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1 # Immunogenicity of convalescent and vaccinated sera against clinical isolates
2 # of ancestral SARS-CoV-2, beta, delta, and omicron variants

3

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40 **ABSTRACT**

41 The omicron variant of concern (VOC) of SARS-CoV-2 was first reported in November 2021 in
42 Botswana and South Africa. Omicron has evolved multiple mutations within the spike protein
43 and the receptor binding domain (RBD), raising concerns of increased antibody evasion. Here,
44 we isolated infectious omicron from a clinical specimen obtained in Canada. The neutralizing
45 activity of sera from 65 coronavirus disease (COVID-19) vaccine recipients and convalescent
46 individuals against clinical isolates of ancestral SARS-CoV-2, beta, delta, and omicron VOCs
47 was assessed. Convalescent sera from unvaccinated individuals infected by the ancestral virus
48 during the first wave of COVID-19 in Canada (July, 2020) demonstrated reduced neutralization
49 against beta and omicron VOCs. Convalescent sera from unvaccinated individuals infected by
50 the delta variant (May-June, 2021) neutralized omicron to significantly lower levels compared to
51 the delta variant. Sera from individuals that received three doses of the Pfizer or Moderna
52 vaccines demonstrated reduced neutralization of the omicron variant relative to ancestral SARS-
53 CoV-2. Sera from individuals that were naturally infected with ancestral SARS-CoV-2 and
54 subsequently received two doses of the Pfizer vaccine induced significantly higher neutralizing
55 antibody levels against ancestral virus and all VOCs. Importantly, infection alone, either with
56 ancestral SARS-CoV-2 or the delta variant was not sufficient to induce high neutralizing
57 antibody titers against omicron. This data will inform current booster vaccination strategies, and
58 we highlight the need for additional studies to identify longevity of immunity against SARS-
59 CoV-2 and optimal neutralizing antibody levels that are necessary to prevent infection and/or
60 severe COVID-19.

61

62 **INTRODUCTION**

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63 SARS-CoV-2 has continued to evolve since its emergence in December 2019 [1,2]. Variants of
64 SARS-CoV-2 that demonstrate potential for interference with diagnostics, therapies, and vaccine
65 efficacy, along with evidence for increased transmissibility or disease severity are termed
66 variants of concern (VOCs). The most recent VOC, omicron was first reported in November
67 2021 in Botswana and South Africa [3,4]. The omicron variant has evolved multiple mutations
68 within the spike protein and the receptor binding domain (RBD) that raise concerns regarding a
69 possible increased ability to evade pre-existing antibodies, both from prior infection and from
70 vaccination [5]. The omicron variant has demonstrated increased transmission and a higher level of
71 resistance to antibody-mediated neutralization [4,5]. However, little is known about its
72 pathogenicity and whether disease severity is altered in convalescent, vaccinated or unvaccinated
73 individuals. In Canada, long-term care (LTC) residents were prioritized for third vaccine doses
74 against SARS-CoV-2 based on the observation that antibody titers in older adults waned within
75 six months of their second vaccine dose [6,7]. The neutralizing potential of antibodies generated in
76 LTC residents against VOCs, such as delta and omicron after three doses of mRNA vaccines
77 remain unknown. Thus, to better assess the efficacy of antibody-mediated neutralization against
78 ancestral SARS-CoV-2 and VOCs (beta, delta, and omicron) in naturally infected and vaccinated
79 individuals, we collected sera from multiple cohorts and tested their neutralization ability against
80 clinical isolates of ancestral SARS-CoV-2 and VOCs.

81

82 **RESULTS**

83 **Isolation of viruses**

84 Isolates of VOCs used in this study were derived from clinical specimens. Nasopharyngeal
85 swabs were collected from PCR positive patients, and virus isolation was performed on African

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86 Green monkey kidney cells (Vero'76) as previously described [8]. We confirmed the whole
87 genome sequence of the isolates and determined their phylogenetic relationship with other
88 SARS-CoV-2 isolates (Figure 1A and see supplementary Table S1). Beta, delta and omicron
89 isolates used in this study aligned with their expected lineages (Figure 1A). SARS-CoV-2 can
90 rapidly adapt in cell culture and evolve adaptive mutations. We confirmed mutations across the
91 full-length viral genome, including the spike protein for all variants prior to using the viruses in a
92 micro-neutralization assay (Figure 1B and see supplementary Table S2).
93
94 **Neutralization of omicron by convalescent sera from individuals infected with ancestral**
95 **SARS-CoV-2 or the delta variant**
96 To determine the neutralizing titer of sera from individuals that were naturally infected with
97 SARS-CoV-2, we tested convalescent sera from individuals that were infected with ancestral
98 SARS-CoV-2 during the first wave of coronavirus disease (COVID-19) in Canada (July 2020;
99 see supplementary Table S3). Serum samples were collected 1-5 months after the onset of
100 COVID-19 (see supplementary Table S3). Convalescent sera (n=15) from individuals infected
101 with ancestral SARS-CoV-2 during the first wave of COVID-19 in Canada contained
102 significantly lower neutralizing antibodies against both beta (p=0.0058) and omicron (p=0.0019)
103 variants, relative to the ancestral virus (Figure 2A). However, neutralizing antibody titers in these
104 serum samples were not significantly different between ancestral SARS-CoV-2 and the delta
105 variant (p=0.1691; Figure 2A). Next, the neutralizing antibody titers in convalescent sera (n=10)
106 from individuals who were infected with the delta variant in Canada between May and June,
107 2021 were determined (see supplementary Table S3). Serum samples were collected 1-2 months
108 after the date of onset of COVID-19 (see supplementary Table S3). Convalescent delta sera

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109 contained lower levels of neutralizing antibodies against both beta (p=0.0468) and omicron
110 variants (p=0.049) relative to delta variant (Figure 2B), while titers against ancestral SARS-CoV-
111 2 and delta were comparable (p=0.6034; Figure 2B). These data suggest that infection with either
112 ancestral SARS-CoV-2 or the delta variant induces cross-neutralizing antibody with comparable
113 titers against both viruses. However, natural infection with either ancestral SARS-CoV-2 or the
114 delta variant induces significantly lower levels of neutralizing antibodies against both beta and
115 omicron variants.
116
117 **Neutralization of omicron by sera from individuals who received two doses of the Pfizer**
118 **BNT162b2 mRNA vaccine post COVID-19 infection**
119 Our data suggest that natural infection with ancestral SARS-CoV-2 is not sufficient to induce
120 comparable levels of neutralizing antibodies against beta and omicron variants (Figure 2A).
121 Next, we determined levels of neutralizing antibodies in sera (n=10) from individuals who had
122 received two doses of the Pfizer BNT162b2 vaccine after being naturally infected with ancestral
123 SARS-CoV-2 (see supplementary Table S3). Two doses of the Pfizer BNT162b2 vaccine after
124 natural infection led to higher levels of neutralizing antibodies against ancestral SARS-CoV-2
125 that were significantly higher than levels against the beta (p=0.0318) and omicron (p=0.0001)
126 variants (Figure 2C). Infection and subsequent two dose vaccination with Pfizer BNT162b2
127 induced higher levels of neutralizing antibodies against ancestral virus and all VOCs, including
128 omicron than infected only individuals (Figure 2D).
129
130 **Neutralization of omicron by sera from individuals that received one dose of the Pfizer**
131 **BNT162b2 mRNA vaccine**

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132 To determine neutralizing antibody titers in sera from individuals that received one dose of the
133 Pfizer BNT162b2 mRNA vaccine, we collected sera (n=10) one month after the first dose of the
134 vaccine and tested neutralizing antibody titers against ancestral SARS-CoV-2, beta, delta and
135 omicron variants (see supplementary Table S3). Low neutralizing antibody titers against the
136 ancestral virus were detected in some samples; however, no neutralization of the three VOCs
137 was observed, with the exception of one serum sample that had detectable levels of neutralizing
138 antibodies against all VOCs (Figure 3A).
139

140 **Neutralization of omicron by sera from triple vaccinated individuals**

141 Additional booster vaccinations have been deemed critical in protecting us from VOCs in part by
142 inducing higher levels of neutralizing antibodies. Thus, we tested the levels of neutralizing
143 antibodies in sera collected from long-term care residents that received three doses of the Pfizer
144 BNT162b2 (n=10) [9] or the Moderna mRNA-1273 (n=10) [10] vaccines (see supplementary Table
145 S3). For both vaccine recipients, doses 1 and 2 were received 3-4 weeks apart. The third vaccine
146 dose was received ~7 months after dose 2, and serum samples were collected 1 month after the
147 third dose. Sera from individuals that received three doses of the Pfizer BNT162b2 vaccine
148 induced high neutralizing titers against ancestral SARS-CoV-2, but levels of neutralizing
149 antibodies were significantly lower against beta (p=0.0007), delta (p=0.0045) and omicron
150 (p<0.0001) variants, compared to ancestral SARS-CoV-2 (Figure 3B). Sera from individuals that
151 received three doses of the Moderna mRNA-1273 vaccine induced high neutralizing titers
152 against ancestral SARS-CoV-2, but levels of neutralizing antibodies were significantly lower
153 against the omicron variant, relative to ancestral SARS-CoV-2 (p=0.0012; Figure 3C).
154 Neutralizing antibody titers were not significantly different against ancestral SARS-CoV-2, beta

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155 and delta variants. Serum samples from individuals that received 3x doses of the Pfizer
156 BNT162b2 vaccine contained 2.86x, 2.25x and 10.3x lower mean neutralizing antibody titers
157 against beta, delta and omicron VOCs, respectively, relative to ancestral SARS-CoV-2 (Figure
158 4). Serum samples from individuals that received 3x doses of the Moderna mRNA-1273 vaccine
159 contained 1.7x, 1.26x and 3.48x lower mean neutralizing antibody titers against beta, delta and
160 omicron VOCs, respectively, relative to ancestral SARS-CoV-2 (Figure 4).
161

162 **DISCUSSION**

163 The emergence of an yet another SARS-CoV-2 VOC, omicron has led to increasing speculation
164 about the ability of this variant to escape vaccine and natural infection-mediated immunity. The
165 current generation of COVID-19 mRNA vaccines are designed using the *spike* gene sequence of
166 ancestral SARS-CoV-2 [9,10]. The omicron variant has accumulated 29 amino acid substitutions, 3
167 amino-acid deletions and a 3-residue insertion within the spike protein compared to the ancestral
168 SARS-CoV-2 Wuhan isolate [5]. Accumulating data suggest that the omicron variant is at least
169 partially resistant to neutralization by antibodies in vaccinated individuals, along with partial or
170 complete resistance to neutralization by therapeutic monoclonal antibodies [5]. Emerging data
171 demonstrate that T-cell-mediated immunity generated upon infection or vaccination likely
172 remain effective against the omicron variant [11], and an additional booster vaccine dose results in
173 higher levels of antibodies against the omicron variant when tested using pseudotyped viruses [12].
174 Despite these recent advances, considerable gaps currently exist in our knowledge regarding the
175 ability of omicron to cause severe COVID-19 and whether partial or complete escape of vaccine
176 or natural infection-mediated immunity occurs and if escape is age dependent. In addition, it is
177 not known if omicron has altered host range, or if transmissibility is increased and whether there

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178 are changes in cellular tropism. Furthermore, data on neutralizing antibody titers against clinical
179 isolates of omicron are limited. Thus, as part of this study, we determined the levels of
180 neutralizing antibodies in individuals that were naturally infected, infected and subsequently
181 vaccinated, or vaccinated with three doses of mRNA vaccines using clinical isolates of ancestral
182 SARS-CoV-2, beta, delta and omicron variants.

183 When omicron was first detected, multiple laboratories reported difficulties in isolating
184 and generating laboratory stocks of this variant. In this study, we used Vero'76 cells to isolate
185 the omicron variant from a clinical specimen (nasopharyngeal swab) that was collected from a
186 Canadian patient. We also confirmed the whole genome sequences of the omicron variant, along
187 with beta and delta variants (Figure 1 and Table 1). Thus, we report that Vero'76 cells are
188 sufficient to facilitate the isolation and propagation of the omicron variant.

189 Next, we tested the levels of neutralizing antibody titers in convalescent sera against the
190 ancestral virus, beta, delta and omicron variants (Figure 2). Infection with both the ancestral
191 virus and the delta variant induced high levels of neutralizing antibodies against each other.
192 However, the levels of neutralizing antibodies in convalescent sera against the omicron variant
193 were lower compared to both the ancestral virus and the delta variant (Figures 2A and 2B). Thus,
194 our data suggest that infection alone, either with the ancestral virus or the delta variant may not
195 be sufficient to induce high levels of neutralizing antibodies against the omicron variant. Indeed,
196 our data demonstrate that two doses of the Pfizer BNT162b2 mRNA vaccine following infection
197 with ancestral SARS-CoV-2 induced significantly higher levels of neutralizing antibodies against
198 ancestral SARS-CoV-2 and beta, delta and omicron variants (Figure 2D).

199 Our data highlight that one dose of the Pfizer vaccine is not sufficient to induce high
200 levels of neutralizing antibodies against ancestral virus or variants (Figure 3A), thus the second

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201 vaccine doses appears to be critically required to induce neutralizing antibodies. Three doses of
202 either the Pfizer BNT162b2 or Moderna mRNA-1273 vaccine induced comparable neutralizing
203 antibodies against the beta and omicron variants (Figures 3B and 3C). However, levels of
204 neutralizing antibodies against omicron were significantly lower compared to ancestral SARS-
205 CoV-2 in serum samples from individuals vaccinated with 3x doses of either of the mRNA
206 vaccines (Figures 3B, 3C and 4).

207 In summary, our data demonstrate that infection alone, either with ancestral SARS-CoV-
208 2 or the delta variant is not sufficient to induce high levels of neutralizing antibodies against
209 omicron. However, two doses of the Pfizer vaccine in previously infected individuals induces
210 higher levels of neutralizing antibodies. While we did not test the effect of two doses of the
211 Moderna mRNA-1273 vaccine in previously infected and recovered individuals, we speculate
212 that the results will be comparable to the Pfizer BNT162b2 vaccine. Our data also show that
213 while 3x doses of both mRNA vaccines induce neutralizing titers against omicron variant in
214 long-term care residents, the levels of neutralizing antibodies remain significantly lower
215 compared to ancestral SARS-CoV-2. Thus, our data support the ongoing third vaccine dose
216 booster strategy for long-term care residents in Canada. Indeed, there is a need for studies to
217 assess the optimal levels of neutralizing antibodies that are required for protection against
218 infection and/or severe COVID-19, which will inform policies around vaccine boosters and
219 enable equitable distribution of vaccines to end the ongoing pandemic.

220

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233 sequencing. We acknowledge the help of Dr. Akarin Asavajaru who handled shipping and
234 receiving of samples.

235

236 **AUTHOR CONTRIBUTIONS**

237 Conceptualization, A.B., F.M., S.M. and D.F.; Sample Collection and Selection: S.E.S, L.G.,
238 A.X.L., M.M., S.W. and A.C.G; Methodology, A.B., J.L., A.K., K.B., P.A., F.M. and D.F.;
239 Formal analysis, A.B., J.L., A.K. and F.M.; Reagents, J.L., R.K., R.M., A.L., J.L.W., T.M.,
240 A.J.M and S.M.; Funding acquisition, A.B. and D.F.; Writing – reviewing and editing, A.B., J.L.,
241 A.K., F.M., V.G., S.M. and D.F.; All authors reviewed the final manuscript; Supervision, A.B.
242 and D.F.

243

244 **DECLARATION OF INTERESTS**

245 The authors declare no competing interests or conflicts of interest.

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260 **FIGURES AND FIGURE LEGENDS**
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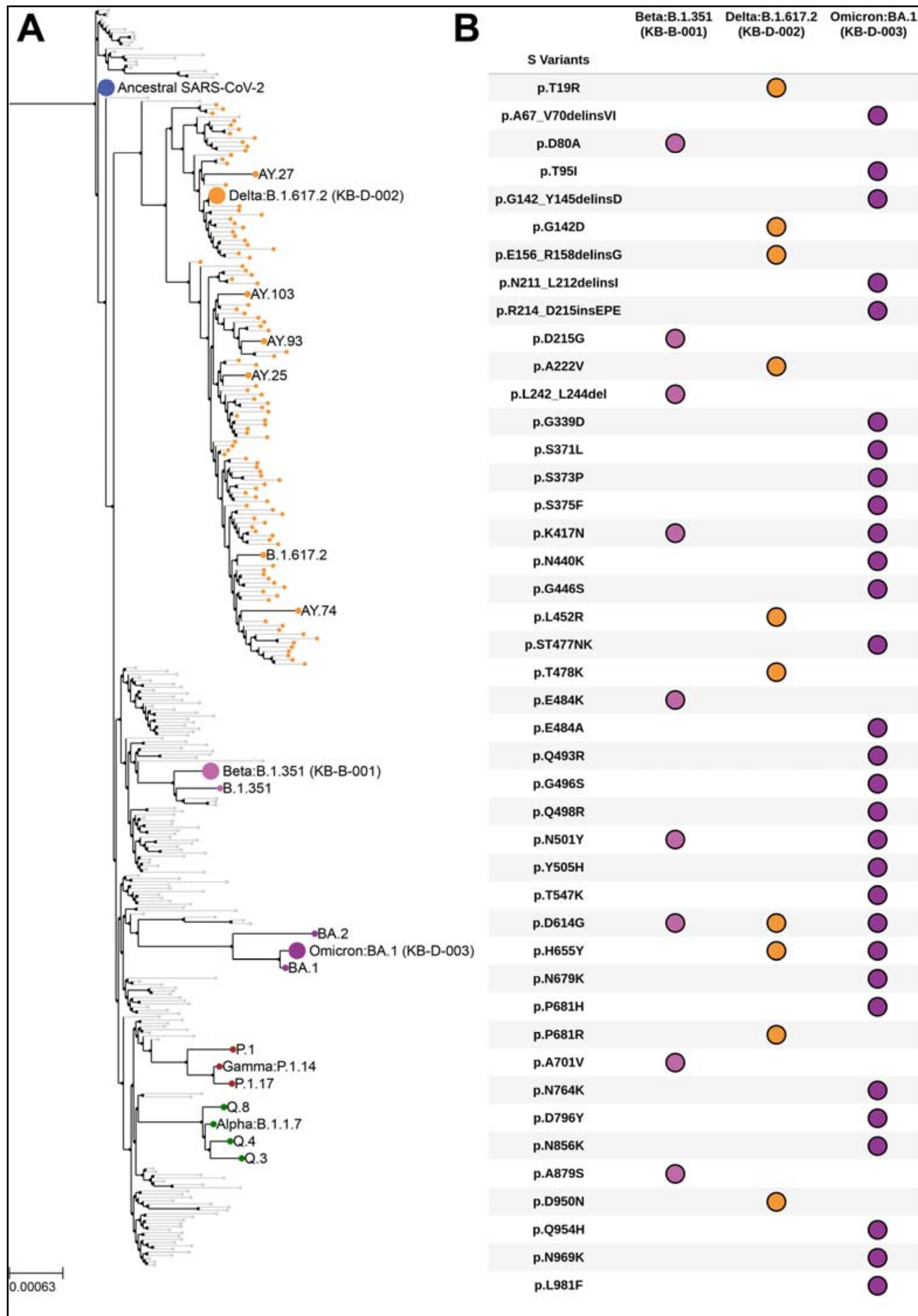

262
263 **Figure 1. Spike mutations and phylogenetic analyses of clinical isolates of VOCs. (A)**
264 Clinical isolates of VOCs used in this study (Delta, B.1.617.2 KB-D-002; Beta, B.1.351 KB-B-
265 001 and Omicron, BA.1 KB-D-003) were sequenced and assigned lineages by phylogeny
266 analysis. **(B)** Mutations within the spike (S) protein of each variant are shown here. Delins,

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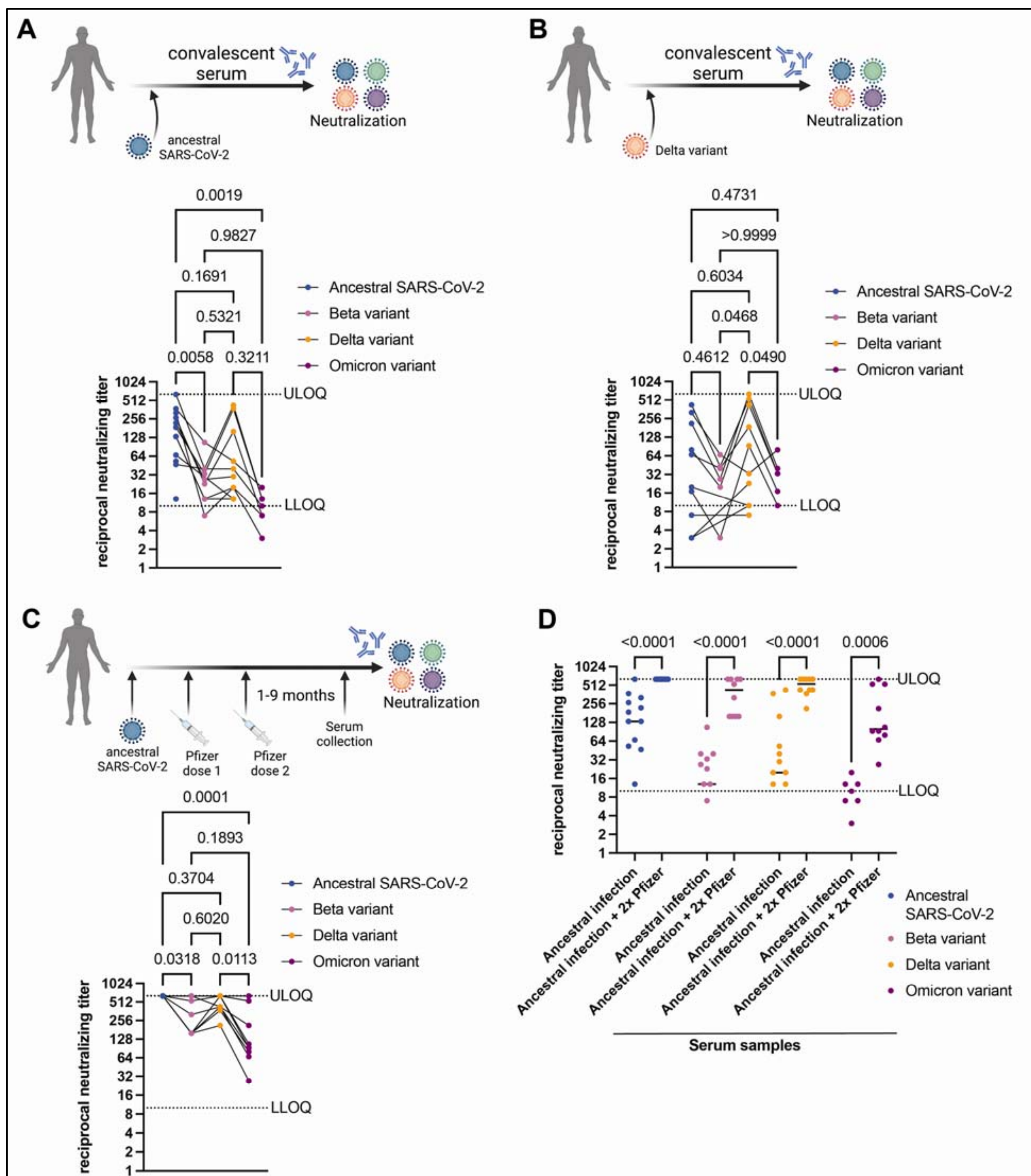
267 deletion+insertion; ins, insertion; p, amino acid position. See also supplementary Tables S1 and
268 S2.
269



270
271 **Figure 2. Detection of neutralizing antibodies in convalescent sera and sera from naturally**
272 **infected individuals who subsequently received two doses of an mRNA vaccine.**

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273 **(A)** Neutralizing antibody titers in convalescent sera collected from individuals that were
274 infected with ancestral SARS-CoV-2 during the first wave in Canada (July 2020; n=15) tested
275 against ancestral SARS-CoV-2, beta, delta and omicron variants.
276 **(B)** Neutralizing antibody titers in convalescent sera collected from individuals that were
277 infected with the delta variant of SARS-CoV-2 in Canada (May-June, 2021; n=10) tested against
278 ancestral SARS-CoV-2, beta, delta and omicron variants.
279 **(C)** Neutralizing antibody titers in sera collected from individuals that were infected with
280 ancestral SARS-CoV-2, followed by two doses of the Pfizer BNT162b2 vaccine tested against
281 ancestral SARS-CoV-2, beta, delta, and omicron variants (n=10). Neutralizing antibody titers
282 against ancestral SARS-CoV-2 reached the upper limit of detection in our assay.
283 **(D)** Neutralizing antibody titers in convalescent sera from individuals infected with ancestral
284 SARS-CoV-2 compared to neutralizing antibody levels in sera from individuals who were
285 infected with the ancestral virus and subsequently received two doses of the Pfizer mRNA
286 vaccine. Data compiled and replotted from panels A and C for comparison. Mean values are
287 indicated by horizontal black bars.
288 Individual data points are shown and titers for matching serum samples are shown across
289 different virus isolates. N = 15 or 10, *p* values are indicated in the figures (Tukey's multiple
290 comparisons test with alpha = 0.05 or Sidak's multiple comparisons test with alpha = 0.05).
291 Samples with neutralizing titer of 0 are not shown. LLOQ, lower limit of quantitation; ULOQ;
292 upper limit of quantitation. See also supplementary Table S3.
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294

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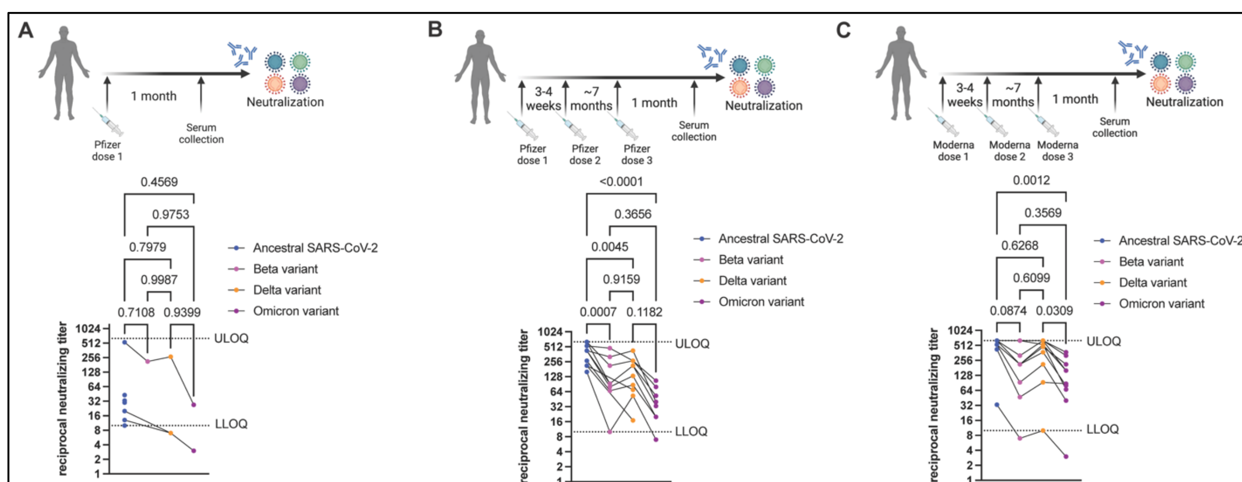

296 **Figure 3. Detection of neutralizing antibodies in sera from vaccinated individuals.**

297 **(A)** Neutralizing antibody titers in sera collected from individuals that received one dose of the
298 Pfizer BNT162b2 vaccine (n=10) tested against ancestral SARS-CoV-2, beta, delta, and omicron
299 variants.

300 **(B)** Neutralizing antibody titers in sera collected from individuals that received three doses of the
301 Pfizer BNT162b2 vaccine (n=10) tested against ancestral SARS-CoV-2, beta, delta, and omicron
302 variants.

303 **(C)** Neutralizing antibody titers in sera collected from individuals that received three doses of the
304 Moderna mRNA-1273 vaccine (n=10) tested against ancestral SARS-CoV-2, beta, delta and
305 omicron variants.

306 Individual data points are shown and titers for matching serum samples are shown across
307 different virus isolates. N = 10, *p* values are indicated in the figures (Tukey's multiple
308 comparisons test with alpha = 0.05). Samples with neutralizing titer of 0 are not shown. LLOQ,
309 lower limit of quantitation; ULOQ; upper limit of quantitation. See also supplementary Table S3.

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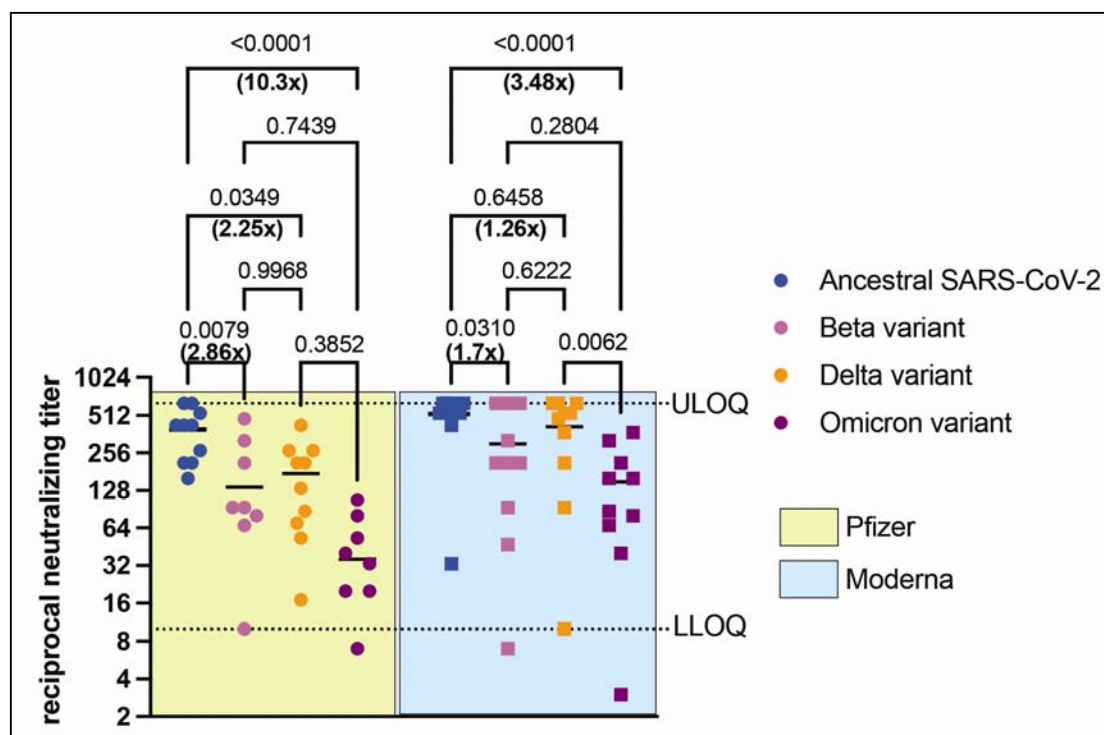

313

314 **Figure 4. Neutralizing antibody levels in individuals vaccinated with 3x doses of the Pfizer**

315 **BNT162b2 or Moderna mRNA-1273 vaccines against ancestral SARS-CoV-2 and VOCs.**

316 To compare neutralizing antibody titers in sera collected from long-term care residents that

317 received three doses of either the Pfizer BNT162b2 or Moderna mRNA-1273 vaccines, we

318 reanalyzed the data in Figure 3. Neutralizing antibody levels against ancestral SARS-CoV-2,

319 beta, delta, and omicron variants are shown here. Mean values are indicated by horizontal black

320 bars.

321 Data are represented as mean, n = 15 or 10, *p* values are indicated in the figure (Sidak's multiple

322 comparisons test with alpha = 0.05). Fold reduction in mean neutralizing antibody titers against

323 VOCs, relative to ancestral SARS-CoV-2 is indicated. Samples with neutralizing titer of 0 are

324 not shown. LLOQ, lower limit of quantitation; ULOQ; upper limit of quantitation. See also

325 supplementary Table S3.

326

327 **SUPPLEMENTARY TABLES**

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328 **Table S1.** Metadata for phylogenetic analysis

329 **Table S2.** Frequency of mutations (percentage read support) across the full genome of clinical

330 isolates of beta, delta and omicron variants used in this study

331 **Table S3.** Serum samples from different cohorts

332

333 **METHODS**

334 **RESOURCE AVAILABILITY**

335 ***Lead Contact***

336 Further information and requests for resources and reagents should be directed to and will be

337 fulfilled by lead contacts, Drs. Darryl Falzarano (Darryl.falzarano@usask.ca) and Arinjay

338 Banerjee (arinjay.banerjee@usask.ca).

339

340 ***Materials availability***

341 This study generated multiple virus isolates. The reagents will be made available on request

342 through institutional Material Transfer Agreements for organizations that have a compliant BSL3

343 laboratory.

344

345 ***Data and code availability***

346 All data and scripts associated with analyses of the virus sequences can be found here:

347 https://github.com/fmaguire/voc_neutralisation_sc2_phylogenomics and DOI

348 10.5281/zenodo.5817727. Full sequences of the viral isolates have been deposited to NCBI

349 BioProject PRJNA794206.

350

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351 **EXPERIMENTAL MODEL**

352 **Cells and viruses.** Vero'76 cells (CRL-1587, ATCC) were used to isolate and/or propagate all
353 virus isolates using a previously published protocol [8]. Briefly, the fluid from PCR-positive
354 nasopharyngeal swabs received from Sunnybrook Research Institute (R.K, S.M. – ancestral
355 SARS-CoV-2), omicron was identified by SPAR-Seq (PMID: 33658502) at the joint MSH/UHN
356 Microbiology clinical diagnostic laboratory (J.L.W., T.M., S.M. – omicron) and beta at the Roy
357 Romanow Provincial Laboratory (A.L., R.M.) were centrifuged at 8000x*g* for 15 minutes and
358 50µl removed and mixed with vDMEM containing 1µg/ml of TPCK trypsin. The mixture was
359 added to a 24 well plate of Vero'76 cells and centrifuged for 1 h at 37°C at 800xg and then
360 placed at 37°C for 30 min. The inoculum was removed and replaced with fresh vDMEM
361 containing 1µg/ml of TPCK trypsin. Cell were monitored daily for cytopathic effect and on day
362 3 or 4, supernatant was passaged to fresh Vero'76 cells in a 6 well plate. Supernatant was
363 subsequently collected on day 3 or 4 and passaged to T175 flasks to generate a p.1 virus stock.
364 Virus stocks were subsequently titered on Vero'76 cells by $TCID_{50}$ assay. Delta was obtained as
365 a virus stock from the National Microbiology Laboratory and used to generate a stock as
366 described.

367 For ancestral SARS-CoV-2, we used SARS-CoV-2/VIDO-1, the sequence for which has
368 been previously reported (>hCoV-19/Canada/ON_ON-VIDO-01-2/2020|EPI_ISL_425177|2020-
369 01-23). All work with infectious SARS-CoV-2 isolates were performed in a containment level 3
370 laboratory at the Vaccine and Infectious Disease Organization using approved protocols. Use of
371 clinical specimen for virus isolation and use of human serum samples for micro-neutralization
372 assays were approved by the University of Saskatchewan's Biomedical Research Ethics Board
373 (REB# 2591).

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374 **Serum samples.** Serum samples were acquired from a series of different cohorts (see
375 supplementary Table S3). Cohort participants provided informed consent for sharing of serum,
376 and studies were approved by the Sunnybrook Research Institute (REB# 149-1994) and/or the
377 Mount Sinai Hospital (REB# 02-0118-U, 20-0339-E, and 21-0069-E) Research Ethics Board [13].
378 For samples from each cohort, samples were selected to have a representative range of anti-spike
379 trimer and anti-RBD antibodies as measured by enzyme-linked immunosorbent assay [14].
380

381 **METHOD DETAILS**

382 **Sequencing and bioinformatic analyses.** cDNA was synthesized from extracted RNA. In brief,
383 4 µL LunaScript RT SuperMix 5X (New England Biolabs, NEB, USA) and 8 µL nuclease free
384 water, were added to 8 µL extracted RNA. cDNA synthesis was performed using the following
385 conditions: 25 °C for 2 min, 55 °C for 20 min, 95 °C for 1 min, and holding at 4 °C.

386 Amplicons were generated from cDNA using ARTIC V4 primer pools
387 (https://github.com/artic-network/artic-ncov2019). Two multiplex PCR tiling reactions were
388 prepared by combining 2.5 µL cDNA with 12.5 µL Q5 High-Fidelity 2X Master Mix (NEB,
389 USA), 6µL nuclease free water, and 4 µL of respective 10 µM ARTIC v4 primer pool
390 (Integrated DNA Technologies). PCR cycling was then performed in the following manner: 98
391 °C for 30 s followed by 35 cycles of 98 °C for 15 s and 63 °C for 5 min.

392 Both PCR reactions were combined and cleaned with 1X ratio Sample Purification Beads
393 (Illumina) at a 1:1 bead to sample ratio. The quantity of amplicons was measured with the Qubit
394 4.0 fluorometer using the 1X dsDNA HS Assay Kit (Thermo Fisher Scientific, USA) and the
395 sequencing libraries were prepared using the Nextera DNA Flex Prep kit (Illumina, USA) as per
396 manufacturer's instructions. Paired-end (2x150 bp) sequencing was performed on a MiniSeq

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397 with a 300–cycle reagent kit (Illumina, USA) with a negative control library with no input
398 SARS-CoV-2 RNA extract.

399 Raw reads underwent adapter/quality trimming (trim-galore v0.6.5) [15], host filtering and
400 read mapping to reference [bwa v0.7.17 [16], samtools v.1.7 [17,18]] trimming of primers (iVar v1.3
401 [19]) and variant/consensus calling (freebayes v1.3.2 [20]) using the SIGNAL workflow [13]
402 (https://github.com/jaleezyy/covid-19-signal) v1.4.4dev (#60dd466) with the ARTICv4 amplicon
403 scheme (from https://github.com/artic-network/artic-ncov2019) and the MN908947.3 SARS-
404 CoV-2 reference genome and annotations. Additional quality control and variant effect
405 annotation (SnpEff v5.0-0 [21]) was performed using the ncov-tools v1.8.0
406 (https://github.com/jts/ncov-tools/). Finally, PANGO lineages were assigned to consensus
407 sequences using pangolin v3.1.17 (with the PangoLEARN v2021-12-06 models) [22], scorpio
408 v0.3.16 (with constellations v0.1.1) [citation: https://github.com/cov-lineages/scorpio], and
409 PANGO-designations v1.2.117 [23]. Variants were summarised using PyVCF v0.6.8 [citation:
410 https://github.com/jamescasbon/PyVCF] and pandas v1.2.4 [24]. Phylogenetic analysis was
411 performed using augur v13.1.0 [25] with IQTree (v2.2.0_beta) [26] and the resulting phylogenetic
412 figure generated using ETE v3.1.2 [27]. Contexual sequences were incorporated into the
413 phylogenetic analysis by using Nexstrain's ingested GISAID metadata and pandas to randomly
414 sample a representative subset of sequences (jointly deposited in NCBI and GISAID) that
415 belonged to lineages observed in Canada (see also Supplemental Tables S1 and S2 for metadata).
416

417 **Micro-neutralization assay.** Serum samples were heat-inactivated at 56°C for 30 minutes and
418 then serially diluted 1:2 in DMEM supplemented with 2% FBS and 1% P/S (vDMEM) in 96-
419 well blocks. The final volume of diluted serum per well was 180 μL. Each virus was diluted to

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420 500 $TCID_{50}$/mL (25 $TCID_{50}$ per well) in vDMEM, from which 180 µL was added to each well
421 containing serum. Positive controls containing only media and negative controls containing only
422 virus were also included. The serum-virus mixture was incubated at 37°C for 1 h and then added
423 to cultured Vero'76 cells in 96 well plates in triplicate. After five days the cells were evaluated
424 for cytopathic effect (CPE), and the endpoint neutralization titer (the highest dilution of sera
425 without CPE) was recorded. The final neutralization titer is reported as the average of the three
426 replicates per sample. As control, each virus isolate that was diluted for our micro-neutralization
427 assay was also back-titered using 2-fold serial dilutions.

428 **Statistical analysis.** Statistical analyses were performed using Tukey's multiple comparisons
429 test with alpha = 0.05 or Sidak's multiple comparisons test with alpha = 0.05.

430

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