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Research Article

Lack of Correlation between Tuber Number and Cognitive Level in Mild TSC Patients: a Clinical and Genetic Study

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Abstract

Several reports have suggested that the tuber number may contribute to cognitive impairment occurring in Tuberous Sclerosis Complex (TSC) patients.

As the detection of prognostic factors for cognitive impairment in TSC is particularly important for its high degree of intellectual involvement we aimed to define if cortical tuber number may play a role in cognitive level in mild TSC patients.

Methods: To assess a possible relationship between tuber number and cognitive level we exhaustively studied 30 TSC patients from clinical radiological and genetic point of view. We selected a subgroup of 14 patients showing a mild clinical phenotype, with a complete seizures control, IQ in the average range and normal behavior. All patients underwent brain Magnetic Resonance Imaging (MRI) using a specific protocol for TSC disorder. The tuber number in the brain was correlated with cognitive level in each patient.

Finding: Surprisingly half of the patients constituting the selected subgroup showed a high tuber count (n>9) and all of them have a good control of seizures and a normal cognitive level. From the genetic point of view 4 patients showed a TSC1 mutation, 5 showed a TSC2 mutation, five showed no mutation for TSC1 or TSC 2 genes. There was no apparent difference and/or severity of neurological manifestations or tuber number between patients with TSC1 and TSC2 mutations.

Interpretation: These data suggest that the relationship between tuber number and intelligence may be not so obvious, and that other factors may be involved, such as tuber's epileptogenic activity, age of onset and infantile spasm. This data suggest that tuber count alone cannot be used to predict neurological outcome in TSC. Furthermore, our results pointed out that a homogeneous mild neurological phenotype could be subtended by a genetic heterogeneity.

Keywords: Cognitive impairment; Tuberous sclerosis complex; Tumor

Introduction

Tuberous Sclerosis Complex (TSC) is an autosomal dominant disorder characterized by abnormal cellular proliferation and development of tumor- like growths hamartomas involving multiple organs, typically the brain, skin, kidneys heart and other organs. TSC affects 1/7000 individual's live births. Two third of cases is sporadic, without antecedent family history.

TSC occurs owing to germline or mosaic mutation in either two genes: TSC1 and TSC2. Although there is not a clear correlation, patients with mutations in TSC1 have showed a phenotype there is milder than patients with TSC2 mutation.

The TSC1 gene encode a protein, hamartin, extent on 9q34 and TSC2 encode a protein, tuber in, extent on 16p13 [1,2]. Inactivating mutation in TSC1 or TSC2 cause this suppressor-gene syndrome [1,2]. The major clinical consequences of TSC are seen in the brain with epilepsy, mental retardation and behavior disorder. Nevertheless, clinical neurological phenotype is highly variable ranging from almost normal to severe neurological phenotype.

Cortical tubers in the brain are regions of disorganized cortical lamination and are directly related to the presence of cortical seizures and to intellectual impairment.

An impairment of cognitive abilities is often associated with TSC. The main explanation invoked to interpret cognitive impairment in TSC is the number of tubers in the brain [3-9] and the presence of spasms in the first year of the life [10-14].

The explanation of the intellectual impairment in TSC is debated and number of cortical tubers and history of infantile spasms were proposed together to explain cognitive impairment in patients with TSC [13].

The main aim of this study is to evaluate the relationship between brain tuber count and Intelligence (IQ). We selected, out of greater 30 affected TSC populations. A subpopulation of 14 mild TSC patients with normal intelligence and tubers number was assessed by brain MRI.

The second aim of our study was to verify whether there were some specific cognitive deficits in presence of normal intelligence level in patients with mild TSC.

Methods

Patients

We included 30 patients (17 women, 13 men, mean age 24 years) of south Europe descent diagnosed with a definite diagnosis

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of TSC (as defined by the Tuberous Sclerosis Complex Consensus Conference,1998) [15].

We conducted a medical, genetic and radiological investigation of all the affected people described above and we actively recorded their follow up (30 patients actively follow-up) in The Tuberous Sclerosis Centre at S. Paolo Hospital in Milano.

All patients underwent an attentive history and physical examination with specific emphasis on organs typically involved by TSC. Imaging study included brain MRI, abdominal MRI, renal ultrasound and lung TC, cardiac ultrasound and ophthalmologic imaging. Laboratory test evaluating renal function (serum creatine and urea)

We selected from 30 TSC patients a sub group of 14 showing a mild clinical phenotype (Table 1 and 2).

The mild phenotype group was defined on the basis of the following clinical criteria:

- Complete control of epileptic seizure
- Normal IQ
- Normal behavior
- Mild skin involvement (only 2 different skin features)
- Mild kidney and hepatic involvement (none or 1-2 small (<1 cm) diameter AML
- Mild kidney cyst evaluation (none or 1-2 small (<1 cm) diameter cysts and normal renal function
- Mild eye involvement (none or one asymptomatic retinal hamartomas)
- Mild oral (v trade odontoiatrico) involvement none or one oral features)

Regarding the dermatological evaluation all patients were examined by dermatologists belonging to the TSC study group. They described the number and the type of skin lesions, distinguishing 2 different groups: mild and severe, as summarized in the Table 3. This distinction was based considering the skin criteria used for the diagnosis of TSC. The mild dermatological phenotype was assigned to those patients that showed only 2 different TSC skin features with a single manifestation and/or a single type of lesion with less than 20 elements. For the severe phenotype the criteria were 2 or more skin features characteristic of TSC and/or a single type of lesion with more than 20 elements. This classification and the propose of a dermatological severity scale is arbitrary, based only on the clinical observation of TSC patients, but there is no report of an international scale severity up to now.

This attempt of dermatological classification was made in order to establish a possible correlation between neurological and dermatological phenotypes. Patients were subjected also to rigorous dental examinations to evaluate TSC oral lesions.

All patients underwent a careful history and neurological examination, EEG analysis, detailed brain MRI and neuropsychological assessment were performed too. Intelligence was assessed using the Wechsler Adult Intelligence Scale (WAIS-R) [16] or the Wechsler Intelligence Scale for Children (WISC-III) [17] and Raven's Colored Progressive Matrices [18], attention by the Attentional Matrices [18], and Trail Making test A and B [19], short-term verbal memory by Digit Span [20] long-term verbal memory by Word Paired Test [21], long-term visual memory by Rey Osterrieth Complex Figure [22], language by Token Test [23] and Fluency Test [24]. All tests were standardized on the Italian population.

Brain Magnetic Resonance Imaging (MRI) was employed with all patients and a specific protocol for TSC patient, including T1, T2, and Inversion Recovery (IR) e Fluid Attenuated Inversion Recovery (FLAIR) sequences were applied. Two neuro radiologists described the number and the site of tubers, while the correlation between the sites of tubers with eventual lobe related cognitive deficits and with seizures type and frequency in their clinical history were performed by one neuropsychologist and one epileptologist.

Statistical analysis

We performed a statistical analysis using the Statistical Package for the Social Science - SPSS (13.0). A correlation (Spearman's r) was performed between the tubers' number and the neuropsychological tests' results. No correlation was found between intelligence and tubers' count ($p>0.05$); the only significant result was the correlation between tubers' number and Attentional Matrices ($p=0.031$), while the other cognitive domains showed no significant relation ($p>0.05$).

It is not possible to analyze test's results in relation to a specific cerebral area due to the fact that tubers spread in all brain's area we divided (stratified) patients with mild TSC neurological phenotype in two groups (A and B) according to the number of tubers identified by MRI. A cut off value was arbitrary set at 9 tubers so that mean tuber number for group A was 2 and 14.8 for cluster B (averaging cutoff values of tuber's number of 9, as follow: Group A<9 (mean tuber's count 2), Group B>9 tubers (mean tuber's count 14, 8).

Genotyping

Blood DNA samples were obtained from TSC patients who given informed consent according to Declaration of Helsinki. The study was approved by the Institutional Review Board of Milan's San Paolo Hospital. DNAs were extracted from peripheral lymphocytes using Wizard Genomic DNA purification kit (Promega, Madison, USA). Polymerase Chain Reaction (PCR) was used to amplified all exons of TSC1 and TSC2 from genomics DNAs using standard methods with previously described primers [25] PCR amplification was performed in 25 µl final volume mix containing: 10 ng to 50 ng of genomic DNA, 200 µM dNTPs, 1.5 mM MgCl2, 10pmoli each primer and 1.25 U AmpliTaq Gold (Applied Bio system, Foster City, USA). TSC1 and TSC2 amplimers were divided into those that amplified successfully at annealing temperature of 55°C, 60°C and 65°C. The PCR products were subjected Denaturating High Performance Liquid Chromatography Technique (DHPL; Transgenomic Inc, Nebraska USA) analysis for mutation detection. To enhance heteroduplex formation, the untreated PCR product was Denaturating at 94°C for 5 min followed by gradual reannealing to 35° for 1h. Samples were analyzed at the melt temperatures™ determinate by using the DHPLC melt software. Amplicons displaying a different chromatogram profile from a wild type control were subjected to direct sequence using BigDye terminator cycle sequencing kit (Applied Biosystem, Foster City, CA). Data analysis was carried out using sequence analysis 3.4.1 software (Applied Biosystem). The sequencing reactions in which mutations were identified were repeated at least twice.

Findings

Clinical features: Fourteen neurological mild patients affected by

TSC were selected. Among them, physical examination showed from 13 patients with mild dermatological phenotype (2 different TSC skin features with a single manifestation and /or a single type of lesion with less 20 elements) and one with severe dermatological phenotype (2 or more different TSC skin features with a single manifestation and/ or a single type of lesion with more than 20 elements). In this way 13 patients showed a mild dermatological phenotype.

No significant cardiac, ophthalmologic or lung lesions were observed on imaging studies of the heart and lung and by fundoscopic examination. Oral lesions were present in 8 out of 14 mild TSC patients.

All patients were asymptomatic for renal pain, hematoma or retroperitoneal hemorrhage. They were normotensive too.

Renal lesions were no significant in all patients, except for one patient that showed a significant renal involvement to renal ultrasound and abdominal MRI, but did not cause abdominal symptoms or renal function impairment.

From the neurological point of view 6 patients had history of seizures completely managed with antiepileptic drugs. 2 patients had a spontaneous remission of seizures and 6 never had epileptic seizures. In two family case (pz 4, 5) a some phenotypic differences exist in the absence of epilepsy in patient 5.

All patients with mild TSC phenotype showed a normal IQ and normal cognitive functions at all the neuropsychological tests. The result at all the administered neuropsychological tests which assessed specific cognitive functions was within normal range (Table 1).

Those results suggest that the relation between number of tubers and IQ is not "linear" and a lower cognitive functioning could be related more to tuber's activity and a history of infantile spasms than tuber's number.

Clinical features of TSC patients are summarized in Table 2 and 3. By the Spearman's pointed out that there is no correlation between intelligence and tubers' count ($p>0.05$); the only significant result was a link between tubers' number and Attentional Matrices ($p=0.031$), while the other cognitive domains showed no significant relation ($p>0.05$).

Neuroimaging features

In mild TSC population brain MRI showed a rate of 9 tubers (mean 2, 1) in seven patient and more 9 tubers (mean 14, 8) in the others seven patients (50% of mild TSC sample). Detailed tubers count obtained by brain MRI is summarized in Table 2.

Molecular study

Of the 13 patients in this study, germline mutations were identified in seven (54%). Four mutations were in the TSC2 gene, 3 mutations in TSC1 and no mutations were found in five patients. The TSC1 mutations consisted in a 1 Base Pair (bp) substitution that is predicted to change amino acid 786 from an arginine to a stop codon, one a 1-bp insertion and one was 1-bp deletion and insertion of 23 bp. Examination of exons 1-41 of TSC2 revealed four alterations of nucleotide sequence, 3 were missense mutations, one was deletion an 7 bp. We found one familial case that had exact same TSC2 mutation (Table 4). We found also a second family (pz 6; 7 Table 4) with no mutation for TSC1 or TSC2 genes. There was no apparent difference and/or severity of neurological manifestations between patients with TSC1 and TSC2 mutations.

Table 1:

	Mean N=14	(S.D.)
Raven's colored progressive matrices	36.4	16.3
Digit span	6	1
Story recall	10.4	4.2
Paired associated words	15.3	3.3
Corsi span	5	1
Rey's figure copy	30.8	4
Rey's figure memory	16.2	5.5
Trail making test A	44.4	6.1
Trail making test B	86.3	30.2
Attentional matrices	56.1	2.5
Oral fluency	35.1	12.7
Token test	34	1.2

Table 2:

Number of patients mild				
patient	epilepsy	Type of seizures	IQ level	Cortical tubers number
1	+	Remission	107	15
2	+	pc	97	16
3	+	Remission spont	100	1
4	+	pc+sec gen	85	13
5	-	-	100	26
6	+	pc	106	2
7	-	-	106	1
8	+	pc	100	13
9	-	-	108	1
10	-	-	110	2
11	-	-	80	12
12	-	-	80	12
13	+	pc	80	1
14	+	ps+psgs	100	9

- indicates the absence of epilepsy; + indicates the presence of epilepsy; pc: partial complex seizures; simple partial seizures; psgs: partial secondary generalized seizures.

Discussion

Our study of the mild TSC patients shows that tuber count is not a reliable hallmark of prognosis of cognitive impairment in TSC. In this way give evidence the data of high patient number showing a normal cognitive level (IQ>90), although with high cortical tuber's count (mean 11, 4; DS 7, 5) in our patient's sample.

Other author's related MR findings in TSC with a seizure development and a mental impairment, 4 while others showed that mental and behavioral outcome of epilepsy in TSC patients is strongly conditioned by spasm during the first year of life. The relation between infantile spasms and learning difficulty remains strong [26].

It has been shown that the cessation of spasms treated with vigabatrin is therefore associated with significant improvement of cognition and behavior in patients affected by TSC.

None of our patients with mild TSC phenotype with high tuber count presented infantile spasm seizures, and none had their first seizure before 1 year of age. This data testifies indirectly the major role of infantile spasm on poor cognition development rather than high tuber number. The fifty percent of mild TSC patients showed to high resolution brain MRI more than 9 tubers (mean 14, 8) and all have a normal cognitive level and a good control of seizures.

It is well known that tubers are epileptogenic [27], but on the other hand TSC patients with tubers do not always develop epileptic seizures.

It is a common opinion that there are epileptogenic and non epileptogenic tubers [28]. The point is controversial, but some authors

Table 3:

Mild TSC patients	Skin lesion	Renal Cysts	Renal angiomyiolipoma	Liver angiolipomas	Retinal hamartomas	Cardiac rhabdomyoma	LAM	Oral Lesion
1	+	+	+	+	-	-	-	+
2		-	-	-	-	-	-	-
3	+	-	+	-	-	-	-	+
4	+	-	+	+	-	-	-	+
5	+	-	+	+	-	-	-	+
6	+	-	+	-	-	-	-	+
7	+	-	-	-	-	-	-	+
8	+	-	-	-	-	-	-	+
9	-	-	-	-	-	-	-	-
10		-	-	-	-	-	-	-
11	++	+	++	++	-	-	+	-
12	+	+	-	-	-	-	-	+
13	+	-	+	-	-	-	-	-
14	+	-	+	-	-	-	-	-

– indicates absence of organ involvement; +: mild phenotype; ++: mild to severe phenotype

Table 4:

Number of patients mild					
	TSC1	**TSC2**			
Patient	**Exon**	**Exon**	**Mutation**	**familial**	**sporadic**
1		39	5079_5088delCCGTGGA		yes
2		9	G8899T, G299V		yes
3	17		2142_2144delCins23		
4		33	G4493A, S1498N	yes	
5			G4493A, S1498N	yes	
6	No mutation	No mutation		yes	
7	No mutation	No mutation		yes	
8	18		C2356T, R786X		yes
9	No mutation	No mutation			
10	No mutation	No mutation			
11	No mutation	No mutation			
12		1	156+5G>T		yes
13	6		989insT		yes
14		25	2281C>A		yes

found that in the TSC patients with poorly controlled seizures, the GABAergic tone is significantly lower compare to the tone in seizure-free patients. The decreasing GABAergic tone therefore contributes to the appearance of seizures in TSC patients with epilepsy [29].

We may speculate that non epileptogenic tubers do not interfere with cognitive activity and that cognitive functioning could be related more to tuber epileptogenic activity and to a history of infantile spasms than tuber number. We also would like to underline that a mild neurological phenotype is linked in our cohort to a mild dermatologic phenotype (only two different skin features with a single manifestation or a single type of lesion with less than 20 elements) probably in relation to a common neuroectodermical embryologic origin.

In our opinion a prognosis on cognitive impairment in TSC patients based on tubers' count should be considered with caution and other factor as infantile spasms and tubers' activity should be focused .Our finding suggest that wrong conclusions may be drawn if the number of lesions alone is used to predict neurological outcome in TSC. From the genetic point of view previous studies showed that there is a more severe outcome in patients with TSC2 than with TSC 1 [30] however a recent study [31] described a clinical heterogeneity in spite of identical TSC2 mutation in four patients.

Our results pointed out that a homogeneous mild neurological phenotype could be subtended by a genetic heterogeneity (4 patients with TSC1, 5 TSC2, no mutation in 5) suggesting that the phenotypic differences in TSC patients are more likely to be caused by mechanism such as somatic mosaicism, a second hit. Somatic mosaicism is a frequent phenomenon in disorders and at least 10% of sporadic TSC cases are imputated to somatic mosaicism for in TSC1 or TSC2 genes [32]. Nevertheless Sancak et al. [33] found mosaicism only in the <1%, suggesting that other unknown pathogenetic mechanisms could play a role in phenotypic variability. Otherwise another explanation, concerning technical issues, could be the limitation of DHPLC screening technology for large gene rearrangements or the absence of analysis performed on promoter/enhancer or intronic regions that have critical functional effects on the protein [34]. Furthermore the familial cases showing an identical TSC2 mutation have some clinical difference in spite of identical mutation as recently observed by other [31], confirming that other that genetic factor play a major role in clinical heterogeneity Interestingly the four TSC patients with no mutation for TSC1 or TSC2 showed a less severe phenotype than patients with definite TSC1 or TSC 2 mutation., as recently observed by others [33].

It looks like that patients with milder phenotype and no mutation found could be mosaic for a causative mutation. Finally, our patient represents an appropriate study group of mild TSC patients to depict any genetic contribution to TSC mild phenotype. Furthermore, from a clinical point of view, we show that prognosis on cognitive impairment in TSC based on tuber count alone is not a reliable prognosis factor. Further studies are needed to better clarify the clinical variability of TSC.

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