1. Introduction

Tuberous sclerosis complex (TSC) is an autosomal dominant neurocutaneous disorder with an estimated incidence of 1/6000 to 1/10 000 live births (1.Osborne, 2.O’Callaghan) characterized by striking phenotypic variability, even within the same family. Mutations in either of two tumor-suppressor genes, TSC1 on chromosome 9q34 and TSC2 on chromosome 16p13.3 which encode proteins hamartin and tuberin respectively, disrupt mTOR regulated cellular proliferation and differentiation leading to the development of hamartomas in a number of tissues, most notably the brain, skin, kidneys, eyes and heart. The diagnosis of TSC is primarily made clinically based on recently updated diagnostic criteria, which include eleven major and six minor clinical findings (3.Northrup, Krueger – Table 1). In recent decades an increasing number of children are diagnosed with TSC by genetic testing before the onset of symptoms and some are recognized prenatally allowing for earlier implementation of surveillance or targeted treatment. Nevertheless little is known about the clinical course and outcome of these early diagnosed cases.

Originally the prenatal diagnosis of TSC was considered upon detection of multiple cardiac rhabdomyomas (CRs) at fetal ultrasound in the second or third trimester of pregnancy. With developing imaging techniques this presumed diagnosis can now be confirmed in a proportion of cases when specific cerebral lesions of TSC, namely cortical tubers and subependymal nodules, or, rarely, renal lesions are also recognized on fetal MRI. Genetic confirmation by sequencing the two TSC genes is also available in selected cases (4.Milunsky, Shim SH). The objective of this paper is to provide an overview of the prenatal features suggestive of TSC diagnosis, discuss the existing literature in relation to the outcome of antenatally diagnosed patients and outline the challenges with regards to parent counseling and postnatal management in the era of mTOR inhibitors.

2. Prenatal diagnosis of cerebral lesions in TSC
Prenatal diagnosis of Tuberous Sclerosis

Tuberous sclerosis is a disorder with multi-organ involvement, yet neurologic manifestations including epilepsy, cognitive impairment and pervasive developmental disorder dominate the clinical picture and determine the long-term prognosis. More than three quarters of TSC patients have epilepsy with onset in the first year of life and nearly half show evidence of a learning disorder (5.Webb). Postnataally, four types of specific TSC lesions are recognized, namely cortical tubers, subependymal nodules (SENs), radial migration abnormalities and subependymal giant cell astrocytomas (SEGAs). The neurological features of TSC are believed to reflect structural brain abnormalities, with evidence suggesting that cortical tuber count, size and volume relates to the severity of cerebral dysfunction (6.Orlova 7.Goodman, 8.O’ Callaghan, 9.Kassiri), however it is not yet clear whether the neurological outcome of children with TSC is poorer when cerebral lesions are detected before birth. Nevertheless prenatal diagnosis of cerebral lesions is important for confirmation of TSC diagnosis and parental counseling.

Recent advances in both fetal ultrasonography and magnetic resonance imaging (MRI) have shed new light on antenatal cerebral lesions of TSC in the brain. These imaging studies indicate that cortical tubers and SENs are the most common lesions detected prenatally and may form in the early gestational period, most likely between weeks 10 and 20 of embryonic development (10.Prabowo, 11.Saada). In a recent review of published cases by Wortmann and colleagues, characteristic TSC brain lesions were diagnosed between the second half of the second trimester and the first half of the third trimester (12.Wortmann). No significant difference was found in the time of detection comparing the two methods but fetal brain MRI appears to be more sensitive than fetal brain ultrasound in the prenatal diagnosis of TSC cerebral lesions because a proportion of cases diagnosed by MRI were initially reported as normal on US (12.Wortmann). Single-shot fast spin-echo (SSFSE) T2-weighted imaging in three planes is the standard sequence for evaluation of the fetal brain with fast sequences used to scan the moving fetus. Subependymal nodules (SENs) and cortical tubers are typically T2-hypointense and T1-hyperintense; the former are more clearly identified on fetal imaging than the latter and cortical tubers are occasionally detected only on the postnatal MRI of the brain (13.Goel).

As far as SEGAs are concerned, these benign ventricular lesions tend to appear in the second decade of life, they are usually slow growing but can become aggressive and cause obstructive hydrocephalus in older children and adolescents. SEGAs may also develop in the first months of life and in extremely rare cases there are reports of SEGA growth in newborns and fetuses (14.Oikawa 15.Hussain 16.Raju 17.Phi 18.Kotulska). In a cohort of
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452 TSC patients congenital SEGAs developed in 2.2% of cases presenting with hydrocephalus or rapid tumour growth \( \text{(18.Kotulska)} \). Patients with large genomic TSC2 mutations affecting PKD1 genes were significantly more prone to develop SEGAs early in life than patients with other mutations in TSC2 genes. Consequently neonates with polycystic kidneys and TSC should be followed with frequent neuroimaging studies \( \text{(18.Kotulska)} \). In young infants with large SEGAs, both surgery and mTOR inhibitor could be considered as a treatment option \( \text{(19.Kotulska)} \), although there is not yet much evidence of the use of mTOR inhibitors in infants.

Immunocytochemical analysis on autopsy tissues has provided information on the neuropathological abnormalities of the TSC fetal brain confirming the cell-associated activation of the TORC1 signaling pathway in both the cortical tubers and subependymal lesions, including congenital SEGAs \( \text{(10.Prabowo)} \). Increased S6K, S6 and 4EBP1 phosphorylation and increased expression of c-myc was detected in small clusters of cells and even isolated, single giant cells scattered throughout the fetal TSC brain and in addition there was evidence for prenatal activation of key inflammatory pathways. These findings open a therapeutic window for possible prenatal intervention with mTOR inhibitors during the period of brain lesion formation \( \text{(10.Prabowo)} \). In a well-established neuroglial (Tsc2-hGFAP) model of TSC combined prenatal and postnatal rapamycin treatment resulted in almost complete histologic rescue, with a well-organized cortex and hippocampus almost identical to control animals, nevertheless postnatally treated animals performed better in learning and memory tasks \( \text{(20.Way SW)} \).

3. Cardiac rhabdomyomas as a prenatal sign of TSC

With the estimated incidence of 1/20,000 births cardiac rhabdomyomas (CRs) are the most common fetal cardiac tumours \( \text{(21.Bejiqi)} \) and also the most common prenatal sign of TSC \( \text{(22. Jozwiak)} \). Since the first reported case in 1982 by DeVore et al. \( \text{(23.DeVore)} \) the technical advancement of fetal echocardiography and magnetic resonance imaging has facilitated their prenatal diagnosis and the number of case series reported is increasing \( \text{(24.Pipitone)} \). The detection of multiple cardiac rhabdomyomas combined with a family history of TS is regarded as predictive of a TSC diagnosis. Although the exact incidence of rhabdomyomas coexisting with TS in fetuses is unknown, an estimate of one in 10000 in routine necropsies of all ages has been reported \( \text{(25. Chao)} \). The earliest antenatal sonographic detection of cardiac tumor was reported at 15 weeks of gestation, whereas
most cases are described after 24 weeks of gestation (25.Chao). Fetal cardiac rhabdomyomas are typically benign and asymptomatic with a tendency to regress spontaneously in more than half of cases (26.Sciacca). The same is true when the presence of these lesions is related to TSC diagnosis. Below the age of 2 years the documented incidence of CRs in patients with TSC is as high as 83.3%, decreasing rapidly to 21.4% in children aged 2 - 5 years (27.Jozwiak, 22.Jozwiak).

Sonographically cardiac rhabdomyomas appear as round, homogenous, hyperechogenic, intramural or intracavitary masses, sometimes multiple, usually located in the ventricles and septal wall and occasionally in the atrium or pericardium. Smaller tumours tend to be multiple and therefore meticulous search for further tumours is recommended when the size of the mass is small (25.Chao). Although histologically benign, larger cardiac rhabdomyomas may lead to right or left cardiac outflow tract obstruction depending on their location or disrupt cardiac rhythm and thus carry a greater risk of causing symptoms of hemodynamic disturbance (25.Chao). Tumor size, the occurrence of fetal dysrhythmia, such as ectopic beats, supraventricular tachycardia or bradycardia, and the development of fetal hydrops are considered strong predictors of a negative neonatal outcome (25.Chao).

More than their importance in terms of cardiac complications, the detection of cardiac rhabdomyomas on fetal ultrasound has long been considered of great significance as an early marker for the prenatal diagnosis of tuberous sclerosis (28. Crawford). In the last decades a growing number of case reports have been described in the literature (23, 28, 30-36) along with original case series studies (11, 21, 24, 25, 26, 22, 29) researching the clinical association of cardiac tumors in fetuses with TSC diagnosis. Most studies are small in number and lack long-term follow-up data (22. Jozwiak). Thus questions still remain as to whether all prenatally multiple CRs are a sign of TSC, what should be the extent of investigations carried out to exclude TSC in fetuses and newborns with CRs and what is the recommended postnatal monitoring in those cases where the diagnosis of TS is not confirmed clinically. Indeed, in the event of a negative family history, cardiac rhabdomyomas may be the only clinical feature present in the first months of life as pathognomonic signs and symptoms of TSC can develop at a later stage. Multiple and multifocal fetal cardiac rhabdomyomas are a strong predictors of an underlying diagnosis of TSC (37. Chen); on the other hand, the diagnostic significance of single CRs with respect to TS is unclear and data from larger studies is required (22. Jozwiak). Based on existing evidence experts suggest that TSC should be suspected in all newborns with prenatally
diagnosed multiple CRs (37. Chen). Genetic analysis of both the affected patient and the parents may be considered to enable early implementation of further diagnostic investigations, patient surveillance and family counselling (22. Jozwiak, 4. Milunsky) in cases with no signs of the condition on clinical examination.

In view of the natural history of spontaneous tumor regression during the first year of life the standard management of CRs is observational in all cases that present without severe hemodynamic cardiac complications, fetal hydrops or malignant dysrhythmias (38. Mlczoch). Surgical intervention is reserved for life-threatening conditions caused by the tumor’s location (39. Paladino). Most recently there have been reports that mTOR inhibitor therapy may also be an option for symptomatic giant rhabdomyomas that develop in the neonatal period (38. Mlczoch, 40. Mohamed). With regards to screening and follow-up of asymptomatic CRs in patients with TSC no consistent guidelines are proposed. In everyday clinical practice despite their favorable prognosis CRs are sought in all patients with TSC and in case of antenatal detection their size is monitored with serial echocardiograms every 6 months and cardiac rhythm is examined via Holter monitoring annually, even if patients remain free of symptoms (26. Sciacca).

4. Renal involvement suggestive of TSC in the fetus

In TSC patients, the most common renal anomaly is the presence of angiomyolipomas (AML), which can be found in up to 80% of patients and is included among the major clinical diagnostic criteria for the disease (41. Pradilla, 42. Bissler). Another frequent lesion related to TSC is renal cysts found in 14-32% of patients (43. Rakowski). Renal cell carcinoma has also been described and at a younger age, but the overall risk is reported to be around 2-4% (44. Yang). Although AMLs are usually asymptomatic, lesions >4 cm in diameter or >3 cm and growing rapidly are prone to bleeding and renal complications. In these patients treatment with mammalian target of rapamycin (mTOR) inhibitors is indicated with the aim of preserving kidney function and avoiding the size-related risk of life-threatening bleeding (45. Kingswood). Renal cystic lesions in TSC patients range in severity from microscopic disease, isolated unilateral cysts to a polycystic phenotype. Polycystic disease in patients with TSC results from large contiguous deletions on chromosome 16p13.3 affecting both the TSC2 gene and the immediately adjacent PKD1 gene that is responsible for adult onset polycystic kidney disease (46. Brook-Carter, 47. Sampson, 48. Longa). Typically patients with the TSC2- PKD1 contiguous gene syndrome present with severe
juvenile polycystic disease of early onset usually entering end-stage renal disease in the second or third decade of life (46. Brook-Carter, 47. Sampson), however some patients have milder renal disease due to mosaicism or separate mutations (49. Smulders, 50. Cabrera-Lopez).

Although renal abnormalities are frequent in TSC, most children are born with normal kidneys and renal involvement appears later as they age (51. Dixon). Early detection of TSC associated renal lesions is very rare with only half a dozen cases of prenatal diagnosis reported in the literature and an additional number of cases diagnosed in the neonatal period (52-58). They reported cases had either unilateral cystic formations or diffuse polycystic kidney involvement. The renal lesions were either isolated or accompanied by cardiac or cerebral findings typical of TSC. There are no reported cases of AML developing in the fetal period. In the general neonatal population, renal masses identified on prenatal imaging are usually benign, with hydronephrosis and cystic renal disease accounting for the majority of cases. All can be diagnosed by fetal ultrasound, nevertheless additional magnetic resonance imaging is useful not only in confirming the diagnosis, but also in distinguishing different cystic renal diseases, evaluating the contralateral kidney and investigating other target organs for typical TSC lesions (59. Wood).

5. Prenatal molecular diagnosis of TSC

Over the past decade screening for and diagnosis of genetic abnormalities in the fetus is undergoing an unprecedented rapid evolution in light of genomic medicine advances. The introduction of novel technologies such as genome-wide single-cell array and high-throughput sequencing analysis, are impacting prenatal diagnosis, just like any other medical field (60. Milunski, 61. Van der Veyer). Among the many applications of the emerging genetic techniques is sequencing and MLPA analysis of the two tumor-suppressor genes (TSC1 and TSC2) for the prenatal diagnosis of TSC. Molecular testing of the TSC1 and TSC2 genes yields a positive mutation result for 75-90% of TSC-affected individuals (62. Rosset) making the identification of a pathogenic mutation an independent diagnostic criterion, regardless of the clinical findings (3. Northup). Extensive studies of the TSC1 and TSC2 genes in patients with TSC have revealed a wide spectrum of mutations, the most frequent type being point mutations. The TSC1 gene incorporates mainly small mutations that result in nonsense or frameshifts, which lead to protein truncation, whereas most
TSC2 mutations involve missense mutations and large deletions or rearrangements \( (63. \text{Dabora}, 64. \text{Au KS}) \). TSC2 gene mutations, which are associated with more severe clinical manifestations, are also more common accounting for a ratio of TSC2:TSC1 of almost 2:1 in familial patients and 3.5:1 in sporadic cases \( (63. \text{Dabora}, 64. \text{Au KS}) \).

Taking into account the significant morbidity associated with TSC and the possibility of a devastating course over the years, preconception avoidance or prenatal diagnosis represents an important option for families with a known affected parent, sibling, previous fetus or other relative (clinical and/or molecular diagnosis) or when an abnormality consistent with a diagnosis of TSC is seen on fetal ultrasound \( (60. \text{Milunski}) \). In a series of 50 pregnancies at risk for TSC studied by Millunski and colleagues, sequencing and MLPA analysis of both the TSC1 and TSC2 genes successfully provided a first- or second-trimester molecular prenatal diagnosis in 48 of the 50 tested fetuses \( (60. \text{Milunski}) \). Genetic variants whose functional effect is not definitely pathogenic are not considered a diagnostic criterion for TSC. At the other end, a normal result from TSC1 and TSC2 testing does not exclude the diagnosis, since a small but consistent minority of TSC patients has no mutation identified by conventional genetic testing \( (65. \text{Nellist}, 66. \text{Curatolo}) \). Nevertheless, if the mutation in an affected relative is known, targeted sequencing has a very high predictive value. Overall, a family history of TSC or the detection of fetal cardiac rhabdomyomas should prompt genetic evaluation and counseling of parents and the option of prenatal molecular diagnosis should be offered to the family.

6. **Prognostic significance of prenatal TSC diagnosis**

With advancing fetal neuroradiology and molecular genomics technology and with increased awareness among medical professionals and families, the number of prenatally diagnosed TSC cases based on clinical or genetic criteria is growing rapidly. This generates the need for both obstetricians and pediatric neurologists to provide informed parental counseling before birth not only if termination of pregnancy is considered but also with regards to the nature of the disease and the need for postnatal care and follow-up \( (12. \text{Wortmann}) \). Questions arise about the need for genetic confirmation when only one clinical criterion is present on antenatal screening, about the evolution of prenatally detected cardiac or renal lesions over time and mostly about the neurological outcome of TSC patients when cerebral lesions are detected early on during fetal life. Furthermore, given the existence of an established targeted treatment for many of the clinical features
of the disease, another question that may emerge in the future is whether early use of mTOR inhibitors could be an option for the treatment of prenatally detected SEGAs (19.Kotulska) or symptomatic giant rhabdomyomas (38.Mlczoch, 40.Mohamed) or even as a pre-emptive therapy against epileptogenesis (67.Zeng, 68.Citraro, 69.Julich). To date the evidence to support conclusive answers to these clinically significant and intriguing questions is far from complete (70.Roach). Knowledge about the postnatal neurological outcome of prenatal detected TSC is limited and it remains unclear whether there is a distinct disease phenotype that develops earlier in life and whether a definite advantage can be taken of the acceleration of the diagnostic process (12.Wortmann). Also left unanswered is whether mTOR inhibitors may improve cognition and behavior in some individuals with TSC (70.Roach). Clinical trials now in progress and systematic evaluation of prenatally diagnosed TSC patients should provide answers to these questions in the years to come.

7. Conclusions

Tuberous sclerosis complex is a dominantly inherited disorder of cellular differentiation and proliferation characterized by the development of benign tumors in many tissues and organs. Clinical signs are extremely variable and early diagnosis of TSC is very important to plan appropriate perinatal care and surveillance testing. Using fetal ultrasound and MRI imaging early detection of TSC-associated lesions such as cardiac rhabdomyomas, subependymal nodules, cortical tubers and renal lesions is made possible, often representing an incidental finding during routine prenatal ultrasound. Targeted genetic testing when the causative mutation is known or molecular analysis of the TSC genes in fetuses with suspected tuberous sclerosis can effectively confirm the diagnosis. In recent years our understanding of TSC pathophysiology and our ability to prevent or treat many of its complications with mechanism-based targeted therapy have grown tremendously, nevertheless ongoing research is still necessary in order to address issues with regards to the outcome of prenatally diagnosed TSC and the value of pre-symptomatic therapy.
References


