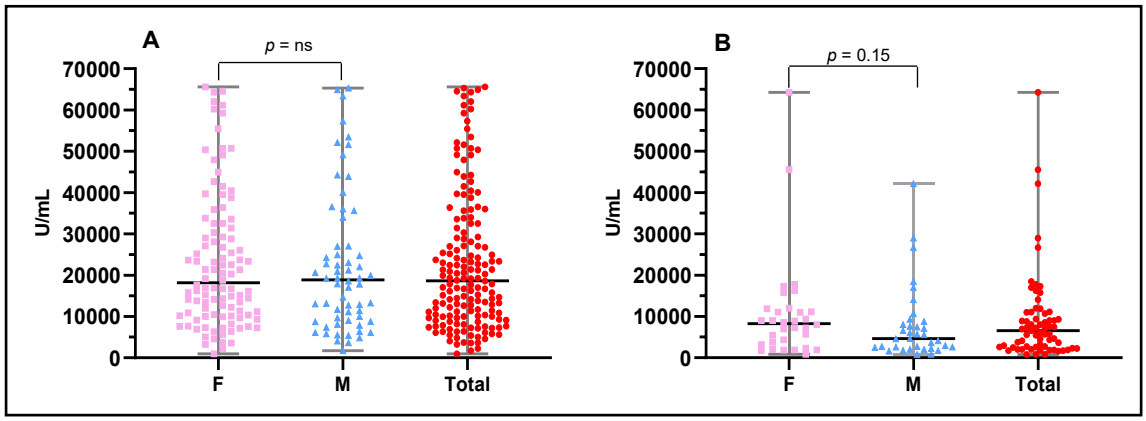


Figure 13. IgG levels distribution. (A) IgG levels distribution stratified by sex in mRNA- based vaccine subjects. **(B)** IgG levels distribution stratified by sex in AdV- based vaccine subjects. ns indicates not significant.

Table 6. Main statistical findings.

IgG – U/mL								
	mRNA				AdV			
	Females	**Males**	**Total**	***P***	**Females**	**Males**	**Total**	***P***
Subjects - n (%)	104 (62.6)	62 (37.3)	166		32 (47.8)	35 (52.2)	67	
25th percentile	13313.96	9165.40	10024.31		3611.87	2531.03	2587.29	
50th percentile	18186.31	18897.76	18644.97		8283.30	4629.94	6589.15	
75th percentile	31698.06	27020.06	31153.88		11316.56	8409.82	10831.01	
Mean ± SD	23214.20± 16536.62	22424.6± 16949.54	22919.32± 16645.15	ns	10721.27± 12764.19	7892.37± 9011.51	9243.48± 10972.06	0.15

ns indicates not significant.

Figure 14 shows the mean of NAbs levels in the whole cohort, stratified by vaccine formulation and sex. We observed a significant difference in NAbs levels mean between mRNA-based vaccine and AdV-based vaccine (Figure 14A, $p< 0.0001$). Considering a sex stratification, the same analysis showed significant higher of NAbs levels in females than in males in the whole cohort of vaccinated subjects (Figure 14B, $p= 0.05$). Table 7 shows in details the main statistical findings of analysed groups.

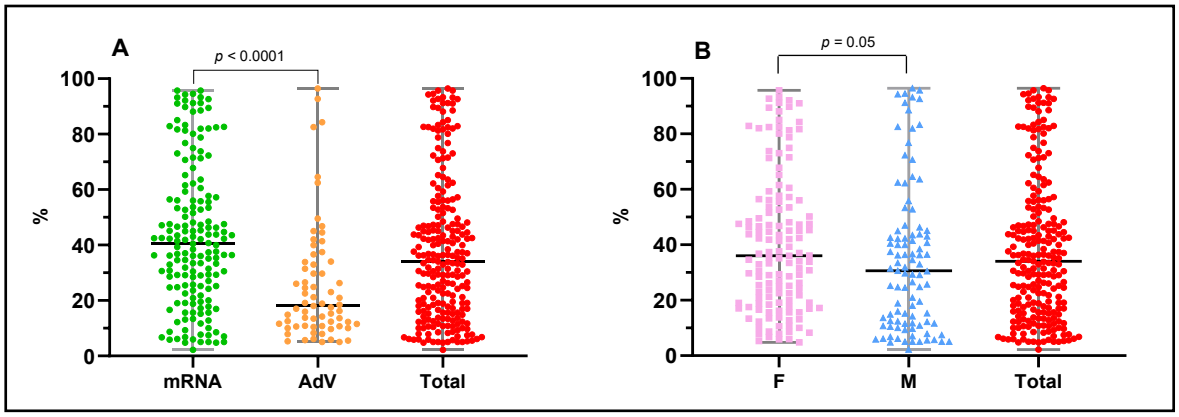


Figure 14. NAbs levels distribution. (A) NAbs levels distribution stratified by sex in mRNA-based vaccine subjects. **(B)** NAbs levels distribution stratified by sex in AdV-based vaccine subjects. Significant differences $p < 0.05$.

Table 7. Main statistical findings.

	NAbs – %							
	mRNA	**AdV**	**Total**	***P***	**Females**	**Males**	**Total**	***P***
Subjects – n (%)	166 (71.2)	67 (28.8)	233		136 (58.4)	97 (41.6)	233	
25th percentile	24.72	10.86	16.62		19.14	11.71	16.62	
50th percentile	40.58	18.00	34.00		36.07	30.59	34.00	
75th percentile	57.57	33.40	52.70		55.65	44.92	52.70	
Mean ± SD	43.41± 25.80	25.47± 21.31	38.25± 25.86	**<0.0001**	40.67± 25.05	34.89± 26.73	38.25± 25.86	**0.05**

In bold significant *p*-value ($p < 0.05$).

Subsequently, we conducted a sub-analysis stratified by sex the NAbs levels for each vaccine formulation. Figure15A shows the mean of NAbs levels stratified by sex in subjects vaccinated with mRNA- based vaccine. There were not differences between the two sexes. Even sex stratification in AdV-based vaccine subjects, did not show significant differences (Figure 15B). The main statistical findings about the differences between the sexes, stratified by vaccine formulation, were showed in Table 8.

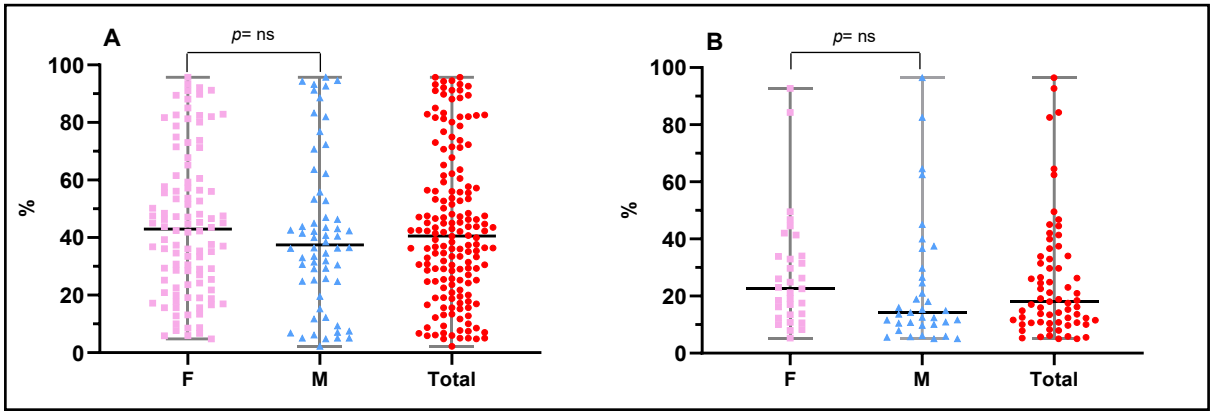


Figure 15. NAbs levels distribution. (A) NAbs levels distribution stratified by sex in mRNA-based vaccine subjects. **(B)** NAbs levels distribution stratified by sex in AdV-based vaccine subjects. ns indicates not significant.

Table 8. Main statistical findings.

NAbs – %								
	mRNA				AdV			
	Females	**Males**	**Total**	**P**	**Females**	**Males**	**Total**	**P**
Subjects– n (%)	104 (62.6)	62 (37.3)	166		32 (47.8)	35 (52.2)	67	
25th percentile	23.94	24.89	24.72		13.69	10.24	10.86	
50th percentile	42.48	37.46	40.58		22.82	14.21	18.00	
75th percentile	58.51	53.18	57.37		33.91	28.11	33.40	
Mean ± SD	44.61± 25.18	41.39± 26.91	43.41± 25.80	ns	27.76± 20.06	23.39± 22.47	25.47± 21.31	ns

ns indicates not significant.

4.3. GENE VARIANTS AND ANTIBODY DISTRIBUTION

We further explored the impact of common genetic variants within genes previously investigated as promising modifiers of the clinical phenotype and progression of COVID-19 or as possible influencers of the antibody response after SARS-CoV-2 infection or vaccination. Some of them have also been investigated in the variability of the humoral and cell-mediated response or as causative of specific mild/common adverse reactions, in particular among Pfizer/BNT162b2 vaccinated subjects.

To investigate possible associations between different genetic determinants and anti-SARS-CoV-2 vaccine-induced antibody levels, the following different statistical assessments were performed in the whole cohort and separately for the mRNA-based vaccine:

i. Distribution of antibody levels (regression analysis) in the whole considered period for each selected gene;
ii. antibody mean levels stratified by selected genotypes (Welch t-test);
iii. genotype or allele frequencies stratified by antibody distributions including possible deviation from the Hardy-Weinberg equilibrium;
iv. Multivariate analyses (multivariate linear regression and multivariate logistic regression).

The same statistical analysis was not carried out for AdV-based vaccine subjects (n= 97) because the number of enrolled subjects was significantly lower than that mRNA-based vaccine subjects (n= 166).
IgG and NAbs levels were analysed as a function of the selected genetic variants (*ABO*, rs657152; *TP53*, rs1042522; *APOE*, rs7412/rs429358; *ACE*, rs1799752; *ACE2*, rs2285666; *CFH*, rs1061170; *CRP*, rs2808635/rs876538; *HLA*, rs2571381/rs2499; *OAS3*, rs10735079; *LZTFL1*, rs35044562 and rs11385942).
Finally, the same approaches were also performed for analysis of IgG and NAbs levels considering the phenotypic sub-group (ABO blood group and Rh system).

4.3.1. REGRESSION ANALYSIS

By regression analyses we performed the trend estimation of IgG and NAbs levels in T1-T2 period of 199 days, accounting the different genotype groups.

***ABO* (rs657152)**. In Figure 16 we reported the regression analyses of IgG and NAbs levels distribution, stratified by *ABO* (rs657152) genotypes, in the whole cohort and in mRNA-based vaccine subgroup.
TT-genotype yielded significant regression equations clustered in the highest part of the scattering compared with the regression obtained by the remaining genotypes, in the whole cohort and in mRNA-based vaccine subgroup (Figures 16A – C – E – G).
Comparing the intercepts of the extreme genotypes (i.e., TT vs GG) in the whole cohort, statistical significance was observed only for IgG levels (p= 0.007), but for NAbs levels we obtained a borderline result (p= 0.06). In mRNA-based vaccine subgroup we obtained the significance only for IgG levels (p=0.02 and p= ns for IgG and NAbs, respectively).
Subsequently, the intercept of TT-genotype was compared with the intercept of GG-coupled with the GT-genotypes (Recessive model: TT vs GG+GT) in the whole cohort and in mRNA-based vaccine subgroup (Figures 16B – D – F – H). The results obtained were similar to those of the extreme genotype comparison. Indeed, significant results were

observed for IgG levels in the whole cohort and in mRNA-based vaccine subgroup (p=0.003 and p= 0.01). On the contrary, not significant differences were observed in NAbs levels. The statistical parameters of regression analyses were reported in Table 9.

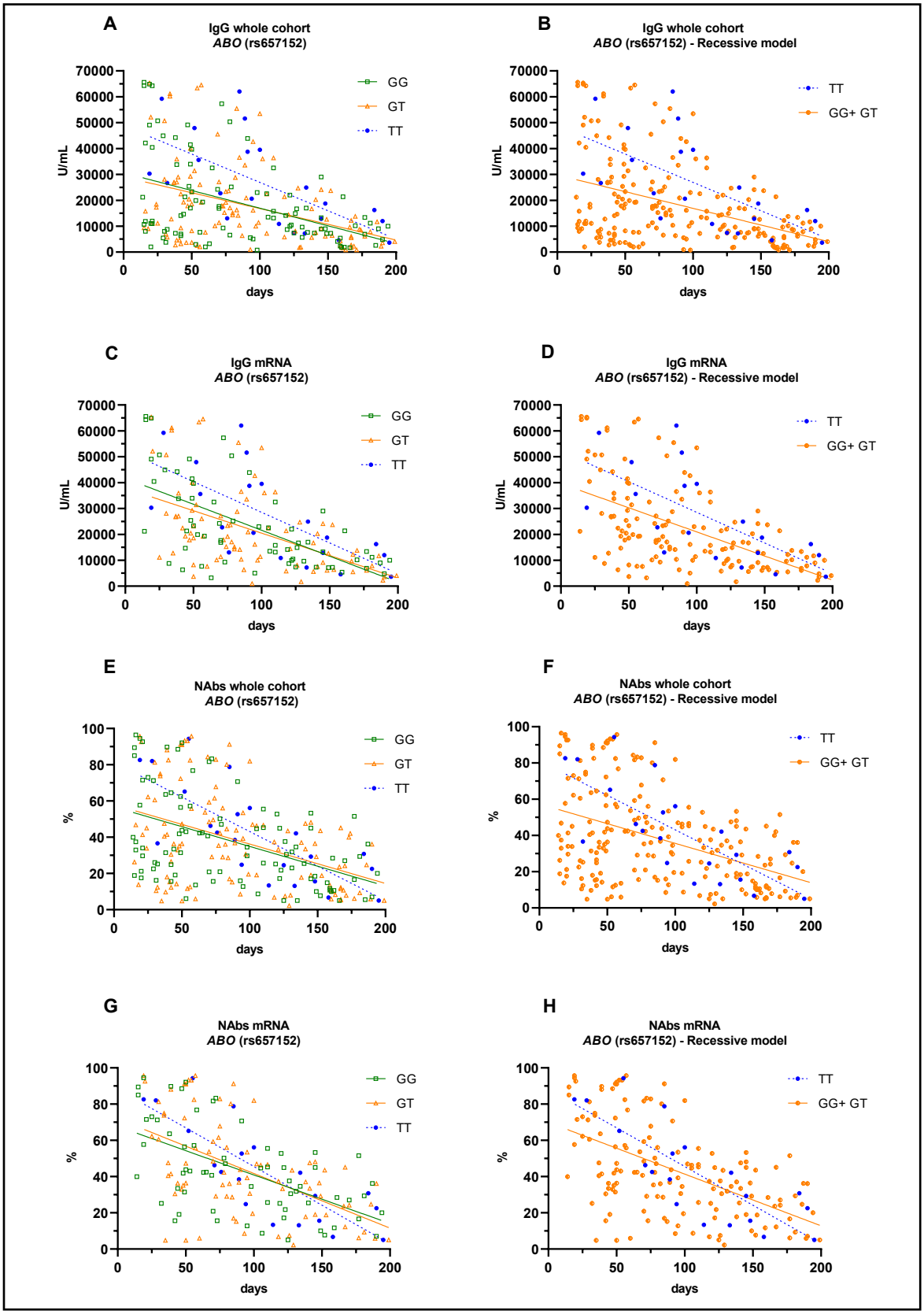


Figure 16. Scatter plots and regression analyses of IgG and NAbs levels stratified by *ABO* (rs6571582) genotypes and genetic model. (A, B) IgG and **(E, F)** NAbs kinetics in the whole cohort. **(C, D)** IgG and **(G, H)** NAbs kinetics in the mRNA-based vaccine. Each panel shows the specific regression lines, according to the indicated genotype and genetic model.

Table 9. Regression analysis and genotype comparison.

***ABO* (rs657152)**										
	Whole cohort (n=233)					**mRNA (n=166)**				
	GG	**GT**	**TT**	**TT**	**GG+ GT**	**GG**	**GT**	**TT**	**TT**	**GG+ GT**
Subjects - n (%)	95 (40.8)	116 (49.8)	22 (9.4)	22 (9.4)	211 (90.6)	64 (38.5)	82 (49.4)	20 (12.1)	20 (12.1)	146 (87.9)
IgG – U/mL										
r^2	0.1999	0.1655	0.4132	0.4132	0.1821	0.3881	0.2653	0.4577	0.4577	0.3191
Slope	-136.44	-121.99	-219.07	-219.07	-128.94	-200.56	-173.29	-236.99	-236.99	-186.33
Intercept	30621.65	29033.81	48840.78	48840.78	29805.06	41662.98	37816.68	52234.94	52234.94	39605.32
***P* comparison** GG vs TT					**0.007**					**0.02**
***P* comparison** TT vs GG+ GT					**0.003**					**0.01**
NAbs - %										
r^2	0.2286	0.1834	0.5718	0.5781	0.2018	0.3248	0.2969	0.6557	0.6557	0.3067
Slope	-0.218	-0.217	-0.381	-0.381	-0.217	-0.268	-0.302	-0.427	-0.4267	-0.2856
Intercept	56.74	57.97	81.25	81.25	57.37	67.73	71.88	88.23	88.23	69.94
***P* comparison** GG vs TT					0.06					ns
***P* comparison** TT vs GG+ GT					0.09					ns

P comparison indicates the statistical assessment between the intercepts obtained from regression analysis. In bold significant *p*-value ($p < 0.05$). ns indicates not significant.

***TP53* (rs1042522).** In Figure 17 we reported the regression analyses of IgG and NAbs levels distribution, stratified by *TP53* (rs1042522) genotypes, in the whole cohort and in mRNA-based vaccine subgroup.

GG-genotype yielded significant regression equations clustered in the lowest part of the scattering compared with the regression obtained by the remaining genotypes, in the whole cohort and in mRNA-based vaccine subgroup (Figures 17A – C – E – G).

Comparing the intercepts of the extreme genotypes (i.e., CC vs GG) in the whole cohort, statistical significance was obtained for both IgG and NAbs levels ($p = 0.02$ and $p = 0.04$ for IgG and NAbs, respectively). In subgroup of mRNA-based vaccinated subjects, the statistical significance was obtained only for IgG ($p = 0.05$), but not for NAbs ($p = 0.10$) levels. Subsequently, the intercept of GG-genotype was compared with the intercept of other genotypes (Recessive model: GG vs CC+CG) in the whole cohort and in mRNA-based vaccine subgroup (Figures 17B – D – F – H). The results obtained were similar to those of the extreme genotype comparison. Indeed, significant results were obtained of IgG levels in the whole cohort and in mRNA-based vaccine subgroup ($p = 0.03$ and $p = 0.05$), and in NAbs levels only in the whole cohort ($p = 0.05$). We did not observe significant differences of NAbs levels in mRNA-based vaccine subgroup.

The statistical parameters of regression analyses were reported in Table 10.

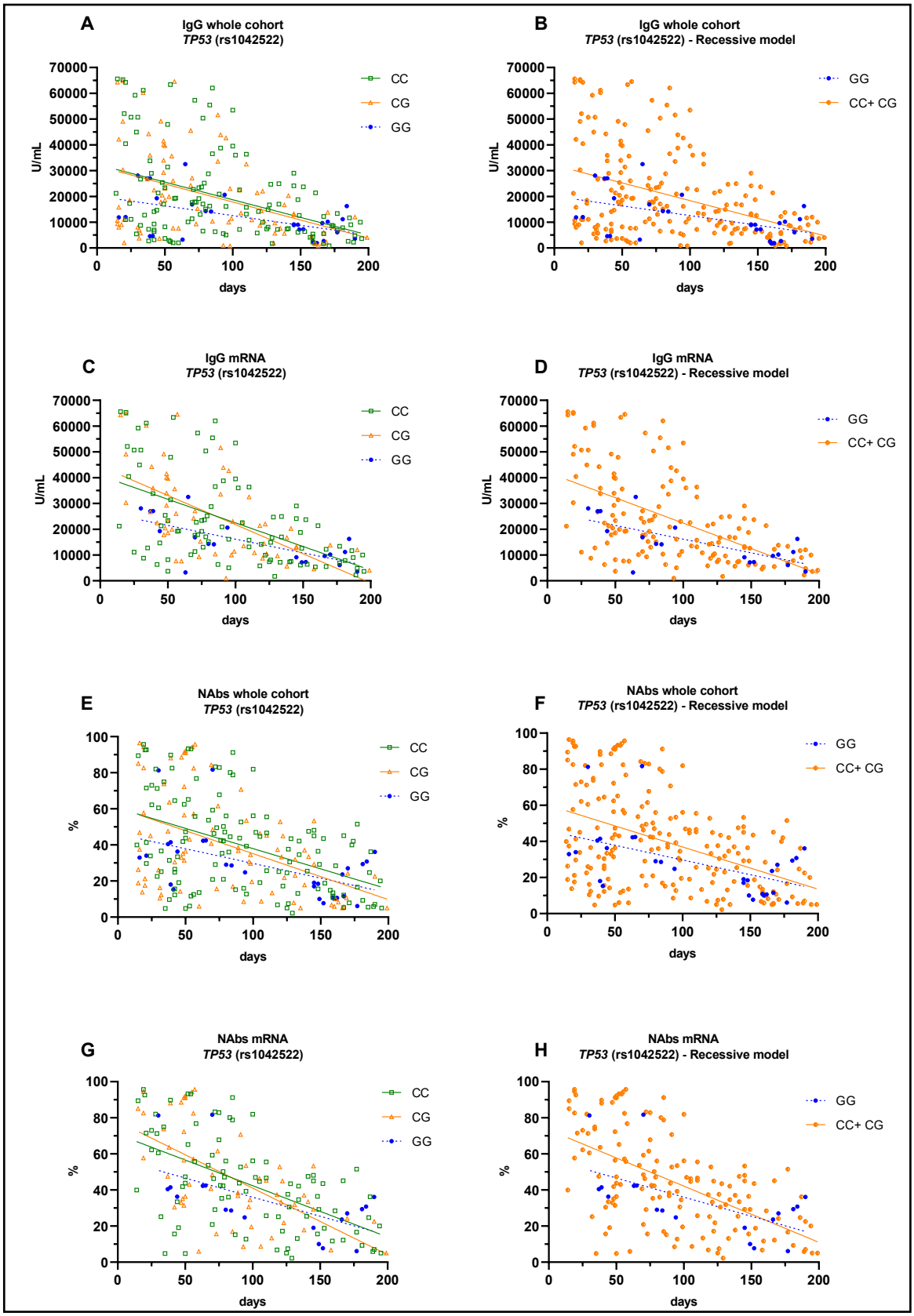


Figure 17. Scatter plots and regression analyses of IgG and NAbs levels stratified by *TP53* (rs1042522) genotypes and genetic model. (A, B) IgG and **(E, F)** NAbs kinetics in the whole cohort. **(C, D)** IgG and **(G, H)** NAbs kinetics in the mRNA-based vaccine. Each panel shows the specific regression lines, according to the indicated genotype and genetic model.

Table 10. Regression analysis and genotype comparison.

TP53 (rs1042522)										
	Whole cohort (n=233)					mRNA (n=166)				
	CC	**CG**	**GG**	**GG**	**CC+ CG**	**CC**	**CG**	**GG**	**GG**	**CC+ CG**
Subjects - n (%)	122 (52.4)	82 (35.2)	29 (12.4)	29 (12.4)	204 (87.6)	90 (54.2)	57 (34.3)	19 (11.4)	19 (11.4)	147 (88.6)
IgG – U/mL										
r^2	0.1752	0.1782	0.2788	0.2788	0.1762	0.2821	0.3713	0.4983	0.4983	0.3140
Slope	- 136.974	- 140.01	- 75.289	- 75.29	- 137.33	- 183.154	- 226.27	- 106.36	- 106.36	- 197.04
Intercept	32409.73	3185.59	20098.32	20098.29	32117.27	40783.13	44432.06	26792.06	26792.06	42069.77
***P* comparison** CC vs GG					**0.02**					**0.05**
***P* comparison** GG vs CC+ CG					**0.03**					**0.05**
NAbs - %										
r^2	0.2103	0.2184	0.2793	0.2793	0.2111	0.3104	0.3804	0.3739	0.3739	0.3360
Slope	- 0.224	- 0.254	- 0.163	- 0.163	- 0.234	- 0.284	- 0.369	- 0.211	0.211	- 0.312
Intercept	60.37	60.60	46.03	46.03	60.30	70.82	77.369	57.18	57.18	73.33
***P* comparison** CC vs GG					**0.04**					0.10
***P* comparison** GG vs CC+ CG					**0.05**					0.15

P comparison indicates the statistical assessment between the intercepts obtained from regression analysis. In bold significant *p*-value ($p < 0.05$).

***APOE* (rs7412/rs429358).** In Figure 18 we reported the regression analyses of IgG and NAbs levels distribution, stratified by *APOE* (rs7412/rs429358) haplotypes, in the whole cohort and in mRNA-based vaccine subgroup.

ε4-carrying haplotypes (i.e., ε2ε4 and ε3ε4) yielded significant regression equations clustered in the lowest part of the scattering compared with the regression obtained by the rest of ε3-carrying haplotypes (i.e., ε2ε3 and ε3ε3) (Figures A – B – C – D). Overall, comparing the intercepts, we found statistically significant differences of IgG and NAbs levels in the whole cohort and in mRNA-based vaccine subgroup ($p = 0.03$ and $p = 0.007$, respectively for IgG and NAbs in the whole cohort, and $p = 0.02$ and $p < 0.0001$, respectively for IgG and NAbs in mRNA-based vaccine subgroup).

The statistical parameters of regression analyses were reported in Table 11.

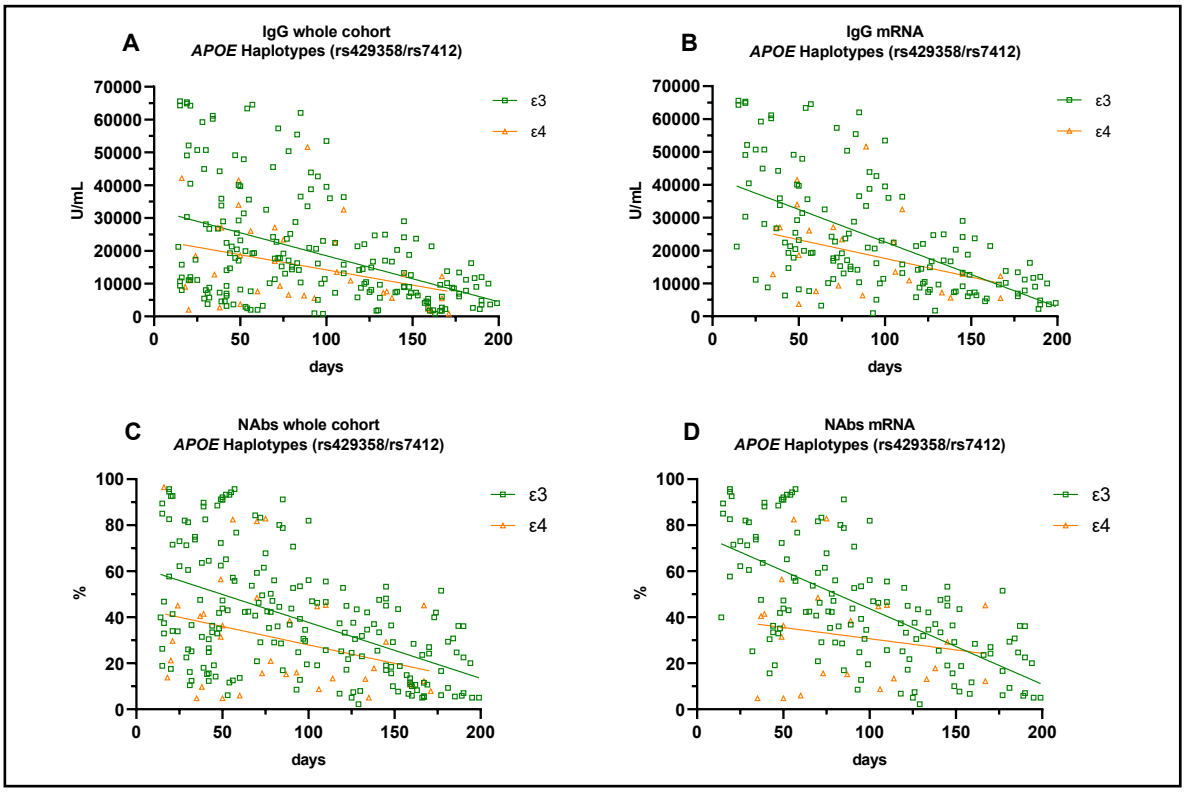


Figure 18. Scatter plots and regression analyses of IgG and NAbs levels stratified by *APOE* (rs7412/rs429358) haplotypes. (A) IgG and **(C)** NAbs kinetics in the whole cohort. **(B)** IgG and **(D)** NAbs kinetics in the mRNA-based vaccine. Each panel shows the specific regression lines, according to the indicated haplotypes.

Table 11. Regression analysis and genotype comparison.

	***APOE* (rs7412/rs429358)**			
	Whole cohort (n=233)		**mRNA (n=166)**	
	ε3	**ε4**	**ε3**	**ε4**
Subjects -n (%)	197 (84.6)	36 (15.4)	143 (86.1)	23 (13.9)
IgG – U/mL				
r^2	0.2012	0.1224	0.3524	0.1380
Slope	-140.10	-92.55	-197.91	-113.745
Intercept	32506.76	23439.03	42400.33	28983.25
***P* comparison** ε3 vs ε4	**0.03**		**0.02**	
NAbs - %				
r^2	0.2599	0.0995	0.4262	0.0321
Slope	-0.24	-0.1606	-0.33	-0.10
Intercept	62.05	44.04	76.56	40.41
***P* comparison** ε3 vs ε4	**0.007**		**< 0.0001**	

P comparison indicates the statistical assessment between the intercepts obtained from regression analysis. In bold significant *p*-value ($p < 0.05$).

***ACE* (rs1799752)** In Figure 19 we reported the regression analyses of IgG and NAbs levels distribution, stratified by *ACE* (rs1799752) genotypes, in the whole cohort and in mRNA-based vaccine subgroup. II-genotype yielded regression equations clustered in the lowest part of the scattering compared with the regression obtained by the remaining genotypes, in the whole cohort and in mRNA-based vaccine subgroup (Figures 19A – C – E – G).

Comparing the intercepts of the extreme genotypes (i.e., DD vs II) in the whole cohort, statistical significance was obtained only for NAbs levels (p= 0.05). We did not obtain statistical differences in the subgroup of mRNA-based vaccinated subjects.

Subsequently, the intercept of II-genotype was compared with the intercept of other genotypes (Recessive model: II vs DD+ID) in the whole cohort and in mRNA-based vaccine subgroup (Figures 19B – D – F – H). The results obtained were similar to those of the extreme genotype comparison. Indeed, significant results were obtained only for NAbs levels in the whole cohort. We did not obtain statistical differences in the subgroup of mRNA-based vaccinated subjects.

The statistical parameters of regression analyses were reported in Table 12.

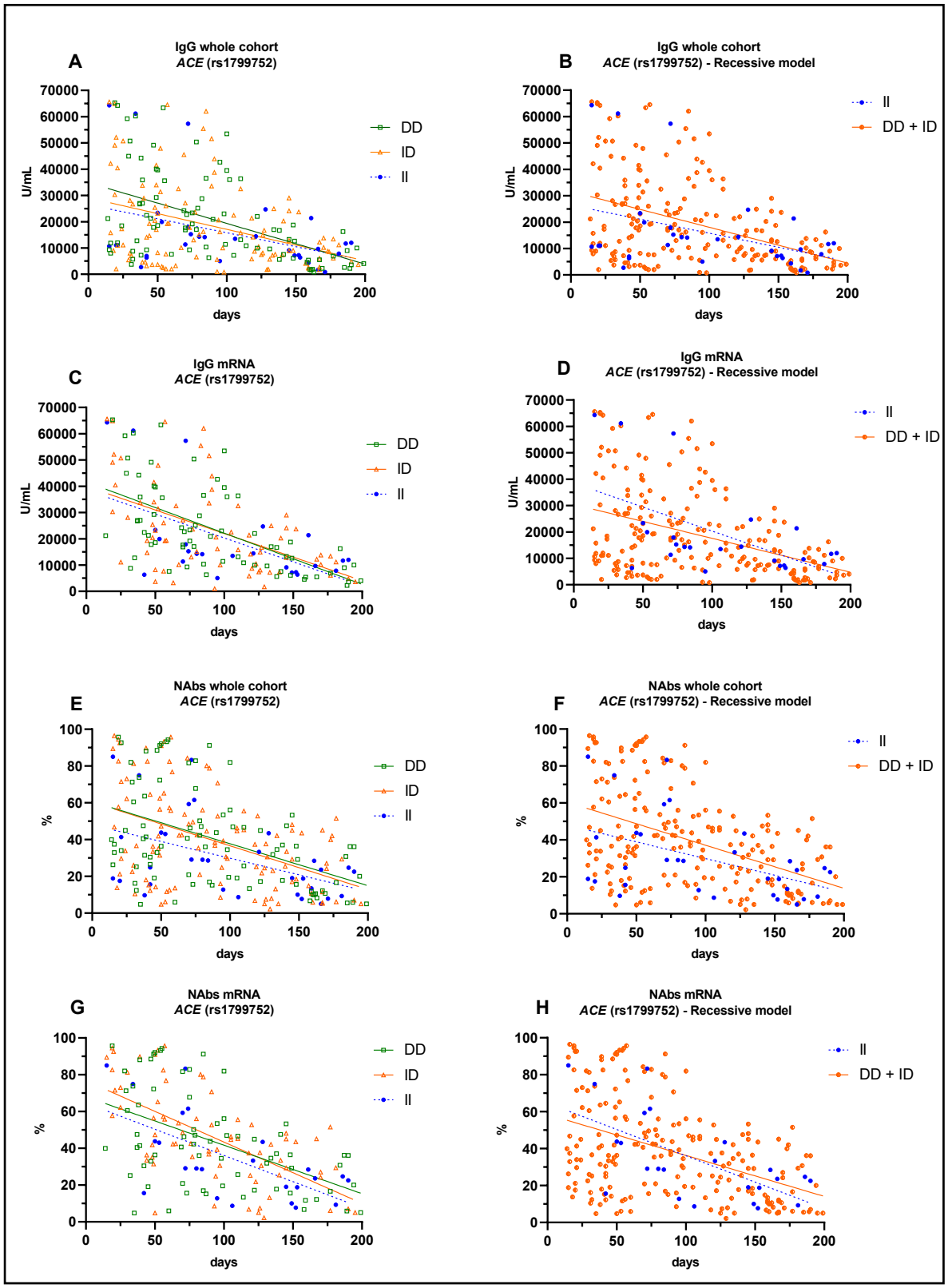


Figure 19. Scatter plots and regression analyses of IgG and NAbs levels stratified by *ACE* (rs1799752) genotypes and genetic model. (A, B) IgG and **(E, F)** NAbs kinetics in the whole cohort. **(C, D)** IgG and **(G, H)** NAbs kinetics in the mRNA-based vaccine. Each panel shows the specific regression lines, according to the indicated genotype and genetic model.

Table 12. Regression analysis and genotype comparison.

ACE (rs1799752)										
	Whole cohort (n=233)					mRNA (n=166)				
	DD	**ID**	**II**	**II**	**DD + ID**	**DD**	**ID**	**II**	**II**	**DD + ID**
Subjects - n	86	115	32	32	201	69	73	24	24	142
(%)	(36.9)	(49.4)	(13.7)	(13.7)	(86.3)	(41.5)	(44.0)	(14.5)	(14.5)	(85.5)
IgG – U/mL										
r^2	0.251	0.145	0.151	0.151	0.188	0.367	0.227	0.312	0.312	0.315
Slope	-154.88	-119.75	-106.33	-106.33	- 0.135.72	-194.92	-179.38	-181.89	-181.89	- 187.29
Intercept	34853.06	29084.55	26446.82	26446.82	31628.30	41646.55	39947.07	38525.29	38525.29	40803.21
***P* comparison** DD vs II					0.12					ns
***P* comparison** II vs DD + ID					ns					ns
NAbs - %										
r^2	0.222	0.218	0.226	0.226	0.219	0.255	0.408	0.410	0.410	0.325
Slope	-0.23	-0.24	-0.18	-0.18	- 0.23	-0.26	-0.33	-0.28	-0.28	- 0.30
Intercept	60.57	60.38	47.99	47.99	60.42	68.04	76.76	64.61	64.61	72.39
***P* comparison** DD vs II					**0.05**					0.14
***P* comparison** II vs DD + ID					**0.05**					0.08

P comparison indicates the statistical assessment between the intercepts obtained from regression analysis. In bold significant *p*-value ($p < 0.05$). ns indicates not significant.

***ACE2* (rs2285666)**. In Figure 20 we reported the regression analyses of IgG and NAbs levels distribution, stratified by *ACE2* (rs2285666) genotypes, in the whole cohort and in mRNA-based vaccine subgroup. This variant is locates on the X chromosome, for this reason it was first analysed in females and males separately. From results of this analysis, we decided to compare the GG+G genotypes (GG-homozygous females plus G-hemizygous males) with the remaining A-carriers (AA-homozygous and AG- heterozygous females plus A-hemizygous males).

GG+G genotypes yielded significant regression equations clustered in the lowest part of the scattering compared with the regression obtained by the remaining A-carriers, in the whole cohort and in mRNA-based vaccine subgroup (Figures 20A – B – C – D).

Comparing the intercepts of the genotypes (i.e., GG+G vs A-carriers) in the whole cohort, statistical significance was obtained only for IgG levels ($p = 0.04$). In subgroup of mRNA-based vaccinated subjects, the statistical significance was obtained for both antibody types (IgG: $p = 0.009$ and NAbs: $p = 0.02$).

The statistical parameters of regression analyses were reported in Table 13.

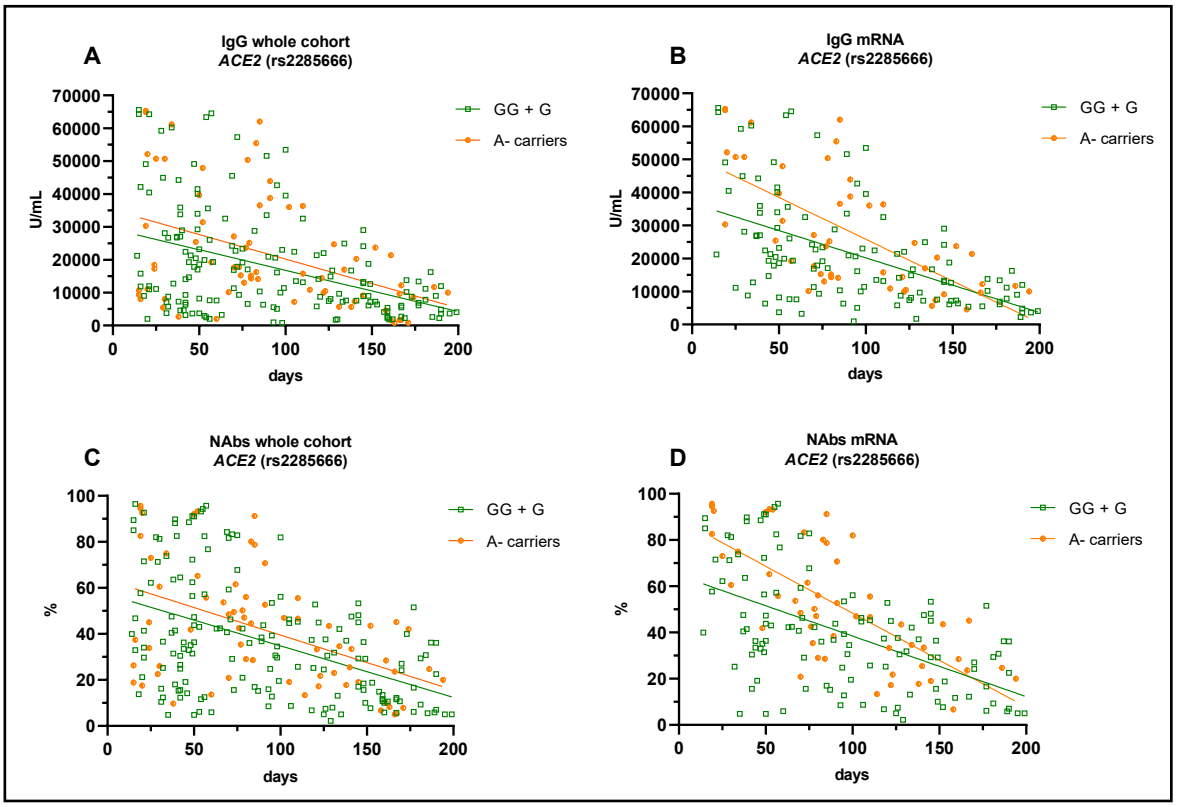


Figure 20. Scatter plots and regression analyses of IgG and NAbs levels stratified by *ACE2* (rs2285666) genotypes. (A) IgG and **(C)** NAbs kinetics in the whole cohort. **(B)** IgG and **(D)** NAbs kinetics in the mRNA-based vaccine. Each panel shows the specific regression lines, according to the indicated groups of genotypes.

Table 13. Regression analysis and genotype comparison.

	***ACE2* (rs2285666)**			
	Whole cohort (n=233)		**mRNA (n=166)**	
	GG + G	**A-carriers**	**GG + G**	**A-carriers**
Subjects – n (%)	165 (70.8)	68 (29.2)	118 (71.1)	48 (28.9)
IgG – U/mL				
r^2	0.1845	0.1942	0.2903	0.4450
Slope	-125.46	- 149.24	- 165.62	- 253.42
Intercept	29323.94	35131.96	36733.94	51142.28
***P* comparison** GG+ G vs A- carriers	**0.04**		**0.009**	
NAbs - %				
r^2	0.2166	0.2434	0.2905	0.5443
Slope	-0.22	-0.24	- 0.27	- 0.39
Intercept	57.19	63.41	66.39	86.36
***P* comparison** GG+ G vs A- carriers	0.07		**0.02**	

P comparison indicates the statistical assessment between the intercepts obtained from regression analysis. In bold significant *p*-value ($p < 0.05$).

***CFH* (rs1061170).** In Figure 21 we reported the regression analyses of IgG and NAbs levels distribution, stratified by *CFH* (rs1061170) genotypes, in the whole cohort and in mRNA-based vaccine subgroup. We did not observe significant differences in the regression equations of the three different genotypes in all groups analysed (Figures 20A – B – C – D). Similarly, comparing the intercepts, no statistically significant results were observed. For this reason, no genetic model was carried out.

The statistical parameters of regression analyses were reported in Table 14.

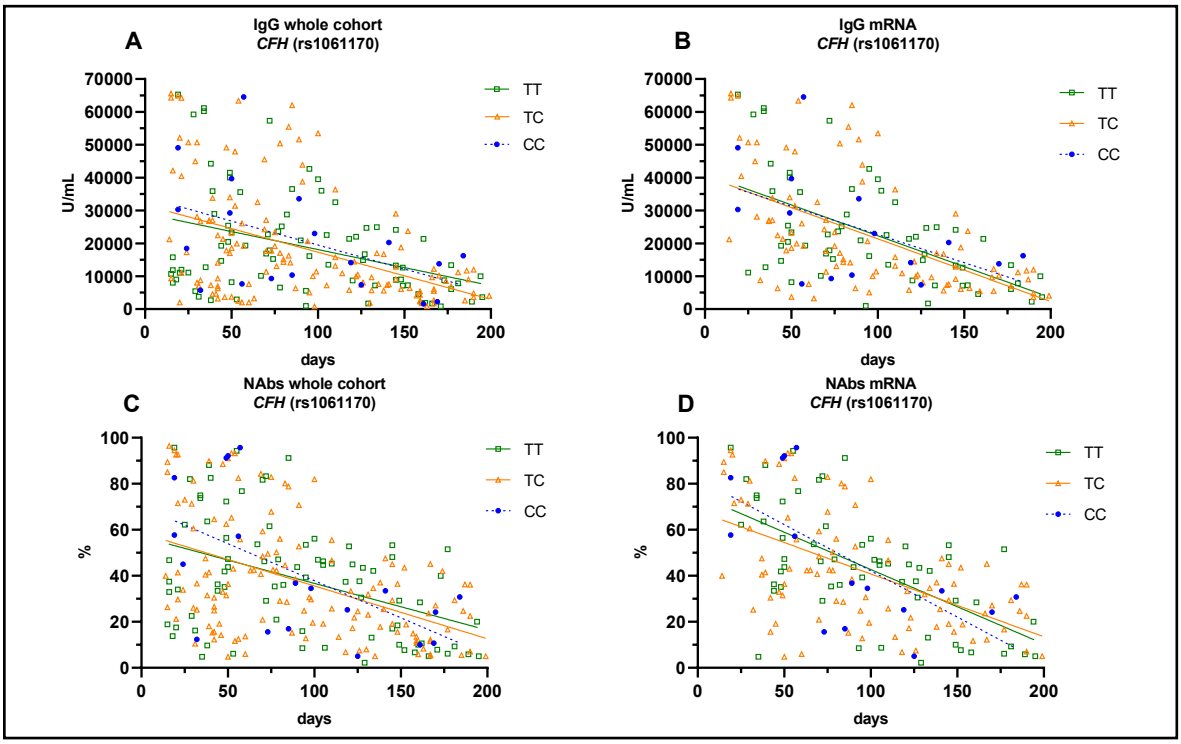


Figure 21. Scatter plots and regression analyses of IgG and NAbs levels stratified by *CFH* (rs1061170) genotypes. (A) IgG and **(C)** NAbs kinetics in the whole cohort. **(B)** IgG and **(D)** NAbs kinetics in the mRNA-based vaccine. Each panel shows the specific regression lines, according to the indicated genotype.

Table 14. Regression analysis and genotype comparison.

CFH (rs1061170)						
	Whole cohort (n=233)			**mRNA (n=166)**		
	TT	**TC**	**CC**	**TT**	**TC**	**CC**
Subjects- n (%)	81 (34.8)	133 (57.1)	19 (8.1)	60 (36.1)	91 (54.8)	15 (9.1)
IgG – U/mL						
r^2	0.1439	0.2040	0.2312	0.3319	0.3215	0.2703
Slope	- 109.67	- 143.61	- 134.94	- 187.90	- 190.39	- 171.38
Intercept	29084.00	31682.53	34095.15	41102.83	40413.64	39823.99
***P* comparison** TT vs CC		ns			ns	
NAbs - %						
r^2	0.1860	0.2276	0.3483	0.3897	0.3021	0.4387
Slope	- 0.20	- 0.23	- 0.32	- 0.32	- 0.27	- 0.40
Intercept	57.00	58.57	70.09	75.12	68.11	82.33
***P* comparison** TT vs CC		ns			ns	

P comparison indicates the statistical assessment between the intercepts obtained from regression analysis. ns indicates not significant.

***CRP* (rs2808635/rs876538).** In Figure 22 we reported the regression analyses of IgG and NAbs levels distribution, stratified by *CRP* (rs2808635/rs876538) haplotypes, in the whole cohort and in mRNA-based vaccine subgroup. We compared the common double homozygotes (TT/CC) versus those with more than one polymorphic allele in at least one of the two loci (i.e., GG rs2808635 plus any combination of rs876538 and TT rs876538 plus any combination of rs2808635). We did not observe significant difference in the regression equations of the different haplotypes in both cohort of vaccinated subjects (whole cohort and mRNA-based vaccine subjects) (Figures 20A – B – C – D). Similarly, comparing the intercepts, no statistically significant results were observed.

The statistical parameters of regression analyses were reported in Table 15.

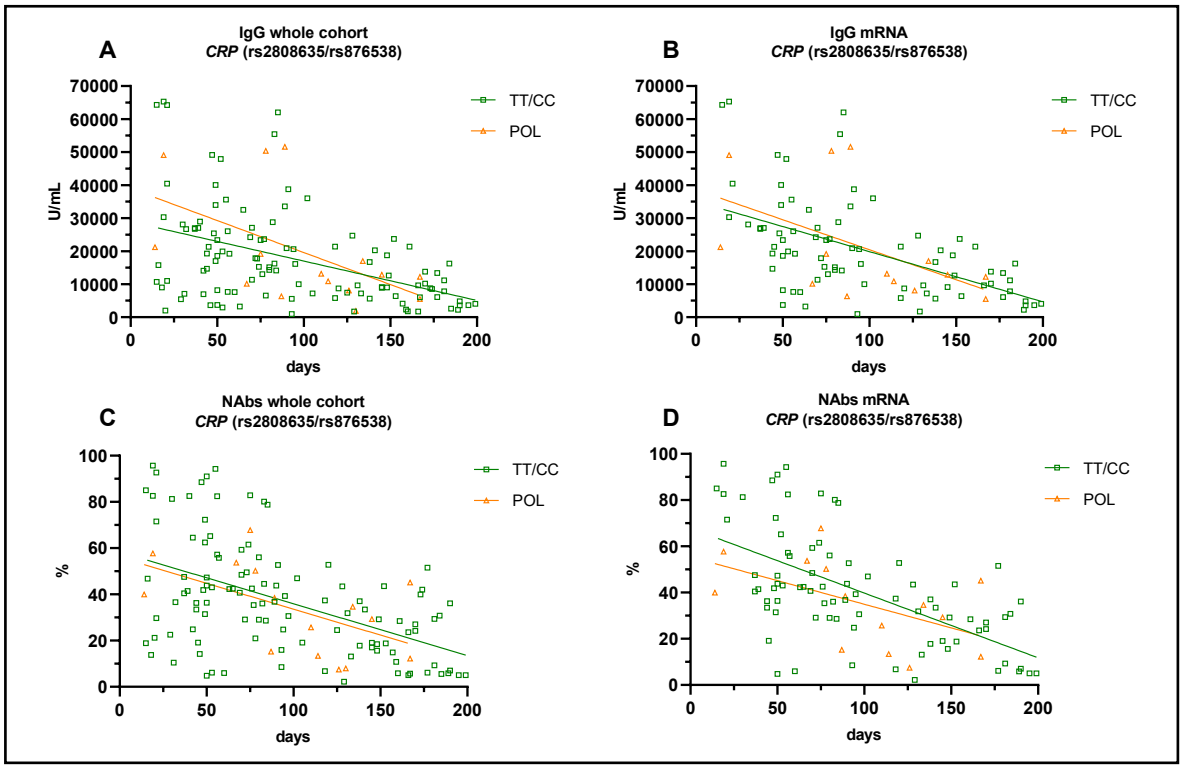


Figure 22. Scatter plots and regression analyses of IgG and NAbs levels stratified by *CRP* (rs2808635/rs876538) haplotypes. (A) IgG and **(C)** NAbs kinetics in the whole cohort. **(B)** IgG and **(D)** NAbs kinetics in the mRNA-based vaccine. Each panel shows the specific regression lines, according to the indicated haplotypes.

Table 15. Regression analysis and genotype comparison.

***CRP* (rs2808635/rs876538)**				
	Whole cohort (n=126)		**mRNA (n=94)**	
	TT/CC	**POL**	**TT/CC**	**POL**
Subjects – n (%)	111 (88.1)	15 (11.9)	80 (85.11)	14 (14.89)
IgG – U/mL				
r^2	0.2007	0.2875	0.2942	0.2668
Slope	- 119.17	- 193.89	- 152.02	- 181.62
Intercept	28915.52	38975.08	35061.81	38602.93
***P* comparison** TT/CC vs POL	ns		ns	
NAbs - %				
r^2	0.2516	0.2907	0.3621	0.2705
Slope	-0.22	-0.22	-0.28	-0.20
Intercept	58.36	55.98	67.80	55.39
***P* comparison** TT/CC vs POL	ns		ns	

P comparison indicates the statistical assessment between the intercepts obtained from regression analysis. ns indicates not significant.

***HLA-A* (rs2571381/rs2499).** In Figure 23 we reported the regression analyses of IgG and NAbs levels distribution, stratified by *HLA-A* (rs2571381/rs2499) haplotypes, in the whole cohort and in mRNA-based vaccine subgroup. In detail, we compared the common double homozygotes (i.e. CC/GG) versus those globally carrying at least two variant alleles (i.e. CT/GT, TT/GG, CC/TT, CT/TT and TT/GT). Due to low frequency of double carriers, we found only double heterozygotes (i.e., CT/GT) and very few CT/TT. We did not observe significant difference when comparing regression equations of the distribution of IgG and NAbs levels in both cohort of vaccinated subjects (whole cohort and mRNA-based vaccine subjects) (Figures 20A – B – C – D). Similarly, comparing the intercepts, no statistically significant results were observed.

The statistical parameters of regression analyses were reported in Table 16.

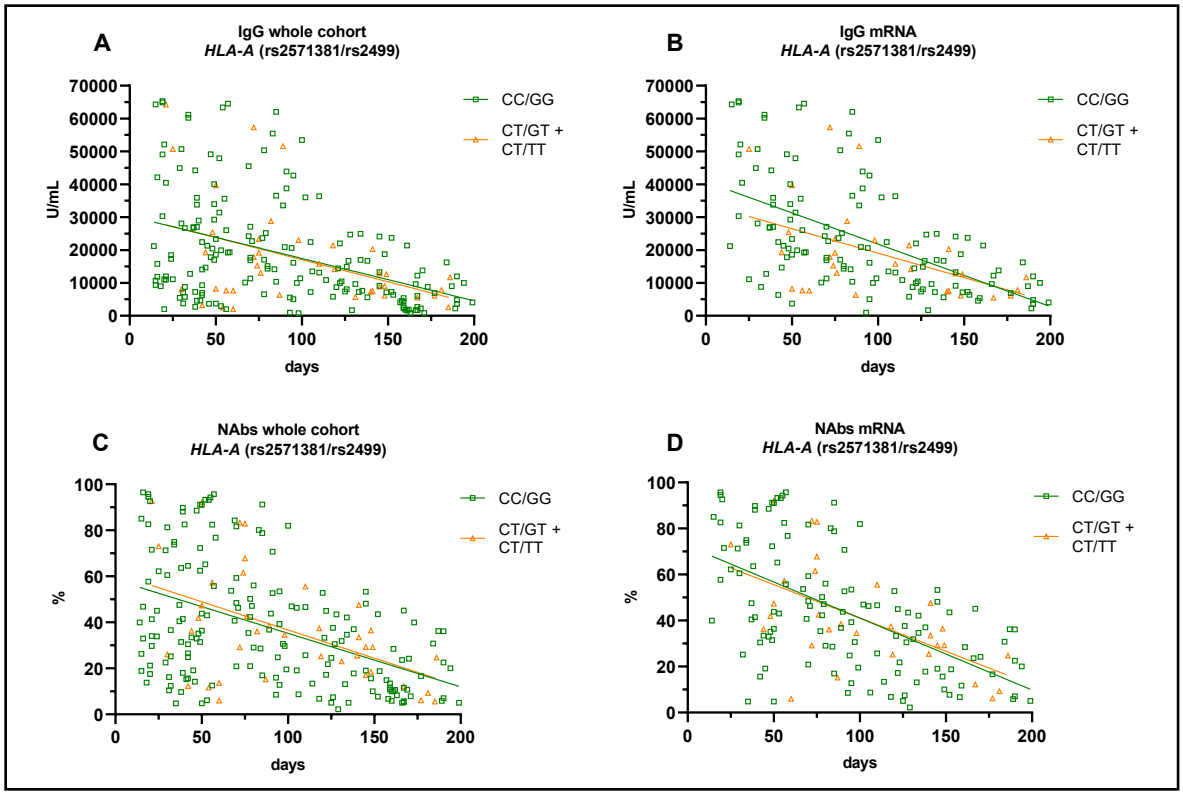


Figure 23. Scatter plots and regression analyses of IgG and NAbs levels stratified by *HLA-A* (rs2671381/rs2499) haplotypes. (A) IgG and **(C)** NAbs kinetics in the whole cohort. **(B)** IgG and **(D)** NAbs kinetics in the mRNA-based vaccine. Each panel shows the specific regression lines, according to the indicated haplotypes.

Table 16. Regression analysis and genotype comparison.

HLA-A (rs2571381/rs2499)				
	Whole cohort (n=214)		mRNA (n=152)	
	CC/GG	CT/GT+ CT/TT	CC/GG	CT/GT+ CT/TT
Subjects – n (%)	175 (81.8)	39 (18.2)	122 (80.3)	30 (19.7)
IgG – U/mL				
r^2	0.1784	0.1794	0.3207	0.2347
Slope	- 128.78	- 134.47	- 189.99	- 148.87
Intercept	30318.10	30517.07	40796.62	33940.59
P comparison CC/GG vs CT/GT+ CT/TT	ns		ns	
NAbs - %				
r^2	0.2186	0.2464	0.3379	0.3401
Slope	-0.23	-0.24	-0.31	-0.29
Intercept	58.56	61.19	72.39	70.08
P comparison TT/CC vs POL	ns		ns	

P comparison indicates the statistical assessment between the intercepts obtained from regression analysis. ns indicates not significant.

***LZTFL1* (rs35044562)** and **(rs11385942).** SNPs (rs35044562 and rs11385942) of the *LZTFL1* gene were analysed only in the whole cohort, because the number of rare homozygotes was very low (GG or TT: 1.7%). The distribution of the three different regression equations for both IgG and NAbs did not permit to create any genetic models and also comparing any subgroups of genotypes with a single genetic counterpart, did not yield appreciable findings. In Figure 24 and 25 we reported the regression analyses of IgG and NAbs levels distribution. In both SNPs, we observed no differences in the regression equations of the three different genotypes. Similarly, comparing the intercepts, no statistically significant results were observed.

The statistical parameters of regression analyses were reported in Table 17.

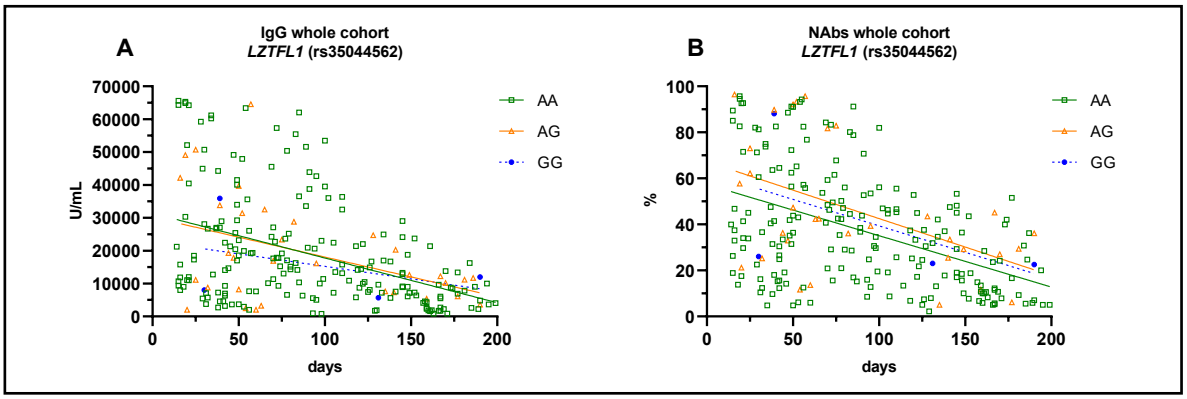


Figure 24. Scatter plots and regression analyses of IgG and NAbs levels stratified by *LZTFL1* (rs35044562) genotypes. (A) IgG and **(B)** NAbs kinetics in the whole cohort. Each panel shows the specific regression lines, according to the indicated genotype.

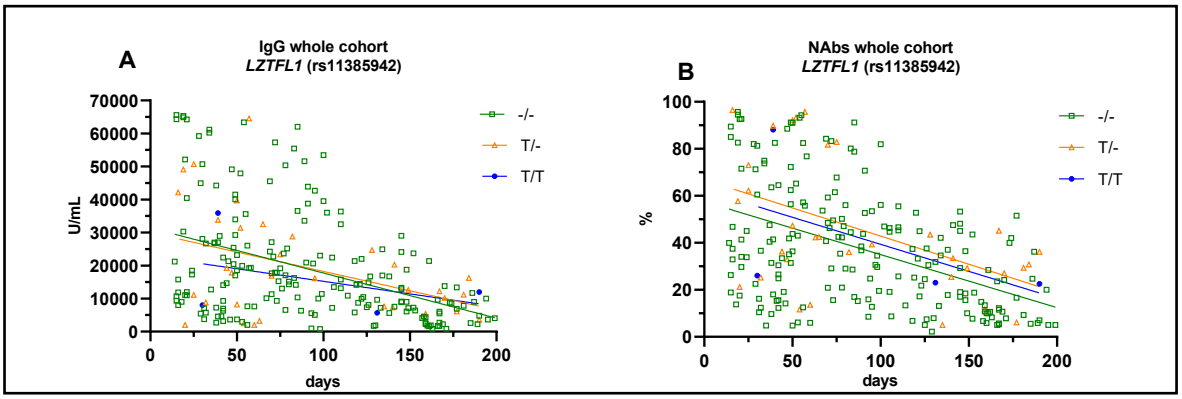


Figure 25. Scatter plots and regression analyses of IgG and NAbs levels stratified by *LZTFL1* (rs11385942) genotypes. (A) IgG and **(B)** NAbs kinetics in the whole cohort. Each panel shows the specific regression lines, according to the indicated genotype.

Table 17. Regression analysis and genotype comparison.

	***LZTFL1* (rs35044562)**			***LZTFL1* (rs11385942)**		
	AA	**AG**	**GG**	**-/-**	**-/T**	**T/T**
Subjects - n (%)	197 (84.55)	32 (13.73)	4 (1.72)	196 (84.12)	33 (14.16)	4 (1.72)
IgG – U/mL						
r^2	0.1850	0.1895	0.1759	0.1867	0.1819	0.1759
Slope	- 135.06	- 125.04	- 76.09	- 0.136.75	- 117.26	- 76.09
Intercept	31315.99	30389.13	22841.11	31420.30	29960.30	22841.11
***P* comparison** AA vs GG		ns			ns	
NAbs - %						
r^2	0.2170	0.2427	0.3006	0.2185	0.2464	0.3006
Slope	-0.22	-0.25	-0.23	-0.22	-0.24	-0.23
Intercept	57.38	67.25	62.36	57.53	66.81	62.36
***P* comparison** AA vs GG		ns			ns	

P comparison indicates the statistical assessment between the intercepts obtained from regression analysis. ns indicates not significant.

OAS3 (rs10735079). In Figure 26 we reported the regression analyses of IgG and NAbs levels distribution, stratified by *OAS3* (rs10735079) genotypes, in the whole cohort and in mRNA-based vaccine subgroup. We observed not differences in the regression equations of the three different genotypes in all groups analysed (Figures 20A – B – C – D). Similarly, comparing the intercepts, no statistically significant results were observed. For this reason, no genetic model was carried out.

The statistical parameters of regression analyses were reported in Table 18.

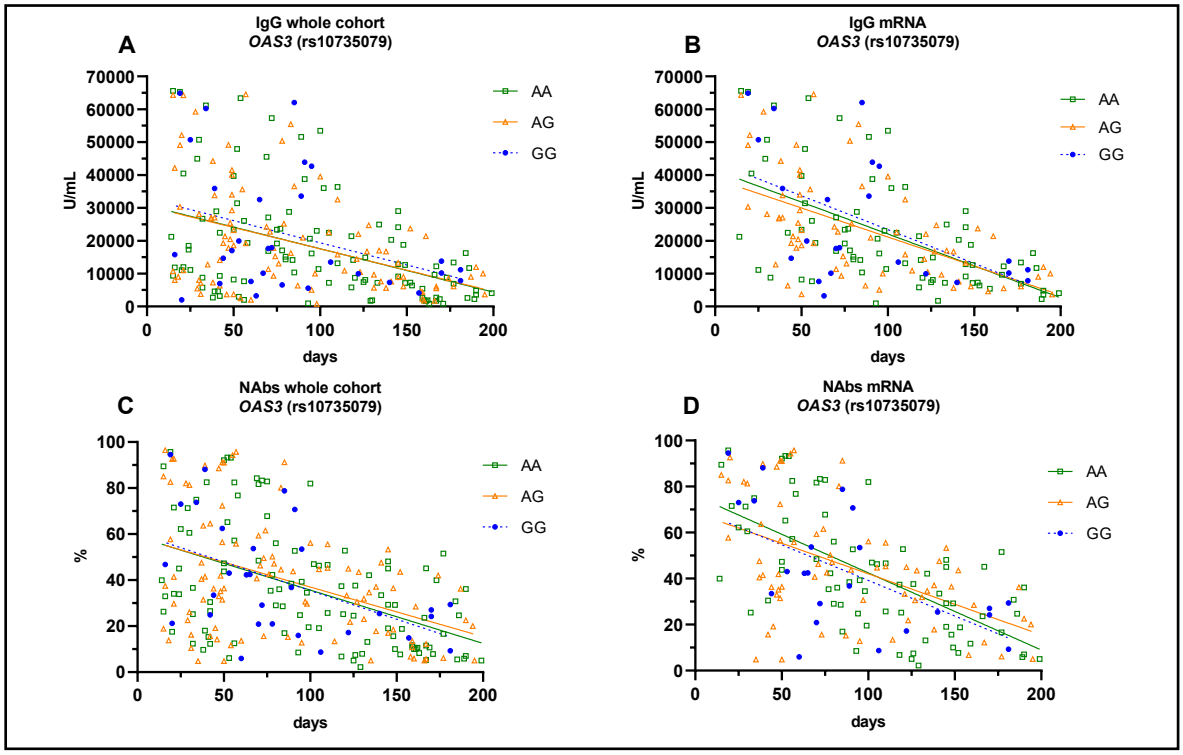


Figure 26. Scatter plots and regression analyses of IgG and NAbs levels stratified by *OAS3* (rs10735079) genotypes. (A) IgG and **(C)** NAbs kinetics in the whole cohort. **(B)** IgG and **(D)** NAbs kinetics in the mRNA-based vaccine. Each panel shows the specific regression lines, according to the indicated genotype.

Table 18. Regression analysis and genotype comparison.

OAS3 (rs10735079)						
	Whole cohort (n=233)			mRNA (n=166)		
	AA	AG	GG	AA	AG	GG
Subjects - n (%)	105 (45.06)	98 (42.06)	30 (12.88)	74 (44.58)	69 (41.57)	23 (13.85)
IgG – U/mL						
r^2	0.1982	0.1844	0.1277	0.3384	0.3185	0.2745
Slope	- 132.09	- 129.88	-134.74	- 194.03	- 176.76	- 205.89
Intercept	30768.46	30539.34	32856.73	41463.87	38810.62	43981.89
***P* comparison** GG vs AA		ns			ns	
NAbs - %						
r^2	0.2410	0.1864	0.2512	0.4216	0.2603	0.3496
Slope	- 0.23	- 0.22	- 0.25	- 0.33	- 0.26	- 0.31
Intercept	58.86	58.57	60.25	76.01	68.59	70.11
***P* comparison** GG vs AA		ns			ns	

P comparison indicates the statistical assessment between the intercepts obtained from regression analysis. ns indicates not significant.

ABO blood group and Rh system. For ABO blood group, we conducted an initial analysis in which we separately examined the four blood groups (O, A, AB, B). The regression equation of B-blood group clustered in the highest part of the scattering, separating significantly from the remaining sub-groups which instead assembled together. Therefore, we decided to compare B-blood group versus the remaining blood groups (No B-group).
In Figure 27 we reported the regression analyses of IgG and NAbs levels distribution, stratified by ABO blood group, in the whole cohort (Figures A – C) and in mRNA-based vaccine subgroup (Figures B – D). B-blood group yielded significant regression equations clustered in the highest part of the scattering compared with the regression obtained by the remaining No B- group. Comparing the intercepts, statistical significance was obtained in IgG levels in the whole cohort ($p = 0.005$) and in mRNA-based vaccine subgroup ($p = 0.04$). Not significant differences were observed in NAbs levels both in the whole cohort and in mRNA-based vaccine subgroup. The statistical parameters of regression analyses were reported in Table 19.

Regarding the Rh system, in Figure 28 we reported the regression analyses of IgG and NAbs levels distributions, stratified by Rh system in the whole cohort (Figures A – C) and in mRNA-based vaccine subgroup (Figures B – D). Rh negative subjects (Rh-) yielded significant regression equations clustered in the highest part of the scattering compared with the regression obtained by Rh positive subjects (Rh+). Comparing the intercepts of regression equations, statistical significance was obtained in IgG and NAbs levels only in the whole cohort ($p = 0.02$ and $p = 0.03$, respectively).

The statistical parameters of regression analyses were reported in Table 20.

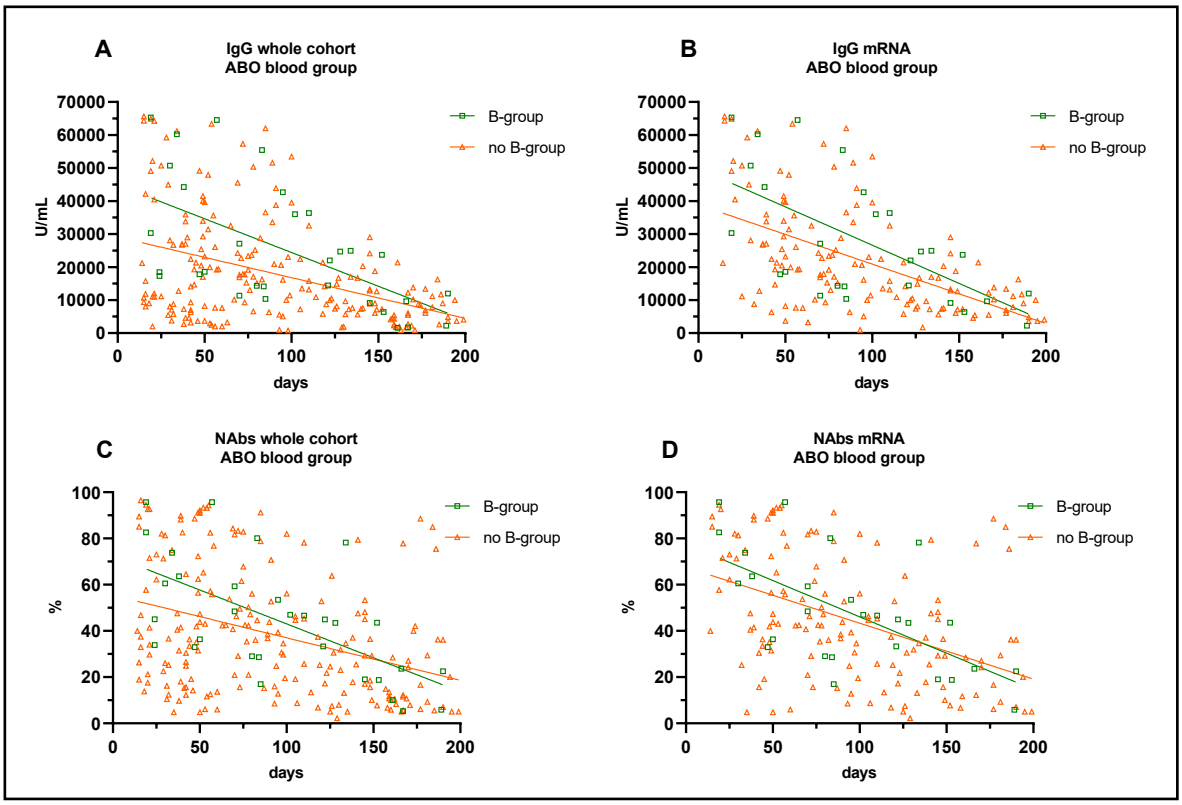


Figure 27. Scatter plots and regression analyses of IgG and NAbs levels stratified by ABO blood group. (A) IgG and **(B)** NAbs kinetics in the whole cohort. **(C)** IgG and **(D)** NAbs kinetics in the mRNA-based vaccine. Each panel shows the specific regression lines, according to the indicated genotype.

Table 19. Regression analysis and genotype comparison.

ABO blood group				
	Whole cohort (n=233)		**mRNA (n=166)**	
	No B-group	**B-group**	**No B-group**	**B-group**
Subjects - n (%)	202 (86.7)	31 (13.3)	139 (83.7)	27 (16.3)
IgG – U/mL				
r^2	0.1725	0.3422	0.3147	0.3915
Slope	- 123.09	- 204.46	- 179.86	- 231.85
Intercept	29107.22	44866.19	38883.10	49829.06
***P* comparison** No B-group vs B-group	**0.005**		**0.04**	
NAbs - %				
r^2	0.3895	0.1323	0.1991	0.4124
Slope	- 0.2933	- 0.1839	- 0.2419	- 0.3143
Intercept	72.36	55.40	67.52	77.51
***P* comparison** No B-group vs B-group	0.16		ns	

P comparison indicates the statistical assessment between the intercepts obtained from regression analysis. In bold significant *p*-value ($p < 0.05$). ns indicates not significant.

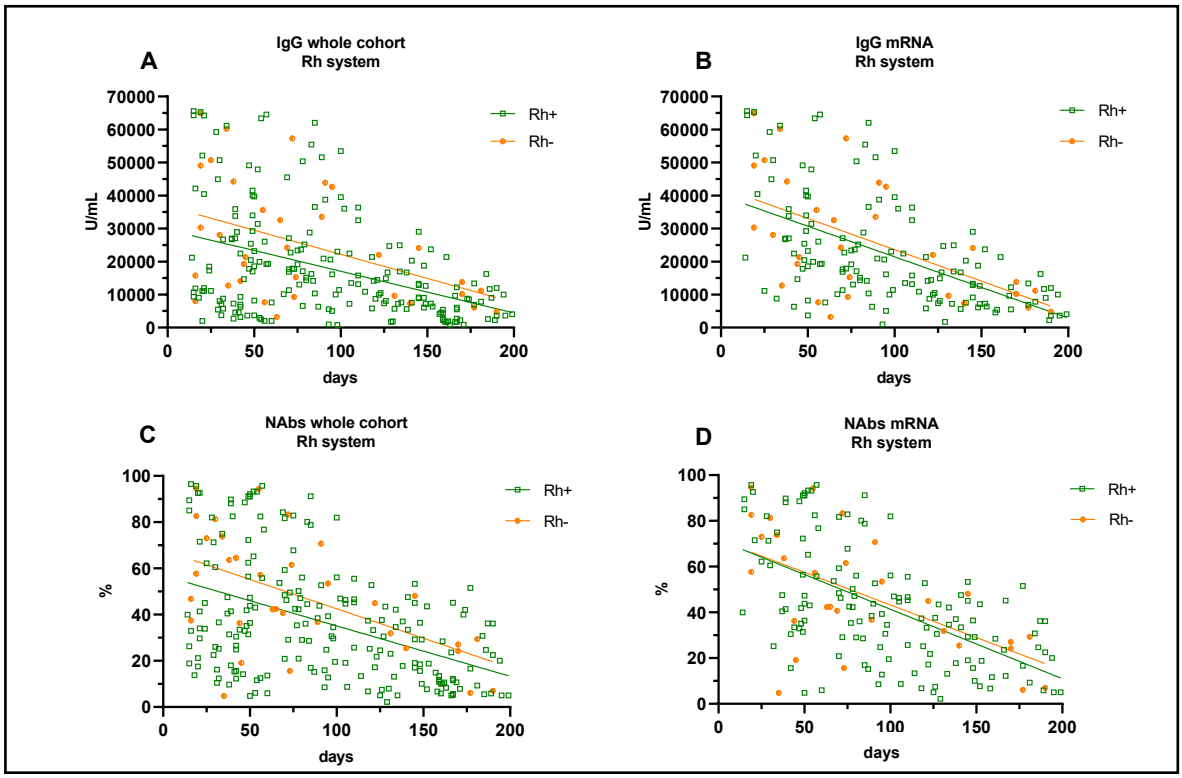


Figure 28. Scatter plots and regression analyses of IgG and NAbs levels stratified by Rh system. (A) IgG and **(B)** NAbs kinetics in the whole cohort. **(C)** IgG and **(D)** NAbs kinetics in the mRNA-based vaccine. Each panel shows the specific regression lines, according to the indicated genotype.

Table 20. Regression analysis and genotype comparison.

Rh system				
	Whole cohort (n=233)		**mRNA (n=166)**	
	Rh +	**Rh -**	**Rh +**	**Rh -**
Subjects – n (%)	200 (85.84)	33 (14.16)	136 (81.93)	30 (18.07)
IgG – U/mL				
r^2	0.1742	0.2048	0.3125	0.3274
Slope	- 125.89	- 145.91	- 185.36	- 189.54
Intercept	29639.46	36790.73	39977.14	42535.15
***P* comparison** Rh+ vs Rh-	**0.02**		ns	
NAbs - %				
r^2	0.2029	0.3088	0.3357	0.3536
Slope	-0.22	-0.25	-0.30	-0.28
Intercept	56.91	67.94	71.72	71.74
***P* comparison** Rh+ vs Rh-	**0.03**		ns	

P comparison indicates the statistical assessment between the intercepts obtained from regression analysis. In bold significant *p*-value ($p < 0.05$). ns indicates not significant.

4.3.2. MEAN OF IgG AND NAbs LEVELS STRATIFIED BY GENOTYPES

The results obtained by the regression analysis performed on the distribution of antibodies according to specific genetic variants, were appreciable and consistent. For this reason, we specifically analysed the mean antibody levels (IgG and NAbs) as a function of the same genetic variants, both in the whole cohort and in the subgroup of mRNA-based vaccine subjects. The same analyses were performed for subgroup of mRNA-based vaccine stratified by sex.
In the next tables will be also shown the data on mean antibody levels in the three different genotypic conditions, the different genetic models, and the main *p*-values.

***ABO* (rs657152)**. TT-genotype yielded significant higher mean of IgG levels when compared with the counterpart GG-genotype only in the whole cohort (TT: 25758.39 U/mL and GG: 18766.87 U/mL, *p*= 0.04). Also comparing the TT-genotype with the GG- coupled with GT- genotypes (Recessive model: TT vs GG+GT) we obtained significant results (Whole cohort, TT: 25758.39 U/mL and GG+GT: 18280.74 U/mL, *p*= 0.02). While in the remaining subgroups, borderline or not significant results were obtained (Table 21).

Table 21. IgG and NAbs levels stratified by vaccine formulation and genotypes.

		ABO (rs657152)							*p*-values		
		GG	**GT**	**TT**	**GG**	**GT + TT**	**TT**	**GG + GT**	***P₁***	***P₂***	***P₃***
Whole cohort (n=233)	**n (%)**	95 (40.77)	116 (49.79)	22 (9.44)	95 (40.77)	138 (59.23)	22 (40.77)	210 (40.77)			
	IgG										
	Mean ± SD	18766.87 ± 16765.86	17882.61 ± 15715.85	25758.39 ± 17737.33	18766.87 ± 16765.86	19138.17 ± 16245.73	25758.39 ± 17737.33	18280.74 ± 16163.93	**0.04**	ns	**0.02**
	NAbs										
	Mean ± SD	37.78 ± 25.08	38.10 ± 26.60	41.07 ± 26.25	37.78 ± 25.08	38.57 ± 26.47	41.07 ± 26.25	37.95 ± 25.87	ns	ns	ns
mRNA vaccine (n= 166)	**n (%)**	64 (38.55)	82 (49.40)	20 (12.05)	64 (38.55)	102 (61.45)	20 (12.05)	146 (87.95)			
	IgG										
	Mean ± SD	23183.26 ± 16806.74	21808.72 ± 16210.09	26628.14 ± 18143.90	23183.26 ± 16806.74	22753.71 ± 16624.09	26628.14 ± 18143.90	22411.26 ± 16431.14	ns	ns	0.14
	NAbs										
	Mean ± SD	43.02 ± 24.56	44.02 ± 26.67	42.12 ± 27.29	43.02 ± 24.56	43.65 ± 26.67	42.12 ± 27.29	43.58 ± 25.69	ns	ns	ns
Females mRNA vaccine (n= 104)	**n (%)**	42 (40.38)	50 (48.08)	12 (11.54)	42 (40.38)	62 (59.61)	12 (11.54)	92 (88.46)			
	IgG										
	Mean ± SD	22992.06 ± 16541.71	22022.68 ± 15616.36	28956.35 ± 20291.54	22992.06 ± 16541.71	23364.68 ± 16666.51	28956.35 ± 20291.54	22465.23 ± 15963.51	0.15	ns	0.10
	NAbs										
	Mean ± SD	41.89 ± 24.31	47.21 ± 25.09	43.30 ± 29.45	41.89 ± 24.31	46.45 ± 25.77	43.30 ± 29.45	44.78 ± 24.75	ns	0.18	ns
Males mRNA vaccine (n= 62)	**n (%)**	22 (35.48)	32 (51.61)	8 (12.90)	22 (35.48)	40 (64.52)	8 (12.90)	54 (87.10)			
	IgG										
	Mean ± SD	23548.27 ± 17690.69	21474.40 ± 17347.13	23135.83 ± 14942.69	23548.27 ± 17690.69	21806.69 ± 16724.98	23135.83 ± 14942.69	22319.31 ± 17351.45	ns	ns	ns
	NAbs										
	Mean ± SD	45.19 ± 25.45	39.03 ± 28.68	40.36 ± 25.55	45.19 ± 25.45	39.30 ± 27.77	40.36 ± 25.55	41.54 ± 27.33	ns	ns	ns

P₁ indicates *p-value* of extreme genotype, *P₂* indicates *p-value* of dominant model and *P₃* indicates *p-value* of recessive model. In bold significant *p*-value ($p < 0.05$). ns indicates not significant.

***TP53* (rs1042522).** GG-genotype yielded significant lower mean of IgG levels when compared with the counterpart CC-genotype in the whole cohort (GG: 11847.67U/mL and CC: 19074.61 U/mL, $p = 0.01$), in the subgroups of mRNA-based vaccine (GG: 14924.00 U/mL and CC: 23057.86 U/mL, $p = 0.03$) as well as in females mRNA-based vaccine (GG: 13110.43 U/mL and CC: 24841.33 U/mL, $p = 0.01$). Significant results were also obtained in the same subgroups comparing the GG-genotype with the CC-coupled with CG-genotypes (Recessive model: GG vs CC+CG) (Whole cohort, GG: 11847.67 U/mL and CC+CG: 20001.66 U/mL, $p = 0.01$; mRNA-based vaccine, GG: 14924.00 U/mL and CC+CG: 23952.73 U/mL, $p = 0.01$; females mRNA-based vaccine, GG: 13110.43 U/mL and CC+CG: 24785.90 U/mL, $p = 0.01$). Instead in NAbs levels comparison, GG-genotype yielded significant lower mean NAbs levels compared with the counterpart CC-genotype only in the whole cohort (GG: 28.16 % and CC: 39.56 %, $p = 0.01$). In the recessive model of Nabs analysis, we obtained significant results in both the whole cohort (GG: 18.58 % and

CC+CG: 26.46 %, *p*= 0.01) and in subgroups of mRNA-based vaccine (GG: 33.58 % and CC+CG: 44.68 %, *p*= 0.04). While in the remaining subgroups, borderline or not significant results were obtained (Table 22).

Table 22. IgG and NAbs levels stratified by vaccine formulation and genotypes.

		***TP53* (rs1042522)**							***p*-values**		
		CC	**CG**	**GG**	**CC**	**GG + CG**	**GG**	**CC + CG**	P_1	P_2	P_3
Whole cohort (n=233)	**n (%)**	122 (52.36)	82 (35.19)	29 (12.45)	122 (52.36)	111 (47.64)	29 (12.45)	204 (87.55)			
	IgG										
	Mean ± SD	19704.61 ± 17183.87	20535.72 ± 16797.66	11847.67 ± 8586.09	19704.61 ± 17183.87	18197.82 ± 15587.48	11847.67 ± 8586.09	20001.66 ± 17024.44	**0.01**	ns	**0.01**
	NAbs										
	Mean ± SD	39.56 ± 25.69	40.36 ± 27.93	28.16 ± 18.58	39.56 ± 25.69	36.81 ± 26.09	28.16 ± 18.58	39.68 ± 26.46	**0.01**	ns	**0.01**
mRNA vaccine (n= 166)	**n (%)**	90 (54.22)	57 (34.33)	19 (11.45)	90 (54.22)	76 (45.78)	19 (11.45)	147 (88.55)			
	IgG										
	Mean ± SD	23057.86 ± 17519.76	25365.67 ± 16607.79	14924.00 ± 8800.66	23057.86 ± 17519.76	22755.25 ± 15660.33	14924.00 ± 8800.66	23952.73 ± 17151.53	**0.03**	ns	**0.01**
	NAbs										
	Mean ± SD	43.30 ± 25.93	46.85 ± 26.77	33.58 ± 20.21	43.30 ± 25.93	43.53 ± 25.82	33.58 ± 20.21	44.68 ± 26.23	0.06	ns	**0.04**
Females mRNA vaccine (n= 104)	**n (%)**	55 (52.88)	35 (33.65)	14 (13.46)	55 (52.88)	49 (47.12)	14 (13.46)	90 (86.54)			
	IgG										
	Mean ± SD	24841.33 ± 17179.63	24698.80 ± 16846.74	13110.43 ± 8445.37	24841.33 ± 17179.63	21387.84 ± 15758.43	13110.43 ± 8445.37	24785.90 ± 16955.86	**0.01**	0.14	**0.01**
	NAbs										
	Mean ± SD	45.80 ± 23.36	46.61 ± 28.66	34.93 ± 22.15	45.80 ± 23.36	43.27 ± 27.26	34.93 ± 22.15	46.11 ± 25.39	0.06	ns	0.06
Males mRNA vaccine (n= 62)	**n (%)**	35 (56.45)	22 (35.48)	5 (8.07)	35 (56.45)	27 (43.55)	5 (8.07)	57 (91.93)			
	IgG										
	Mean ± SD	20255.28 ± 17929.98	26426.59 ± 16555.65	20001.98 ± 8540.32	20255.28 ± 17929.98	25236.85 ± 15461.85	20001.98 ± 8540.32	22637.19 ± 17525.91	ns	0.13	ns
	NAbs										
	Mean ± SD	39.36 ± 29.46	47.24 ± 24.12	29.81 ± 14.80	39.36 ± 29.46	44.01 ± 23.48	29.81 ± 14.80	42.40 ± 27.57	ns	ns	0.15

P_1 indicates *p-value* of extreme genotype, P_2 indicates *p-value* of dominant model and P_3 indicates *p-value* of recessive model. In bold significant *p*-value ($p< 0.05$). ns indicates not significant.

***APOE* (rs7412/rs429358).** ε4-carrying haplotypes (i.e., ε2ε4 or ε3ε4) yielded significant lower mean of NAbs levels when compared with counterpart haplotypes (i.e., ε2ε3+ε3ε3), in the whole cohort (ε4: 30.62 % and ε3: 39.64 %, *p*= 0.03) and in the subgroup of mRNA-based vaccine (ε4: 31.93 % and ε3: 45.25 %, *p*= 0.01) as well as in males mRNA-based vaccine (ε4: 27.74 % and ε3: 43.71 %, *p*= 0.05). Conversely, mean of IgG levels yielded borderline or not significant results, remaining higher the mean values ascribed to ε3-carriers (Table 23).

Table 23. IgG and NAbs levels stratified by vaccine formulation and genotypes.

		***APOE* (rs7412/rs429358)**		
		ε3	**ε4**	***p*-values**
Whole cohort (n=233)	**n (%)**	197 (84.55)	36 (15.45)	
	IgG			
	Mean ± SD	19586.36 ± 16948.22	15705.72 ± 12906.59	0.09
	NAbs			
	Mean ± SD	39.64 ± 25.86	30.62 ± 24.83	**0.03**
mRNA vaccine (n= 166)	**n (%)**	143 (86.14)	23 (13.85)	
	IgG			
	Mean ± SD	23538.02 ± 17142.91	19072.58 ± 1 2772.65	0.12
	NAbs			
	Mean ± SD	45.25 ± 25.87	31.93 ± 22.65	**0.01**
Females mRNA vaccine (n= 104)	**n (%)**	89 (85.58)	15 (14.42)	
	IgG			
	Mean ± SD	24205.55 ± 17136.74	17332.20 ± 11074.88	0.07
	NAbs			
	Mean ± SD	45.76 ± 24.51	37.76 ± 28.81	0.13
Males mRNA vaccine (n= 62)	**n (%)**	53 (85.48)	9 (14.52)	
	IgG			
	Mean ± SD	22542.14 ± 17404.66	21732.88 ± 14863.97	ns
	NAbs			
	Mean ± SD	43.71 ± 27.99	27.74 ± 13.59	**0.05**

In bold significant *p*-value (*p*< 0.05). ns indicates not significant.

***ACE* (rs1799752).** DD-genotype yielded significant higher mean of NAbs levels when compared with the counterpart II-genotype in the whole cohort (DD: 40.05 % and II: 29.82 %, *p*= 0.03), and in the subgroups of mRNA-based vaccine (DD: 43.69 % and II: 33.99 %, *p*= 0.05). Also comparing the II-genotype with the DD-coupled with ID genotypes (Recessive model: II vs DD+ID) we obtained significant results (Whole cohort, II: 29.82 % % and DD+ID: 39.59 %, *p*= 0.02; mRNA-based vaccine, II: 33.99 % and DD+ID: 45.00 %, *p*= 0.03). In females mRNA-based vaccine subgroup we obtained the significance only in recessive model (II: 32.38 % and DD+ID: 47.17 %, *p*= 0.01) While in the remaining subgroups, borderline or not significant results were obtained (Table 24).

Table 24. IgG and NAbs levels stratified by vaccine formulation and genotypes.

		ACE (rs1799752)							*p*-values		
		DD	**ID**	**II**	**DD**	**II+ ID**	**II**	**DD+ID**	***P*$_1$**	***P*$_2$**	***P*$_3$**
Whole cohort (n=233)	n (%)	86 (36.91)	115 (49.36)	32 (13.73)	86 (36.91)	147 (63.09)	32 (13.73)	201 (86.27)			
	IgG										
	Mean ± SD	20923.26 ± 16950.89	18431.60 ± 16120.80	15777.69 ± 15914.11	20923.26 ± 16950.89	17853.87 ± 16059.33	15777.69 ± 15914.11	19497.69 ± 16485.61	0.07	0.08	0.11
	NAbs										
	Mean ± SD	40.05 ± 26.59	39.25 ± 26.03	29.82 ± 22.13	40.05 ± 26.59	37.20 ± 25.46	29.82 ± 22.13	39.59 ± 26.21	**0.03**	ns	**0.02**
mRNA vaccine (n= 166)	n (%)	69 (41.57)	73 (43.97)	24 (14.46)	69 (41.57)	97 (58.43)	24 (10.30)	142 (60.94)			
	IgG										
	Mean ± SD	23640.62 ± 16424.11	23532.85 ± 16750.72	18979.41 ± 17110.64	23640.62 ± 16424.11	22406.22 ± 16866.67	18979.41 ± 17110.64	23585.22 ± 16534.03	0.12	ns	0.11
	NAbs										
	Mean ± SD	43.69 ± 26.66	46.23 ± 25.35	33.99 ± 23.37	43.69 ± 26.66	43.20 ± 25.32	33.99 ± 23.37	45.00 ± 25.93	**0.05**	ns	**0.03**
Females mRNA vaccine (n= 104)	n (%)	36 (34.61)	50 (48.08)	18 (17.31)	36 (34.61)	68 (65.38)	18 (17.31)	86 (82.69)			
	IgG										
	Mean ± SD	23281.46 ± 16405.23	25200.68 ± 16261.68	17561.71 ± 17178.12	23281.46 ± 16405.23	23178.60 ± 16727.17	17561.71 ± 17178.12	24397.28 ± 16253.28	0.12	ns	0.06
	NAbs										
	Mean ± SD	42.66 ± 27.10	50.42 ± 22.98	32.38 ± 23.26	42.66 ± 27.10	45.64 ± 24.24	32.38 ± 23.26	47.17 ± 24.93	0.09	ns	**0.01**
Males mRNA vaccine (n= 62)	n (%)	33 (53.23)	23 (37.10)	6 (9.67)	33 (53.23)	29 (46.77)	6 (9.67)	56 (90.32)			
	IgG										
	Mean ± SD	24032.44 ± 16689.98	19907.13 ± 17585.03	23232.53 ± 17734.70	24032.44 ± 16689.98	20595.14 ± 17349.71	23232.53 ± 17734.70	22338.11 ± 17028.08	ns	ns	ns
	NAbs										
	Mean ± SD	44.82 ± 26.54	37.14 ± 28.29	38.82 ± 25.22	44.82 ± 26.54	37.49 ± 27.26	38.82 ± 25.22	41.66 ± 27.29	ns	0.14	ns

P$_1$ indicates *p-value* of extreme genotype, *P*$_2$ indicates *p-value* of dominant model and *P*$_3$ indicates *p-value* of recessive model. In bold significant *p*-value (*p*< 0.05). ns indicates not significant.

***ACE2* (rs2285666).** G/GG genotypes yielded lower mean of IgG and NAbs levels when compared with the opposite respective genotypes (i.e. A-carriers) in the mRNA-based vaccine subgroup (IgG, G/GG: 21118.34 U/mL and A-carriers: 27346.72 U/mL, *p*=0.03; NAbs, G/GG: 40.97 % and A-carriers: 49.38 %, *p*=0.01) and in males mRNA-based vaccine subgroup(IgG, G/GG: 19671.42 U/mL and A-carriers: 36741.6 U/mL, *p*=0.001; NAbs, G/GG: 36.69 % and A-carriers: 65.83 %, *p*=0.0006). However, borderline or not significant results were obtained in the whole cohort and in female subgroup for both antibody types, (Table 25).

Table 25. IgG and NAbs levels stratified by vaccine formulation and genotypes.

		ACE2 (rs2285666)		
		G/ GG	**A- carriers**	***p*-values**
Whole cohort (n=233)	**n (%)**	165 (70.82)	68 (29.18)	
	IgG			
	Mean ± SD	17915.38 ± 15786.17	21586.51 ± 17733.45	0.06
	NAbs			
	Mean ± SD	36.82 ± 26.00	41.71 ± 25.38	0.09
mRNA vaccine (n= 166)	**n (%)**	118 (71.08)	48 (28.92)	
	IgG			
	Mean ± SD	21118.34 ± 15854.87	27346.72 ± 17853.02	**0.01**
	NAbs			
	Mean ± SD	40.97 ± 25.80	49.38 ± 25.09	**0.03**
Females mRNA vaccine (n= 104)	**n (%)**	66 (63.46)	38 (36.54)	
	IgG			
	Mean ± SD	22258.34 ± 16489.10	24874.38 ± 16707.70	ns
	NAbs			
	Mean ± SD	44.35 ± 26.43	45.05 ± 23.18	ns
Males mRNA vaccine (n= 62)	**n (%)**	52 (83.87)	10 (16.13)	
	IgG			
	Mean ± SD	19671.42 ± 15044.77	36741.6 ± 19824.13	**0.001**
	NAbs			
	Mean ± SD	36.69 ± 24.56	65.83 ± 26.44	**0.0006**

In bold significant *p*-value ($p < 0.05$). ns indicates not significant.

***CFH* (rs1061170).** TT- genotype produced not significant different mean of IgG and NAbs levels compared with the counterpart CC-genotype in the whole cohort and in all subgroups. For this reason, none genetic models investigated yielded significant differences in any group (Table 26).

Table 26. IgG and NAbs levels stratified by vaccine formulation and genotypes.

		CFH (rs1061170)							*p*-values		
		TT	**TC**	**CC**	**TT**	**CC + TC**	**CC**	**TT + TC**	***P*$_1$**	***P*$_2$**	***P*$_3$**
Whole cohort (n=233)	**n (%)**	81 (34.76)	133 (57.08)	19 (8.16)	81 (34.76)	152 (65.24)	19 (8.16)	214 (91.84)			
	IgG										
	Mean ± SD	19044.52 ± 15446.83	18680.58 ± 17047.72	20884.07 ± 16760.02	19044.52 ± 15446.83	18956.01 ± 16972.78	20884.07 ± 16760.02	18812.33 ± 16424.17	ns	ns	ns
	NAbs										
	Mean ± SD	38.41 ± 25.15	37.77 ± 25.82	40.91 ± 30.16	38.41 ± 25.15	38.16 ± 26.16	40.91 ± 30.16	38.01 ± 25.51	ns	ns	ns
mRNA vaccine (n= 166)	**n (%)**	60 (36.14)	91 (54.82)	15 (9.04)	60 (36.14)	106 (63.86)	15 (9.04)	151 (90.96)			
	IgG										
	Mean ± SD	22920.47 ± 15842.73	22644.35 ± 17307.91	24582.83 ± 16684.90	22920.47 ± 15842.73	22918.67 ± 17156.57	24582.83 ± 16684.90	22754.07 ± 16687.72	ns	ns	ns
	NAbs										
	Mean ± SD	43.79 ± 25.20	42.63 ± 25.60	46.60 ± 30.70	43.79 ± 25.20	43.19 ± 26.26	46.60 ± 30.70	43.09 ± 25.36	ns	ns	ns
Females mRNA vaccine (n= 104)	**n (%)**	36 (34.62)	55 (52.88)	13 (12.50)	36 (34.62)	68 (65.38)	13 (12.50)	91 (87.50)			
	IgG										
	Mean ± SD	22458.64 ± 15638.85	23043.89 ± 17164.95	26027.1 ± 17250.48	22458.64 ± 15638.85	23614.21 ± 17092.74	26027.1 ± 17250.48	22812.36 ± 16491.70	ns	ns	ns
	NAbs										
	Mean ± SD	41.32 ± 22.43	45.31 ± 25.70	50.72 ± 30.42	41.32 ± 22.43	46.34 ± 26.51	50.72 ± 30.42	43.73 ± 24.41	0.12	0.16	0.17
Males mRNA vaccine (n= 62)	**n (%)**	24 (38.71)	36 (58.06)	2 (3.23)	24 (38.71)	38 (61.29)	2 (3.23)	60 (96.77)			
	IgG										
	Mean ± SD	23613.22 ± 16457.14	22033.95 ± 17750.83	15195.04 ± 11093.98	23613.22 ± 16457.14	21674.10 ± 17429.33	15195.04 ± 11093.98	22665.66 ± 17120.41	ns	ns	ns
	NAbs										
	Mean ± SD	47.48 ± 28.96	38.53 ± 25.26	19.80 ± 20.82	47.48 ± 28.96	37.54 ± 25.16	19.80 ± 20.82	42.11 ± 26.93	0.1	0.08	0.13

P$_1$ indicates *p-value* of extreme genotype, *P*$_2$ indicates *p-value* of dominant model and *P*$_3$ indicates *p-value* of recessive model. In bold significant *p*-value ($p< 0.05$). ns indicates not significant.

***CRP* (rs2808635/rs876538)** Common double homozygotes (TT/CC) produced not significant different mean of IgG and NAbs levels compared those with more than one polymorphic allele in at least one of the two loci (i.e., GG rs2808635 plus any combination of rs876538 and TT rs876538 plus any combination of rs2808635) in the whole cohort and in all subgroups. For this reason, none genetic models investigated yielded significant differences in any group (Table 27).

Table 27. IgG and NAbs levels stratified by vaccine formulation and genotypes.

		***CRP* (rs2808635/rs876538)**		
		TT/CC	**POL**	***p*-values**
Whole cohort (n=233)	**n (%)**	111 (88.10)	15 (11.90)	
	IgG			
	Mean ± SD	17776.52 ± 14484.60	19301.67 ± 16861.16	ns
	NAbs			
	Mean ± SD	37.40 ± 24.34	33.25 ± 19.38	ns
mRNA vaccine (n= 94)	**n (%)**	80 (85.11)	14 (14.89)	
	IgG			
	Mean ± SD	20413.2 ± 14577.73	20544.43 ± 16769.58	ns
	NAbs			
	Mean ± SD	40.80 ± 24.22	35.06 ± 18.75	ns
Females mRNA vaccine (n= 58)	**n (%)**	51 (87.93)	7 (12.07)	
	IgG			
	Mean ± SD	20462.07 ± 14567.29	24128.36 ± 17808.00	ns
	NAbs			
	Mean ± SD	42.49 ± 23.37	46.09 ± 17.71	ns
Males mRNA vaccine (n=36)	**n (%)**	29 (80.56)	7 (19.44)	
	IgG			
	Mean ± SD	20327.24 ± 14853.65	16960.51 ± 16192.97	ns
	NAbs			
	Mean ± SD	37.83 ± 25.79	24.03 ± 12.81	ns

ns indicates not significant.

***HLA-A* (rs2571381/rs2499)** Common double homozygotes (i.e. CC/GG) produced not significant different mean of IgG and NAbs levels compared those carrying at least two variant alleles (i.e. CT/GT, TT/GG, CC/TT, CT/TT and TT/GT) in the whole cohort and in all subgroups.

In the male subgroup, double-homozygotes (i.e., CC/GG), showed higher mean levels of NAbs when compared with the other genotypes (CC/GG: 45.07 % and CT/GT+CT/TT: 30.58 %, *p*= 0.04). While in the remaining subgroups, borderline or not significant results were obtained (Table 28).

Table 28. IgG and NAbs levels stratified by vaccine formulation and genotypes.

		***HLA* (rs2571381/rs2499)**		
		CC/GG	**CT/GT + CT/TT**	***p*-values**
Whole cohort (n=233)	**n (%)**	175 (81.78)	39 (18.22)	
	IgG			
	Mean ± SD	19020.11 ± 16379.54	17170.24 ± 15644.75	ns
	NAbs			
	Mean ± SD	38.06 ± 26.71	36.86 ± 24.34	ns
mRNA vaccine (n=152)	**n (%)**	122 (80.26)	30 (19.74)	
	IgG			
	Mean ± SD	23507.27 ± 16772.65	18780.47 ± 14145.11	ns
	NAbs			
	Mean ± SD	43.90 ± 26.92	40.57 ± 22.87	ns
Females mRNA vaccine (n= 91)	**n (%)**	74 (81.32)	17 (18.68)	
	IgG			
	Mean ± SD	23240.83 ± 16817.39	19106.27 ± 12064.49	0.17
	NAbs			
	Mean ± SD	43.14 ± 26.59	48.20 ± 22.58	ns
Males mRNA vaccine (n=61)	**n (%)**	48 (78.69)	13 (21.31)	
	IgG			
	Mean ± SD	23918.03 ± 16872.74	18354.43 ± 17003.55	0.14
	NAbs			
	Mean ± SD	45.07 ± 27.66	30.58 ± 19.85	**0.04**

In bold significant *p*-value (*p*< 0.05). ns indicates not significant.

LZTFL1 **(rs35044562)** and **(rs11385942).** In both SNPs, comparing AA-genotype (rs35044562) or -/- -genotype (rs11385942) with respective counterpart (GG, rs35044562 and TT, rs11385942), borderline and not significant statistically differences were observed in IgG and NAbs levels in all groups. Instead, statistical significance was observed in the dominant model for NAbs mean levels in the whole cohort (rs35044562, AA: 36.99% and GG+AG: 45.13 %, *p*= 0.04; rs11385942, -/-: 37.02 % and TT+T/-: 44.74 %, *p*= 0.05) and in the subgroup of females vaccinated with mRNA-based vaccine (rs35044562, AA: 41.82 % and GG+AG: 55.00 %, *p*= 0.01; rs11385942, -/-: 41.96 % and TT+T/-: 53.94 %, *p*= 0.02). While in the remaining subgroups, borderline or not significant results were obtained (Tables 29-30).

Table 29. IgG and NAbs levels stratified by vaccine formulation and genotypes.

		***LZTFL1* (rs35044562)**							***p*-values**		
		AA	**AG**	**GG**	**AA**	**AG + GG**	**GG**	**AG + AA**	P_1	P_2	P_3
Whole cohort (n=233)	n (%)	197 (84.55)	32 (13.73)	4 (1.72)	197 (84.55)	36 (15.45)	4 (1.72)	229 (98.28)			
	IgG										
	Mean ± SD	18969.66 ± 16570.96	19537.78 ± 16189.87	15422.00 ± 13917.67	18969.66 ± 16570.96	19080.46 ± 15826.56	15422.00 ± 13917.67	19049.05 ± 16484.35	ns	ns	ns
	NAbs										
	Mean ± SD	36.99 ± 25.26	45.78 ± 28.29	39.93 ± 32.18	36.99 ± 25.26	45.13 ± 28.31	39.93 ± 32.18	38.22 ± 25.82	ns	**0.04**	ns
mRNA vaccine (n= 166)	n (%)	137 (82.54)	27 (16.26)	2 (1.20)	137 (82.54)	29 (17.47)	2 (1.20)	164 (98.80)			
	IgG										
	Mean ± SD	23268.00 ± 16893.49	21072.52 ± 15817.10	23966.16 ± 16922.71	23268.00 ± 16893.49	21272.08 ± 15591.52	23966.16 ± 16922.71	22906.55 ± 16694.01	ns	ns	ns
	NAbs										
	Mean ± SD	42.17 ± 25.57	48.78 ± 25.97	55.32 ± 46.41	42.17 ± 25.57	49.23 ± 26.58	55.32 ± 46.41	43.26 ± 25.67	ns	ns	ns
Females mRNA vaccine (n= 104)	n (%)	82 (78.85)	20 (19.23)	2 (1.92)	82 (78.85)	22 (21.15)	2 (1.92)	102 (98.08)			
	IgG										
	Mean ± SD	23055.73 ± 16577.59	23788.76 ± 17183.31	23966.16 ± 16922.71	23055.73 ± 16577.59	23804.89 ± 16756.65	23966.16 ± 16922.71	23199.46 ± 16614.09	ns	ns	ns
	NAbs										
	Mean ± SD	41.82 ± 24.27	54.97 ± 25.55	55.33 ± 46.42	41.82 ± 24.27	55.00 ± 26.33	55.33 ± 46.42	44.40 ± 24.96	ns	**0.01**	ns
Males mRNA vaccine (n= 62)	n (%)	55 (88.71)	7 (11.29)	0	-	-	-	-			
	IgG										
	Mean ± SD	23584.49 ± 17503.29	13311.83 ± 7365.34	-	-	-	-	-	0.07		
	NAbs										
	Mean ± SD	42.70 ± 27.61	31.12 ± 17.09	-	-	-	-	-	0.14		

P_1 indicates *p-value* of extreme genotype, P_2 indicates *p-value* of dominant model and P_3 indicates *p-value* of recessive model. In bold significant *p*-value (*p*< 0.05). ns indicates not significant.

Table 30. IgG and NAbs levels stratified by vaccine formulation and genotypes.

		LZTFL1 (rs11385942)							*p*-values		
		-/-	T/-	TT	-/-	T/- + TT	TT	-/- + T/-	P_1	P_2	P_3
Whole cohort (n=233)	**n (%)**	196 (84.12)	33 (14.16)	4 (1.72)	196 (84.12)	37 (15.88)	4 (1.72)	229 (98.28)			
	IgG										
	Mean ± SD	18983.48 ± 16612.26	19438.46 ± 15945.10	15422.00 ± 13917.67	18983.48 ± 16612.26	19004.25 ± 15612.08	15422.00 ± 13917.67	19049.05 ± 16484.35	ns	ns	ns
	NAbs										
	Mean ± SD	37.02 ± 25.33	45.33 ± 27.97	39.93 ± 32.18	37.02 ± 25.33	44.74 ± 28.01	39.93 ± 32.18	38.22 ± 25.82	ns	**0.05**	ns
mRNA vaccine (n= 166)	**n (%)**	136 (81.93)	28 (16.87)	2 (1.20)	136 (81.93)	30 (18.07)	2 (1.20)	164 (98.80)			
	IgG										
	Mean ± SD	23319.53 ± 16945.13	20900.65 ± 15548.05	23966.16 ± 16922.71	23319.53 ± 16945.13	21105.02 ± 15347.64	23966.16 ± 16922.71	22906.55 ± 16694.01	ns	ns	ns
	NAbs										
	Mean ± SD	42.26 ± 25.64	48.14 ± 25.72	55.33 ± 46.42	42.26 ± 25.64	48.62 ± 26.33	55.33 ± 46.42	43.26 ± 25.67	ns	0.11	ns
Females mRNA vaccine (n= 104)	**n (%)**	81 (77.88)	21 (20.19)	2 (1.92)	81 (77.88)	23 (22.12)	2 (1.92)	102 (98.08)			
	IgG										
	Mean ± SD	23139.62 ± 16663.35	23430.26 ± 16828.60	23966.16 ± 16922.71	23139.62 ± 16663.35	23476.86 ± 16446.80	23966.16 ± 16922.71	23199.46 ± 16614.09	ns	ns	ns
	NAbs										
	Mean ± SD	41.96 ± 24.39	53.81 ± 25.46	55.32 ± 46.42	41.96 ± 24.39	53.94 ± 26.22	55.32 ± 46.42	44.40 ± 24.96	ns	**0.02**	ns
Males mRNA vaccine (n= 62)	**n (%)**	55 (88.7)	7 (11.3)	0 (0)	-	-	-	-			
	IgG										
	Mean ± SD	23584.49 ± 17503.29	13311.83 ± 7365.34	-	-	-	-	-	0.07	-	-
	NAbs										
	Mean ± SD	42.70 ± 27.61	31.12 ± 19.09	-	-	-	-	-	0.14	-	-

P_1 indicates *p-value* of extreme genotype, P_2 indicates *p-value* of dominant model and P_3 indicates *p-value* of recessive model. In bold significant *p*-value ($p< 0.05$). ns indicates not significant.

***OAS3* (rs10735079).** AA-genotype produced not significant different mean of IgG and NAbs levels compared with the counterpart GG-genotype in the whole cohort and in all subgroups. For this reason, none genetic models investigated yielded significant differences in any group (Table 31).

Table 31. IgG and NAbs levels stratified by vaccine formulation and genotypes.

		***OAS3* (rs10735079)**							***p*-values**		
		AA	**AG**	**GG**	**AA**	**GG + AG**	**GG**	**AA + AG**	P_1	P_2	P_3
Whole cohort (n=233)	**n (%)**	105 (45.06)	98 (42.06)	30 (12.88)	105 (45.06)	128 (54.93)	30 (12.88)	203 (87.12)			
	IgG										
	Mean ± SD	17738.05 ± 16175.26	19510.51 ± 15943.21	21646.50 ± 18888.64	17738.05 ± 16175.26	20011.14 ± 16619.80	21646.50 ± 18888.64	18593.72 ± 16044.17	0.13	0.14	0.17
	NAbs										
	Mean ± SD	35.99 ± 25.75	40.25 ± 26.33	39.62 ± 24.88	35.99 ± 25.75	40.10 ± 25.90	39.62 ± 24.88	38.05 ± 26.06	ns	0.11	ns
mRNA vaccine (n= 166)	**n (%)**	74 (44.58)	69 (41.57)	23 (13.85)	23 (13.85)	143 (86.14)	74 (44.58)	92 (55.42)			
	IgG										
	Mean ± SD	21649.38 ± 16712.27	23350.85 ± 15575.70	25710.61 ± 19676.69	25710.61 ± 19676.69	23940.79 ± 16611.53	21649.38 ± 16712.27	22470.37 ± 16139.63	0.17	0.19	0.19
	NAbs										
	Mean ± SD	41.76 ± 25.88	45.41 ± 25.84	42.68 ± 26.18	42.68 ± 26.18	44.73 ± 25.81	41.76 ± 25.88	43.52 ± 25.83	ns	ns	ns
Females mRNA vaccine (n= 104)	**n (%)**	47 (45.19)	40 (38.46)	17 (16.35)	47 (45.19)	57 (54.81)	17 (16.35)	87 (83.65)			
	IgG										
	Mean ± SD	21518.69 ± 15196.94	24518.41 ± 16941.04	24833.07 ± 19529.01	21518.69 ± 15196.94	24613.26 ± 17574.46	24833.07 ± 19529.01	22897.87 ± 15998.17	ns	ns	ns
	NAbs										
	Mean ± SD	44.16 ± 24.32	46.56 ± 26.58	41.13 ± 25.23	44.16 ± 24.32	44.98 ± 26.07	41.13 ± 25.23	45.26 ± 25.26	ns	ns	ns
Males mRNA vaccine (n= 62)	**n (%)**	27 (43.55)	29 (46.77)	6 (9.68)	27 (43.55)	35 (56.45)	6 (9.68)	56 (90.32)			
	IgG										
	Mean ± SD	21876.88 ± 19377.96	21740.41 ± 13593.95	28196.97 ± 21750.79	21876.88 ± 19377.96	22847.25 ± 15094.80	28196.97 ± 21750.79	21806.21 ± 16480.11	ns	ns	ns
	NAbs										
	Mean ± SD	37.60 ± 28.39	43.82 ± 25.16	46.70 ± 30.86	37.60 ± 28.39	44.31 ± 25.74	46.70 ± 30.86	40.82 ± 26.70	ns	0.18	ns

P_1 indicates *p-value* of extreme genotype, P_2 indicates *p-value* of dominant model and P_3 indicates *p-value* of recessive model. In bold significant *p*-value ($p < 0.05$). ns indicates not significant.

ABO blood group and **Rh system.** In blood groups analysis, B-blood group yielded significant higher mean of IgG levels when compared with No-B blood groups in the whole cohort (B-group: 25416.44 U/mL and No B-group: 18000.05 U/mL, *p*= 0.01) and in all subgroups (mRNA-based vaccine, B-group: 27734.48 U/mL and No B-group: 21984.00 U/mL, *p*= 0.05; males mRNA-based vaccine, B-group: 316950.1 U/mL and No B-group: 21051.29 U/mL, *p*= 0.05), except for the female mRNA-based vaccine subgroup. We did not obtain statistical differences in the mean of levels NAbs analyses (Table 32).

Regarding the analysis of Rh system, Rh negative subjects (Rh-) yielded significant higher mean of antibody levels when compared with Rh positive subjects (Rh+), only in the whole cohort (IgG, Rh-: 25250.80 U/mL and Rh+: 17953.22 U/mL, *p*< 0.009; NAbs, Rh-: 47.82% and Rh+: 36.67%, *p*= 0.01) ascribing to the remaining comparisons borderline and not significant results (Table 33).

Table 32. IgG and NAbs levels stratified by vaccine formulation and genotypes.

		ABO BLOOD GROUPS		
		NO B-group	**B-group**	***p*-value**
Whole cohort (n=233)	**n (%)**	202 (86.7)	31 (13.3)	
	IgG			
	Mean ± SD	18000.05 ± 15861.50	25416.44 ± 18744.67	**0.01**
	NAbs			
	Mean ± SD	37.47 ± 26.05	43.30 ± 24.41	0.12
mRNA vaccine (n= 166)	**n (%)**	139 (83.7)	27 (16.3)	
	IgG			
	Mean ± SD	21984.00 ± 16109.66	27734.48 ± 18763.24	**0.05**
	NAbs			
	Mean ± SD	42.86 ± 26.18	46.22 ± 24.02	ns
Females mRNA vaccine (n= 104)	**n (%)**	85 (81.7)	19 (18.3)	
	IgG			
	Mean ± SD	22576.55 ± 15758.73	26066.88 ± 19884.12	ns
	NAbs			
	Mean ± SD	44.67 ± 25.21	44.31 ± 25.69	ns
Males mRNA vaccine (n= 62)	**n (%)**	54 (87.1)	8 (12.9)	
	IgG			
	Mean ± SD	21051.29 ± 16753.89	31695.01 ± 16293.75	**0.05**
	NAbs			
	Mean ± SD	40.00 ± 27.637	50.76 ± 20.31	0.15

Table 33. IgG and NAbs levels stratified by vaccine formulation and genotypes.

		Rh SYSTEM		
		Rh+	**Rh-**	***p*-value**
Whole cohort (n=233)	**n (%)**	200 (85.84)	33 (14.16)	
	IgG			
	Mean ± SD	17953.22 ± 16004.78	25250.80 ± 17770.41	**0.009**
	NAbs			
	Mean ± SD	36.67 ± 25.68	47.82 ± 25.24	**0.01**
mRNA vaccine (n= 166)	**n (%)**	136 (81.93)	30 (18.07)	
	IgG			
	Mean ± SD	22126.61 ± 16260.83	26512.90 ± 18143.32	0.09
	NAbs			
	Mean ± SD	42.47 ± 25.71	47.64 ± 26.26	0.16
Females mRNA vaccine (n= 104)	**n (%)**	86 (82.69)	18 (17.31)	
	IgG			
	Mean ± SD	19581.30 ± 16502.28	24544.53 ± 17058.50	ns
	NAbs			
	Mean ± SD	39.57 ± 25.94	47.26 ± 21.58	ns
Males mRNA vaccine (n= 62)	**n (%)**	50 (80.65)	12 (19.35)	
	IgG			
	Mean ± SD	15658.22 ± 15927.94	26209.32 ± 20227.15	0.08
	NAbs			
	Mean ± SD	32.58 ± 25.32	48.59 ± 33.13	0.18

In bold significant *p*-value (*p*< 0.05). ns indicates not significant.

4.3.3. DYNAMIC DISTRIBUTION OF GENOTYPE, ALLELE OR HAPLOTYPE FREQUENCY STRATIFIED BY ANTIBODY LEVELS

Finally, in this analysis, we computed how the genotype, haplotype, or allele frequency of the investigated gene variants stratified above and below the trend lines of the IgG or NAbs distribution in the whole cohort, in the mRNA vaccine subgroup (further stratified by sex) and also tested for possible deviation from the expected Hardy–Weinberg equilibrium.
Based on the results of the previous analyses, we decided to include in this one only those genes that reported statistical significance comparisons.

***ABO* (rs657152).** The three genotypes were differently distributed in the area above and below the trend line of the IgG levels in the whole cohort (p= 0.03). Also comparing the homozygous TT-genotype, it clustered in the area above the trend line, accordingly TT-genotype (p= 0.007) were overrepresented when compared with the rest of genotypes (i.e., GG+GT) (Table 34). We did not find any statistical significances in the other subgroups analysed.
Moreover, borderline or not significant results were obtained in any subgroups of NAbs (Table 35).

Table 34. Genotype, allele and genetic models frequency of the gene variant in the area above and below the trend lines of the IgG distribution.

Gene		*ABO* (rs657152)									*p*-values			
		GG	GT	TT	GG	TT+GT	TT	GG+GT	G-Allele	T-Allele	P_1	P_2	P_3	P_4
Whole cohort (n= 233)	n (%)													
	High	38 (39.2)	44 (45.4)	15 (15.4)	38 (39.2)	59 (60.8)	15 (15.4)	82 (84.6)	120 (61.9)	74 (38.1)	**0.03**	0.14	ns	**0.007**
	Low	57 (41.9)	72 (52.9)	7 (5.2)	57 (41.9)	79 (58.1)	7 (5.2)	129 (94.8)	186 (68.4)	86 (31.6)				
mRNA (n= 166)	n (%)													
	High	31 (42.5)	31 (42.5)	11 (15.0)	31 (42.5)	42 (57.5)	11 (15.0)	62 (85.0)	93 (63.7)	53 (36.3)	ns	ns	ns	ns
	Low	33 (35.5)	51 (54.8)	9 (9.7)	33 (35.5)	60 (64.5)	9 (9.7)	84 (90.3)	117 (62.9)	69 (37.1)				
Females mRNA vaccine (n= 104)	n (%)													
	High	20 (44.4)	18 (40.0)	7 (15.6)	20 (44.4)	25 (55.6)	7 (15.6)	38 (84.4)	58 (64.4)	32 (35.6)	ns	ns	ns	ns
	Low	22 (37.3)	32 (54.2)	5 (8.5)	22 (37.3)	37 (62.7)	5 (8.5)	54 (91.5)	76 (64.4)	42 (35.6)				
Males mRNA vaccine (n= 62)	n (%)													
	High	11 (42.3)	11 (42.3)	4 (15.4)	11 (42.3)	15 (57.7)	4 (15.4)	22 (84.6)	33 (63.5)	19 (35.5)	ns	ns	ns	ns
	Low	11 (30.6)	21 (58.3)	4 (11.1)	11 (30.6)	25 (69.4)	4 (11.1)	32 (88.9)	43 (59.7)	29 (40.3)				

P_1 indicates *p-value* of genotypes, P_2 indicates *p-value* of alleles, P_3 indicates *p-value* of dominant model and P_4 indicates *p-value* of recessive model. In bold significant *p*-values ($p< 0.05$). ns indicates not significant.

Table 35. Genotype, allele and genetic models frequency of the gene variant in the area above and below the trend lines of the NAbs distribution.

Gene		*ABO* (rs657152)									*p*-values			
		GG	GT	TT	GG	TT+GT	TT	GG+GT	G-Allele	T-Allele	P_1	P_2	P_3	P_4
Whole cohort (n= 233)	n (%)													
	High	40 (37.0)	54 (50.0)	14 (13.0)	40 (37.0)	68 (63.0)	14 (13.0)	94 (87.0)	134 (62.0)	82 (38.0)	ns	0.12	ns	0.09
	Low	55 (44.0)	62 (49.6)	8 (6.4)	55 (44.0)	70 (56.0)	8 (6.4)	117 (93.6)	172 (68.8)	78 (31.2)				
mRNA (n= 166)	n (%)													
	High	31 (37.3)	41 (49.4)	11 (13.3)	31 (37.3)	52 (62.79)	11 (13.3)	72 (86.7)	103 (62.0)	63 (38.0)	ns	ns	n	ns
	Low	33 (39.8)	41 (49.4)	9 (10.8)	33 (39.8)	50 (60.2)	9 (10.8)	74 (89.2)	107 (64.5)	59 (35.5)				
Females mRNA vaccine (n= 104)	n (%)													
	High	21 (36.2)	29 (50.0)	8 (13.8)	21 (36.2)	37 (63.8)	8 (13.8)	50 (86.2)	71 (61.2)	45 (38.8)	ns	ns	ns	ns
	Low	21 (45.7)	21 (45.7)	4 (8.6)	21 (45.7)	25 (54.3)	4 (8.6)	42 (91.4)	63 (68.5)	29 (31.5)				
Males mRNA vaccine (n= 62)	n (%)													
	High	11 (39.29)	13 (46.43)	4 (14.28)	11 (39.29)	17 (60.71)	4 (14.28)	24 (85.72)	35 (62.50)	21 (37.50)	ns	ns	ns	ns
	Low	11 (32.35)	19 (55.88)	4 (11.77)	11 (32.35)	23 (67.65)	4 (11.77)	30 (88.23)	41 (60.29)	27 (39.71)				

P_1 indicates *p-value* of genotypes, P_2 indicates *p-value* of alleles, P_3 indicates *p-value* of dominant model and P_4 indicates *p-value* of recessive model. In bold significant *p*-values ($p< 0.05$). ns indicates not significant.

***TP53* (rs1042522).** The three genotypes were differently distributed in the area above and below the trend line of NAbs levels in the whole cohort (*p*= 0.02). The homozygous GG-genotype (p= 0.01) as well as G-allele (*p*= 0.05) clustered in the area below the trend line and were significantly overrepresented when compared with the rest of genotypes (i.e., CC+CG) and the counterpart C-allele, respectively (Table 37). In contrast, in IgG distribution, the significance was observed only when GG-genotype was compared with the rest of genotypes (i.e., CC+CG) in the mRNA-based vaccine subgroup (*p*= 0.03) (Table 36).

Table 36. Genotype, allele and genetic models frequency of the gene variant in the area above and below the trend lines of the IgG distribution.

		TP53 (rs1042522)									*p*-values			
		CC	**CG**	**GG**	**CC**	**GG+CG**	**GG**	**CC+CG**	**C-Allele**	**G-Allele**	***P*$_1$**	***P*$_2$**	***P*$_3$**	***P*$_4$**
Whole cohort (n= 233)	n (%)													
	High	52 (53.6)	36 (37.1)	9 (9.3)	52 (53.6)	45 (46.9)	9 (9.3)	88 (90.7)	140 (72.2)	54 (27.8)	ns	ns	ns	ns
	Low	70 (51.5)	46 (33.8)	20 (14.7)	70 (51.5)	66 (48.5)	20 (14.7)	116 (85.3)	186 (68.4)	86 (31.6)				
mRNA (n= 166)	n (%)													
	High	40 (54.8)	29 (39.7)	4 (5.5)	40 (54.8)	33 (45.2)	4 (5.5)	69 (94.5)	109 (74.6)	37 (25.4)	0.07	ns	ns	**0.03**
	Low	50 (53.8)	28 (30.1)	15 (16.1)	50 (53.8)	43 (46.2)	15 (16.1)	78 (83.9)	128 (68.8)	58 (31.2)				
Females mRNA vaccine (n= 104)	n (%)													
	High	26 (57.8)	15 (33.4)	4 (8.8)	26 (57.8)	19 (42.1)	4 (8.8)	41 (91.1)	67 (74.4)	23 (25.6)	ns	ns	ns	ns
	Low	29 (49.2)	20 (33.9)	10 (16.9)	29 (49.2)	30 (50.8)	10 (16.9)	49 (83.1)	78 (66.1)	40 (33.9)				
Males mRNA vaccine (n= 62)	n (%)													
	High	13 (50.0)	13 (50.0)	0	13 (50.0)	13 (50.0)	0	26 (100.0)	39 (75.0)	13 (25.0)	-	ns	ns	-
	Low	22 (61.1)	9 (25.0)	5 (13.9)	22 (61.1)	14 (38.9)	5 (13.9)	31 (86.1)	53 (73.6)	19 (26.4)				

P$_1$ indicates *p-value* of genotypes, *P*$_2$ indicates *p-value* of alleles, *P*$_3$ indicates *p-value* of dominant model and *P*$_4$ indicates *p-value* of recessive model. In bold significant *p*-values (*p*< 0.05). ns indicates not significant.

Table 37. Genotype, allele and genetic models frequency of the gene variant in the area above and below the trend lines of the NAbs distribution.

		TP53 (rs1042522)									*p*-values			
		CC	**CG**	**GG**	**CC**	**GG+CG**	**GG**	**CC+CG**	**C-Allele**	**G-Allele**	***P*$_1$**	***P*$_2$**	***P*$_3$**	***P*$_4$**
Whole cohort (n= 233)	n (%)													
	High	64 (59.3)	37 (34.3)	7 (6.4)	64 (59.3)	44 (40.7)	7 (6.4)	101 (93.6)	165 (76.4)	51 (23.6)	**0.02**	**0.05**	0.05	**0.01**
	Low	58 (46.4)	45 (36.0)	22 (17.6)	58 (46.4)	67 (53.6)	22 (17.6)	103 (82.4)	161 (64.4)	89 (35.6)				
mRNA (n= 166)	n (%)													
	High	47 (56.6)	29 (34.9)	7 (8.4)	47 (56.6)	36 (43.4)	7 (8.4)	76 (91.6)	123 (74.1)	43 (25.9)	ns	ns	ns	ns
	Low	43 (51.8)	28 (33.7)	12 (14.5)	43 (51.8)	40 (48.2)	12 (14.5)	71 (85.5)	114 (68.7)	52 (31.3)				
Females mRNA vaccine (n= 104)	n (%)													
	High	33 (56.9)	18 (31.0)	7 (12.1)	33 (56.9)	25 (43.1)	7 (12.1)	51 (87.9)	84 (72.4)	32 (27.6)	ns	ns	ns	ns
	Low	22 (47.8)	17 (37.0)	7 (15.2)	22 (47.8)	24 (52.2)	7 (15.2)	39 (84.8)	61 (66.3)	31 (33.7)				
Males mRNA vaccine (n= 62)	n (%)													
	High	17 (60.7)	11 (39.3)	0	17 (60.7)	11 (39.3)	0	28 (100.0)	45 (80.4)	11 (19.6)	-	0.15	ns	-
	Low	18 (52.9)	11 (32.3)	5 (14.7)	18 (52.9)	16 (47.6)	5 (14.7)	29 (85.3)	47 (69.1)	21 (30.9)				

P$_1$ indicates *p-value* of genotypes, *P*$_2$ indicates *p-value* of alleles, *P*$_3$ indicates *p-value* of dominant model and *P*$_4$ indicates *p-value* of recessive model. In bold significant *p*-values ($p< 0.05$). ns indicates not significant.

***APOE* (rs7412/rs429358).** Haplotype distribution gave an overrepresentation of the ε4-carrying haplotypes (i.e., ε2ε4 + ε3ε4) in the area below the trend line of the NAbs levels in the whole cohort (*p*= 0.04), mRNA-based vaccine (*p*= 0.03) and males mRNA-based vaccine (*p*= 0.03) subgroups when compared with the counterpart haplotypes (i.e., ε2ε3+ε3ε3) (Table 39). We did not find any statistical significances in any subgroups of IgG (Table 38).

Table 38. Haplotype frequency of the gene variant in the area above and below the trend lines of the IgG distribution.

		APOE (rs7412/rs429358)		
		ε3	**ε4**	***p*-values**
Whole cohort (n= 233)	n (%)			ns
	High	85 (87.6)	12 (12.4)	
	Low	112 (82.4)	24 (17.6)	
mRNA (n= 166)	n (%)			ns
	High	65 (89.0)	8 (11.0)	
	Low	77 (82.8)	16 (17.2)	
Females mRNA vaccine (n= 104)	n (%)			ns
	High	41 (91.1)	4 (8.9)	
	Low	48 (81.4)	11 (18.6)	
Males mRNA vaccine (n= 62)	n (%)			ns
	High	23 (88.5)	3 (11.5)	
	Low	30 (83.3)	6 (16.7)	

Table 39. Haplotype frequency of the gene variant in the area above and below the trend lines of the NAbs distribution.

		APOE (rs7412/rs429358)		
		ε3	**ε4**	***p*-values**
Whole cohort (n= 233)	n (%)			**0.04**
	High	97 (89.8)	11 (10.2)	
	Low	100 (80.0)	25 (20.0)	
mRNA (n= 166)	n (%)			**0.03**
	High	76 (91.6)	7 (8.4)	
	Low	66 (79.5)	17 (20.5)	
Females mRNA vaccine (n= 104)	n (%)			ns
	High	52 (89.7)	6 (10.3)	
	Low	37 (80.4)	9 (19.6)	
Males mRNA vaccine (n= 62)	n (%)			**0.03**
	High	27 (96.4)	1 (3.6)	
	Low	26 (76.5)	8 (23.5)	

In bold significant *p*-values ($p < 0.05$). ns indicates not significant.

***ACE* (rs1799752).** The three genotypes were differently distributed in the area above and below the trend line of the IgG levels in the females mRNA-based vaccine subgroup (*p*= 0.05). Also comparing the homozygous II-genotype, it clustered in the area below the trend line, accordingly II-genotype (*p*= 0.05) were overrepresented when compared with the remaining genotypes (i.e., DD+ID) (Table 40). We did not find any statistical significances in any subgroups of NAbs (Table 41).

Table 40. Genotype, allele and genetic models frequency of the gene variant in the area above and below the trend lines of the IgG distribution.

		*ACE (*rs1799752)									*p*-values			
		DD	**ID**	**II**	**DD**	**II+ID**	**II**	**DD+ID**	**D-Allele**	**I-Allele**	***P*$_1$**	***P*$_2$**	***P*$_3$**	***P*$_4$**
Whole cohort (n= 233)	n (%)													
	High	38 (39.2)	50 (51.5)	9 (9.3)	38 (39.2)	59 (60.8)	9 (9.3)	88 (90.7)	126 (64.9)	68 (35.1)	ns	ns	ns	0.09
	Low	48 (35.3)	65 (47.8)	23 (16.9)	48 (35.3)	88 (64.7)	23 (16.9)	113 (83.1)	161 (59.2)	111 (40.8)				
mRNA (n= 166)	n (%)													
	High	28 (38.4)	37 (50.7)	8 (10.9)	28 (38.4)	45 (61.6)	8 (10.9)	65 (89.1)	93 (63.7)	53 (36.3)	ns	ns	ns	ns
	Low	41 (44.10)	36 (38.7)	16 (17.2)	41 (44.10)	52 (55.9)	16 (17.2)	77 (82.8)	118 (63.4)	68 (36.6)				
Females mRNA vaccine (n= 104)	n (%)													
	High	14 (31.1)	27 (60.0)	4 (8.9)	14 (31.1)	31 (68.9)	4 (8.9)	41 (91.1)	55 (61.1)	35 (38.9)	**0.05**	ns	ns	**0.05**
	Low	22 (37.3)	23 (39.0)	14 (23.7)	22 (37.3)	37 (62.7)	14 (23.7)	45 (76.3)	67 (56.8)	51 (43.2)				
Males mRNA vaccine (n= 62)	n (%)													
	High	14 (53.8)	8 (30.8)	4 (15.4)	14 (53.8)	12 (46.2)	4 (15.4)	22 (84.6)	36 (69.2)	16 (30.8)	ns	ns	ns	ns
	Low	19 (52.8)	15 (41.7)	2 (5.5)	19 (52.8)	17 (47.2)	2 (5.6)	34 (94.4)	53 (73.6)	19 (26.4)				

P$_1$ indicates *p-value* of genotypes, *P*$_2$ indicates *p-value* of alleles, *P*$_3$ indicates *p-value* of dominant model and *P*$_4$ indicates *p-value* of recessive model. In bold significant *p*-values (*p*< 0.05). ns indicates not significant.

Table 41. Genotype, allele and genetic models frequency of the gene variant in the area above and below the trend lines of the NAbs distribution.

		*ACE (*rs1799752)									*p*-values			
		DD	**ID**	**II**	**DD**	**II+ID**	**II**	**DD+ID**	**D-Allele**	**I-Allele**	***P*$_1$**	***P*$_2$**	***P*$_3$**	***P*$_4$**
Whole cohort (n= 233)	n (%)													
	High	40 (37.0)	57 (52.8)	11 (10.2)	40 (37.0)	68 (63.0)	11 (10.2)	97 (89.8)	137 (63.4)	79 (36.6)	ns	ns	ns	0.14
	Low	46 (36.8)	58 (46.4)	21 (16.8)	46 (36.8)	79 (63.2)	21 (16.8)	104 (83.2)	150 (60.0)	100 (40.0)				
mRNA (n= 166)	n (%)													
	High	33 (39.8)	40 (48.2)	10 (12.0)	33 (39.8)	50 (60.2)	10 (12.0)	73 (88.0)	106 (63.8)	60 (36.2)	ns	ns	ns	ns
	Low	36 (43.4)	33 (39.8)	14 (16.8)	36 (43.4)	47 (56.6)	14 (16.8)	69 (83.2)	105 (63.3)	61 (36.7)				
Females mRNA vaccine (n= 104)	n (%)													
	High	20 (34.5)	31 (53.4)	7 (12.1)	20 (34.5)	38 (65.5)	7 (12.1)	51 (87.9)	71 (61.2)	45 (38.8)	ns	ns	ns	0.11
	Low	16 (34.8)	19 (41.3)	11 (23.9)	16 (34.8)	30 (65.2)	11 (23.9)	35 (76.1)	51 (55.4)	41 (44.6)				
Males mRNA vaccine (n= 62)	n (%)													
	High	16 (57.2)	9 (32.1)	3 (10.7)	16 (57.1)	12 (42.9)	3 (10.7)	25 (89.3)	41 (73.2)	15 (26.8)	ns	ns	ns	ns
	Low	19 (52.8)	15 (41.7)	2 (5.5)	19 (52.8)	17 (47.2)	2 (5.6)	34 (94.4)	53 (73.6)	19 (26.4)				

P$_1$ indicates *p-value* of genotypes, *P*$_2$ indicates *p-value* of alleles, *P*$_3$ indicates *p-value* of dominant model and *P*$_4$ indicates *p-value* of recessive model. In bold significant *p*-values ($p< 0.05$). ns indicates not significant.

***ACE2* (rs2285666).** The G/GG genotypes (i.e., GG-homozygous females and G-hemizygous males) were overrepresented in the area below the trend line of the NAbs levels scattering when compared with the counterpart A-carriers (i.e., AA-homozygous plus to AG-heterozygous females and A-hemizygous males) in the whole cohort (*p*= 0.03), mRNA-based vaccine (*p*= 0.02), and males mRNA-based vaccine (*p*= 0.002) subgroups (Table 43). We observed significant statistical difference only for IgG in males mRNA-based vaccine subgroup (*p*= 0.008) (Table 42).

Table 42. Genotype frequency of the gene variant in the area above and below the trend lines of the IgG distribution.

		ACE2 (rs2285666)		
		G/GG	**A-carriers**	***p*-values**
Whole cohort (n= 233)	n (%)			
	High	65 (67.0)	32 (33.0)	ns
	Low	100 (73.5)	36 (15.5)	
mRNA (n= 166)	n (%)			
	High	48 (65.7)	25 (34.3)	ns
	Low	70 (75.3)	23 (24.7)	
Females mRNA vaccine (n= 104)	n (%)			
	High	28 (62.2)	17 (37.8)	ns
	Low	38 (64.4)	21 (35.6)	
Males mRNA vaccine (n= 62)	n (%)			
	High	18 (69.2)	8 (30.8)	**0.008**
	Low	34 (94.4)	2 (5.6)	

Table 43. Genotype frequency of the gene variant in the area above and below the trend lines of the NAbs distribution.

		ACE2 (rs2285666)		
		G/GG	**A-carriers**	***p*-values**
Whole cohort (n= 233)	n (%)			
	High	69 (63.9)	39 (36.1)	**0.03**
	Low	96 (76.8)	29 (23.2)	
mRNA (n= 166)	n (%)			
	High	52 (62.7)	31 (37.3)	**0.02**
	Low	66 (79.5)	17 (20.5)	
Females mRNA vaccine (n= 104)	n (%)			
	High	36 (62.1)	22 (37.9)	ns
	Low	30 (65.2)	16 (34.8)	
Males mRNA vaccine (n= 62)	n (%)			
	High	19 (67.86)	9 (32.14)	**0.002**
	Low	33 (97.06)	1 (2.94)	

In bold significant *p*-values (*p*< 0.05). ns indicates not significant.

ABO blood group and Rh system. The ABO blood group was differently distributed in the area above and below the trend lines of the IgG levels in the males mRNA-based vaccine subgroup (*p*= 0.04). We did not observe significant differences in the remaining sub-groups in both IgG and NAbs levels (Tables 44 – 45).

Rh system was differently distributed in the area above and below the trend lines of the IgG levels only in the whole cohort (*p*= 0.04) (Tables 46 – 47).

Table 44. ABO frequency in the area above and below the trend lines of the IgG distribution.

		ABO blood group		
		NO B-group	B-group	*p*-values
Whole cohort (n= 233)	n (%)			
	High	80 (82.5)	17 (17.5)	0.11
	Low	122 (89.7)	14 (10.3)	
mRNA (n= 166)	n (%)			
	High	59 (80.8)	14 (19.2)	ns
	Low	80 (86.0)	13 (14.0)	
Females mRNA vaccine (n= 104)	n (%)			
	High	8 (17.8)	37 (82.2)	ns
	Low	11 (18.6)	48 (81.4)	
Males mRNA vaccine (n= 62)	n (%)			
	High	20 (76.9)	6 (23.1)	**0.04**
	Low	34 (94.4)	2 (5.6)	

Table 45. ABO frequency in the area above and below the trend lines of the NAbs distribution.

		ABO blood group		
		NO B-group	B-group	*p*-values
Whole cohort (n= 233)	n (%)			
	High	89 (82.4)	19 (17.6)	0.07
	Low	113 (90.4)	12 (9.6)	
mRNA (n= 166)	n (%)			
	High	67 (80.7)	16 (19.3)	ns
	Low	72 (86.7)	11 (13.3)	
Females mRNA vaccine (n= 104)	n (%)			
	High	48 (82.8)	10 (17.2)	ns
	Low	38 (82.6)	8 (17.4)	
Males mRNA vaccine (n= 62)	n (%)			
	High	23 (82.1)	5 (17.9)	0.14
	Low	32 (94.1)	2 (5.9)	

In bold significant *p*-values ($p < 0.05$). ns indicates not significant.

Table 46. Rh frequency in the area above and below the trend lines of the IgG distribution.

		Rh system		
		Rh +	Rh -	*p*-value
Whole cohort (n= 233)	n (%)			
	High	78 (80.4)	19 (19.6)	**0.04**
	Low	122 (89.7)	14 (10.3)	
mRNA (n= 166)	n (%)			
	High	56 (76.7)	17 (23.3)	0.12
	Low	80 (86.0)	13 (14.0)	
Females mRNA vaccine (n= 104)	n (%)			
	High	35 (77.8)	10 (22.2)	ns
	Low	51 (86.4)	8 (13.6)	
Males mRNA vaccine (n= 62)	n (%)			
	High	19 (73.1)	7 (26.9)	ns
	Low	31 (86.1)	5 (13.9)	

Table 47. Rh frequency in the area above and below the trend lines of the NAbs distribution.

		Rh system		
		Rh +	Rh -	*p*-value
Whole cohort (n= 233)	n (%)			
	High	88 (81.5)	20 (18.5)	0.08
	Low	112 (89.6)	13 (10.4)	
mRNA (n= 166)	n (%)			
	High	66 (79.5)	17 (20.5)	ns
	Low	70 (84.3)	13 (15.7)	
Females mRNA vaccine (n= 104)	n (%)			
	High	47 (81.0)	11 (19.0)	ns
	Low	39 (84.8)	7 (15.2)	
Males mRNA vaccine (n= 62)	n (%)			
	High	21 (75.00)	7 (25.00)	ns
	Low	29 (85.29)	5 (14.71)	

In bold significant *p*-values ($p < 0.05$). ns indicates not significant.

4.3.4. MULTIVARIATE ANALYSES

To complete our statistic investigations, we finally performed a multivariate analysis of data. We first performed a multivariate linear regression considering IgG and Nabs levels as continuous variables. We then calculated the changes (if any) of the circulating antibody levels (IgG and Nabs) in subjects with a specific genotype (i.e. common homozygotes "0"; heterozygotes "1"; rare homozygotes "2"; according to the number of polymorphic alleles) and the different genetic models by comparing subjects with opposite class of genotype for each significant variant investigated. All estimates, were previously adjusted for covariates, i.e., age, sex, vaccine type (mRNA- and AdV-based vaccine), different time points, ABO blood group, and Rh system.

Table 48 shows that *ABO gene* variant (rs657152) TT-genotype compared with the remaining classes of genotype by the recessive model (i.e. TT vs GG+GT) has a statistically significant positive correlation with IgG levels (p= 0.006). Similarly, B-blood group compared with No-B blood groups has the same significant relationship with IgG levels (p< 0.001).

Also NAbs levels showed a significant relationship with B-blood group (p= 0.02). A significant positive correlation was also found between NAbs levels and *APOE* ε3-carrying haplotypes (rs7412/rs429358) compared with the counterpart ε4-carrying haplotypes (p= 0.012).

Table 48. Multivariate linear regression estimating the relationship between each selected gene variants and anti-SARS-CoV-2 circulating antibodies (IgG and NAbs).

Gene variants	IgG levels (U/mL)		NAbs levels (%)	
	Adjusted coeff. (95% CI)	***P***	**Adjusted coeff. (95% CI)**	***p***
ABO (rs657152): TT vs GG+GT	**8275 (2368; 14,183)**	**0.006**	7.10 (-2.00; 16.2)	0.13
TP53 (rs1042522): GG vs CC+CG	-5017 (-10,253; 219.4)	0.06	-7.10 (-15.1; 0.88)	0.08
APOE (rs7412/rs429358): e3 vs e4	4006 (-726.7; 8739)	0.09	**9.19 (2.04; 16.3)**	**0.012**
ACE (rs1799752): II vs DD+ID	-2404 (-7412; 2604)	0.3	-6.69 (-14.3; 0.90)	0.08
ACE2 (rs2285666): G/GG vs A-carriers	-609.3 (-4614; 3395)	0.8	0.97 (-5.13; 7.06)	0.8
Blood type (B-group vs NO-B group) *	**17,564 (8395; 26,734)**	**<0.001**	**17.4 (3.14; 31.6)**	**0.02**
Rh blood group (positive vs negative) **	-2744 (-7795; 2307)	0.3	-5.95 (-13.6; 1.74)	0.13

Coeff.: coefficient; CI: confidence interval.
All models (otherwise differently reported) were adjusted for the following covariates: age, sex, vaccine type (mRNA- and AdV-based vaccine), different time points, ABO blood group, and Rh system.
* Adjusted for: age, sex, vaccine type (mRNA- and AdV-based vaccine), different time points, and Rh system.
** Adjusted for: age, sex, vaccine type (mRNA- and AdV-based vaccine), different time points, ABO blood group.
In bold significant p-values (p< 0.05).

Subsequently, we performed a multivariate logistic regression only for NAbs levels using them as a categorical variable, then considering subjects with a percentage of NAbs >20% as positive. Again, we adjusted the values for covariates, i.e., age, sex, vaccine type (mRNA- and AdV-based vaccine), different time points, ABO blood group, and Rh system. We then estimated the odds of subjects with a specific gene variant achieving NAbs>20% levels.

Table 49, shows that there seems to be a higher probability of having a percentage of NAbs >20% for the *APOE* (rs7412/rs429358) ε3-carrying haplotypes compared with counterpart ε4-carrying haplotypes (p= 0.013), and for B-blood group compared with No-B blood groups (p= 0.04).

Table 49. Multivariate logistic regression estimating the relationship between each selected gene variant and the likelihood of achieving NAbs (expressed as % neutralization) >20%, adjusting for selected potential confounders.

Gene variants	Adjusted Odds Ratio (95% CI)	*p*
ABO (rs657152): TT vs GG+GT	2.28 (0.67-7.75)	0.2
TP53 (rs1042522): GG vs CC+CG	0.69 (0.26-1.85)	0.5
APOE (rs7412/rs429358): e3 vs e4	**3.16 (1.27-7.82)**	**0.013**
ACE (rs1799752): II vs DD+ID	0.51 (0.20-1.27)	0.14
ACE2 (rs2285666): G/GG vs A-carriers	1.10 (0.51-2.37)	0.8
Blood type (B-group vs NO-B group) *	**7.38 (1.14-47.7)**	**0.04**
Rh blood group (positive vs negative) **	0.47 (0.15-1.43)	0.2

CI: confidence interval.
All models (otherwise differently reported) were adjusted for the following covariates: age, sex, vaccine type (mRNA- and AdV-based vaccine), different time points, ABO blood group, and Rh system.
* Adjusted for: age, sex, vaccine type (mRNA- and AdV-based vaccine), different time points, and Rh system.
** Adjusted for: age, sex, vaccine type (mRNA- and AdV-based vaccine), different time points, ABO blood group.
In bold significant p-values (p< 0.05).

5. DISCUSSION

COVID-19 pandemic continues to be a major public health threat. The several number of virus variants identified around the world and the observation of unpredictable extreme clinical and laboratory phenotypes could be explained by the existence of possible combination of hereditary traits and interindividual and population predispositions in terms of demographic peculiar characteristics.

It is largely known by several past studies, that the immune response against a virus is closely related to individual genetic background and different environmental factors in a variety of infectious diseases such as those from hepatitis B virus (HBV) or Epstein-Barr virus (EBV)[149 150].

Moreover, previous studies reported that vaccines may not protect all population or individuals at the same way, due to multiple host- and vaccine-specific factors. The contribution of mutations or polymorphisms in the modulation of innate and adaptive immunity after vaccination was already evident in several studies on the response to measles, rubella, smallpox and influenza vaccines [151 152 149].

Although the genetic basis of clinical variability in response to SARS-CoV-2 infection has currently been widely explored, the host genetic determinants that might be responsible for the different response to the anti-SARS-CoV-2 vaccine remain largely unknown. Better knowledge in this field could help to generate personalized vaccines to optimize the antibody response to each vaccine.

Responses to SARS-CoV-2 infection was so different in the population and among individuals that factors such as age, sex, or comorbidities cannot alone explain this variability. This observation highlight the importance of further investigating host genetics to understand the causes responsible for interindividual differences.

To better understand the genetic causes behind these differences, the COVID-19 Host Genetic Initiative (HGI) consortium has been established [3].

In the studies performed by HGI, has primarily identified novel genes and loci responsible for the different susceptibility and severity characterizing the COVID-19, by evoking molecular and genetics mechanisms that perfectly matched with our previously published and ongoing researches [12 75 91].

In the present study we investigate whether some of these selected genes could be implicated in the observed variability of the individual immune response induced by anti-SARS-CoV-2 vaccine and stratified and disaggregated data and results also by sex [2].

The first result of this study is that the magnitude of the anti-SARS-CoV-2 IgG and NAbs response varies among individuals, vaccine types, time after vaccination and sex also considering wide (i.e. T1 or T2) or small sub-periods of times after vaccination as the first three months or the last three months respectively.

In this context, by dividing the subjects into those whose blood was collected within 90 days (T1) and after 90 days (T2) after the second dose of vaccine, we found that IgG and NAbs levels decrease significantly from 3 months after the second vaccine dose (IgG, $p< 0.0001$ and NAbs $p< 0.0001$), while before 90 days the decrease was not significant (IgG, $p= 0.43$ and NAbs $p= 0.64$). On the contrary, T1 showed the widest difference in levels for both IgGs and NAbs also considering a shorter sub-period (i.e. 30 days). Interestingly, the same analysis did not show significantly differences in the T2 time frame. This is in favour that individual genetics has a stronger role in the initial phases of the vaccine response, less evident in the T2 windows.

We also divided the subjects by vaccine formulation (mRNA-based vaccine and AdV-based vaccine) without considering the distinction between time-points. This result showed a different distribution of antibody levels in the two vaccine formulations, in both IgG and NAbs.

To begin to study individual variability, we firstly performed a sex stratification. The distribution for both IgG and NAbs levels shows that the females tends to have higher antibody levels than males, but the trend of these as a function of time shows not strong statistically significant differences. These results expand what was recently published by our research group coordinated by Prof. Gemmati conducted on a smaller sample of healthy subjects [5].

Subsequently, doing a correlation analysis between the two antibody types levels (IgG and NAbs) in the whole cohort and stratifying by vaccine formulation and sex, we observed a strong positive correlation between IgG and NAbs (Whole cohort, $r^2= 0.809$; mRNA-based vaccine, $r^2= 0.769$; AdV-based vaccine, $r^2= 0.88\ 0$; Females, $r^2= 0.779$; Males $r^2= 0.847$).

By using the same stratification strategy, we evaluated the mean of IgG and NAbs levels, and the results showed that sex does not seem to significantly affect IgG levels, but it seems to play a major role on the neutralizing capacity of NAbs. Regarding the difference in the mean of antibody levels stratified by vaccine formulation, it was found that the mRNA-based vaccine would appear to be responsible for significantly higher production of both IgG and NAbs levels ($p< 0.0001$). Both results would merit further investigation by expanding the number of male and AdV-based vaccine subjects, as they are underrepresented in our cohort.

However, it would be challenging to continue the study in this direction because at the moment the vaccination campaign has gone forward with the administration of additional doses of vaccine, but this might allow in the future to assess the change in antibody levels over longer time frames with respect to additional booster doses of vaccine given to the population. Alternatively, a second round of assessment after a mean period of about 150 days after vaccination could add new insights in the present research considering that after

this time frame no detectable antibodies were detectable in our cohort. This, strategy is however affected by the fact that naïve subjects to vaccine or infection are virtually extremely rare and a completely different approach have to be designed yielding certainly additional informative data and information potentially corroborating and completing our explorative investigation.

As above stated, to further explore the interindividual variability, we focused on studying specific genetic variants that have been identified, from GWAS and HGI studies, as being responsible in changing the clinical phenotype to COVID-19 or as possible influencers of antibody response or negative side effects after SARS-CoV-2 infection [2] [64].

From here to ahead, we will consider a selection of these genetic variants to assess whether they had a significant action in predicting the titre or duration of post-vaccination IgG and NAbs.

Significant differences were found in 5 genes carrying the following gene variants: *ABO* (rs657152), *TP53* (rs1042522), *APOE* (rs7412/rs429358), *ACE* (rs1799752), *ACE2* (rs2285666). Interestingly, significant differences were also found in the phenotypic analysis of blood group and Rh system.

To evaluate possible genetic differences that discriminate the levels of antibodies produced after vaccination, we used three complementary approaches that evaluated trend estimates (regression analysis), mean levels stratified by genotype (Welch's t-test), and genotype or allele frequencies stratified by antibody distribution, including possible deviation from Hardy-Weinberg equilibrium.

These analyses allowed a defined genotype or haplotype to be assigned to predict the titre or duration of post-vaccination IgG and NAbs.

ABO (rs657152) was initially described as mainly responsible for hypercoagulability, arterial embolism/thrombosis and other circulatory system issues [81]. With the onset of COVID-19, the GWAS authors found that the T-allele of this genetic variant was strongly associated with the severity of COVID-19 [64]. In initial contrast to the findings in the disease, in vaccination response evaluation it appears that the TT-genotype is instead responsible for increased production of IgG antibodies, so it may have a possible positive relationship with regard to the increased immune response.

On the other hand, and in agreement with recent GWAS on inflammation determinants [153], authors found novel signals including *IL6* and *ABO* genes (rs657152, $p = 2.13\times10^{-29}$). In detail, some *IL6* SNPs with strong p-values ($p<5\times10^{-8}$) were found to be located in the *ABO* locus on chromosome 9q34.1-q34.2 with the strongest signal at rs643434. This SNP is in strong linkage disequilibrium with another associated variant (rs687289) which tags the O-allele of the *ABO* locus ($r^2=0.931$ in HapMap CEU). Finally, and according to our results, as for the circulating C-reactive protein (CRP) levels positive associations between CRP and immune response after SARS-CoV-2 infection or vaccine

have been recently reported, hypothesizing basal or unresolved inflammation as causative reasons for the best performers in immune response [53] [115] [5] . A next follow-up assessment of the top SNP rs643434 in ABO showed a strong replication of the signal (p= 4.07×10−05) supporting a role for this gene in regulating the levels of IL6 [153].

TP53 (rs1042522) is the most common SNP occurring in the P72R codon (CCC>CGC). The function of p53 as a "guardian of the genome" capable of activating the apoptosis pathway in the event of cellular damage has long been known [88]. At the same time, it appears to play a key role in innate immunity [89]. Recently, an important correlation with COVID-19 has been suggested in that the responsible virus is able to alter p53 function by inhibiting the type I IFN response and at the same time altering NF-kB signalling pathways, activating the expression of inflammatory genes as recently reposted also by our group [90] [91]. Lodhi et. al, have shown that the population carrying the G allele is more susceptible to viral infections because the pro-inflammatory immune response is not as strong as that of the population carrying the C allele [88]. These results are in line with what we observed in this study, which is that GG subjects have lower production of IgG and NAbs antibodies following vaccination. This finding is further enhanced in the subgroup of females vaccinated with mRNA-based vaccine, mainly considering IgG levels.

APOE ε4 haplotype (rs7412/rs429358) has been studied mainly in neurodegenerative diseases, but also for cardiovascular disease, type 2 diabetes, and cerebral vascular disease, which have been identified as comorbidities to the severity of COVID-19 [99] [102] [103]. Some recent studies have since found a positive association between APOE ε4 and severe form of COVID-19. Accordingly, in vitro study demonstrated that Astrocytes and neurons expressing the 4-allele were more vulnerable to SARS-CoV-2 infection than those expressing the 3-allele, inducing an increase of COVID-19 severity [105].

An association between APOE and response to infection has been found before, such as ε4 haplotype increases susceptibility to HIV-1 infection and aggravates the AIDS disease course [104]. In addition, APOE has been shown to modulate important innate immune responses and consequently the inflammatory response. This response is unbalanced in subjects carrying the *APOE* ε4 variant, which induces increased release of pro-inflammatory cytokines, including IL-6, IL-1b, IFN-g and TNFa, the principal responsible for the COVID-19 worse prognosis [107].

In our study, we were able to observe that, in line with what has been described as a function of disease, the ε4 haplotype also seems to play a negative role in immunity given by vaccination; in fact, it seems to correlate with lower production of both IgG and NAbs antibodies. Corroborating what we observed in the subgroup of males vaccinated with mRNA-based vaccine.

ACE (rs1799752) is the most studied SNP as it modulates ACE expression and activity. In general, the D allele is related to diseases associated with RAS activity such as

hypertension, coronary artery disease, stroke and nephropathy [69]. Also in COVID-19, the D/D genotype is responsible for a worse prognosis, with higher mortality rates than the I/I genotype [72]. Contrary to what has been observed in the disease, in the case of antibody response following vaccination, in our study we observed that the I/I genotype seems to be deputed to a lower production of NAbs than the D/D genotype. However, there is no significant association with IgG levels. A further sex stratification, yielded a significant result in the subgroup of females vaccinated with mRNA-based vaccine

ACE2 (rs2285666) is one of the most relevant SNPs in the gene that influences receptor activity and levels; it is a G8790A transition with the GG genotype characterized by a reduction in expression of about 50% compared with the AA genotype [12 73]. ACE2 is the receptor for SARS-CoV-2 entry into the cell by interacting with SARS-CoV-2 RBD proteins. The ACE2 gene is located on the X chromosome, as a result males and females are necessarily characterized by different genotypic architectures that attribute males only the G or A hemizygosity condition compared to females who have the ability to carry AG heterozygosity. This has suggested possible benefits for females against the risk of infection, considering them more protected than males, who are considered the risk sex [71]. Some studies showed that individuals carrying the A allele were more prone to develop the severe form of COVID-19, especially in the male population. After vaccination, carriers of the A-allele seem to have a better outcome in antibody production. In fact, we observed that subjects carrying the A-allele and vaccinated with mRNA-based vaccine produced more IgG and NAbs than G/GG subjects. This significance is further enhanced in the subgroup of males vaccinated with mRNA-based vaccine, contrary to what has been seen so far in the disease.

ABO blood group and Rh system. Studies on ABO blood group and correlation with SARS-CoV-2 infection classify O- blood group as protective against the disease, compared with other blood groups [85]. Regarding antibodies production after infection and/or vaccination, the data in the literature are controversial, in fact some suggest that O-blood group and B-blood group produce lower antibodies than A and AB-blood groups, while other studies suggest that B-blood group is the one associated with more NAbs production than the rest [86 87]. Our data are partially in line with what Bloch et al. demonstrated, as we found that subjects belonging to group B produced higher levels of IgG than the remaining groups, even in mRNA-vaccinated males. However, we could not demonstrate the same result for NAbs. To further investigate the role of blood group in the context of vaccination, we tried to analyse the Rh system. Our results show that Rh negative is responsible for a higher production of IgG and NAbs antibodies.

Finally, as for the other genes considered in the initial study design, they did not yield findings that would suggest their possible involvement in the vaccine-induced antibody

response, or at least the comprehensive number of analysed cases was not enough to disclose additional associations.

On the other hand, interesting are also the results of the multivariate statistical analyses that was performed. Multivariate linear regression showed a significant association between antibody levels (IgG and NAbs) adjusted for covariates (i.e.: age, sex, vaccine type, different time points, ABO blood group and Rh system) with both *ABO* variant (rs657152) and B-blood group. This result is in line with previous analyses of this variant, and we can therefore assume that carriers of the TT-genotype have increased production of IgG levels following vaccination. The same result is also evident for B-blood group compared with No-B blood group, which, however, seems to show a significant increase in both IgG and NAbs levels.

APOE (rs7412/rs429358) ε3-carrying haplotypes, compared with the ε4-carrying haplotypes, shows a significant increase in NAbs levels. This, in line with what has been shown previously for this variant, suggesting that the ε4-carrying haplotype is disadvantaged and exhibits lower NAbs production.

In order to predict the probability that subjects with a specific gene variant may achieve NAbs levels >20%, a multivariate logistic regression was performed for NAbs levels, taking in account a percentage > 20% as cut-off (NAbs levels were set as the categorical variable).

Also from the results of this analysis, it can be hypothesized that *APOE* (rs7412/rs429358) ε3-carrying haplotypes, compared with ε4-carrying haplotypes, produce higher NAbs levels (p= 0.013). The same result was obtained when compared the B-blood group compared with No-B blood group (p= 0.04). This again confirms what has been described in previous analyses for this genetic variant and ABO blood group. In conclusion, we would like to highlight the importance of these results because they may lead us to develop a predictive model of stronger or weaker antibody response in subjects undergoing anti-SARS-CoV-2 vaccination.

This study is still ongoing, and it aims at further retrospectively assess the risk of SARS-CoV-2 infection after complete vaccine dose in symptomatic cases. In this regard, we will enlarge the recruitment number of subjects and send questionnaires to collect data on their health status following anti-SARS-CoV-2 vaccination also including if any vaccine negative side effects. Finally, the recruited data will also be stratified by the genetic variants considered.

6. CONCLUSION

“Variability”, has been the key word in the COVID-19 pandemic. The poor predictable symptoms and the high mutation rate of SARS-CoV-2, creating virus variants, combined together rendered the handling of COVID-19 hard to treat. Altogether, this favoured the pandemic spreading in a very short time causing the wide and extreme phenotypes we observed in the general population around the world strongly suggesting different and targeted treatments for each individuals according to the personalized precision medicine also considering the different sex.

From the beginning, it was shown that COVID-19 was influenced by factors that could affect the evolution of the disease. Sex, age, genetic background, demographic and environmental factors (comorbidities included) were the main variables that determined who was at higher risk of SARS-CoV-2 infection or severe forms of COVID-19. To combat SARS-CoV-2, it was necessary to quickly study and develop vaccines that can stop the pandemic, and fortunately the scientific community succeeded in this objective by producing several vaccine formulations that were administered to the population. However, the result was not exactly what it was expected; in fact, not all the population responded to the vaccination in the same way, and important though limited, number of severe negative side effects have been recorded. For this reason, host genetics could explain both the different responses to vaccines and predict possible negative side effect. However, it must be considered that different candidate vaccines also induce different immune response in terms of circulating antibody titre, and therefore, the vaccine response if influenced by host genetic factors could be tuned in advance according to pharmacogenomics.

Hoping that studying host genetics could help to identify in advance those individuals who are less responsive to specific vaccines, in terms of titre and duration, a prognostic genetic screening campaign might be very useful to improve vaccine efficacy and effects to create and personalize an individual vaccine approach.

Equally important would be to focus on that part of the population to be expected more likely to develop the severe form of COVID-19, such as the elderly and vulnerable individuals in which to prioritize the vaccination. This application should be approached in a translational way should also be applied and extended for all current and future vaccine campaigns.

Certainly, there will exist additional important genetic influences, heritable traits, acquired and concomitant situations that could be responsible for the observed individual variability.

Larger, multicentre future studies may help to better understand the underlying mechanisms that regulate the immune response associated with individual characteristics as well as interindividual genetic variability. This could enable a more efficient and personalized approach to the selection, formulation and planning of vaccination campaigns.

Studying host genetics associated with sex differences, demographic and environmental variables may be the best strategy to identify useful markers in the study of treatment of complex diseases not only by utilizing classical pharmacological treatments but also by employing novel tools as the recently applied mRNA or AdV vaccines. The identification of predictive molecular markers, would yield significant improvements in the translational medical. Prior stratification of patients would lead to a refinement of the effectiveness of treatments and management of the course of disease, and this could shorten the recovery time while greatly improving the patients' quality of life.

The interplay between information from polygenic predictive markers and serological screening stratified by demogeographic information might help to recognize the individual humoral response, also accounting for ethnic and geographical differences.

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