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TSC2 Deletions and Duplications: A Descriptive Study in Iranian Patients Affected with Tuberous Sclerosis

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Abstract

Tuberous sclerosis complex (TSC) is an autosomal dominant disorder characterized by formation of benign tumors called hamartomas. Although the TSC is diagnosed based on clinical findings but approximately 85% of individuals who meet diagnostic criteria for TSC a mutation can be identified in TSC2 (69%) and TSC1 (31%). A review of mutation type in TSC1 & TSC2 genes reveals that deletion/duplication assay could be a good screening strategy as a first step in TSC molecular diagnosis. All 41 exons and 5' untranslated region of TSC2 gene in addition to adjacent PKD1 gene were screened for deletion/duplication in 81 patients DNA samples using multiplex ligation dependent probe amplification (MLPA) method. Deletion/duplication was found in 29 (35.8%) patients, including deletions in 26 (32.0%) patients and duplication in 3 (3.8%). Genotype/phenotype analysis, showed five patients with renal function impairment who have large deletions including PKD gene area. Approximately 65% of cases were sporadic, while the remaining have familial positive history. Deletions/duplications of TSC2 gene were seen in 35.8% of patients with TSC. So it could be concluded that MLPA is a useful testing strategy for molecular screening in sporadic forms of TSC patients. MLPA increased the detection of TSC mutations. MLPA is less expensive, quicker and more precise than direct sequencing and southern blot in the characterization of TSC deletions. This technique is recommended as a standard part of TSC clinical molecular diagnosis.

Keywords

Tuberose Sclerosis Complex, TSC2, MLPA, Iran

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1. Introduction

Tuberous sclerosis complex (TSC) is a multisystem disorder associated with hamartomas formation in the brain, skin, heart, and other viscera [1] [2], with Epilepsy as the most common presenting symptom [3]. The incidence of TSC is estimated to be 1 in 6000 to 1 in 10,000 live birth [4]. The autosomal dominant pattern of TSC inheritance comes from a mutation in either the TSC1 or TSC2 gene [5] [6]. Both serve as tumor suppressors and inactivating mutations in either gene is associated with the formation of hamartomas [7]. Although familial TSC shows an equivalent representation of mutations in TSC1 and TSC2, sporadic cases are more associated with mutations in the TSC2 gene and have severe symptoms [1] [5] [8].

Missense mutations in TSC2 which disrupt its ability to bind to TSC1 have been identified in TSC patients [1] [9]. TSC2 located on chromosome 16p13.3.38, contains 41 exons and encodes a 200-kDa protein called tuberin [1] [4]. Mutations in TSC2 are four times more common and have more severe clinical symptoms than those of TSC1 [5] [10]. According to Human Genome Mutation Data Base (www.HGMD.org) roughly more than 45% of TSC1 & TSC2 mutations are deletions/duplications.

Renal problems have been observed in some sporadic patients as a relatively sever phenotype caused by a large deletion involving both TSC2 and PKD1 locus which is located 60bp downstream of TSC2 in a tail-to-tail orientation [6] [11].

Scientists are looking for a genius method to screen TSC2 and TSC1 gene mutations through populations as a fast cost benefit screening test before entire gene direct sequencing. Multiplex ligation dependent probe assay (MLPA) has been recently developed by MRC Co. as a method to identify deletion/duplication mutations as copy number variations (CNVs). This method enables accurate copy number determination of multiple (up to 40) genomic regions, by utilizing an oligonucleotide ligation assay with embedded universal primer sequences [10] [11].

In this study we applied commercially available TSC2 MLPA probe sets to enable comprehensive analysis of all exons within TSC2 for deletions and duplications in 81 Iranian TSC patients.

2. Experimental Section

2.1. Patients

All 81 TSC patients enrolled in our study were admitted to the Mofid Hospital, Shahid Beheshti University of medical sciences, Tehran, Iran. The study was approved by the local ethical committee of Mofid Hospital. Informed consent was obtained from all study participants prior to their inclusion in the study. Clinical criteria for a TSC diagnosis of Patients was determined by clinicians which has been previously published [3].

2.2. DNA Extraction Techniques and MLPA Method

DNA was extracted from peripheral blood samples by standard methods and qualified by Thermo fisher Nano drop, 2000 model. Multiplex ligation-dependent probe amplification was performed following the directions provided by MRC Holland, Amsterdam, The Netherlands (www.mlpa.com), using the probe set for TSC2 (SALSA MLPA kit P046-C1-1011) covering each of the 41 exons of the TSC2 gene. In addition, it contains one probe for the PKD1 gene next to TSC2 and 8 reference probes for sequences located on other chromosomes. To complete and verify our investigations we also used another probe set for TSC2 (SALSA MLPA kit P337-A2-0510) containing one probe for PKD1, 10 reference probes, 37 probes for exons and two for upstream regions of TSC2. In order to initial denaturation, 5 µl genomic DNA (20 ng/µl) was incubated at 98˚C for 5 minutes. Samples were cooled to room temperature and were mixed with 1.5 µl of probes mix (containing 1.5 µmol of each probe) and 1.5 µl of SALSA hybridization buffer. The procedure followed by 2 min denaturation at 95˚C and 16 hours hybridization at 60˚C. 32 µl ligation mixture added to the hybridized probe to be ligated at 54˚C for 15 min. After heat inactivation, 30 µl of PCR buffer added to 10 µl of ligation reaction heated to 60˚C. PCR amplification for 30 cycles was done for each sample after adding 10 µl PCR mixture (Salsa polymerase, dNTPs, and universal primers, one of which was labeled with Xuorescein). MLPA result was considered uninterpretable when the SD of normalized signal for probes was >0.15; in those cases, MLPA analysis was repeated. Fragment analysis was done for all amplification products by DNA sequencer machine CAC13130ABI. Finally fragment analysis outputs were evaluated using gene marker software V. 1.85.

3. Results and Discussion

Clinical and Para clinical Manifestations of 28 girls and 53 boys affected with TSC showed a range of 7 - 180 months for age of the patients. The most common manifestation were Hypo pigmented malecules (82.7%). About 29.6% of the patients have positive familial history of TSC but only sixteen families (19.8%) had a consanguineous marriage. In the other word about 70.4% showed a sporadic form without positive history of TSC in pedigree.

We used MLPA technique to screen 81 TSC patient DNA samples for genomic deletions and duplications of TSC2 gene and contiguous deletions of TSC2 and the adjacent PKD1. 29 cases (35.8%) were identified with Deletions/duplications in TSC2 gene (**Figure 1**). Only three of the identified mutations were genomic duplications, while all others were deletions. Considering 26 deletions, 18 (69.2%) were defined as large deletions, of which two of them extended to 5'UTR, 4 (15.4%) were deletions of two adjacent exons, 3 (11.5%) were deletions of just a single exon, there were also one sample (3.8%) showing deletion only in 5'UTR. Exons 21 - 41 were defined as mutational hotspots. Five samples showed deletion in PKD1 gene, these samples had no retinal cysts. Four of these were sporadic and one of them had a familial history of TSC. Genotype-phenotype analysis showed that none of five patients with retinal problems had deletions in PKD1. 57 (70.37%) patients had no familial history of Tuberous Sclerosis, including those cases with only one of symptoms of TSC, and 24 (29.63%) with at least one affected family member (**Table 1**). All clinical aspects of studied patients have been previously announced [3].

Tuberous sclerosis complex is an autosomal dominant disorder caused by mutations in either TSC1 or TSC2 gene. Screening these mutations in different populations could help to unravel complexities of the disease and find a new and faster way of diagnosis for TSC. Studies on TSC and many other human diseases have shown that MLPA is a highly sensitive technique to identify many aspects of widespread deletions/duplications [12] [13]. We used MLPA to detect deletion/duplication mutation in TSC2 gene of 81 patients with TSC. We also checked for probable contiguous deletions of TSC2 and the adjacent PKD1. We found that 64.5% of all patients had mutations in TSC2; these data are similar to those previously done in different populations and showed strong relation between TSC2 deletions/duplications and Tuberous Sclerosis [7] [10] [14]. The samples with no mutations in TSC2 are going to be analyzed for mutations in TSC1 gene. Those with deletions duplications in TSC2 are going to undergo target analysis for detection of the main area of mutation, in our future studies.

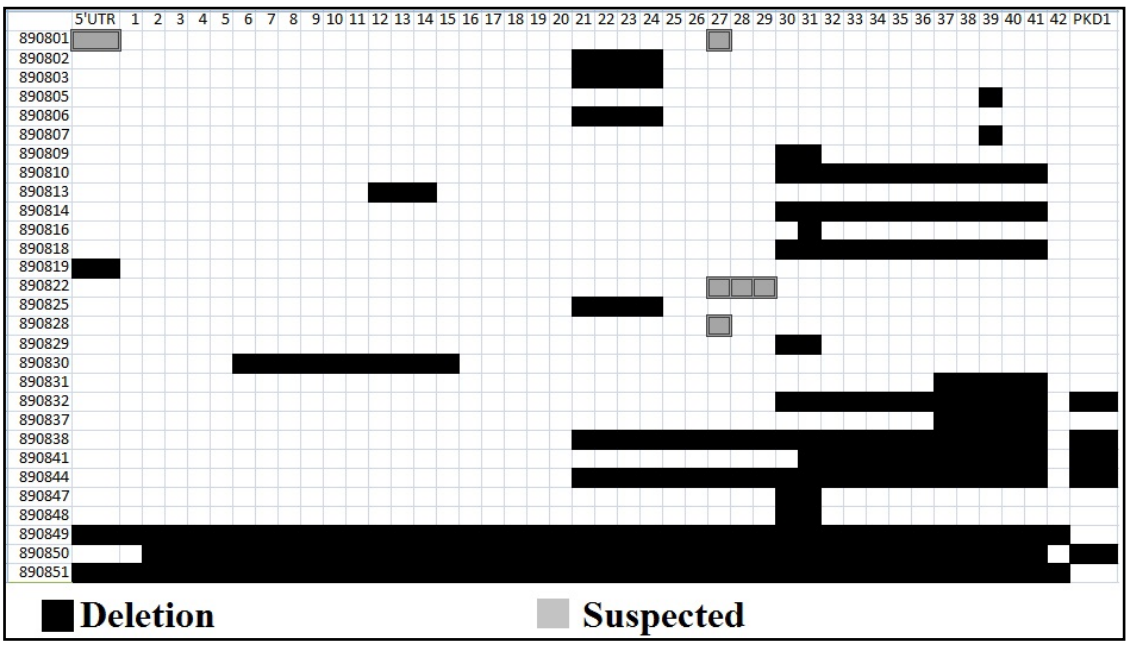


Figure 1. Deletion/duplication mutations in TSC2 gene. Deletions in each exon are shown in black and duplications are shown in gray. Numbers at the left are the specific code for each case and at the top numbers indicating numbers of exons.

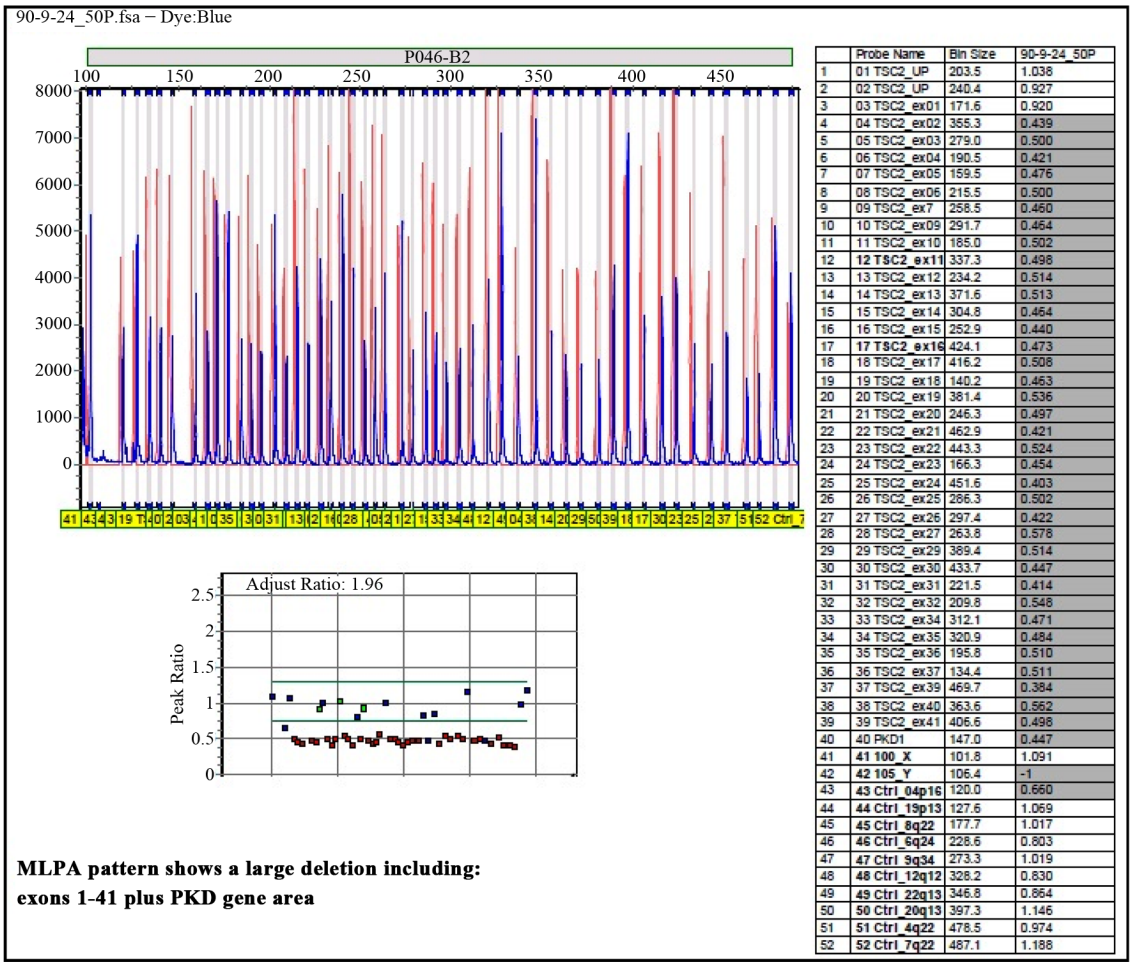
In the present study we found that duplications are much less common than deletions (only 10.3% with duplications). Our finding is consistent with the results that Kozlowski *et al*. revealed; they had a comprehensive study on large deletions/duplications of TSC1 and TSC2 in patients with TSC, which could find only two duplications in TSC2 [10].

From all patients of our study 8 were diagnosed with renal problems, but none of them had deletions in PKD1, and all PKD1 deletions were found in patients with no signs of kidney disorders, not considering one sample whose father had renal function impairment. According to previous studies, which have shown a strong relation between PKD1 deletions and kidney problems [10] [11], we should also consider the patients with deletion in PKD1 for kidney disorders in following studies (**Figure 2**).

Sporadic cases covering 70.37% of our study, which is in consistent with previous studies that estimated two thirds of tuberous sclerosis patients are sporadic [7].

Table 1. Distribution of TSC2 deletion/duplication in TSC patients.

Group	Total		Del		Dup	
Normal for TSC2	N	%	N	%	N	%
	52	0.64	0	0.0	0	0.0
Abnormal For TSC2	29	0.36	26	32	3	4
Total	81	100	26	32	3	4



	Probe Name	Bin Size	90-9-24_50P
1	01 TSC2_UP	203.5	1.038
2	02 TSC2_UP	240.4	0.927
3	03 TSC2_ex01	171.6	0.920
4	04 TSC2_ex02	355.3	0.439
5	05 TSC2_ex03	279.0	0.500
6	06 TSC2_ex04	190.5	0.421
7	07 TSC2_ex05	159.5	0.476
8	08 TSC2_ex06	215.5	0.500
9	09 TSC2_ex7	258.5	0.460
10	10 TSC2_ex09	291.7	0.464
11	11 TSC2_ex10	185.0	0.502
12	12 TSC2_ex11	337.3	0.498
13	13 TSC2_ex12	234.2	0.514
14	14 TSC2_ex13	371.6	0.513
15	15 TSC2_ex14	304.8	0.464
16	16 TSC2_ex15	252.9	0.440
17	17 TSC2_ex16	424.1	0.473
18	18 TSC2_ex17	416.2	0.508
19	19 TSC2_ex18	140.2	0.463
20	20 TSC2_ex19	381.4	0.536
21	21 TSC2_ex20	246.3	0.497
22	22 TSC2_ex21	462.9	0.421
23	23 TSC2_ex22	443.3	0.524
24	24 TSC2_ex23	166.3	0.454
25	25 TSC2_ex24	451.6	0.403
26	26 TSC2_ex25	286.3	0.502
27	27 TSC2_ex26	297.4	0.422
28	28 TSC2_ex27	263.8	0.578
29	29 TSC2_ex29	389.4	0.514
30	30 TSC2_ex30	433.7	0.447
31	31 TSC2_ex31	221.5	0.414
32	32 TSC2_ex32	209.8	0.548
33	33 TSC2_ex34	312.1	0.471
34	34 TSC2_ex35	320.9	0.484
35	35 TSC2_ex36	195.8	0.510
36	36 TSC2_ex37	134.4	0.511
37	37 TSC2_ex39	469.7	0.384
38	38 TSC2_ex40	363.6	0.552
39	39 TSC2_ex41	406.6	0.498
40	40 PKD1	147.0	0.447
41	41 100_X	101.8	1.091
42	42 105_Y	106.4	-1
43	43 Ctrl_04p16	120.0	0.660
44	44 Ctrl_19p13	127.6	1.069
45	45 Ctrl_8q22	177.7	1.017
46	46 Ctrl_6q24	228.6	0.803
47	47 Ctrl_9q34	273.3	1.019
48	48 Ctrl_12q12	328.2	0.830
49	49 Ctrl_22q13	346.8	0.864
50	50 Ctrl_20q13	397.3	1.146
51	51 Ctrl_4q22	478.5	0.974
52	52 Ctrl_7q22	487.1	1.188

Figure 2. The report shows a large deletion covered entire gene including PKD1 gene. Comparisons of the red picks (Control probes) compared to the blue picks (Exon probes) show about 2-fold higher in the length which mean a large deletion. The results confirm a normalized adjusted pick ratio as well.

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