



HAL
open science

Olfactory function in congenital cytomegalovirus infection: a prospective study

Françoise Lazarini, Sarah Levivien, Yoann Madec, Fabien Taieb, Estelle Mottez, Tan-Phuc Buivan, Audrey Maudoux, Sylvette Wiener-Vacher, Jérôme Nevoux, Thierry van den Abbeele, et al.

► To cite this version:

Françoise Lazarini, Sarah Levivien, Yoann Madec, Fabien Taieb, Estelle Mottez, et al.. Olfactory function in congenital cytomegalovirus infection: a prospective study. *European Journal of Pediatrics*, 2022, 10.1007/s00431-022-04375-1 . pasteur-03527304

HAL Id: pasteur-03527304

<https://pasteur.hal.science/pasteur-03527304>

Submitted on 17 Jan 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

Olfactory function in congenital cytomegalovirus infection: a prospective study

Françoise Lazarini¹, Sarah Levivien², Yoann Madec³, Fabien Taieb^{3,4}, Estelle Mottez⁴,
Tan-Phuc Buivan⁵, Audrey Maudoux^{2,6,10}, Sylvette Wiener-Vacher^{2,6}, Jérôme Nevoux^{7,8,9},
Thierry Van Den Abbeele^{2,6}, Pierre Gressens⁶, Pierre-Marie Lledo^{1,*}, Natacha Teissier^{2,6,*,#}

1 Institut Pasteur, Perception and Memory Unit, Paris, Centre National de la Recherche Scientifique, Unité Mixte de Recherche 3571, Paris, France

2 Pediatric ENT department, Robert Debré Hospital, Neurodiderot Inserm, Université de Paris, Paris, France

3 Institut Pasteur, Epidemiology of Emerging Diseases Unit, Paris, France

4 Institut Pasteur, Centre de Recherche Translationnelle, Paris, France

5 Institut Pasteur, Pôle Intégré de Recherche Clinique, Paris, 75724, Cedex 15, Paris, France

6 Université de Paris, NeuroDiderot, Inserm, Paris, France

7 Service d'ORL et Chirurgie Cervico-Faciale, CHU Kremlin-Bicêtre, Le Kremlin-Bicêtre, France

8 Faculté de Médecine, Université Paris-Saclay, Le Kremlin-Bicêtre, France

9 INSERM, U955; CNRS, ERL 7240, Créteil, France

10 Institut Pasteur, Institut de l'Audition, Progressive Sensory Disorders, Pathophysiology and Therapy Unit, INSERM-UMRS 1120, Sorbonne Université, Paris, France

* Pierre-Marie Lledo and Natacha Teissier contributed equally to this article.

Françoise Lazarini: Institut Pasteur, Perception and Memory Unit, Paris, Centre National de la Recherche Scientifique, Unité Mixte de Recherche 3571, Paris, France; francoise.lazarini-serandour@pasteur.fr

Sarah Levivien: Pediatric ENT department, Robert Debré Hospital, Neurodiderot Inserm, Université de Paris, Paris, France; sarah.levivien@aphp.fr

Yoann Madec: Institut Pasteur, Epidemiology of Emerging Diseases Unit, Paris, France; yoann.madec@pasteur.fr

Fabien Taieb: Institut Pasteur, Epidemiology of Emerging Diseases Unit, Paris, France; Institut Pasteur, Centre de Recherche Translationnelle, Paris, France ; fabien.taieb@pasteur.fr

Estelle Mottez: Institut Pasteur, Centre de Recherche Translationnelle, Paris, France; estelle.mottez@pasteur.fr

Tan-Phuc Buivan: Institut Pasteur, Pôle Intégré de Recherche Clinique, Paris, 75724, Cedex 15, Paris, France ; tan-phuc.bui-van@pasteur.fr

Audrey Maudoux: Pediatric ENT department, Robert Debré Hospital, Neurodiderot Inserm, Université de Paris, Paris, France ; Université de Paris, NeuroDiderot, Inserm, Paris, France; Institut Pasteur, Institut de l'Audition, Progressive Sensory Disorders, Pathophysiology and Therapy Unit, INSERM-UMRS 1120, Sorbonne Université, Paris, France; audrey.maudoux@aphp.fr

52
53 **Sylvette Wiener-Vacher:** Pediatric ENT department, Robert Debré Hospital, Neurodiderot Inserm,
54 Université de Paris, Paris, France · Université de Paris, NeuroDiderot, Inserm, Paris, France; sylvette.wiener@gmail.com
55
56
57 **Jérôme Nevoux:** Service d'ORL et Chirurgie Cervico-Faciale, CHU Kremlin-Bicêtre, Le Kremlin-
58 Bicêtre, France; Faculté de Médecine, Université Paris-Saclay, Le Kremlin-Bicêtre, France; IN-
59 SERM, U955; CNRS, ERL 7240, Créteil, France; jerome.nevoux@aphp.fr
60
61 **Thierry Van Den Abbeele:** Pediatric ENT department, Robert Debré Hospital, Neurodiderot In-
62 serm, Université de Paris, Paris, France · Université de Paris, NeuroDiderot, Inserm, Paris, France;
63 thierry.van-den-abbeele@aphp.fr
64
65 **Pierre Gressens:** Université de Paris, NeuroDiderot, Inserm, Paris, France, [pierre.gressens@in-](mailto:pierre.gressens@inserm.fr)
66 [serm.fr](mailto:pierre.gressens@inserm.fr)
67
68 **Pierre-Marie Lledo:** Institut Pasteur, Perception and Memory Unit, Paris, Centre National de la
69 Recherche Scientifique, Unité Mixte de Recherche 3571, Paris, France; [pierre-marie.lledo@pas-](mailto:pierre-marie.lledo@pasteur.fr)
70 [teur.fr](mailto:pierre-marie.lledo@pasteur.fr)
71
72 **Natacha Teissier:** Pediatric ENT department, Robert Debré Hospital, Neurodiderot Inserm, Uni-
73 versité de Paris, Paris, France · Université de Paris, NeuroDiderot, Inserm, Paris, France; [nata-](mailto:natacha.teissier@inserm.fr)
74 [cha.teissier@inserm.fr](mailto:natacha.teissier@inserm.fr)
75
76
77 **# Corresponding author:** Pr Natacha Teissier Pediatric ENT department, Robert Debré Hospital,
78 Neurodiderot Inserm, Université de Paris, 48 Boulevard Sérurier, 75019, Paris, France; E-mail: [nata-](mailto:natacha.teissier@inserm.fr)
79 [cha.teissier@inserm.fr](mailto:natacha.teissier@inserm.fr)
80

81
82 **Abstract**

83
84 Congenital cytomegalovirus (CMV) infection leads to olfactory bulb lesions in the fetus, yet
85 little is known about its impact on olfaction after birth. Here, we have assessed in a prospec-
86 tive study conducted on children in two French hospitals from 2016 to 2019, infection sever-
87 ity and olfactory performance after congenital CMV infection. Children with congenital CMV
88 infection aged 3 to 10 years and healthy controls (CTL) matched for age and sex to CMV
89 children symptomatic at birth (sCMV) were enrolled. Olfactory discrimination was assessed
90 using mono-odorants and binary mixtures. Data were analyzed for 54 children with PCR-con-
91 firmed congenital CMV infection, including 34 sCMV (median [IQR] age, 6 [5-8] years; 19
92 [55.9%] male) and 20 CMV asymptomatic at birth (aCMV, median [IQR] age, 4 [3-6] years;
93 12 [60.0%] male). sCMV were compared to 34 CTL children. Olfactory scores in CMV-

94 infected children were independent from vestibular deficit and hearing loss. The olfactory
95 score was efficient to discriminate between CTL and sCMV for children >6 years (area under
96 the receiver-operating characteristic curve (AUC, 0.85; $P=0.0006$), but not for children <7
97 years. For children >6 years, the proportion of children with total olfactory score <4 differed
98 between sCMV and CTL groups (91.2% and 18.7%, $P < 0.001$), but not between aCMV and
99 age-matched healthy control groups.

100

101 *Conclusion:* Congenital CMV infection is associated with reduced olfactory performance in
102 children with infection symptoms at birth.

103

104 **Key words:** diagnosis, olfaction, smell, discrimination, odorant mixture, children

105

106

107 **Introduction**

108

109 Cytomegalovirus (CMV) is a herpes type 5 virus that can affect the fetal and neonatal brain
110 after *in utero* infection [1]. CMV affects 0.5-2% of newborns and is the leading infectious
111 cause of congenital deafness. Depending on neonatal clinical presentation, children are either
112 categorized as having a symptomatic (sCMV) (presenting with growth retardation, prema-
113 turity, jaundice, petechiae, liver and/or hematological anomalies) or asymptomatic (aCMV)
114 (no clinical sign of infection other than possible hearing loss) infection at birth. Prognostic
115 factors for neurosensory sequelae comprise gestational age at infection and sCMV [2, 3]. 40-
116 60% of sCMV and 10-20% of aCMV children will manifest varying degrees of hearing loss,
117 which can be present at birth or may occur in the first months or years [4]. Although 90% of
118 clinical presentations are silent at birth, no systematic newborn screening has been established
119 to identify aCMV children who are at risk of hearing loss. Human CMV has a specific olfac-
120 tory receptor expressed on olfactory neurons in the olfactory system that may define viral

121 olfactory cell tropism [5]. Congenital CMV exhibits tropism for neural stem cells of the olfac-
122 tory system of fetuses, thus lesioning the olfactory bulb [6-8]. This infection leads to both ol-
123 factory and hearing impairments in a mouse model [9]. However, little is known about olfac-
124 tory dysfunction in CMV-infected children, partly because it is challenging to assess olfaction
125 in toddlers. Many studies have shown the difficulty to reliably test children under 5 years
126 [10-13] because of the cognitive and verbal involvement. Discrimination tasks are the most
127 relevant because they are rapid to perform, unlike threshold tasks, and they are requiring min-
128 imal cognitive and verbal skills, contrary to identification tasks. New tests based on percep-
129 tion level could constitute useful tools to address olfaction in children. In this regard, mixture
130 based olfactory discrimination tests perform better than standard smell tests in adult humans
131 and in adult and pup animal models [9, 14]. Here, we report the olfactory performance of chil-
132 dren with a confirmed congenital CMV infection, using a new psychophysical test we have
133 developed. This test aims at measuring the discrimination of monomolecular odorants from
134 the Sniffin' test battery [15] and the discrimination of mixture odorants presented in Sniffin'
135 pens. It is non-invasive and rapid to perform, even in very young children, thus requiring lit-
136 tle attention and concentration.

137

138 **Methods**

139

140 **Study Overview and ethical considerations**

141 The main objective of this study was to investigate the association between hearing loss and
142 olfactory performance in children with a congenital CMV infection followed in Robert Debré
143 (Paris) and Bicêtre (Le Kremlin- Bicêtre) hospitals, in France. This prospective study is a
144 nontrial, nondrug study, qualified as exploratory, multicenter, in a paediatric population (Clin-
145 icalTrials.gov number, NCT02782988). It received ethical approval (N° 3372) from Comité

146 de Protection des Personnes (CPP IDF-3). Children were included in the study after explana-
147 tion of the study and obtaining of written informed consent from both parents.

148

149 **Enrolment Criteria**

150 Children with confirmed congenital CMV, aged 3 to 10 years, were enrolled in this study dur-
151 ing a standard care visit. Proof of congenital infection was ascertained by positive CMV poly-
152 merase chain reaction (PCR) in urine and/or blood in the first 3 postnatal weeks, or retrospec-
153 tive diagnosis for the presence of positive PCR on dried blood spots collected at postnatal day
154 3 to 7.

155 Exclusion criteria included clinical conditions that may interfere with the study, such as
156 chronic rhinosinusitis, allergic rhinitis, primary ciliary dyskinesia, Kallmans syndrome or
157 other neurologic issues that can impact olfaction.

158 CMV infected children were divided into two groups according to neonatal characteristics
159 consistent with recognized clinical definitions: sCMV and aCMV at birth. Healthy controls
160 (CTL) matched for age and sex to the sCMV group were enrolled among children consulting
161 for other ear, nose, throat (ENT) non-rhinological pathologies, anaesthesiology or orthopaedic
162 appointments. CTL children had no history of congenital infection and presented with transi-
163 ent evoked otoacoustic emissions <20 dB for each ear.

164

165 **Clinical and Radiologic Symptoms**

166 Prenatal and neonatal clinical signs and virological data in favour of congenital CMV infec-
167 tion were recorded. Postural developmental milestones, vestibular canal and otolithic function
168 were assessed as previously described [16]. Magnetic resonance imaging of the brain and the
169 inner ear was performed to assess cerebral lesions (see the Supplemental Information for de-
170 tails).

171

172 **Hearing Evaluation**

173 Children with congenital CMV underwent either objective auditory brainstem response or
174 subjective behavioral audiometry tests to assess auditory thresholds. Hearing deficit was de-
175 fined by an auditory threshold of the most affected ear ≥ 25 dB. In CTL, normality of hearing
176 was assessed using evoked otoacoustic emissions.

177

178 **Olfactory Evaluation**

179 Olfaction was assessed in a 15-minute session with 18 pen-like odour-dispensing devices
180 (Sniffin' Sticks, Burghardt, Wedel, Germany) [15]. Two series of 3-odorant discrimination
181 tasks were performed: the first with simple odorants (monomolecular odorant test), and the
182 second with binary mixtures of odorants (mixture odorant test). For each task, 3 Sniffin'
183 Sticks were sequentially presented to the subject, two contained the same odorant and one
184 contained a different associated odorant. The child was requested to smell each stick and indi-
185 cate the stick that smells differently (forced choice between three possibilities). A correct or
186 incorrect answer resulted in a score of 1 or 0, respectively.

187

188 ***Monomolecular Odorant Test***

189 The sticks for the first task contained isoamylacetate (one stick) and anethol odorant (two
190 sticks). The sticks for the second task contained limonene (one stick) and citronellal odorant
191 (two sticks). The sticks for the third task contained anethol (one stick) and eugenol odorant
192 (two sticks). The total score for this test ranged from 0 (no correct response) to 3 (all correct
193 responses). Binary variables were defined using the threshold of 2.

194

195 ***Mixture Odorant Test***

196 The sticks for the first task contained a mixture of L-carvone and D-carvone at a 2:8 propor-
197 tion (one stick) and mixture of L-carvone and D-carvone at a an 8:2 proportion (two sticks).
198 The sticks for the second task contain a mixture of isoamylacetate and anethol in an 8:2 pro-
199 portion (one stick) and mix of isoamylacetate and anethol at a 2:8 proportion (two sticks). The
200 sticks for the third task contain a mixture of anethol and eugenol at an 8:2 proportion (one
201 stick) and mix of anethol and eugenol at a 2:8 proportion (two sticks). The total score for this
202 test ranged from 0 (no correct response for the 3 problems) to 3 (correct responses for the 3
203 problems). Again, binary variables were defined using the threshold of 2.

204

205 ***Olfactory Score Calculation***

206 The total olfactory score (TOS) was calculated by adding the monomolecular odorant score to
207 the mixture score. It ranged from 0 (no correct response for the 6 problems) to 6 (correct re-
208 sponses for the 6 problems). Binary variables were defined by a total score <4, this threshold
209 was retained as it corresponds to a majority of incorrect responses.

210

211 **Statistical Analysis**

212 Quantitative variables were summarized as median with interquartile range (IQR) and com-
213 pared across groups using Mann-Whitney non-parametric test. Categorical data were ex-
214 pressed as percentages and compared between groups using Fisher exact test. The accuracy of
215 olfactory tests was evaluated by applying data to receiver-operating characteristic (ROC)
216 curves. To study the associations between children characteristics and olfaction, the Spearman
217 non-parametric test was used. Statistical analyses were performed using Stata 16 (StataCorp
218 LLC, Texas, USA) and Prism software (GraphPad, version 9, San Diego, USA), significance
219 was considered at the level 5%.

220

221 **Results**

222

223 **Child Characteristics**

224 From May 2016 to December 2019, we recruited 34 sCMV children (median [IQR] age, 6 [5-
225 8] years; 19 [55.9%] male, Tables 1, S1, S2). We also recruited 34 healthy matched-CTL. As
226 a supplementary control, we included aCMV children. However, due to absence of CMV
227 newborn screening in France, enrolment of aCMV was complex, particularly in the 7-10 year
228 age group, and only 20 aCMV were enrolled (median [IQR] age, 4 [3-6] years (only 5 chil-
229 dren aged 7-10); 12 [60.0%] male. Thus, we ultimately essentially compared sCMV to CTL
230 children because we did not reach the targeted number of aCMV children. Fig. 1 shows the
231 flow chart of the selection process.

232 Among the 54 children with congenital CMV infection, 23 presented hearing or vestibulo-
233 lar deficit at inclusion. Hearing deficits were reported in 19 children (12 in the sCMV group
234 and 7 in the aCMV group). Three presented with profound congenital hearing loss at birth (1
235 in the sCMV group and 2 in the aCMV group).

236

237 **Olfactory Performance**

238 Among CTL, both the monomolecular odorant discrimination score and the TOS were
239 positively correlated with age ($r=0.42$, $P=0.012$; and $r=0.48$, $P=0.004$, respectively). In
240 CTL, TOS was significantly higher in children 7-10 years than in those 3-6 years (median
241 (IQR): 4.0 [4.0-5.0] and 3.0 [1.0-4.0], $P=0.002$), and in consequence the proportion with a
242 TOS <4 was significantly lower in CTL 7-10 years than in CTL 3-6 years (18.75% and
243 66.7%, respectively; $P=0.007$, Table 2). Considering the monomolecular odorant discrimina-
244 tion score, the proportion with a score <2 was significantly lower in controls aged 7-10 years
245 than in controls aged 3-6 years (6.3% and 55.6%, respectively; $P=0.003$). Considering the

246 mixture odorant discrimination score, the proportion with a score <2 was not different be-
247 tween CTL aged 7-10 and 3-6 years (37.5% and 61.1%, respectively; $P=0.30$). There was no
248 association between olfactory scores and sex or with passive smoking.

249 ROC curve analysis revealed that the TOS was efficient to discriminate between CTL
250 and sCMV for children 7-10 years (area under the ROC curve [AUC]=0.857, $P=0.0006$, Fig.
251 2b), but not for children 3-6 years (AUC=0.519, Fig. 2a). Moreover, for children >6 years, the
252 mixture score alone was efficient to discriminate between CTL and sCMV (AUC=0.809,
253 $P=0.003$, Fig. 2d), but not the monomolecular odorant score (AUC=0.588, Fig. 2c).

254 Overall, the proportion of children with a TOS <4 was significantly higher in the
255 sCMV group than in the CTL group (73.5% and 44.1%; $P=0.025$). Considering only the
256 monomolecular odorant discrimination score, there was no difference between the two groups
257 (Fig. 3b). For the only mixture scores, the proportion of children with a score <2 was signifi-
258 cantly higher in the sCMV group than in the CTL group (76.5% and 50.0%, respectively, P
259 =0.043).

260 Stratifying by age, the difference in the proportion of children with a TOS <4 was
261 highly significant between sCMV and CTL in children 7-10 years of age (91.2% and 18.7%,
262 $P<0.001$), but not in younger children (Fig. 3d).

263 In sCMV children, there was no difference in the TOS between children presenting
264 with and those without neurological involvement (Fig. S1). There was no difference for the
265 TOS between sCMV children presenting with hearing loss and those with normal hearing
266 (Fig. 3e).

267 There was no difference in the proportion of children with a TOS<4 between aCMV,
268 subset of age-matched sCMV and subset of age-matched CTL children in the 7-10 year age
269 group as well as in younger children (Fig. S2).

270 There was no difference in the olfactory scores between children who received anti-
271 ral treatment after CMV detection (n=7) and those without treatment (n=38) (Table 2).

272

273 **Discussion**

274

275 This is the first study to assess olfactory function in children with congenital CMV infection
276 and to report the severity of their altered olfaction ability. The strengths of this study are: i)
277 PCR-confirmed congenital CMV infection, ii) the documentation of clinical, radiologic and
278 vestibular symptoms as well as concomitant evaluation of hearing and iii) enrolment of age
279 and sex-matched CTL.

280 Reduced olfactory score was frequent in congenital CMV infection, occurring in
281 91.2% of our sCMV patients aged 7-10 years, thus becoming the most frequent sensorineural
282 deficit in our series. 44.1 % of these patients experience other sensorineural deficits (hearing
283 loss in 35.3%, vestibular deficit in 38.2%). Conversely, 5 aCMV children aged 7-10 years
284 demonstrated normal olfaction. The most likely explanation of this observation is the proba-
285 ble link between olfactory performance and the severity of congenital CMV infection. A re-
286 cent retrospective study demonstrated that 67% of children with olfactory dysfunction were of
287 congenital origin, whereas 12% were due to head trauma [13]; the role of congenital infection
288 being to date unknown, the responsibility of CMV has certainly not yet been evaluated. In
289 previous studies, loss of smell in infants has been linked to neurodevelopmental disorders, in-
290 cluding attention deficit/hyperactivity disorders and autism spectrum [10, 17]. Olfaction is
291 essential for food information, safety, emotion regulation, scaffolds environment perception
292 and memory, mother-child attachment, and social cognition [18]. However, there is no abso-
293 lute correlation between neurodevelopmental disorders and olfactory scores, as we do not find
294 a link between these two in our present series.

295 Olfactory loss can also be observed after other post-viral infections such as rhinovirus,
296 parainfluenza virus, coronavirus (CoV) 229E and Epstein-Barr virus [19]. Olfactory discrimi-
297 nation and thresholds were preserved in these latter infections, compared to identification
298 [20]. Olfactory loss can be an early sign of coronavirus disease 2019 (COVID-19) due to se-
299 vere acute respiratory syndrome CoV-2; this dysfunction can persist several months and be
300 associated to an olfactory bulb hypometabolism [21-23]. Fetopathological studies have
301 demonstrated the presence of CMV in neural stem cells of the olfactory bulb underlining the
302 specific targeting of the pluripotent cells, rather than olfactory neurons [8].

303 Olfactory scores in our CMV-infected children were independent from age, con-
304 trasting with CTL children. Improved olfactory performance in healthy children is correlated
305 with the maturation of the olfactory system with better ability to discriminate with age. This is
306 not observed in sCMV-infected children, possibly due to the viral targeting of pluripotent
307 cells [8]. Olfactory scores in our CMV-infected children were independent from hearing loss
308 or vestibular deficit. These findings contrast with an epidemiological study where a correla-
309 tion was found between hearing loss and olfactory dysfunction, but infection, in particular
310 congenital, was not considered as an influential factor [24]. The incidence of cranial neuropat-
311 hies is higher in patients with post-viral olfactory loss compared to a control population [25];
312 however, we found no difference for the olfactory score between children presenting neuro-
313 logical manifestations and those without neurological involvement. These findings suggest
314 that peripheral (audiovestibular) and central (cerebral) lesions are independent and that neuro-
315 logical damage did not induce vulnerability to olfactory dysfunction in our sCMV infants.
316 CMV host entry is probably systemic, associated with macrophage infection [26]. To date,
317 there is no evidence of CMV spread to the brain through the cribriform plate.

318 Another insight of our study is the greater efficiency of the mixture discrimination
319 tests in assessing olfactory function in children compared to the mono-odorant testing. While

320 the monomolecular test evaluates the ability to discriminate between two single odorants of
321 similar concentration, the mixture test is a more difficult perception test with discrimination
322 of mixtures presenting the same two odorants but in different concentration. Of note, the
323 odorant mixture discrimination score only discriminates between CTL and CMV from the age
324 of 7, which strongly limits its use in clinics. The lower discrimination efficacy in younger
325 children may be due to the subtler olfactory difference between scent pens that children 3-6
326 may be less attentive to.

327 Limitations of our study include the use of olfactory tests that have not been validated
328 for children in this version before and a predefined cut-off value that was not based on previ-
329 ous observations in a control group. The cut-off value first appears in the initial statistical
330 plan of the study's protocol, that was subject to no change. This cut-off of 4 points to distin-
331 guish between normosmia and olfactory dysfunction was retained in the initial statistical plan
332 of this study as it corresponds to a majority of incorrect responses. This cut-off leads to a high
333 percentage of children in the control group with reduced olfactory function. Another limit of
334 our study is the small sample size of the human cohort, especially for aCMV patients. Extend-
335 ing these investigations to a larger group of children, including controls, would allow specify-
336 ing these first findings. Moreover, this study would benefit from additional approaches to
337 characterize the olfactory function, by using tests of perception and identification of odorants.

338 In conclusion, this study highlighted the high incidence of olfactory impairment in
339 children with congenital sCMV infection. As olfactory loss can impact nutrition, social inter-
340 action, safety and quality of life, early detection of olfactory disorders may lead to olfactory
341 rehabilitation programs in order to limit neurodevelopmental consequences: recent studies
342 have demonstrated the importance of olfactory training to improve the olfactory function in
343 adults [27, 28] and children [29].

344

345 **Supplementary information**

346 **Supplementary methods.**

347 **Table S1.** Congenital CMV diagnostic confirmation of the 54 cytomegalovirus-infected chil-
348 dren.

349 **Table S2.** Neonatal viral symptom characteristics of the 34 cytomegalovirus-infected children
350 symptomatic at birth.

351 **Fig. S1.** Olfactory scores in control children and children with congenital cytomegalovirus in-
352 fection symptomatic at birth, according to neurological involvement.

353

354 **Competing interests**

355 The odorant mixtures are the subject of a patent (WO2017198816A1 published on November
356 23, 2017) by Institut Pasteur, Centre National de la Recherche Scientifique, and Assistance
357 Publique–Hôpitaux de Paris on which Drs Lazarini, Lledo, Teissier and Levivien are named
358 as inventors. Drs Lazarini, Madec, Taieb, Mottez, Lledo and Mr Buivan are employees of In-
359 stitut Pasteur of Paris that sponsored this research. The remaining authors declare no other
360 disclosures.

361

362 **References**

363

- 364 1. Coyne CB, Lazear HM (2016) Zika virus - reigniting the TORCH. *Nat Rev Microbiol*
365 14(11):707-715
- 366 2. Nicloux M, Peterman L, Parodi M, Magny JF (2020) Outcome and management of
367 newborns with congenital cytomegalovirus infection. *Arch Pediatr* 7(3):160-165

- 368 3. Dahle AJ, Fowler KB, Wright JD, Boppana SB, Britt WJ, Pass RF (2000) Longitudinal
369 investigation of hearing disorders in children with congenital cytomegalovirus. *J Am*
370 *Acad Audiol* 11(5):283-290
- 371 4. Goderis J, Keymeulen A, Smets K, et al. (2016) Hearing in Children with Congenital
372 Cytomegalovirus Infection: Results of a Longitudinal Study. *J Pediatr* 172:110-115.e2
- 373 5. E X, Meraner P, Lu P, et al. (2019) OR14I1 is a receptor for the human cytomegalovirus
374 pentameric complex and defines viral epithelial cell tropism. *Proc Natl Acad Sci U S A*
375 116(14):7043-7052.
- 376 6. van Den Pol AN, Mocarski E, Saederup N, et al (1999) Cytomegalovirus cell tropism,
377 replication, and gene transfer in brain. *J Neurosci* 19(24):10948-10965
- 378 7. Odeberg J, Wolmer N, Falci S, Westgren M, Seiger A, Söderberg-Nauclér C (2006) Hu-
379 man cytomegalovirus inhibits neuronal differentiation and induces apoptosis in human
380 neural precursor cells. *J Virol* 80(18):8929-8939
- 381 8. Teissier N, Fallet-Bianco C, Delezoide AL, et al (2014) Cytomegalovirus-induced brain
382 malformations in fetuses. *J Neuropathol Exp Neurol* 73(2):143-158
- 383 9. Lazarini F, Katsimpardi L, Levivien S, et al (2018) Congenital cytomegalovirus infection
384 alters olfaction prior to hearing deterioration in mice. *J Neurosci* 38(49):10424-10437
- 385 10. Doty RL (2001) Olfaction. *Annu Rev Psychol* 52:423-452
- 386 11. Hummel T, Bensafi M, Nikolaus J, et al (2007) Olfactory function in children assessed
387 with psychophysical and electrophysiological techniques. *Behav Brain Res* 180(2):133-
388 138
- 389 12. Cameron EL, Doty RL (2013) Odor identification testing in children and young adults
390 using the smell wheel. *Int J Pediatr Otorhinolaryngol* 77(3):346-350
- 391 13. Schriever VA, Hummel T (2020) Etiologies of olfactory dysfunction in a pediatric pop-
392 ulation: based on a retrospective analysis of data from an outpatient clinic. *Eur Arch*

393 Otorhinolaryngol 277(11):3213-3216

394 14. Hsieh JW, Keller A, Wong M, Jiang RS, Vosshall LB (2017) SMELL-S and SMELL-R:

395 Olfactory tests not influenced by odor-specific insensitivity or prior olfactory experience.

396 Proc Natl Acad Sci U S A 114(43):11275-11284

397 15. Hummel T, Sekinger B, Wolf SR, Pauli E, Kobal G (1997) 'Sniffin' sticks': olfactory

398 performance assessed by the combined testing of odor identification, odor discrimination

399 and olfactory threshold. Chem Senses 22(1):39-52

400 16. Maudoux A, Teissier N, Francois M, et al (2020) Vestibular impact of Friedreich ataxia

401 in early onset patients. Cerebellum Ataxias 7:6

402 17. Rozenkrantz L, Zachor D, Heller I, et al (2015) A Mechanistic Link between Olfaction

403 and Autism Spectrum Disorder. Curr Biol 25(14):1904-1910

404 18. Schaal B, Saxton TK, Loos H, et al (2020) Olfaction scaffolds the developing human

405 from neonate to adolescent and beyond. Phil Trans R Soc B 375(1800):20190261

406 19. Suzuki M, Koichi Saito K, Wei-Ping Min, et al (2007) Identification of viruses in patients

407 with postviral olfactory dysfunction. Laryngoscope 117(2):272-277

408 20. Whitcroft KL, Cuevas M, Haehner A, et al (2017) Patterns of olfactory impairment re-

409 flect underlying disease etiology. Laryngoscope 127(2):291-295

410 21. Niklassen AS, Draf J, Huart C, et al (2021) COVID-19: Recovery from Chemosensory

411 Dysfunction. A Multicentre study on Smell and Taste. Laryngoscope 131(5):1095-1100

412 22. de Melo GD, Lazarini F, Levallois S, et al (2021) COVID-19-related anosmia is associ-

413 ated with viral persistence and inflammation in human olfactory epithelium and brain

414 infection in hamsters. Sci Transl Med 13(596):eabf8396

415 23. Guedj E, Lazarini F, Morbelli S, et al (2021) Long COVID and the brain network of

416 Proust's madeleine: targeting the olfactory pathway. Clin Microbiol Infect S1198-

417 743X(21)00238-X

- 418 24. Park JH, Byeon HK, Park KN, et al (2017) Epidemiological association of olfactory dys-
419 function with hearing loss and dysphonia in the Korean population: A cross-sectional
420 study. *Medicine (Baltimore)* 96(47):e8890
- 421 25. Jitaroon K, Wangworawut Y, Ma Y, Patel ZM (2020) Evaluation of the Incidence of
422 Other Cranial Neuropathies in Patients With Postviral Olfactory Loss. *JAMA Otolaryn-
423 gol Head Neck Surg* 146(5):465-470
- 424 26. Farrell HE, Stevenson PG (2019) Cytomegalovirus host entry and spread. *J Gen Virol*
425 100(4):545-553
- 426 27. Addison AB, Wong B, Ahmed T, et al (2021) Clinical Olfactory Working Group Con-
427 sensus Statement on the Treatment of Post Infectious Olfactory Dysfunction. *J Allergy
428 Clin Immunol* 147(5):1704-1719
- 429 28. Zhang Y, Mei T, Chen Y, et al (2021) Smell disorders in COVID-19 patients: role of
430 olfactory training: A protocol for systematic review and meta-analysis. *Medicine (Balti-
431 more)* 100(8):e24862
- 432 29. Mahmut MK, Pieniak M, Resler K, et al (2021) Olfactory training in 8-year-olds increases
433 odour identification ability: a preliminary study. *Eur J Pediatr* 180(7):2049-2053

434

435

436

Table 1. Characteristics of the children with congenital, PCR-confirmed, CMV infection

Demographics	Total (n=54)	Severity of congenital CMV infection		P Value
		Viral symptoms ^a at birth: sCMV (n=34)	Asymptomatic at birth: aCMV (n=20)	
Boys, No. (%)	31 (57.4)	19 (55.9)	12 (60.0)	0.77
Age at inclusion (years), Median (IQR)	5 (4-8)	6 (5-8)	4 (3-6)	0.27
No. of children with age ≤6 years	34	18	15	0.15
No. of children with age >6	21	16	5	
Confirmed maternal CMV reactivation with neuro sequelae, No.	1	1	0	
Including hearing deficit ^b	0	0	0	
Maternal primary CMV infection ^c , No. (%)	21 (38.9)	9 (26.5)	12 (60.0)	0.020
Including confirmed, No. / suspected, No.	14 / 7	8 / 1	6 / 6	
Timing of CMV congenital infection: known, No. (%)				
Periconceptional or during first trimester (<14 weeks)	12 (57.1)	6 (66.7)	6 (50.0)	
Confirmed	7	6	1	
Including hearing deficit ^b	6	4	2	
Including neurosequelae	6	6	0	
Including intrauterine growth retardation	2	2	0	
Second (≥14 weeks and <28 weeks)	6 (28.6)	1 (10.0)	5 (41.7)	
Confirmed, No. / suspected, No.	4 / 2	0 / 1	4 / 1	
Including hearing deficit ^b : confirmed, No. / suspected, No.	1 / 1	0 / 1	1 / 0	
Neurosequelae: confirmed, No. / suspected, No.	1 / 1	0 / 1	1 / 0	
Third (>28 weeks)	2 (1.0)	1 (10.0)	1 (8.3)	
Confirmed	1	0	1	
Including hearing deficit and neurosequelae	0	0	0	
Antiviral treatment after detection of CMV infection, No./No. of data (%)	7/45 (15.6)	6/30 (20.0)	1/15 (6.7)	0.40
Postuomotor development, No./No. of data (%)				
Head control at age >4 months	5/46 (10.9)	4/30 (13.3)	1/16 (6.3)	0.64
Unsupported sitting at age >9 months	7/51 (13.7)	3/32 (9.4)	4/19 (21.1)	0.40
Unaided walking at age >17 months	12/52 (23.1)	8/33 (24.2)	4/19 (21.1)	0.99
Transcranial Doppler sonography assessment, No. (%)	26 (48.1)	20 (58.8)	6 (30.0)	0.09
Abnormal, No. (%)	8 (30.8)	8 (40.0)	0 (0.0)	0.08

Table 1. Characteristics of the children with congenital, PCR-confirmed, CMV infection (continued).

Demographics	Total (n=54)	Severity of congenital CMV infection		P Value
		Viral symptoms ^a at birth: sCMV (n=34)	Asymptomatic at birth: aCMV (n=20)	
Cerebral computed tomography and MRI assessment, No. (%)	35 (64.8)	23 (67.6)	12 (60.0)	0.52
Abnormal, No. (%)	25 (71.4)	20 (87.0)	5 (41.7)	0.024
including microcephaly	1	1	0	
Intracerebral calcifications	2	1	1	
Hyperintense signals in the white matter	17	13	4	
Ventricular dilations	5	5	0	
Ischemic lesions	1	1	0	
Olfactory bulb agenesis	1	1	0	
Cerebellar abnormalities	3	3	0	
Sensorineuro and neurocognitive disorders at inclusion, No. (%)	30 (55.6)	21 (38.9)	9 (16.7)	0.18
CNS only	7	6	1	
PNS only	4	-	4	
Including hearing loss ^b	3	-	3	
Mixed	19	15	4	
Including hearing loss ^b	16	12	4	
Behavioural disorders	5	4	1	
Hyperactivity	4	3	1	
Autism	1	1	0	
Hearing ^b or vestibular dysfunctions at inclusion, No. (%)	23 (42.6)	15 (44.1)	8 (40.0)	>0.99
Hearing deficit ^b at birth	3	1	2	
including boys, No.	3	1	2	
Hearing deficit ^b at enrolment, No. (%)	19 (35.2)	12 (35.3)	7 (33.3)	0.61
in boys, No.	13	8	5	0.64
Bilateral symmetric	1	3	1	
Bilateral asymmetric (10dB)	3	2	1	
Unilateral	6	4	2	
Auditory threshold of the most affected ear ^d - dB, median (IQR)		100 (60-100)	70 (40-100)	0.60
Auditory threshold of the least affected ear ^d - dB, median (IQR)		15 (10-35)	20 (15-40)	0.71
Profound and severe hearing loss: No. (%) with auditory threshold \geq 61dB	16 (29.6)	10 (29.4)	6 (30.0)	

Table 1. Characteristics of the children with congenital, PCR-confirmed, CMV infection (continued).

Demographics	Severity of congenital CMV infection			P Value
	Total (n=54)	Viral symptoms ^a at birth: sCMV (n=34)	Asymptomatic at birth: aCMV (n=20)	
Cochlear implants ^e , No. (%)	9 (16.7)	5 (14.7)	4 (20.0)	
Bilateral implants, No. (%)	5 (9.3)	2 (5.9)	3 (25)	
Vestibular deficit, No. (%)	20 (37.0)	13 (38.2)	7 (35.0)	>0.99
Complete and bilateral (areflexia)	2	1	1	
Partial and bilateral	5	2	3	
Canalar disorders alone	-	-	-	
Otolithic disorders alone	-	-	-	
Mixed disorders	5	2	3	
Partial and Unilateral	13	10	3	
Canalar disorders alone	1	1	-	
Otolithic disorders alone	-	-	-	
Mixed disorders	12	9	3	
Severity scale for vestibular dysfunction				
0, No. (%)	35 (64.8)	21 (38.9)	14 (25.9)	
1 (unilateral), No. (%)	13 (24.1)	10 (18.5)	3 (5.6)	
2 (bilateral), No. (%)	6 (11.1)	3 (5.6)	3 (5.6)	
Both hearing and vestibular deficit, No. (%)	15 (27.8)	10 (18.5)	5 (9.26)	
Including bilateral symmetric hearing loss	1	1	0	
Including profound and severe hearing loss (>61 dB)	13	8	5	
Including bilateral vestibular dysfunction	5	2	3	
Including both bilateral hearing and vestibular dysfunction	1	1	0	

^aViral symptoms at birth: one at least of the following neonatal symptoms: intrauterine growth retardation, prematurity, petechiae, organomegaly, icteriae, thrombocytopenia;

^bMaternal primary infection: Cases with high IgG avidity in the first trimester were considered as non-primary infections. Cases with seroconversion and/or positive IgG positive IgM, and low or intermediate IgG avidity in first trimester were considered as primary infections in the first trimester. Cases with negative IgG and IgM levels in the first trimester (at 12 to 14 weeks) were classified in either the second or third trimester groups, depending on the date of seroconversion.

^cHearing deficit: auditory threshold of the most affected ear ≥ 25 dB.

^dIn those with hearing deficit and no implant.

^eCochlear implants were usually performed in the early infancy, before 6.

Table 2. Olfactory scores by characteristics in controls and CMV-infected children

Variable	No. (%)		
	Monomolecular odorant discrimination Score <2	Mixture odorant discrimination Score <2	Total olfactory Score <4
Controls (n=34)			
Age group, y			
≤6 years (n=18)	10 (55.6)	11 (61.1)	12 (66.7)
>6 years (n=16)	1 (6.3)	6 (37.5)	3 (18.75)
<i>P</i> Value	0.003	0.30	0.007
Sex			
Girls (n=15)	5 (33.3)	6 (40.0)	6 (40.0)
Boys (n=19)	6 (31.6)	11 (57.9)	11 (57.9)
<i>P</i> Value	>0.999	0.49	0.49
Passive smoking			
Yes (n=8)	2 (25.0)	2 (25.0%)	1 (12.5)
No (n=26)	9 (34.6)	15 (57.7%)	14 (53.9)
<i>P</i> Value	>0.999	0.22	0.053
CMV-infected children (n=54)			
Age group, y			
≤6 years (n=34)	20 (60.6)	19 (57.6)	22 (66.7)
>6 years (n=21)	5 (23.8)	18 (85.7)	15 (71.4)
<i>P</i> Value	0.012	0.038	0.772
Sex			
Girls (n=23)	11 (47.8)	19 (82.6)	20 (86.7)
Boys (n=31)	14 (45.2)	18 (58.1)	17 (54.8)
<i>P</i> Value	>0.999	0.077	0.017
Passive smoking			
Yes (n=10)	4 (40.0)	7 (70.0)	6 (60.0)
No (n=44)	24 (54.6)	30 (68.2)	31 (70.5)
<i>P</i> Value	0.49	>0.999	0.71
Antiviral treatment after CMV detection			
Yes (n=7)	3 (42.9)	6 (85.7)	7 (100.0)
No (n=38)	18 (47.4)	25 (65.8)	23 (60.5)
<i>P</i> Value	>0.999	0.407	0.077
Hearing deficit			
Yes (n=19)	11 (57.9)	14 (73.7)	14 (73.7)
No (n=35)	16 (45.7)	26 (74.3)	25 (71.4)
<i>P</i> Value	0.57	>0.999	>0.999
Vestibular deficit			
Yes (n=20)	9 (45.0)	1 (75.0)	13 (65.0)
No (n=31)	13 (41.9)	19 (61.3)	20 (66.7)
<i>P</i> Value	>0.999	0.37	>0.999

Olfactory score is the sum of monomolecular and mixture odorant discriminations. Passive smoking is defined by exposition to more than a tobacco pack per day; Hearing deficit is defined by auditory threshold of the most affected ear ≥ 25 dB; Controls had normal hearing (inclusion criterion).

Legends of the figures

Fig. 1. Enrolment in the INFECSMELL-CLIN study. This study was performed between May 2016 and December 2019 in two hospital centers in Paris, France.

Fig. 2. ROC curves for the discrimination of children with congenital cytomegalovirus infection and controls using the olfactory scores. Panels a-d show the ROC curves for the discrimination of sCMV and matched controls between 3-6 years (a) and 7-10 years (b-d) using the olfactory score (a, b), the monomolecular odorant score (c) and the mixture score (d). N=34 sCMV; N=34 CTL.

Fig. 3. Olfactory scores in children with congenital cytomegalovirus infection and controls. Panels a-e show the total olfactory score (a, d, e), the monomolecular odorant (b) and mixture (c) scores. Box and whiskers showing median, 10 percentile, 25 percentile, 75 percentile, and 90 percentile in bar graphs. $P < 0.05$ are shown. N=54 CMV including 34 sCMV and 20 aCMV. N=34 CTL.