

RESEARCH LETTER

## Severe congenital toxoplasmosis due to a *Toxoplasma gondii* strain with an atypical genotype: case report and review

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Infection due to the protozoan parasite *Toxoplasma gondii* can be devastating in congenitally infected children and in immunodeficient individuals. In congenital toxoplasmosis (CT), transmission to the fetus occurs predominantly in women who acquire their primary infection during pregnancy. The clinical outcome of CT is highly variable, ranging from subclinical (retinochoroiditis may occur at birth or later during childhood) to severe (including fetal death, spontaneous abortion and severe neuro-ophthalmic involvement). The main factor determining the severity of CT is the gestational age at the time of fetal infection. A fetal infection in early pregnancy has more severe consequences than an infection in late pregnancy, but the parasite genotype may also play a role (Ajzenberg *et al.*, 2002; Elbez-Rubinstein *et al.*, 2009). We report a case of severe CT associated with an atypical multilocus genotype of *T. gondii* and a fatal outcome despite suitable management, and we discuss the role of *Toxoplasma* atypical genotype in CT outcome.

A 29-year-old pregnant woman, Caucasian and native to France, with one previous normal pregnancy, was tested seronegative for toxoplasmosis at 9.5 weeks of pregnancy by an enzyme-linked immunosorbent assay (Enzygnost; Behring, Marburg, Germany) for IgM and IgG antibodies as previously described (Simon *et al.*, 2004). She was then informed of dietetic and hygienic measures for the prevention of *Toxoplasma* infection, and she underwent monthly serologic testing according to the recommendations of the French health authorities. The serological follow-up was consistent with a primary *Toxoplasma* infection between 20 and 23 weeks of gestation with absence of IgG and IgM at week 20, presence of IgM and absence of IgG at week 23 and presence of

IgM associated with a rising IgG titer between 25 and 27 weeks of gestation (25 and 39 IU/mL, respectively). Spiramycine treatment (9 MUI/day) was started on the 23rd week; prenatal consultations and fetal ultrasonographies were made monthly. At 27 weeks of gestation, *in utero Toxoplasma* infection was documented with a positive real-time polymer chain reaction (PCR) amplification of the 35-fold repetitive B1 gene for detection of *T. gondii* DNA in an amniotic fluid sample (Simon *et al.*, 2004). The amniotic fluid was not inoculated into mice; the genotyping analysis has been performed directly from DNA extracted from the amniotic fluid sample. An atypical genotype was identified using direct genotyping at six *T. gondii* microsatellite loci (*Tub2*, *TgM-A*, *W35*, *B17*, *B18* and *M33*) in a multiplex PCR assay performed at the Centre National de Référence Toxoplasmose, Limoges, France (Ajzenberg *et al.*, 2009). This atypical genotype was divergent from the prototypic genotypes I, II and III, which are representative of European strains. Details concerning the geographical origin of our patient, the existence of a stay abroad, as well as patient dietary habits (especially any consumption of imported meat) were documented retrospectively as negative. Despite an anti-*Toxoplasma* treatment combining pyrimethamine and sulfadiazine with folic acid supplementation initiated as soon as the end of the 27th week of gestation, fetal ultrasound done 3 weeks later demonstrated bilateral ventricular enlargement and calcifications, confirming a severe CT (Figure 1). Termination of pregnancy was decided on the 31st week of gestational age; the placenta examination showed focal low-grade chronic villitis without *Toxoplasma* cysts.

*T. gondii* displays a population structure consisting of three clonal lineages that are predominant in North America and Europe (Ajzenberg *et al.*, 2002; Ajzenberg *et al.*, 2009; Elbez-Rubinstein *et al.*, 2009). However, sampling from South America has revealed that strains from this region are highly divergent (Ajzenberg *et al.*, 2004). In French Guiana, some of these atypical

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Figure 1—Bilateral ventricular enlargement and calcifications, confirming a severe CT at fetal ultrasound

genotypes are associated with severe toxoplasmosis in immunocompetent patients suggesting that differences in clinical severity may be influenced by the parasite genotype (Demar *et al.*, 2007). These highly virulent strains, poorly adapted to humans, emerged from the forest-based cycle involving wild felids and their preys.

In Europe, mainly in France, various studies conducted in animals and humans showed a tremendous predominance (>90%) of only one *Toxoplasma* genotype, type II (Ajzenberg *et al.*, 2002). Most of the atypical genotypes identified in France are collected from immunocompromised patients who acquired toxoplasmosis outside Europe, especially in sub-Saharan Africa (Ajzenberg *et al.*, 2009). Atypical genotypes collected in CT are very unusual in France where more than 96% of the consecutive cases of CT are due to type II strains (Ajzenberg *et al.*, 2002). The distribution of type II strains between asymptomatic and severe CT cases is strongly related to the time of gestation when maternal infection occurs: infection in early pregnancy with type II strains results in more severe outcome than infection in later pregnancy with type II strains, following the general rule (Ajzenberg *et al.*, 2002). If these results indicate that identifying a type II strain is not considered as a risk factor for severity of fetal infection, there are some data indicating that atypical genotypes of *T. gondii* may be implicated in the outcome of CT, *T. gondii* being responsible for more severe ocular disease in congenitally infected children in Brazil compared with Europe (Gilbert *et al.*, 2008). The marked differences in the frequency, size and multiplicity of retinochoroidal lesions may be due to infection with more virulent genotypes of the parasite that predominate in Brazil but are rarely isolated in Europe.

We reviewed the literature reporting CT cases and *Toxoplasma* genotyping data. We excluded studies without usable data on clinical finding, gestational age at maternal infection, receipt of specific anti-*Toxoplasma* treatment and outcome. We also excluded studies that used a single genetic marker for the genotyping analysis as multilocus markers are needed for the detection

of strains with atypical genotypes. To the best of our knowledge, the case described in this article is the eighth case of documented CT as a consequence of maternal infection with an atypical strain (Table 1) that has been reported in the literature (Ajzenberg *et al.*, 2002, 2009; Cneude *et al.*, 2003; Demar *et al.*, 2007). Although no final conclusions can be drawn from this small number of cases, this review strongly suggests a higher virulence of atypical strains than type II strains in CT. First, all CT cases due to atypical strains were severe and resulted in medical termination of pregnancy or death few days after birth in six of eight cases (Table 1). This poor outcome does not appear being linked with therapy management, since among the 4 cases that had received specific anti-*Toxoplasma* therapy 2 cases had a favorable outcome (cases 1 and 5, Table 1) and 2 patients presented an unfavorable outcome (case 7 and the present case, Table 1). The fact that all reported CT cases due to atypical strains were diagnosed because they were symptomatic may introduce a bias in the apparent distribution of atypical strains in asymptomatic CT, even if no atypical genotypes were observed in the 45 subclinical or benign CT identified among the 86 patients with CT previously analyzed in France (Ajzenberg *et al.*, 2002). Second, severe CT is exceptional when maternal infection occurs during late pregnancy and was never observed with type II strains in 86 patients with CT in France (Ajzenberg *et al.*, 2002). In three of eight cases reviewed here (cases 4, 5 and 7 in Table 1), the maternal infection occurred at the beginning of the third trimester and resulted in disseminated and life-threatening disease in offsprings, which is a strong argument toward a higher virulence of strains with atypical genotype than type II strains. Factors that might support the virulence of atypical strains seem to be complex, more probably associated with fitness than with drug sensitivity of *Toxoplasma* strains. A high degree of parasite multiplication within host cells can be suspected as parasitemia over a prolonged period has been proposed to explain severe toxoplasmosis (Elbez-Rubinstein *et al.*, 2009). The ability of atypical strains to reinfect immunocompetent host (mice) and

Table 1—Bibliographic data of documented cases of CT with atypical *Toxoplasma* strains

Patient [reference] <sup>b</sup>	Clinical finding	Gestational age at maternal infection (in weeks)	Year, country	Isolate designation	Receipt of specific anti- <i>Toxoplasma</i> treatment	Outcome
1 [1]	Disseminated toxoplasmosis diagnosed <i>in utero</i>	20	1987, France	GPHT	Yes	Favorable
2 [1]	Disseminated toxoplasmosis diagnosed <i>in utero</i>	18	1991, France	MAS	No	Medical termination of pregnancy
3 [1]	Disseminated toxoplasmosis diagnosed <i>in utero</i>	13	1992, France	TONT	No	Medical termination of pregnancy
4 <sup>a</sup> [2]	Disseminated toxoplasmosis diagnosed at birth	27–33	2003, France	TgH22003	No	Death on day 4
5 [3]	Disseminated toxoplasmosis diagnosed at birth	27–32	2002, France	TgH20005	Yes	Favorable
6 [4]	Disseminated toxoplasmosis diagnosed <i>in utero</i>	17–22	2004, Suriname	TgH18011	No	<i>In utero</i> fetal death
7 [4]	Disseminated toxoplasmosis diagnosed at birth	30–35	2004, Suriname	TgH18014	Yes	Death on day 6
8 [present case]	Severe neurological impairment diagnosed <i>in utero</i>	20–23	2008, France	TgH22010	Yes	Medical termination of pregnancy

<sup>a</sup> For patient 4, the parasite genotype has been determined at the Centre National de Référence de la Toxoplasmose after the publication of the case;

<sup>b</sup> The patient references are: [1] for Ajzenberg *et al.*, 2002; [2] for Cneude *et al.*, 2007; [3] for Gilbert *et al.*, 2008; and [4] for Demar *et al.*, 2009.

to produce cysts that coexist with type II cysts in mice brain has been recently demonstrated (Elbez-Rubinstein *et al.*, 2009). On the contrary, no relationship has been found between drug susceptibility and *Toxoplasma* genotype (Meneceur *et al.*, 2008).

Currently, factors that regulate the pathogenesis of *T. gondii* in humans are poorly understood. Relationship between parasite and host seems to be a complex equilibrium, composed of parasite phenotype and genotype, as well as host factors. Beside the host immune status that represents a well known factor (immunocompromised patients being at high risk to develop disseminated toxoplasmosis), host genetic predisposition (such as Human Leukocyte Antigen: HLA-DQ3 in Caucasians and polymorphisms at the loci: *COL2A1* (for collagen type II alpha 1) and *ABCA4* (for ATP-binding cassette, subfamily A, member 4) have been described as associated with toxoplasmosis outcome (Jamienson *et al.*, 2008; Maubon *et al.*, 2008).

In conclusion, there is a growing body of data indicating that strains with an atypical genotype are associated with a worse outcome than type II strains in CT. The number of cases reviewed from the literature is insufficient to make recommendations based on clinical practice. Further research is needed to confirm these results and we strongly suggest physicians to seek for genotyping analysis of *T. gondii* strains or DNA samples from severe cases of CT. A *Toxoplasma* genotype

approach should be integrated in any clinical research on drug CT management.

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