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A novel missense mutation in the GTPase activating protein homology region of TSC2 in two large families with tuberous sclerosis complex

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Tuberous sclerosis complex (TSC) is an autosomal dominant disorder (OMIM 191092) characterised by autism, seizures, mental retardation, benign tumours of the brain, heart, kidney, lung, and skin, and malignant tumours of the kidney. TSC has a wide range of phenotypic variability, with some subjects severely affected and others only mildly affected. There are two TSC genes, TSC1 on chromosome 9q34 and TSC2 on chromosome 16p13.3. Approximately two thirds of cases of TSC appear to result from de novo germline mutations.

In 1994, an extended four generation family with 19 affected members was reported in which 34 members (17 affected with TSC and 17 unaffected) underwent both physical and psychiatric assessments. The majority of the affected subjects had mild physical expression of TSC, but there was significant clustering of neuropsychiatric disorders among affected subjects compared with their unaffected relatives. The disorders that were over-represented included mood disorder, anxiety disorder, and autism. The largest difference was observed in anxiety disorder, which was seen in 10 of the affected subjects and in two of the unaffected subjects (p=0.016). One affected child had pervasive developmental disorder and one had autism. Analysis of this family suggested that TSC could present phenotypically with mild physical signs and symptoms, but with significant neuropsychiatric disease. Linkage to the TSC2 gene locus on chromosome 16p13.3 was shown with a lod score of over 3.

We report here the identification of a missense mutation in exon 34 of the TSC2 gene in affected members of this family. We also examined a second four generation family from the same geographical area as the first family but not known to be related to them. The same exon 34 mutation was found in affected members of the second family.

Methods

To search for mutations in the coding regions of the TSC2 gene we used single strand conformation analysis (SSCP). The primers amplifying each of the 41 exons of TSC2 and the PCR conditions have been described previously. The PCR products were run on MDE gels (AT Biochem). To maximise the detection of each variant band, each PCR product was run on two gels, one without glycerol and one with 5% glycerol. Samples in which variant bands were detected were reamplified and sequenced. This
study was approved by the Institutional Review Boards of Fox Chase Cancer Center, the University of California at Los Angeles, and the University of California, Irvine.

Results

We found variant bands in exon 34 in DNA from the index patient (patient 355) from the first family (fig 1A). Variant bands were not found in any other exon of TSC2. DNA sequencing of exon 34 showed an A to C change at position 4508 (fig 1B). We then analysed 12 affected family members and three unaffected family members from the first family. All of the affected subjects, and none of the unaffected family members, had the exon 34 change (fig 1C).

We next tested affected and unaffected members of a second, four generation, TSC family, TS-15. This family, from the same geographical area as the first family, includes 24 affected subjects. The same exon 34 change found in the first family was found in both of the affected members that we tested and none of six unaffected members. We also analysed TSC2 exon 34 from 57 unrelated subjects without a personal or family history of TSC. None of these controls had the A4508C change, indicating that this is not a common genetic polymorphism.

Discussion

The A4508C mutation is predicted to change amino acid 1503 from glutamine to proline. This amino acid is identical among the human, mouse, rat, fugu, and Drosophila homologues of tuberin, the product of the TSC2 gene (fig 2). Exons 34 to 38 of TSC2 encode a region of tuberin with homology to rap1 GTPase activating protein (GAP). Tuberin has been shown to have GAP activity for both rap1 and rab5. The A4508C is the first missense mutation identified in exon 34 of TSC2. Ten TSC2 missense mutations in the GAP domain have been previously reported (one in exon 35, one in exon 36, five in exon 37, and three in exon 38). These are described in detail in the online TSC Variation Database (http://expmed.bwh.harvard.edu/ts/). Only one of the previously reported GAP domain missense mutations (in exon 38) was found in a familial, rather than a sporadic, case of TSC.

In the first family with the exon 34 mutation, mild physical signs of TSC were associated with significant neuropsychiatric symptoms including pervasive developmental disorder and autism. The second family has not yet had formal neuropsychiatric evaluations. However, like the first family, many affected subjects appear to have a mild form of TSC. For example, hypomelanotic skin macules (white spots) or white skin freckles were the only known manifestation of TSC in 10 of the 19 affected subjects in the first family (52%) and 12 of the 23 affected subjects in the second family (52%).

Several of the mildly affected subjects in these families might not have been recognised as having TSC had they not been related to the index patients. This raises the possibility that people in the general population with neuropsychiatric disease who lack the classical signs of TSC could also have germline missense changes within the GAP domain of TSC2. TSC is associated with autism, hyperactive behaviour, sleep disorders, and aggressive behaviour. Both mutations and polymorphisms in the TSC genes could therefore be considered candidate susceptibility or genetic modifier alleles for any of these disorders. TSC2 may be of particular interest as a possible susceptibility locus for autism because autism appears to have a strong genetic component.

Multiple genome wide screens have been performed, two of which have identified
potential autism susceptibility loci on chromosome 16p, 11q; the location of \( TSC2 \).

In summary, we have identified a novel missense change at a highly conserved residue within the region of GTPase activating domain homology of the \( TSC2 \) gene in two four generation TSC pedigrees with a total of more than 40 affected members. This is, to our knowledge, by far the largest known group of TSC patients carrying the same mutation. Therefore, we anticipate that these families will be important in the future identification of modifier gene effects in TSC. In one family, an association of TSC with significant neuropsychiatric disease has already been documented. Further studies will be required to understand biochemically the functional consequences of this exon 34 missense mutation and to characterise more completely the clinical and neuropsychiatric manifestations of TSC in these families. Understanding the relationship between naturally occurring germine \( TSC2 \) mutations and neuropsychiatric disease could elucidate the underlying biology of TSC and potentially facilitate studies aimed at prevention and/or early diagnosis.

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3 Autosomal deletions or chromosomal haploinsufficiency syndromes are observed in 1 in 7000 live born infants and may cause multiple malformations, growth failure, and mental retardation. Deletions on the short arm of chromosome 3 have been reported in 35 cases and have been divided into two groups: deletion 3p syndrome with breakpoints between 3p23 and 3p25 and proximal deletion 3p syndrome with different breakpoints between 3p11 and 3p21.2. The first reported case of an interstitial deletion of chromosome 3p22.2-p24.2 in a 6 year old male with developmental delay is presented here.

**Case report**

The proband was the fourth child born, in England, to healthy, unrelated, white parents. There was no family history of note. He was born vaginally following spontaneous onset of labour at 41 weeks of gestation after an uneventful pregnancy and weighed 3140 g (10th centile). A murmur was noted shortly after delivery and echocardiography confirmed the presence of a small, perimembranous ventricular septal defect. His early milestones were reported as normal, but he was referred for assessment of developmental delay when aged 16 months. He made good progress following input from a child development unit. He walked at 23 months and had speech delay. He was reassessed three months after arrival in New Zealand at the age of 3.5 years. He had global developmental delay and he also had some hearing impairment. His language skills were poor, only speaking occasional two to three word sentences by the age of 4 years, although his comprehension was felt to be good. He was a sociable child with no behavioural difficulties. He needed nappies at