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Transcriptomic profiling of different responder types in adults after a Priorix® vaccination

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1 Transcriptomic profiling of different responder types in adults after a

2 Priorix® vaccination

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41

42 **Abstract**

43 Thanks to the recommendation of a combined Measles/Mumps/Rubella (MMR) vaccine, like Priorix®,
44 these childhood diseases are less common now. This is beneficial to limit the spread of these diseases
45 and work towards their elimination. However, the measles, mumps and rubella antibody titers show a
46 large variability in short- and long-term immunity. The recent outbreaks worldwide of measles and
47 mumps and previous studies, which mostly focused on only one of the three virus responses, illustrate
48 that there is a clear need for better understanding the immune responses after vaccination. Our
49 healthy cohort was already primed with the MMR antigens in their childhood. In this study, the adult
50 volunteers received one Priorix® vaccine dose at day 0. First, we defined 4 different groups of
51 responders, based on their antibody titers' evolution over 4 time points (Day 0, 21, 150 and 365). This
52 showed a high variability within and between individuals. Second, we determined transcriptome
53 profiles using 3'mRNA sequencing at day 0, 3 and 7. Using two analytical approaches, "one response
54 group per time point" and "a time comparison per response group", we correlated the short-term gene
55 expression profiles to the different response groups. In general, the list of differentially expressed
56 genes is limited, however, most of them are clearly immune-related and upregulated at day 3 and 7,
57 compared to the baseline day 0. Depending on the specific response group there are overlapping
58 signatures for two of the three viruses. Antibody titers and transcriptomics data showed that an
59 additional Priorix vaccination does not facilitate an equal immune response against the 3 viruses or
60 among different vaccine recipients.

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65 Introduction

66 Measles, mumps and rubella (MMR) used to be common childhood diseases. Thanks to the current 2-
67 dose vaccination schedule for all children (In Belgium: first dose at 12 months and second dose
68 between 11 and 12 years [1]), both recommended by the WHO (World Health Organization) and most
69 National Immunization Technical Advisory Group (NITAGs), the incidence of MMR cases has decreased
70 with 99% to on average only a few hundred cases reported per year in Belgium. Immunization against
71 MMR is currently combined in one vaccine (for example Priorix® or MMR VAX PRO®), containing three
72 live-attenuated virus strains. This vaccine type is considered to induce the strongest possible immune
73 response without causing disease. Nevertheless, the short- and long-term immune responses after
74 MMR vaccination are highly variable and inconsistent over time. A single dose of the MMR vaccine has
75 been shown to be 93% effective against measles, 78% effective against mumps, and 97% effective
76 against rubella (CDC) [2]. Following the CDC-recommended childhood vaccination schedule, two doses
77 of the MMR vaccine are at least 97% effective against measles and 88% effective against mumps
78 (rubella numbers not available, CDC) [2]. Children who underwent and survived a natural infection are
79 considered to be permanently protected for life. However, individuals who have never been
80 vaccinated, who remain non-responders after both doses or who were never exposed to the natural
81 infection in their childhood, are at risk for infection and complications at a later age. These infections
82 during adulthood are known to have a higher severity. Therefore, MMR vaccination is still
83 recommended for adults at high risk: a second dose for adults who only received one primary dose or
84 a full 2-dose vaccination for unvaccinated individuals
85 (<https://www.cdc.gov/vaccines/vpd/mmr/hcp/recommendations.html>) (Belgian NITAG). Offering a
86 second dose at any age can also help to limit the spread of MMR infections. Nowadays, 88% of measles,
87 mumps or rubella outbreaks are due to unvaccinated individuals (CDC) [2]. The remaining 12% are due
88 to vaccine non-responders or individuals that have lost their protective immunity over time [3,4].

89 Given the recurring outbreaks in recent years [5,6] and the fact that previous studies mostly focused
90 on only one of the three virus responses [3,7–16], there is a need to assess the complete transcriptome
91 profile in recipients of the MMR vaccine. The combination of this profile with the three separate MMR
92 antibody titers could give us more insights in the variation of the immune response after MMR
93 vaccination and may eventually result in a guideline for a new vaccination strategy.

94 In this MMR vaccination study, we correlate short term gene expression profiles of 40 healthy
95 individuals, before (day 0) and after vaccination (day 3 and 7), with the corresponding evolution of the
96 humoral immunity against measles, mumps and rubella, measured by the three separate antibody
97 titers at day 0, 21, 150 and 365. Measuring the effect of a vaccination on the gene expression level can
98 teach us how the immune system responds to three different viruses at the same time.

99 **Material and methods**

100 Study cohort

101 Forty healthy individuals (between 20 and 30 years) whom have received at least one MMR vaccine
102 dose earlier in life, were recruited for this study (Supplementary table 1: data previous vaccinations).
103 These individuals were administered one vaccination dose of Priorix[®] (a live attenuated MMR vaccine).
104 Before vaccination (day 0) and at days 3, 7, 21, 150 and 365, blood samples were collected from each
105 individual. At days 0, 3 and 7, blood samples were collected in two PaxGene blood RNA tubes
106 (PreAnalytiX GmbH). These tubes contain a buffer that stabilizes the *in vivo* gene expression profile by
107 minimalizing *in vitro* RNA degradation. At days 0, 21, 150 and 365, serum samples were collected and
108 immediately stored at -80°C. The serum samples were sent in batch to the Antwerp University Hospital.
109 The antibody (Ab) titers for measles, mumps and rubella were measured by the LIAISON[®] using the
110 chemiluminescence immunoassay (CLIA) technology. Figure 1 shows the detailed timeline.

111 RNA extraction

112 RNA extraction from blood collected in PaxGene tubes was performed via a column-based RNA
113 extraction using the PaxGene blood RNA extraction kit (Qiagen). To optimize RNA concentrations, we
114 used the RNA clean & concentrator-5 kit (Zymo research). We verified the RNA quality using a fragment
115 analyzer (Advanced Analytical, DNF-471 Standard Sensitivity RNA Analysis Kit). No RNA samples had to
116 be excluded based on low quality.

117 3'mRNA sequencing

118 The experimental set-up is similar to the workflow reported by Bartholomeus et al., 2018. In brief, all
119 RNA samples were prepared with the QuantSeq 3'mRNASeq Library Prep Kit FWD for Illumina (Lexogen
120 GmbH) following the standard protocol for long fragments. The resulting cDNA libraries were
121 equimolarly pooled, up to 40 samples for one NextSeq 500 sequencing run (high output v2 kit, 150
122 cycles, single read, Illumina).

123 NGS data processing

124 Raw data from the NextSeq was demultiplexed and further processed through an in-house developed
125 3'mRNA sequencing pipeline. The quality of all reads was evaluated using FastQC (v0.11.5) before and
126 after processing with Trimmomatic (v0.36). Trimmomatic was used to remove the leading 20 bases
127 from reads, ensure a minimum quality score of 15 over a sliding window of 4 bases and require a
128 minimum read length of 30 bases. Usage of oligodT primers could cause poly-A stretches at the 3' end.
129 To remove these poly-A stretches, the 3' read end was trimmed with our own in-house poly-A removal
130 script. All remaining sequences were mapped against the human reference genome build 38
131 (polymorph variants excluded) with HISAT2 (v2.0.4). HTseq (v0.6.1) was used to count all reads for
132 each gene and set up a read count table.

133 Differential gene expression analysis and gene ontology enrichment analysis

134 Differential gene expression analysis was performed using the DESeq2 Bioconductor package [17]. For
135 any given differential gene expression analysis, genes were removed prior to the analysis if they had

136 less than 200 read counts (5 times the number of individuals included in the study) over all samples
137 considered during the analysis. Gene ontology enrichment analysis was performed on significantly
138 differentially expressed genes using GOA tools [18]. To determine significantly enriched/depleted gene
139 ontology terms related to biological processes, a Fisher's exact test was performed with Benjamini-
140 Hochberg correction for multiple testing (FDR < 0.05). As a reference background set of genes, we used
141 all genes (total number: 18180) measured during the 3'-mRNAseq experiment.

142 All codes used for the preprocessing and analysis of the data within this manuscript as well as the codes
143 used to generate results and the DESeq2 results itself, are publicly available on github at
144 https://github.com/NDeNeuter/mmr_rnaseq.

145 Ethical approval

146 This non-commercial investigator-driven study was approved by the medical ethical committee of the
147 Antwerp University Hospital/Antwerp University, Belgium (EC15_19_210). All participants signed an
148 informed consent form.

149 **Results**

150 Response groups

151 With the MMR study vaccine, the three live-attenuated MMR viruses were administered
152 simultaneously at day 0, leading to one "combined" immune response. The humoral immune response
153 against the vaccine were determined by measuring three separate antibody titers: anti-Measles IgG
154 (AU/mL), anti-Mumps IgG (AU/mL) and anti-Rubella IgG (IU/ml). For classification of the immune
155 responses, a hierarchical clustering method was applied on the antibody (Ab) titers at day 0 (pre-
156 vaccination baseline) and days 21, 150 and 365 (post-vaccination). This clustering technique groups
157 individuals with similar patterns in Ab titer evolution. This avoids using cut-off values, which may be
158 biased. As shown in Figure 2, four different response groups were identified for each antibody titer:
159 (a) High Ab: individuals with a relatively high Ab titer before vaccination that remained stable or further

160 increased after vaccination (b) Low Ab: individuals with a relatively low Ab titer before vaccination that
161 remained low after vaccination (c) Long response: individuals with a relatively low Ab titer before
162 vaccination that increased after vaccination and stayed stable within the first year (d) Peak response:
163 individuals with a relatively low Ab titer before vaccination that increased at day 21, and then
164 decreased by day 150 and 365. Table 1 shows the antibody titer response for each individual. The
165 humoral immune response to each of the three attenuated vaccine viruses clearly varies between and
166 within individuals, as shown earlier [19,20]. For example, individual 8 is a high Ab responder for
167 measles, a long responder for mumps and a low Ab responder for rubella.

168 For both measles and mumps, 12/40 (30%) of the individuals had a high Ab titer at day 0 (before
169 vaccination). In addition, 17/40 (42.5%) and 21/40 (52.5%) were able to gain high Ab titers for measles
170 and mumps respectively without a loss of protection within the first year (long response). In contrast,
171 the high Ab group for rubella contained only 3 individuals, whereas the majority of individuals (23/40)
172 were classified in the low Ab group. While the remaining 35% (14/40) of the individuals had a peak
173 response for rubella, measured at day 21, they were not able to sustain these higher anti-rubella IgG
174 levels, which had declined by day 150 and 365. The peak response profile was thus mainly observed
175 within the rubella Ab titer measurements.

176 Short term gene expression profiling

177 Since the three live-attenuated viruses are administered in a single vaccine, gene expression to each
178 virus cannot be measured independently. However, gene expression changes can be determined in
179 relation to the three different Ab titers at a given time point for each individual. We therefore
180 performed two different analyses, one where the gene expression for a particular response group was
181 compared to the other response groups and this for each Ab (virus) at a given time point (response
182 group per time point) and one where the gene expression within a particular response group was
183 compared at different time points and this for each Ab (virus) (time comparison per response group).
184 These analyses resulted in a list of differentially expressed genes (DEGs) with a positive or negative

185 log2 fold change and an adjusted p-value, which can be related to biological processes by testing for
186 gene ontology enrichment.

187 Response group per time point analysis

188 In this first (vertical) approach, one response group is selected for a specific Ab (virus) at a single time
189 point. The gene expression profile of that response group is compared to the profile of the remaining
190 groups for that specific virus at the same time point. For example, one analysis compared the
191 expressed genes within the high Ab response group of measles at day 0 with the expressed genes of
192 the remaining cohort of measles (low Ab, long response and peak response) at day 0. Results are shown
193 in Table 2. Overall, this response group per time point analysis results in a very limited list of DEGs,
194 with only a few immune-related genes (See supplementals, Supplementary table 2).

195 For measles, the high Ab group did not show any differential expression compared to the 3 other Ab
196 groups at any time point. In the low Ab group four interferon (IFN) inducible genes were upregulated
197 at day 0 before vaccination, compared to the 3 other Ab groups. These four IFN-inducible genes
198 contribute to type I IFN responses and signaling and are related to functional anti-viral GO terms.
199 Comparisons with the measles low Ab group showed no DEGs at day 3 and 7 at all. The measles long
200 response and the peak response comparisons both featured no immune-related DEGs at day 0 and 3.
201 However at day 7, alpha defensins (DEFA1/1B/3) were downregulated in the long response group and
202 (DEFA1/1B/3/4) upregulated in the peak response group. These defensins are antimicrobial and
203 cytotoxic peptides, involved in host defense mechanism, which are highly abundant in the granules of
204 neutrophils, the largest group of granulocytes. Further, IFITM3, another IFN-inducible protein with
205 antiviral capacities, is also significantly downregulated in the measles peak response group compared
206 to the other groups.

207 For mumps, no immune-related DEGs were found in comparison with the high Ab group. In the low Ab
208 group, similarly as for measles, the IFN-inducible gene, MX1, was upregulated at day 0. Also, CASP5,
209 coding for a caspase in the lectin complement cascade, is upregulated in the mumps low Ab group at

210 day 0. Furthermore, 5 genes were downregulated in the low Ab group compared to the other Ab
211 groups at day 7. Those 5 genes contribute to general defense response (including IFN), NK cells
212 chemotaxis and cytotoxicity and granzyme-mediated apoptosis GO terms. The mumps long response
213 group at day 7 showed downregulation for 3 genes, including MX1. SIGLEC1 is a lectin-like adhesion
214 molecule that binds different lymphocytes and is considered as member of the immunoglobulin (Ig)
215 family. FCGR2B is part of the Fc receptor of IgG and contributes to different B-cell GO terms.

216 For rubella, the high Ab group, consisting of only 3 individuals, showed immune-related DEGs at each
217 time point: upregulation of CD40 at day 0 (involved in B-cell isotype switching); upregulation of XCL1
218 at day 3 (chemotactic activity towards lymphocytes); upregulation of IGH (the heavy locus of IgG) and
219 downregulation of both HLA-DRB5 and APOBEC3A (an anti-viral DNA deaminase) at day 7. The rubella
220 low Ab group, which is the largest group, had no significant DEGs related to the immune system. The
221 rubella peak response comparison showed downregulation of IFITM3 at day 3.

222 Time comparison per response group analysis

223 In this second (horizontal) approach only individuals in a particular response group per Ab (virus) are
224 considered. This analysis compared the gene expression profile at a post-vaccination time point to the
225 baseline profile (Day 0 – before administration of the vaccine). The results are shown in Table 3
226 (Supplementary table 2: functions).

227 For measles, the high Ab group showed no significant differences at day 3 and 7, compared to day 0.
228 In the low Ab group, only the day 7 comparison revealed three upregulated genes related to immunity:
229 UBB (Ubiquitin B), IFITM3, and ACKR1 (atypical chemokine receptor 1). In contrast, several DEGs were
230 observed in the longitudinal comparison of the measles long response group. The day 3 vs day 0
231 comparison revealed upregulated genes related to interleukin regulation, Toll-like signaling and IFN
232 related terms. The day 7 vs day 0 comparison showed additional upregulation of genes involved in IFN
233 and cytokine signaling pathways, cellular responses and Ab processing/presentation, viral transcription
234 and replication processes, and T-cell signaling. Time point comparison of the measles peak response

235 group only showed 2 upregulated genes for the day 3 vs day 0 comparison: IFI6 (IFN inducible protein)
236 and TUSC2.

237 For mumps, in the high Ab group only upregulated genes could be found between day 7 and day 0.
238 These 11 genes are related to viral processes, IFN and cytokine mediated signaling GO terms. The
239 mumps low Ab group longitudinal comparison only showed a downregulation of PRTN3, which is
240 related to neutrophil extravasation. Similar to what was observed for measles, the long response group
241 showed the most immune-related DEGs at both post-vaccination time points. These DEGs contained
242 mostly defensins and were all consistently upregulated at the later time point. Gene ontology analysis
243 reveals the following upregulated processes for the mumps long response group after vaccination:
244 Innate responses incl. cytokines, IFN, ILs and TNF, immune effector responses, viral
245 transcription/replication and neutrophil activation and degranulation.

246 For rubella, the high Ab group showed a similar pattern to that observed for mumps high Ab group:
247 immune-related DEGs are found only between day 7 and day 0, all upregulated, except for IRF3. Gene
248 ontology analysis reveals enrichment for cellular response to virus, different viral processes and toll-
249 like receptor signaling among these genes. The rubella low Ab group on the other hand was more
250 comparable with the measles low Ab group: two upregulated DEGs at day 7 vs 0, including ACKR1
251 (related to chemokine/cytokine binding) and DEFA4 (part of innate immune system). In the rubella
252 peak response group, 10 upregulated immune-related DEGs were observed between day 7 and day 0.
253 Those 10 DEGs featured the following enriched GO processes: innate and humoral immune response,
254 cytokine stimulus and inflammatory response and neutrophil activation and degranulation.

255 **Discussion**

256 Live-attenuated virus vaccines are known to trigger a strong immune response, as they are most similar
257 to a natural infection. As the MMR vaccine is known to generate a robust cell-mediated immune
258 response, it should result in a sustainable protection level. However, our results indicate that

259 vaccination with the 3 different live-attenuated MMR viruses does not result in a consistent and shared
260 immune response against the three viruses [19–23]. Indeed each one of the vaccinees showed a
261 different response profile for measles, mumps and rubella (Table 1). These results correspond well
262 with the reported variability of the MMR vaccine response [2]. However, there are two aspects to be
263 considered here. First, this was an additional dose of the vaccine given to adults, who had already
264 received one or two doses during childhood (Supplementary table 1). There is thus a large baseline
265 variability present between and within individuals caused by the previous MMR vaccination. The
266 acquired immunity from the previous vaccine doses could have declined at a different rate for each of
267 the three viruses. Second, there are no clinical thresholds set for MMR Ab titers to determine non-,
268 low and strong responders. To handle the differences in variability, we used a formal clustering
269 strategy to define 4 different types of response based on the evolution of each Ab titer over 1 year
270 post-vaccination. This led to non-uniform group sizes, where the measles peak response and rubella
271 high response group only had 4 and 3 individuals, respectively. The observation of these different
272 antibody titer profiles captured by these groups suggest that there is a large individual variability in
273 the immune response against the different viral components. Most of the cohort only showed
274 increased antibody titers for one or at most two components post-vaccination. Long-term increases
275 could be observed mainly for either measles or mumps, while in 92.5% of the cohort, the increase of
276 the rubella Ab titer was of short duration (low Ab and peak response group). The exact cause of this
277 inability to build up consistent high Ab protection levels against all 3 MMR viruses could not be defined
278 based on the gene expression profiles. The difference in immunogenicity for each vaccine component
279 within monovalent vaccines compared to combined vaccines is less studied for MMR [24–27]. As
280 monovalent vaccines require more shots and create a delay in protection for all three infections due
281 to separate vaccination schedules, health care providers and pharma industry are focused on
282 developing combined vaccines [28]. For example, the production of ATTENUVAX® (Measles Virus
283 Vaccine Live), MUMPSVAX® (Mumps Virus Vaccine Live), and MERUVAX®II (Rubella Virus Vaccine Live)
284 was shut down in 2009 (Merck).

285 Prior studies of the MMR vaccines have mostly been based on the serological responses [3,8,29].
286 Rager-Zisman et al. added also flow cytometry data with a focus on CD4/CD8 lymphocyte ratio and NK
287 cells activity [30]. Poland et al., studied the MMR vaccine extensively, but mostly focused on only one
288 of the 3 viral components: cytokine data [12,16], measles specific or rubella specific humoral immunity
289 with proteome microarrays [11,15] and HLA typing [13–15]. Transcriptomic analyses have been
290 performed on PBMCs of previously vaccinated highest responders and lowest non-responders after *in*
291 *vitro* measles virus stimulation [10], or with the live rubella virus [7]. These studies found that genes
292 related to plasma cell survival and CD93 were associated with measles-specific antibody response after
293 vaccination, which corresponds with the gene expression difference found in the study. Further,
294 antigen presentation genes and inflammation-related genes were postulated in these studies to
295 explain rubella vaccine-induced immune response variations. There is a certain overlap of GO
296 enrichments with our study results. However, these studies focused on one virus response at a time in
297 preselected MMR responders using a cell culture reactivation set-up with only one of the three virus
298 strains [7,10,29]. Thus the trivalent combined immune response measured in this study cannot be
299 directly compared to these single immune response.

300 The seroconversion against the 3 viruses in children after vaccination has been reported to be more
301 straightforward [12,30]. Our cohort of healthy individuals was unintentionally limited in age
302 categories. All participating individuals were born between 1987 and 1997, after the start of the MMR
303 vaccination program in Belgium. The cohort thus contained individuals between 20 and 30 years old
304 when the study took place in 2017. Due to this young cohort, we did not expect any age-related
305 immune system and health issues. Half of the volunteers completed the two dose vaccination schedule
306 [1], based on reporting and childhood records. The other 50% of the cohort reported at least one MMR
307 vaccination during their childhood (12 months) or at adolescence (10-12 years). The full overview of
308 previous MMR vaccination history for our cohort is provided in Supplementary material 1. However,
309 the MMR vaccination history (1 or 2 doses) does not seem to have an impact on the response types of
310 this study vaccination (Pearson's Chi-squared test, $p = 0.8136$). It is notable that there is seemingly no

311 relationship between those that have completed the two dose vaccination schedule and those that
312 were categorized as High Ab by clustering. This result is unexpected as this schedule is currently
313 deemed as sufficient for long-term protection in most individuals. Indeed, this lack of association may
314 be due to the small sample size of this study, the age limits of the study cohort, incomplete vaccination
315 records/recall or the biased clustering approaches used to define the High Ab group. What also could
316 lead to such insignificance, is that volunteers who recall only one dose probably received a second
317 dose as well, if we look to the age categories. On the other hand, recent outbreaks show that about
318 12% of the infected consisted of vaccinated individuals [5,6]. The low number of volunteers in the High
319 Ab groups may suggest that there is a need to increase protection levels in the population should
320 outbreaks continue. This could be accomplished by optimization of the vaccination schedule, also for
321 adults, or to adapt the MMR vaccine to increase the long-term immunogenicity.

322 Few immune-related DEGs were found when comparing the different responder groups across
323 different time points (Table 2). Twenty out of the 30 different comparisons showed no immune-related
324 or no significant DEGs at all. Lack of DEGs may suggest that any gene expression changes were
325 consistent across response groups for each specific viral component, or that any gene expression
326 differences may have been not large enough to be picked up because of the small sample size. The
327 gene expression profiles for rubella are the most divergent, as expected because of the large number
328 of low rubella Ab titers at day 0, prior to vaccination. While it is important to not overly conjecture
329 based on specific DEGs due to the known high false positive rate, two main observations from the
330 overall cross-responder group analysis can be considered. Firstly, at day 0 (baseline), we found
331 upregulated genes (IFN inducible genes/caspase 5) in the measles and mumps low Ab groups and an
332 upregulated CD40 marker (TNF receptor on antigen presenting cells) in the rubella high Ab group. It
333 was unexpected to find differences in the gene expression at baseline in healthy individuals prior to
334 vaccination. However, our previous hepatitis B vaccine study also found significant immune related
335 differences in non-responders at the day 0 baseline [31]. As such, baseline gene expression may play
336 a larger role in determining vaccine outcomes than is currently appreciated. Secondly, while rubella is

337 a single-stranded RNA virus, we found a downregulated DNA cytidine deaminase, APOBEC3A, at day 7
338 in the rubella high Ab group. However, latest literatures suggest also a role in RNA editing and acting
339 on the pathogen itself and their host RNA [32,33]. What the relation is between the live-attenuated
340 rubella virus and the downregulation of APOBEC3A is unknown. And finally, it seems that alpha
341 defensins are still expressed at the peak response: DEFA1, 3 and 4 are transcribed to cytotoxic proteins,
342 stored in neutrophilic granules. Expression of these genes is upregulated in the peak response and
343 downregulated in the long response. This could indicate that neutrophilic degranulation is still ongoing
344 and/or the granules have to be refilled. A follow-up study could focus on the protein load of
345 neutrophils.

346 Using day 0 as an uninfluenced immune baseline to determine significant changes in gene expression
347 at day 3 and 7 resulted in a larger amount of DEGs, except for the high Ab measles group. The gene
348 expression stability in the high Ab measles group could be the result of the high level of previously
349 established immunity against the measles virus. All immune-related DEGs found for the other groups
350 are upregulated both at day 3 and day 7 when compared to the baseline (day 0) situation, except for
351 PRTN3 (Low Ab Mumps, 0 vs 3), related to neutrophil extravasation, and IRF3 (high Ab rubella, 0 vs 7),
352 an IFN regulatory transcription factor. In general, all DEGs are known to be IFN-related or are known
353 to play a role in the innate immune system, like the alpha defensins. This matches prior knowledge
354 that a large part of the antiviral immune response might be IFN-based [34–36].

355 The complexity of the diverse responses to the different components of the vaccine, even within a
356 single individual, makes a straightforward vaccine-related gene expression pattern not possible.
357 Furthermore, this vaccination was an additional dose given to adults, following their childhood
358 vaccinations. This means that the immune system has already been exposed to these viruses, and the
359 elicited “secondary” immune response could be faster or lower than would be the case with a “de
360 novo” vaccination. Comparatively however, there are far fewer or even no DEGs in all low Ab groups
361 than found with the other response groups. This is expected as the low Ab groups are expected to have

362 a low immune response to the viral component before and after vaccination. Overall the day 3 vs 0
363 analysis tended to find less DEGs than the day 7 vs 0, but both remained small compared to overall
364 gene expression changes occurring in healthy individuals on a daily basis. Furthermore, we only see
365 significant DEGs associated with the innate immune system, but none related to the adaptive cell-
366 mediated or the humoral immunity. Given the mechanisms of vaccination, one would expect these to
367 be present. Thus these results indicate that an additional dose does not elicit a strong immunogenic
368 response reflected by gene expression changes. The small numbers of DEGs could also suggest that
369 the impact of the adaptive immune response on the gene expression was restricted to an earlier time
370 point than was measured.

371 Conclusion

372 Antibody titers and gene expression levels varied greatly within and across individuals in MMR vaccine-
373 induced immune responses against the 3 viruses. The largest difference in gene expression was
374 observed for those individuals who had a significant change in antibody titers following vaccination,
375 compared to smaller gene expression changes in those individuals that maintained consistent Ab titers
376 (either low or high). However, any gene expression changes stayed limited in size and in time compared
377 to other possible interventions, largely independent of the previously established immune protection.
378 This indicates that the effect of additional MMR vaccinations on the immune system is very minor and
379 that any measurable effects subside in a matter of days. As a consequence, our results highlight a need
380 to further study the individual host's and public health benefits of extra doses at different stages of
381 adult life. Such insights will become critical as the MMR vaccinated population gains in size and grows
382 older.

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Figure legends

Figure 1: Overview of the MMR study time line.

Figure 2: Overview of all antibody titers. A hierarchical clustering method is used to define four response groups: High Ab, Low Ab, Peak response and long response.

Keywords: Priorix, Measles-mumps-rubella vaccination, RNA-sequencing, gene expression profiling,