An atypical strain associated with congenital toxoplasmosis in Tunisia

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We report the identification and typing of a congenital toxoplasmosis case in a diabetic pregnant young woman living in Tunis. The Toxoplasma DNA extracted from amniotic fluid was detected by Real Time PCR and subjected to a multilocus genetic characterisation of the strain at markers: 3'SAG2, 5'SAG2, New SAG2, SAG3, GRA6, BTUB, APICO, PK1, KT850 and UPRT1. An atypical genotype of T.gondii with unusual genetic composition was revealed. It is the first time that an atypical strain has been associated with congenital toxoplasmosis in Africa. Atypical strains are associated with severe clinical manifestations so systematic genotyping should be investigated with the amniocentesis.

KEY WORDS: Congenital toxoplasmosis, Multilocus genotyping, Atypical strain

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outer primers of PK1 and KT850 genes respectively. For the new SAG2 marker, primer combinations were used. Outer primers were SAG2.F4 (Howe et al., 1997) and SAG2.Ra (Su et al., 2006) whereas inner primers were SAG2.Fa (Su et al., 2006) and the new designed SAG2.Rin (CTCGGGAGTACCGTCGTAAG). Amplification and enzymatic restriction conditions were in agreement with methods described elsewhere (Boughattas et al., 2010). Several control measures were taken to avoid contamination: separate spaces to set up PCRs, electrophoresis and RFLP, filter tips, and UV radiation. Different negative controls (no DNA, uninfected sample, and extracted no DNA) were also used. Positive controls were carried out from RH, Prugniaud and NED strains kindly provided by Pr. Marie Laure Dardé. Sequencing was also investigated whenever there were unclear enzyme digestion results and at the polymorphic uracil phosphoribosyl transferase (UPRT) intron 1 marker as described previously. Phylogenetic network analysis was performed for UPRT1 locus using the program SplitsTree 4 (Huson and Bryant, 2006). All sequences were submitted to GenBank, their accession numbers are cited below.

The multilocus genotypic characterization of the *Toxoplasma gondii* strain yielded a new genotype with unusual combination of type I, II, III and nonarchetypal alleles.

Both digestions in 3’ and 5’ extremities of SAG2 marker concluded for a mixture of types II and III. For the new SAG2 marker, final amplification size, with the new association of primers, was 486bp. Its RFLP pattern revealed type II allele. The restriction profile of SAG3 product suggested a mixture of types I and III which was confirmed by sequencing. In fact, superposed picks were observed at the level of polymorphic sites generating two sequences: one with both NciI restriction sites (Type I: GU249509) and the other with just one site (Type III: GU249510). Restriction profiles of Apico and PK1 markers were identical to type I, whereas restriction profiles of GRA6 and KT850 were identified as type II. For this last marker, sequences of reference strains were unavailable in GenBank. So, RH (GU249505), Prugniaud (GU249506) and NED (GU249507) strains were subjected to sequencing in addition to AF20/04 (GU249508). The alignment of different sequences showed 15 nucleotide positions polymorphism. It also confirmed type II of our isolate.

By BTUB marker, amplification product gave a band with unexpected size of 400pb instead of 411pb. Restriction of the product gave a unique profile (u-1), different from those of the reference strains (Figure 1). This result was reproduced in at least ten experiments.

Analysis of sequences of the Intron 1 revealed double peaks at known polymorphic sites thus giving two sequences for the strain (GU249511, GU249512). The presence of multiple alleles was observed as well as the presence of an exotic nucleotide which is well demonstrated by phylogenetic network analysis (Figure 2).

Primary maternal *Toxoplasma* infection exposes the fetus to the risk of parasite transmission, leading to congenital toxoplasmosis (Garcia-Méric et al., 2010). In this situation, it is important to check for the presence or absence of the parasite in the amniotic fluid by amniocentesis. DNA amplification provides a fast and sensitive result. A positive reaction indicates parasite transmission to fetus. Amniocentesis is usually practised in early pregnancy, and it has been established that the toxoplasmic fetal transmission rate is lower when the infection is in this period. However, when ac-

![FIGURE 1 - 3% Agarose gel electrophoresis of BTUB RFLP products. G1 corresponds to RH strain, G2 to Prugniaud strain, G3 to NED strain, MM to molecular DNA ladder 50bp (Invitrogen), ND to 411bp undigested control and B20 to AF20/04.](image-url)
quired then, the congenital toxoplasmosis is more severe, ranging from in utero death to severe neuro-ophthalmic involvement. The damage could increase drastically in the presence of atypical/exotic strains of the parasite (Delhaes et al., 2010). In our case, due to the early and specific parasite detection, others analyses were not required and the patient's pregnancy was terminated. In retro-

FIGURE 2 - Phylogenetic network (NeighborNet) of Toxoplasma gondii isolates analyzed at the locus UPRT1.
spect, this seems to have been the best taken especially with the identification of the atypical strain. Such strains are often isolated from exotic areas, however in Africa, as far as we know, it is the first time that an exotic strain has been associated with congenital toxoplasmosis. Different methods have been described to type toxoplasmal strains. Microsatellites are the most polymorphic, however it requires at least 50 parasites/sample (Ajzenberg et al., 2005). RFLP's show a good sensitivity level (5 parasites/sample) permitting direct typing on clinical samples which is why we used it (Khan et al., 2005). To enhance the polymorphism information we used 10 independent markers. Some markers were adapted to our multiplex PCR by primers design. Sequencing analysis improved our result. This was not as described in our previous study in which mixed infections were frequent without any observation of exotic atypical strains (Boughattas et al., 2010). This pattern could indicate that African strains of T. gondii undergo more frequent sexual recombination, resulting in more unique genotypes. Such emergent epidemiological data concerning circulating of atypical T. gondii strains in Africa needs special awareness on the part of physicians. A systematic genotyping of the parasite strain should be investigated with amniocentesis.

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REFERENCES


