Human Cytomegalovirus UL144 Gene Polymorphisms in Congenital Infections

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The human cytomegalovirus (HCMV) UL144 gene is a tumor necrosis factor-like receptor with the potential to affect HCMV virulence. HCMV strains display genetic variability in the UL144 region, and the analysis of a potential link between UL144 gene polymorphisms and disease severity has scarcely been studied. However, a correlation between the UL144 genotype and congenital-disease outcome has been reported in one previous study, with the observation that all asymptomatic infants had a single UL144 genotype. In order to confirm or refute this finding, we determined the UL144 polymorphisms of HCMV strains recovered from the amniotic fluids of 38 infected fetuses and compared them to HCMV strains obtained from 30 viremic adult controls. The UL144 sequences were distributed among five genotypes (A, B, C, AC, and AB), as previously described. We observed similar percentages of the three major genotypes A (37%), B (33%), and C (27%) in our population. The UL144 genotype distributions were similar among the group of infected adults and the group of infected fetuses and among asymptomatic and symptomatic fetuses (P < 0.05). In our series, all five UL144 genotypes could be vertically transmitted from mothers to fetuses, and all could cause symptomatic congenital infection. We concluded that determination of UL144 polymorphisms in cases of congenital infection is not relevant, since it is unlikely to help predict the outcome of the infection.

Materials and Methods

Patients and samples. Between February 2001 and May 2002, 505 amniotic fluid samples were sent to our laboratory for real-time HCMV PCR assays. Forty-four samples were found to be positive, and 38 of these samples were available for UL144 polymorphism testing. The geographical origins of the 38 pregnant women with primary HCMV infection were Paris and the surrounding area (n = 32; 84%) and Reunion Island (n = 6; 16%).

The control group consisted of 30 blood samples obtained from 30 infected adults. These 30 adults were either organ or bone marrow transplant recipients diagnosed with active HCMV disease between May 2002 and January 2003 (12). All of the patients were followed in Necker Hospital and lived in Paris and the surrounding area.

Informed consent was obtained from all patients or their parents.

Clinical-data collection. Ultrasonographic reports, outcome, and/or postmortem examination reports were collected. Some of these cases have also been described elsewhere (15). We classified the fetuses into two groups. Group 1 was the group of severely symptomatic fetuses showing the presence of cerebral ultrasounds feature(s), the presence of at least two extracerebral features, or subsequent abnormal neurological development. Group 2 was the group of non-severely symptomatic fetuses with the presence of, at most, only one extra cerebral ultrasonad feature or of subsequent normal neurological development.
UL144 gene amplification. Total DNA was extracted from 200 μL of amniotic fluid or blood plasma with the QIamp DNA minikit (QIAGEN S.A., Courtaboeuf, France). The extracted DNA was amplified in 10 mM Tris HCl, 50 mM KCl, 1.5 mM MgCl₂ (Applied Biosystems), 1 mM deoxynucleoside triphosphate (Applied Biosystems), 10 μM forward primer (5'-TCG TAT TAC AAA CCG CGG AGA GGA T-3') and reverse primer (5'-ACT CAG ACA CGG TTC CGT AA-3') (3), and 1U of Taq polymerase (Applied Biosystems). A nested PCR was performed with forward primer (5'-CTT CCG GTA GGC ATG AA-3') and reverse primer (5'-GAC TTC ATC GTA CCG TGA-5'). Amplification was carried out with a Perkin-Elmer Gene Amp PCR system 2400. The conditions for amplification with all primers sets were 94°C for 5 min, followed by 30 cycles at 94°C for 1 min, 55°C for 45 s, and 72°C for 1 min. The 30 cycles were followed by a single extension cycle at 72°C for 5 min. The PCR products were purified using a QIAquick PCR Purification kit (QIAGEN).

DNA sequencing. The purified PCR products were sequenced using a fluorescent-dideoxyterminator method (Big Dye Terminator Sequencing kit; Applied Biosystems). The sequencing products were analyzed on a model 377 automated DNA sequencer (Applied Biosystems). The sequences obtained were aligned with Sequence Navigator software and compared to five reference sequences from Arav-Boger et al. (1) (GenBank accession numbers AF498086 to AF498090) and 12 sequences from Lurain et al. (14) (GenBank accession numbers AF084994, AF084999, AF084996, AF084990, AF179208, AF084994, AF085003, AF085004, AF084996, AF084999, and AF085002).

Pairwise evolutionary distances were estimated using Kimura's two-parameter method, and the trees were then constructed by a neighbor-joining method (implemented in PHYLIP (Phylogeny Inference Package version 3.6)).

RESULTS

Congenital CMV infection-related symptoms and outcome. Congenital-infection outcome data were obtained in 36 out of 38 cases: 2 cases (numbers 2 and 7) were lost for follow-up, 23
pregnancies were terminated because of ultrasonographic CMV-related severe abnormalities, and 11 pregnancies went to term. Among the 11 neonates, 9 were asymptomatic at birth and had not developed neurological problems at 6 months to 3 years of life. Two neonates were symptomatic at birth and developed severe neurological handicaps.

Detailed ultrasonographic data are reported in Table 1. The main ultrasonographic findings were cerebral ventriculomegaly ($n = 12$), echogenic bowel ($n = 12$), intrauterine growth retardation (IUGR) ($n = 8$), microcephaly ($n = 6$), cerebral calcifications ($n = 4$), and oligohydramnios ($n = 4$). Hydrops, hepatomegaly, cerebral cysts, and hyperechogenic ventricles were less frequent findings. According to our selection criteria, 25 fetuses were considered severely symptomatic and were
classified in group 1, 11 fetuses were asymptomatic and were classified in group 2, and 2 fetuses were not classified because of the absence of follow-up.

**UL144 genotyping results.** The UL144 gene sequences of the 68 samples studied clustered in five genotypes, as previously described by Arav-Boger et al. and Lurain et al. (1, 14) (Fig. 1). The classification in genotypes A, B, C, AB, and AC used by Arav-Boger et al. (1) was also used in this series. These results were confirmed by a bootstrap value of ≥60%. Variabilities between genotypes A and C, C and B, and A and B were 15, 19, and 22%, respectively. Intravariability within each genotype varied between 0 and 5% and was mainly confined to the 5’ extremity of the gene.

The distribution of UL144 genotypes in the group of infected adults was as follows: 11 (37%), 10 (33%), 8 (27%), 0, and 1 (3%) for genotype B, A, C, AB, and AC, respectively. The UL144 genotype distribution was not significantly different among the three populations studied (symptomatic fetuses, asymptomatic fetuses, and control population) (P > 0.05) (Table 2).

**DISCUSSION**

The UL144 gene encodes a homologue of the herpesvirus entry mediator, which is a member of the TNF receptor superfamily (6), and the UL144 protein might act as a lure to divert the host immunity response. Variability in the UL144 gene nucleotide sequence, therefore, may affect HCMV virulence. Based on this hypothesis, Arav-Boger et al. (1) determined the UL144 genotypes in a population of infected fetuses and reported that infection with the least common UL144 genotypes (A, C, AC, and AB) was associated with an unfavorable outcome of the disease. They therefore suggested that polymorphisms in the UL144 gene may be associated with congenital HCMV disease.

In this study, we could identify the five UL144 genotypes previously described (1, 4, 14). Genotype B was also the most prevalent genotype recovered among French HCMV strains, as it had been previously described in the United States (1, 4, 14). However, the B genotype was less frequent in our population than in the U.S. population: 37% in our control group versus 51 (23 of 45) and 69% (34 of 49) in U.S. studies (4, 14). Alternatively, the prevalences of genotypes A and C were higher in the French population than in the United States, with 33 and 27%, respectively, in our control group versus 22 (10 of 45) and 9% (4 of 45) in the first U.S. study (14) and 4 (2 of 49) and 10% (5 of 49) in the second U.S. study (4). The prevalences of genotypes AB and AC were as low among French strains as in the United States (4, 14).

In our study, UL144 genotype distributions were similar among strains recovered from infected fetuses and among those obtained from infected adults. Thus, the information obtained on UL144 genotypes suggests that the strains recovered in congenital infection reflect the strains circulating in the French population and that no UL144 genotype is particularly associated with congenital HCMV infection. All genotypes except AC, which was found in only 4% of all strains, could be recovered both in asymptomatic and in symptomatic cases of congenital infection. Our results are therefore different from those of Arav-Boger et al. (1), who described an association between UL144 genotypes A and C and the severity of congenital infection. In our study, 45 (5 of 11) and 60% (15 of 25) of the strains detected in 11 asymptomatic fetuses and in 25 symptomatic fetuses, respectively, were genotype A or C. In the U.S. study, no strains of genotypes A and C were recovered from 10 asymptomatic infected fetuses, although 46% of the strains found in 13 symptomatic fetuses were genotype A or C. These results are probably explained by the conjunction of a lower prevalence of genotypes A and C in the U.S. population (4 to 22 and 9 to 10%, respectively) and the small size of the study groups. However, collecting samples from congenitally HCMV-infected fetuses in the absence of national screening policies is challenging, explaining the difficulty in gathering a larger series of samples.

In conclusion, the UL144 genotype does not seem to carry any definite prognostic value in infected fetuses. All five UL144 genotypes can be vertically transmitted from mothers to fetuses, and all can cause symptomatic congenital infection. It is therefore not relevant to test for UL144 genotypes in this context. Other HCMV polymorphisms, such as glycoprotein B genotypes, have been evaluated in congenital infection and also proved to be disappointing (2, 3, 5, 13, 15, 21). Moreover, it was recently demonstrated that the inter- and intragenic variability of HCMV clinical isolates leads to an infinite number of genetic combinations, probably explaining the failure to use sequence information to predict disease outcome (16). Alternatively, specific host genetic factors probably explain the severity of congenital HCMV infection in some individuals (11), and more efforts probably should target this approach.

**REFERENCES**


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**TABLE 2. UL144 genotype distribution among French HCMV strains**

<table>
<thead>
<tr>
<th>Case</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>AB</th>
<th>AC</th>
</tr>
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<tr>
<td>Congenital infection (n = 38)</td>
<td>10 (26)</td>
<td>13 (34)</td>
<td>12 (32)</td>
<td>2 (5)</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Asymptomatic (n = 11)</td>
<td>2 (18)</td>
<td>5 (45)</td>
<td>8 (32)</td>
<td>3 (27)</td>
<td>1 (10)</td>
</tr>
<tr>
<td>Symptomatic (n = 25)</td>
<td>6 (24)</td>
<td>8 (32)</td>
<td>9 (36)</td>
<td>1 (4)</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Unclassified (n = 2)</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Control group (n = 30)</td>
<td>10 (33)</td>
<td>11 (37)</td>
<td>8 (27)</td>
<td>0</td>
<td>1 (3)</td>
</tr>
</tbody>
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