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Cytomegalovirus DNA detection in dried blood spots and perilymphatic fluids from pediatric and adult cochlear implant recipients with prelingual deafness

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ABSTRACT

Background: Congenital cytomegalovirus (CMV) infection is the leading cause of non-genetic congenital hearing loss. The contribution of congenital CMV to prelingual deafness and the pathophysiology is largely unknown.
Objective: (1) To analyze the prevalence of congenital CMV among cochlear implant (CI) recipients with prelingual deafness. (2) To genotype CMV present in dried blood spots (DBS) and in the inner ear years after birth.
Study design: Children and adults with prelingual deafness who received a CI in 2010–2011 were included prospectively. Perilymphatic fluids were collected during CI surgery and, in the pediatric cases, DBS were retrieved for CMV DNA detection. Furthermore, a cohort of children with prelingual deafness who received a CI between 2003 and 2008 were included retrospectively. CMV detection in DBS and perilymph was followed by gB and gH genotyping.
Results: Seventysix pediatric CI recipients were included. Seventy DBS were tested for CMV DNA, resulting in a prevalence of congenital CMV of 14% (10/70). Perilymphatic fluid was available from 29 pediatric CI recipients. One perilymph fluid, of a 21-month old girl with congenital CMV, asymptomatic at birth, was CMV DNA positive. The CMV strain in the perilymph was genotypically identical to the strain present in her DBS (gB1/gH2). Perilymph samples from 21 adult CI recipients were CMV DNA negative.
Conclusions: Our study stresses the important contribution of congenital CMV among pediatric CI recipients. Furthermore, our genotyping data support the hypothesis that CMV-related hearing loss is associated with ongoing viral replication in the inner ear up to years after birth.

1. Background

Congenital cytomegalovirus (CMV) affects approximately 1 in 200 newborns and is the leading cause of non-genetic childhood sensorineural hearing loss.[1] Hearing loss can be detected at birth in approximately 10% of children with symptomatic congenital CMV. Since the hearing loss is of late-onset character in approximately half of the cases,[2] an additional 10% of the infected children will develop hearing loss in the years after birth.[3]

Bilateral hearing loss has been reported to be attributable to congenital CMV in 15–40% of cases,[4–7] depending on the studied population. We previously found 8% congenital CMV infections among children with hearing loss of >40 dB at the age of 3–5 years in the Netherlands.[8] However, the exact prevalence of congenital CMV among children with cochlear implant (CI) is unknown.

The pathophysiology of CMV-related hearing loss has been studied in animal models. Data from mouse[9] and guinea pig[10] models show the presence of CMV in the inner ear predominantly in the coclear perilymphatic epithelial cells of the scala tympani and the auditory nerve spiral ganglion cells.[11] Besides a direct viral effect, inflammation may play a role in cochlear hair cell death causing hearing loss.[9] Some authors have tried to detect CMV in the inner ear during autopsy of congenitally infected fetuses[12] or

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newborns.[13,14] Data on CMV detection in the inner ear of living children are limited.

2. Objectives

The aim of this study was to analyze the prevalence of congenital CMV infections among CI recipients with prelingual deafness. Therefore, we retrospectively diagnosed congenital CMV in a cohort of pediatric and adult CI recipients with prelingual deafness using dried blood spots (DBS). Furthermore, we analyzed perilymphatic fluid, which is routinely exposed during CI surgery, for the presence of CMV DNA (including CMV gB/gH genotypes), in an attempt to gain insight into the pathophysiology.

3. Study design

3.1. Study population

3.1.1. Pediatric CI recipients

The pediatric study population consisted of profoundly deaf children who received a unilateral CI from October 2010 to December 2011 in the Leiden University Medical Center (LUMC), and from February to December 2011 in the Radboud University Medical Center Nijmegen (UMCN). These children were included prospectively ($n = 31$). Blood and perilymphatic fluid were collected during CI surgery and DBSs were retrieved to diagnose congenital CMV infection retrospectively. Because of the limited national storage duration of DBS, the inclusion was limited to children up to the age of 6 years.

Furthermore, a cohort of profoundly deaf children who received a CI from 2003 to 2008 at the age of 0–5 years were included retrospectively ($n = 45$). These children were part of the previously published DECIBEL study, which included infants with permanent bilateral hearing impairment (≥40 dB in the better ear) at the age of 3–5 years.[8] DBS were retrieved from these children. Since these children were retrospectively included, no blood and perilymphatic fluid samples were available.

3.1.2. Adult CI recipients

The adult study population consisted of patients with profound prelingual deafness who received a unilateral CI from April to December 2011 in the LUMC ($n = 21$). These adults were included prospectively and perilymphatic fluid and blood samples at the time of CI surgery were collected. No DBS could be retrieved from these adults because the national storage duration of DBS is limited to 6 years.

Written (parental) informed consent was given. The study was approved by the Medical Ethics Committee (CME) of the LUMC, the Netherlands.

3.2. Specimen processing

3.2.1. Blood samples

EDTA-anticoagulated blood samples were drawn during CI surgery from the prospectively included pediatric and adult CI recipients. IgG anti-CMV antibodies were measured in plasma using an automated chemiluminescent microparticle immunoassay (Architect, Abbott Laboratories, Abbott Park, IL, USA). A CMV antibody level ≥ 6 AU/ml was considered to be positive.

3.2.2. Dried blood spots

In the Netherlands, dried blood spots (DBS) are routinely collected from all newborns within a few days of birth for the nationwide metabolic and endocrine screening program. These are stored for a maximum of 6 years at the National Institute for Public Health and the Environment (RIVM, Bilthoven, the Netherlands). After parental informed consent, DBS were retrieved from pediatric CI recipients younger than 6 years of age.

DNA was extracted from the DBS using the QIAamp DNA Mini Kit following the protocol "Isolation of total DNA from FTA and Guthrie cards", as described previously.[15]

3.2.3. Perilymphatic fluid

Study participants received standard care cochlear implantation including a mastoidectomy with facial recess approach into the middle ear. During cochleostomy, the scala tympani was entered with a 24 gauge needle attached to a 1 ml syringe, and the routinely exposed perilymphatic fluid was aspirated. The specimen (10–50 μl) was transferred to the laboratory within an hour, where the fluid was supplemented with phosphate buffered saline (PBS) to a total of 100 μl.

DNA was extracted from the perilymphatic fluid using the QIAamp DNA Blood Mini Kit following the protocol "Isolation of total DNA from Blood or Body Fluids (spin protocol)".

3.3. Real-time PCR

3.3.1. CMV DNA amplification

Amplification of a 126-bp fragment from the CMV immediate-early antigen region was performed using an internally controlled quantitative real-time PCR as described previously.[15,16] Ten μl of DNA extract was used. Quantification was performed using a dilution series of titrated CMV (strain AD169; Advanced Biotechnologies Inc., Columbia, MD, USA) as an external standard.

3.3.2. Beta-globin amplification

To control for the presence of human DNA in perilymphatic fluid, a 110-bp fragment of the housekeeping gene beta-globin was amplified by means of a monoplex real-time PCR. Ten μl of DNA extract of perilymphatic fluid was added to 40 μl PCR pre-mixture obtaining final concentrations of 0.5 μM forward beta-globin primer (5′ AAG TGC TCG GTG CCT TTA GTG 3′), 0.5 μM reverse beta-globin primer (5′ ACG TGC AGC TTG TCA CAG TG 3′), 0.2 μM beta-globin TaqMan probe (YAK-5′ TGG CCT GGC TCA CCT GGA CAA CCT 3′-BHQ-1), 4.5 mM $MgCl_2$, and 25 μl HotStar Master Mix (QIAGEN, Hilden, Germany). Template denaturation and activation of HotStar Taq DNA polymerase for 15 min at 95 °C were followed by 50 cycles of denaturation at 95 °C for 5 s, annealing at 55 °C for 15 s, and extension at 72 °C for 15 s. Real-time PCR was performed using a CFX96™ Real-Time PCR Detection System (Bio-Rad, Veenendaal, The Netherlands). Quantification was performed using a 10-fold dilution series of human genomic DNA (Promega Netherlands) (100 ng/μl to 0.1 pg/μl) as standard.

3.3.3. Genotyping of CMV

CMV envelope glycoproteins gB (UL55) and gH (UL75) genotypes were determined by means of two multiplex real-time PCR assays, as described previously.[17]

4. Results

4.1. Pediatric CI recipients

4.1.1. Prevalence of congenital CMV

A total of 76 pediatric CI recipients were included in the study. Thirty-one patients were included prospectively and received a CI in the two university medical centers between 2010 and 2011 (LUMC: $n = 13$, UMCN: $n = 18$). The median age was 1 year (range 8 months–8 years). Forty-five patients received a CI from 2003 to 2008 and were included retrospectively (DECIBEL-study,[8] age range 3–5 years).