Genotype of 86 *Toxoplasma gondii* Isolates Associated with Human Congenital Toxoplasmosis, and Correlation with Clinical Findings

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To study the influence of *Toxoplasma gondii* genotypes on the severity of human congenital toxoplasmosis (asymptomatic, benign, or severe infection or newborn or fetal death), 8 microsatellite markers were used to analyze 86 *T. gondii* isolates collected from patients with congenital toxoplasmosis. Seventy-four different genotypes were detected, some identical genotypes originating probably from the same source of contamination. The 3 less polymorphic microsatellite markers associated with 6 isoenzymatic markers allowed a classification of isolates into the 3 classical types and detected atypical genotypes. Whatever the clinical findings, type II isolates were largely predominant (84.88% in the whole collection and 96.49% in 57 consecutive cases). Type I and atypical isolates were not found in asymptomatic or benign congenital toxoplasmosis. However, in 4 cases in which children were not infected despite isolation of *T. gondii* from placenta, only type I isolates were found.

Toxoplasma gondii infection acquired during pregnancy can lead, in ~30% of cases, to infection of the fetus [1]. The risk and severity of congenital toxoplasmosis largely depend on the gestational age at which the mother acquired infection. For many years, it has been established that the fetal transmission rate is lower when the infection is acquired during early pregnancy, but, when acquired then, the congenital toxoplasmosis is more severe, ranging from in utero death to severe neuro-ophthalmic involvement. Fetal infection acquired during the last trimester gives rise to subclinical toxoplasmosis, to isolated intracranial calcifications, or to chorioretinal disease. The transmission rate during the third trimester of pregnancy increases from 30% at 6 months of pregnancy to nearly 100% during the last weeks [1, 2].

This study aimed to determine the prevalence of each main genotype in congenital toxoplasmosis observed in several French laboratories and whether there is any influence of the genotype on the severity of the disease in this collection of isolates. To approach these problems, the multilocus-typing strategy was based on the study of the polymorphism of 8 microsatellites (MS) present in the genome of *T. gondii* as described elsewhere [3]. Microsatellites are high-resolution markers, allowing an epidemiologic tracking of isolates. The less polymorphic MS markers associated with isoenzyme markers allow a clustering of isolates in the 3 classical types and a better detection of atypical genotypes.

The parasite factors (inoculum, infective parasite stage, and genotype of *T. gondii* isolate) that could contribute to pathogenicity have been insufficiently studied. Recently, quantitative polymerase chain reaction (PCR) for prenatal diagnosis suggested an association between high parasite count in amniotic fluid and ultrasonographic abnormalities, although the main factor remains early maternal infection [4]. The question of relationship between congenital toxoplasmosis and *T. gondii* genotype has been approached in several studies, with sometimes discrepant results. For biologic and epidemiologic studies, 3 main genotypes are generally recognized in the *T. gondii* population—types I, II, and III [5]—although, from a phylogenetic point of view, types II and III belong to the same clonal group [3]. By use of different multilocus-typing strategies on disparate collections of isolates, type II genotype (as determined by PCR–restriction fragment–length polymorphism [RFLP] analysis of 6 single-copy genes) [5] or the equivalent zymodemes 2 and 4 (as determined by isoenzymatic analysis of 6 enzyme systems) [6, 7] was found to be the most prevalent genotype in human disease, including human congenital toxoplasmosis. Non–type I isolates were also found in 35 consecutive amniotic-fluid samples in Paris [8]. However, findings in a study of 106 isolates with different origins of isolation (animals and humans with toxoplasmic reactivation in immunosuppressed patients or with congenital toxoplasmosis) suggested that type I isolates were significantly more often associated with human congenital toxoplasmosis than with animal infection or with reactivation of chronic infections in pa-

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tients with AIDS [5]. More recently, a Spanish study of a limited number of congenital toxoplasmoses found type I isolates in 75% of cases of congenital infection [9]. None of these studies presented detailed clinical information that would have enabled determination of a relation between genotype and the severity of congenital toxoplasmosis.

Materials and Methods

Toxoplasma *isolates.* We studied 86 *T. gondii* isolates originating from proven or suspected cases of congenital toxoplasmosis. They were obtained, after mouse inoculation or cell culture, from amniotic fluid, placenta, cord blood, or tissues of dead fetuses or newborns. They originated from European countries, mainly from France (82 isolates); 2 were from Belgium, 1 was from Italy, and 1 was from the Netherlands (BK strain, 1948 [10]). Apart from 3 historical strains (BK strain, 1948; PRUGNIAUD strain, 1965; and CHAM strain, 1983), they were isolated between 1987 and 2001. Some of these isolates were sent to our laboratory for strain typing because of unusual or severe clinical findings (fetal death or severe cases of congenital toxoplasmosis) or unusual behavior in mice (high virulence to mice). They constitute a disparate collection. Our study also includes 2 collections of isolates originating from consecutive cases of congenital toxoplasmosis observed in 2 French laboratories: 28 isolates consecutively isolated in Limoges, France, during 1987–2001, and 29 isolated during 1994–2000 in Paris (Hôpital Pitié-Salpétrière). Clinical data included time of maternal seroconversion, prenatal echographic findings, histologic and parasitologic analysis of fetuses in cases of spontaneous abortion or medically interrupted pregnancy, and clinical findings at birth (table 1). Benign toxoplasmosis (isolated intracranial calcifications or isolated chorioretinitis) and toxoplasmosis presenting at birth without any clinical symptoms were considered to be in the same clinical group, because chorioretinitis could appear later during the child's life. Severe toxoplasmoses included children born with hydrocephalus, microphthalmy, ascites, or hypotrophy, as well as medically aborted fetuses with hydrocephalus and/or disseminated toxoplasmosis.

DNA extraction. DNA was extracted by the QIAamp DNA Mini Kit (Qiagen) and was stored at 4°C. DNA isolation was done on *T. gondii* stocks stored in liquid nitrogen, as tachyzoites cultivated in mouse peritoneal exudates or culture cells or as bradyzoites in cysts of infected mouse brains.

Genotype analysis. To determine the genotype of the *T. gondii* isolates, we used PCR to amplify 8 MS markers, as described elsewhere [3]. These MS sequences were present in the intron of the genes coding for myosin A *(TgM-A)* and for β-tubulin *(TUB2)* and in 6 expressed sequence tags (ESTs) (GenBank accession numbers *AA519150, W35487, N61191, N82375, N83021,* and *N60608*). Primers are presented in table 2.

The amplification reaction mixture consisted of 10 m*M* Tris-HCl (pH 8.3), 50 m*M* KCl, 1.5 m*M* $MgCl_2$, 0.2 m*M* each dATP, dCTP, and dGTP (Roche Diagnostics), 0.4 m*M* dUTP (Roche Diagnostics), 4 pmol of each primer (GibcoBRL Life Technologies and Applied Biosystems), 5% (vol/vol) dimethyl sulfoxide, 0.6 U of *Taq* DNA polymerase (Amersham Pharmacia Biotech), 0.2 U of uracyl DNA glycosylase (Roche Diagnostics), and 2.4 μL of DNA in a 20-μL reaction volume. After 2 min at 50°C for uracyl DNA glycosylase action, and 3 min at 94°C for initial denaturation, each of the 35 cycles consisted of 30 s of denaturation at 94°C; annealing at 52°C for the *TgM-A* gene, at 54°C for the *TUB2* gene and ESTs *AA519150, W35487, N61191, N82375,* and *N83021,* and at 55°C for EST *N60608,* and extension at 72°C. The final cycle was followed by an additional 10 min at 72°C. The forward primers were 5′ end-labeled with fluorescein (6-FAM or HEX), to allow sizing of PCR products by an automatic sequencer.

PCR products were first separated by electrophoresis in 2% agarose gels to confirm DNA amplification; then they were diluted 1:10, 1:5, or 1:2 or left undiluted, depending of the intensity of the band in the agarose gels. Each PCR product (1 μL) was mixed with 0.5 μL of the red dye–labeled GeneScan size standard ROX 500 (Applied Biosystems) and 24.5 μL of deionized formamide. This mixture was denatured and run on a polyacrylamide gel POP4 (Applied Biosystems) in a 47-cm/50-μm capillary for genetic analysis. Signals were read with an automatic sequencer (Abiprism 310 collection 1.0; Applied Biosystems), and the data were stored and analyzed with GeneScan analysis software (version 2.1; Applied Biosystems).

Isolate clustering. As has been described elsewhere [3], genetic diversity analysis with the 8 MS markers detects a high polymorphism of *T. gondii* isolates structured into 2 main clonal groups: MS groups 1 and 2. However, to compare our results with those of classical strain typing (zymodemes or *SAG2* types I, II, or III),

Table 1. Clinical characteristics of patients with *Toxoplasma gondii* infection, and relationships with the main genotypes of *T. gondii*

Clinical finding(s)	Time of maternal infection, weeks	No. of cases				
		T. gondii type				
		I	II	III	Atypical	Total
Fetal death	2–11[a]	—	6	—	—	6
Newborn death (historical cases)	Unknown	1	2	—	—	3
Severe toxoplasmosis at birth or medical abortion	7–17	2	16	—	3	21
Asymptomatic or benign toxoplasmosis	15–38	—	43	2	—	45
Child not infected, placenta positive	14–20[b]	4	—	—	—	4
No clinical data available	10–31	—	6	—	1	7
Total		7	73	2	4	86

[a] One reactivation during AIDS.
[b] One reinfection or reactivation.

Table 2. Microsatellite markers, polymerase chain reaction (PCR) primers, and allelic polymorphism in 86 isolates originating from human congenital toxoplasmosis.

Marker	Repeat sequence[a]	PCR primers (5′→3′)	No. of alleles	Size range of alleles,[b] bp
TUB2	$(TG)_8$	1: HEX-CCAAGTTCTTCCGTCATTTC 2: CCTCATTGTAGAACACATTGAT	2	124–126
TgM-A	$(TG)_9$	1: 6-FAM-CATGTCCCTGTCGGTTTCTC 2: CGTAAATGCGGATGGAAACT	3	117–121
W35487[c]	$(CT)_{10}$	1: 6-FAM-TGCTGCGGTCTTTTCTCTTC 2: AACATGCCGTTCCCTTCC	2	95–101
N60608[c]	$(TA)_{13}$	1: 6-FAM-GAATCGTCGAGGTGCTATCC 2: AACGGTTGACCTGTGGCGAGT	7	131–145
N82375[c]	$(TA)_{13}$	1: 6-FAM-TGCGTGCTTGTCAGAGTTC 2: GCGTCCTTGACATGCACAT	9	107–123
N83021[c]	$(TA)_{11}$	1: 6-FAM-ACAACGACACCGCTATCTC 2: CTCTCTATACACAGACCGATTGG	8	127–161
N61191[c]	$(TA)_{11}$	1: 6-FAM-CCGTATCACCAGATCATGTT 2: CTCTCACCTGATGTTGATGTAA	16	118–160
AA519150[c]	$(TA)_{13}$	1 HEX-GTTGTCTATGCTGTCGTGCG 2: CACCCATAAACGGTTACTGGTC	15	134–166

[a] Number of dinucleotide repeats, as published in GenBank, for RH strain or ME49 strain.
[b] A complete listing of genotypes for all strains is available from the corresponding author.
[c] GenBank accession number.

we have to consider only the allelic association of our 3 less polymorphic MS markers: EST *W35487, TUB2,* and *TgM-A* (table 3). Three main multilocus genotypes are described according to this association. Because 3 MS markers only may be not sufficient to detect all atypical genotypes, we added the results obtained with isoenzymatic markers for 38 isolates [6, 7] to give a better resolution. The correlation with *SAG2* PCR-RFLP typing has been discussed elsewhere [3]. Atypical genotypes are defined by an unusual allelic association of the 3 less polymorphic MS markers or by an atypical zymodeme (table 3).

Results

In our population of 86 *T. gondii* isolates, 3 MS markers *(TUB2, TgM-A,* and *W35487)* exhibited a low polymorphism, with only 2 or 3 alleles, whereas the 5 other MS markers, with 7–16 alleles, were more polymorphic (table 2). Identical genotypes with the 8 MS markers were detected for only 6 pairs of isolates. Of the 86 isolates, 74 had a unique genotype with the association of the 8 MS markers. All 48 isolates not tested by isoenzyme analysis had an MS profile shared by only 25 isolates belonging to zymodeme 2 or 4. Considering the 3 main types, as defined above (see Materials and Methods), this analysis shows that 73 (84.88%) *T. gondii* isolates are type II, 2 (2.33%) are type III, and 7 (8.14%) are type I; 4 (4.65%) isolates have an atypical genotype (table 3).

If we consider only consecutive cases of congenital toxoplasmosis observed in Limoges during a 14-year period, nearly all (26/28 [92.86%]) of the isolates are type II, 2 are type III, and no type I or atypical genotypes were observed. Similarly, in the Paris laboratory, only type II isolates were observed during a 6-year period.

The correspondence between these genotypes and clinical outcomes of congenital toxoplasmosis is presented in table 1. Fetal death was observed in 6 cases, after maternal infection occurring early during pregnancy in 5 cases and, in 1 case, after toxoplasmic reactivation in a woman with AIDS. In all of these cases of fetal death, the isolates found in amniotic fluid or in fetal tissues are type II. Newborn death occurred in 3 of our cases a few days or weeks after birth. One type I and 2 type II isolates were implicated in these lethal cases.

In 17 cases, a decision to perform medical abortion followed a positive prenatal diagnosis. Mothers were infected during the first or second trimester of pregnancy. The parasite was isolated from amniotic fluid or fetal blood, and pregnancy was terminated between 20 and 33 weeks of gestation. *T. gondii* was found in brain and liver, but disseminated inflammatory lesions were also described in most of the fetuses. Four children were born with severe toxoplasmosis: isolated hydrocephalus (2 cases), hydrocephalus and microphthalmy (1 case), and intracranial calcification, ascites, and hypotrophy (1 case). When medical

Table 3. Allelic combinations of 3 less-polymorphic microsatellite (MS) markers associated with isoenzyme markers, and correspondence with types I, II, III, and atypical types, for a population of 86 isolates from human congenital toxoplasmoses.

Allelic combination of 3 MS markers						
EST *W35487*[a]	*TUB2*	*TgM-A*	Zymodeme(s)	MS group	No. (%) of isolates	Type
101	126	121	Z1	1	7 (8.14)	I
95	124	119	Z2, Z4	2	25 (29.07)	II
95	124	119	ND	2	48 (55.81)	II
95	124	117	Z3	2	2 (2.33)	III
95	124	117	Z8	2	1 (TONT)	Atypical
101	126	117	Z1	1	1 (GPHT)	Atypical
95	126	117	Z5	2	1 (MAS)	Atypical
95	124	121	Z2	2	1 (GANGI)	Atypical

NOTE. EST, expressed sequence tag; ND, not done.
[a] GenBank accession number.

abortion and severe toxoplasmosis at birth were considered together, 21 isolates were found to be considered to be responsible for severe infection; 16 (76.19%) of these isolates were type II, 2 were type I, and 3 had an atypical genotype. Different genotypes would be implicated in the same type of lesions.

Forty-five cases corresponded to subclinical or benign infections (isolated chorioretinitis or cerebral calcifications). Type II isolates were found in 43 cases (95.56%), type III in 2 cases (4.44%). No type I and no atypical types were observed in these subclinical or benign toxoplasmoses.

Four isolates (P, ENT, FAJI and PIL isolates) were isolated, by mouse inoculation, from placentas for ENT, FAJI, and PIL isolates and from placenta and cord blood for P isolate. For 3 of these 4 isolates, the maternal infections occurred relatively early during pregnancy (between 14 and 20 weeks of amenorrhea); 1 case (PIL isolate) could have been due to reinfection or reactivation occurring in a debilitated (tuberculosis, alcoholism) pregnant woman. After detection of maternal infection, women were treated only with spiramycin throughout pregnancy (except for PIL isolate, in which case the mother was not treated), and there was no prenatal diagnosis. At birth, the infants did not exhibit any clinical or serologic signs of infection. Yet, because of the isolation of *T. gondii* from placenta, 3 of them were treated with a sulfonamide-pyrimethamine combination, for congenital infection; 1 of them (PIL isolate) was not treated. All of them became seronegative before 1 year of age (age 7–10 months) and remained seronegative, suggesting that they were not infected. These 4 isolates are type I and are virulent in mice. Finally, of the 7 isolates without any clinical data, 1 exhibits an atypical genotype, whereas the others are type II.

Discussion

The prevalence of the main genotypes in this collection of *T. gondii* isolates originating from human congenital toxoplasmosis is in agreement with the results observed in other studies of disparate collections, which showed that >70% of human disease cases are associated with type II isolates [5–7]. However, as in other collections of isolates present in reference laboratories, the relative prevalence of each main genotype is biased, because some mouse-virulent isolates or isolates responsible for unusual clinical findings are sent to reference laboratories because of their rarity. In addition, it has been suggested that the high prevalence of type II isolates in human congenital toxoplasmosis might be an effect of selection in the process of culture or mouse inoculation, before strain characterization [9, 11]. Given both the high virulence, in mice, of type I strains and their high rate of multiplication in cell culture, one could think that type I strains, and not type II strains, would have been selected. During a 14-year period (1987–2001), we observed 38 consecutive congenital toxoplasmoses in the Limoges laboratory: mouse inoculation of placenta, tissues of aborted fetus, or amniotic fluid yielded positive results in 28 cases, was not done in 8 cases (mainly because of an infection occurring late during pregnancy—and detected only a few days after delivery, so that placenta was no longer available), and yielded negative results in only 2 cases. Thus, in $\geqslant$28 (73.68%) of 38 cases, congenital toxoplasmosis was not due to a type I strain (i.e., 26 cases were type II and 2 were type III). These results are in agreement with those showing that, after amplification of the MS present in the β-tubulin gene, performed directly on 35 consecutive amniotic-fluid samples, all of the isolates had the allele characteristic of non–type I strains (this kind of characterization could not differentiate bewteen types II and III) [8]. Type II is also the only genotype found associated with abortion in sheep, as determined by a direct characterization of infected tissue samples by genetic analysis of the polymorphic surface antigen 2 locus *(SAG2)* [12]. However, more recently, a study from Spain, also using a direct characterization of clinical sample by the genetic analysis of *SAG2,* reported a higher prevalence of non–type II strains in a very limited data set from human congenital toxoplasmosis [9]: 6 of 8 fully characterized samples of congenital toxoplasmosis were the type I *SAG2* genotype. Whether this discrepancy corresponds to true epidemiologic differences between the 2 countries (Spain and France) remains to be determined. Further studies, including a large number of isolates from other parts of the world, are needed. These studies should be based on a multilocus typing, because it has been demonstrated that *SAG2* typing is not enough to identify the *T. gondii* group [3, 13]; for instance, RFLP analysis at only the *SAG2* locus would have misidentified 2 atypical isolates (MAS and TONT) as type I (M.-L.D., unpublished data).

In France, seronegative pregnant women undergo monthly serologic testing to detect seroconversion as early as possible; each maternal infection is first treated with spiramycin and then, in cases of positive prenatal diagnosis, with sulfadiazine-pyrimethamine or sulfadoxine-pyrimethamine. Therapeutic abortion is debated in cases of ultrasonographic demonstration of ventricular dilatation or other ultrasonic abnormalities. Most of our cases of congenital toxoplasmosis followed this management, and the relationship between clinical findings at birth and genotype should be analyzed in this context, which could be different in other parts of the world.

In the 3 cases of newborn death, corresponding to historical cases for which there was no survey of pregnancy and for which the children presented at birth with severe neuroophthalmic lesions (BK strain [10], type I) or a disseminated toxoplasmosis with hemorrhages and hepatic, digestive, and neuroophthalmic involvement (PRUGNIAUD [14] and CHAM strains, type II), 1 type I and 2 type II isolates were implicated, showing that type I as well as type II could be responsible for lethal infection in untreated human congenital toxoplasmosis. The influence of the time of maternal infection is obvious in the 6 cases of fetal death, because they followed infection during the first trimester. All are due to type II isolates.

Similarly, type II isolates predominated in severe lesions observed in fetuses after medical abortion or in children born with

severe neurologic or ocular involvement. The only 2 type I isolates in these fetuses were isolated from liver, whereas, for the other genotypes, *Toxoplasma* species were isolated from brain, liver, and sometimes other organs (kidneys, adrenal glands, and lungs). It should be noted that the 3 isolates with an atypical genotype, all virulent in mice, are in this group of severe congenital toxoplasmosis: GPHT isolate was responsible for ascites, intracranial calcifications, and hypotrophy; MAS isolate was responsible for a disseminated toxoplasmosis in a fetus and an atypical maternal toxoplasmosis (unusually high antibody titers and apparition of persistent lymphadenopathy shortly after medical abortion); and TONT isolate was responsible for hydrocephalus and ascites in the fetus. These 3 isolates were also found to be atypical by other genetic markers [15, 16]. Once again, the importance of multilocus genetic typing is obvious, and even our 3 MS markers are not enough to detect all atypical genotypes (table 3, TONT isolate). New low-polymorphism MS markers are needed to obtain a better resolution. The small number of isolates with atypical genotypes does not allow us to draw any definitive conclusion about their higher pathogenicity in human congenital toxoplasmosis. However, atypical genotypes were also found to have an unusual frequency in acquired ocular toxoplasmosis [13] and in severe acquired toxoplasmosis in immunocompetent patients [17, 18].

No type I isolate was observed in subclinical or benign infections. The 45 subclinical or benign infections that developed (similar to classical) after an infection occuring late during pregnancy were associated mainly with type II isolates. However, 4 isolates, all type I, were isolated from placentas, although, because of persistent negative results of serologic tests for *T. gondii,* the children were considered to be not infected. Since congenitally infected children can present with transiently negative results of serologic tests for *T. gondii* [19], long-term follow-up (up to 15 months of life) was done to eliminate this possibility. Another eventuality to rule out was a false-positive result due to contamination by the RH lines present in the 3 different laboratories where these 4 type I isolates were isolated. This possibility was eliminated by analysis of the full MS profiles of these 4 isolates showing that they were different from those of the RH strains present in these 3 laboratories (data not shown). It has already been reported that placental infection may not systematically imply a fetal infection [20–22]. However, the isolation of *T. gondii* from placenta, without detection of a congenital infection, is quite exceptional: only 3 such cases were reported in the management of 746 *T. gondii* infections occurring during pregnancy [21]. The fact that the 4 cases in our study that have this very unusual outcome are due to isolates that are type I, which is itself an unusual *T. gondii* type, could not be a mere coincidence. Type I strains are characterized by rapidly dividing tachyzoites, with a lower propensity, compared with other isolates, to be transformed into bradyzoites in cell culture or in mice [23, 24]. The high rate of multiplication of type I tachyzoites might imply a high level of parasitemia and a high risk of placental infection, as has been suggested by Howe and Sibley [5]. However, it is possible that, as demonstrated for the RH strain in mice and rats [25, 26], cysts of type I genotype formed during human infection contain immature bradyzoites that will be more sensitive to treatment or to immunologic response, leading to the elimination of these cysts. In our 4 cases in which the placenta was infected without infection of the child, two hypotheses could be formulated: (1) the fetuses or the children were never infected because type I isolates are less capable of crossing the human placental barrier, or (2) the children were infected and eliminated the immature cysts, which were unable to resist the immune system or treatment. This does not mean that type I cysts are always eliminated in humans, as has been shown by cyst reactivation in immunodeficient patients [11, 27], or that type I isolates are never virulent in human congenital toxoplasmosis, as can be seen in untreated congenital toxoplasmosis (BK strain) or in our 2 cases of medically aborted fetus with liver involvement. In addition, the fact that no type I isolate or atypical genotypes were observed in our group of asymptomatic or benign congenital toxoplasmoses suggests that these types of isolate are more pathogenic to the fetus when they cross the placental barrier or when they are not eliminated by treatment or immunity. The higher prevalence of type I isolates in studies in other countries [5, 9] may be explained by a selection of more-severe cases of congenital toxoplasmoses, which is due to the absence of serologic screening during pregnancy.

The highly discriminating power obtained when the 8 MS markers are used allows control of laboratory contamination and epidemiologic tracking [3]: in 3 cases, totally identical genotypes were found for 2 epidemiologically related isolates. For instance, 2 type I strains were isolated in the same town, at 1-week intervals, from 2 fetuses with the same pathologic abnormalities (liver involvement); their MS profile with the 8 MS markers was identical, suggesting a common source of infection. However, this high-resolution classification does not allow us to establish a more precise relationship with clinical findings.

In conclusion, type II is by far the most prevalent genotype in human congenital toxoplasmosis in France and is found in different clinical forms, the main factor for the severity of congenital infection remaining the stage of pregnancy at the time of infection. Except for 4 type I isolates originating from placentas without infection of the child, the few type I and atypical isolates are associated with severe congenital toxoplasmosis. This could lead clinics to reinforce both treatment and ophthalmologic surveillance of these children. Further studies in other parts of the world, including precise clinical histories, are necessary to confirm these findings. Experimental models of congenital toxoplasmosis and in vitro experiments should also be developed with recently isolated strains of different genotypes, to better understand the transplacental transmission of *T. gondii* and the host immune response.

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