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Abstract

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

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

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

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

Introduction

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

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

Methods

Study Overview and ethical considerations

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

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

Enrolment Criteria

Children with confirmed congenital CMV, aged 3 to 10 years, were enrolled in this study during a standard care visit. Proof of congenital infection was ascertained by positive CMV polymerase chain reaction (PCR) in urine and/or blood in the first 3 postnatal weeks, or retrospective diagnosis for the presence of positive PCR on dried blood spots collected at postnatal day 3 to 7.

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

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

Clinical and Radiologic Symptoms

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

Hearing Evaluation

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

Olfactory Evaluation

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

Monomolecular Odorant Test

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

Mixture Odorant Test

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

Olfactory Score Calculation

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

Statistical Analysis

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

Results

Child Characteristics

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

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

Olfactory Performance

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

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

Discussion

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

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

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

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

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

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

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

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

Supplementary information

Supplementary methods.

Table S1. Congenital CMV diagnostic confirmation of the 54 cytomegalovirus-infected children.

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

Fig. S1. Olfactory scores in control children and children with congenital cytomegalovirus infection symptomatic at birth, according to neurological involvement.

Competing interests

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

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

Demographics	Total (n=54)	Severity of congenital CMV infection: Viral symptoms[a] at birth: sCMV (n=34)	Severity of congenital CMV infection: Asymptomatic at birth: aCMV (n=20)	*P* Value
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 sequalae, 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	
Neurosequalae: 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
Posturomotor 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: Viral symptoms[a] at birth: sCMV (n=34)	Severity of congenital CMV infection: Asymptomatic at birth: aCMV (n=20)	*P* Value
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 ≥61dB	16 (29.6)	10 (29.4)	6 (30.0)	

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

Demographics	Total (n=54)	Severity of congenital CMV infection: Viral symptoms[a] at birth: sCMV (n=34)	Severity of congenital CMV infection: Asymptomatic at birth: aCMV (n=20)	*P* Value
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	

[a]Viral symptoms at birth: one at least of the following neonatal symptoms: intrauterine growth retardation, prematurity, petechiae, organomegaly, icteriae, thrombocytopenia;
[b]Maternal 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.
[c]Hearing deficit: auditory threshold of the most affected ear ≥25dB.
[d]In those with hearing deficit and no implant.
[e]Cochlear 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)
P 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)
P 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)
P 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)
P 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)
P 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)
P 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)
P 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)
P 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)
P 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 ≥25dB; 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.