

UC Irvine

UC Irvine Previously Published Works

Title

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

Permalink

<https://escholarship.org/uc/item/5jv3t9fh>

Journal

Journal of Medical Genetics, 38(5)

ISSN

0022-2593

Authors

Khare, Leena

Strizheva, Galina D

Bailey, Julia N

et al.

Publication Date

2001-05-01

DOI

10.1136/jmg.38.5.347

Copyright Information

This work is made available under the terms of a Creative Commons Attribution License, available at <https://creativecommons.org/licenses/by/4.0/>

Peer reviewed

We would like to thank the family for its participation, Dr A Nivelon-Chevalier and Dr C Robinet for their kind collaboration in the clinical study of the patient, and Dr M Mitchell for reviewing the English language. This work was supported by INSERM and the Association pour la Recherche contre le Cancer (ARC).

- 1 Morison IM, Reeve AE. A catalogue of imprinted genes and parent-of-origin effects in humans and animals. *Hum Mol Genet* 1998;7:1599-609.
- 2 Engel E. Uniparental disomy: related syndromes and implications for prenatal diagnosis. *Eur J Hum Genet* 1998;6:29-30.
- 3 Robinson WP, Langlois S. Phenotype of maternal UPD(14). *Am J Med Genet* 1996;66:89.
- 4 Tomkins DJ, Roux AF, Wayne J, Freeman VC, Cox DW, Whelan DT. Maternal uniparental isodisomy of human chromosome 14 associated with a paternal t(13q14q) and precocious puberty. *Eur J Hum Genet* 1996;4:153-9.
- 5 Cotter PD, Kaffe S, McCurdy LD, Jhaveri M, Willner JP, Hirschhorn K. Paternal uniparental disomy for chromosome 14: a case report and review. *Am J Med Genet* 1997;70:74-9.
- 6 Sanlaville D, Aubry MC, Dumez Y, Nolen MC, Amiel J, Pinson MP, Lyonnet S, Munnich A, Vekemans M, Morichon-Delvallez N. Maternal uniparental heterodisomy of chromosome 14: chromosomal mechanism and clinical follow up. *J Med Genet* 2000;37:525-8.
- 7 Cattanach B, Barr J, Jones J. Use of chromosome rearrangements for investigations into imprinting in the mouse. In: Ohlsson R, Hall K, Ritzen M, eds. *Genomic imprinting. Causes and consequences*. Cambridge: Cambridge University Press, 1995:327-41.
- 8 Georgiades P, Chierakul C, Ferguson-Smith AC. Parental origin effects in human trisomy for chromosome 14q: implications for genomic imprinting. *J Med Genet* 1998;35:821-4.
- 9 Sutton VR, Shaffer LG. Search for imprinted regions on chromosome 14: comparison of maternal and paternal UPD cases with cases of chromosome 14 deletion. *Am J Med Genet* 2000;28:381-7.
- 10 Martin RA, Sabol DW, Rogan PK. Maternal uniparental disomy of chromosome 14 confined to an interstitial segment (14q23-14q24.2). *J Med Genet* 1999;36:633-6.
- 11 Robin NH, Harai-Shacham A, Schwartz S, Wolff D. Duplication 14(q24.3q31) in a father and daughter: delineation of a possible imprinted region. *Am J Med Genet* 1997;71:361-5.
- 12 Pinkel D, Straume T, Gray JW. Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. *Proc Natl Acad Sci USA* 1986;83:2934-8.
- 13 Stavropoulou C, Mignon C, Delobel B, Moncla A, Depetris D, Croquette MF, Mattei MG. Severe phenotype resulting from an active ring X chromosome in a female with a complex karyotype: characterisation and replication study. *J Med Genet* 1998;35:932-8.
- 14 Deloukas P, Schuler GD, Gyapay G, Beasley EM, Soderlund C, Rodriguez-Tome P, Hui L, Matise McKusick KB, Beckmann JS, Bentolila S, Bihoreau M, Birren BB, Browne J, Butler A, Castle Chiannikulchai N, Clee C, Day PJ, Dehejia A, Dibbling T, Drouot N, Duprat S, Fizames C, Fox S, Gelling S, Green L, Harrison P, Hocking R, Holloway E, Hunt S, Keil S, Lijnzaad P, Louis-Dit-Sully C, Ma J, Mendis A, Miller J, Morissette J, Muselet D, Nusbaum HC, Peck A, Rozen S, Simon D, Slonim DK, Staples R, Stein LD, Stewart EA, Suchard MA, Thangarajah T, Vega-Czarny N, Webber C, Wu X, Hudson J, Auffray C, Nomura N, Sikela JM, Polymeropoulos MH, James MR, Lander ES, Hudson TJ, Myers RM, Cox DR, Weissenbach J, Boguski MS, Bentley DR. A physical map of 30,000 human genes. *Science* 1998;282:744-6.
- 15 Daniel A. The size of prometaphase chromosome segments. *Clin Genet* 1985;28:216-24.
- 16 Morton NE. Parameters of the human genome. *Proc Natl Acad Sci USA* 1991;88:7474-6.
- 17 Craig JM, Bickmore WA. Chromosome bands: flavors to savor. *Bioassays* 1993;15:349-54.

J Med Genet
2001;38:347-349

Department of
Medical Oncology, Fox
Chase Cancer Center,
7701 Burholme
Avenue, Philadelphia,
PA 19111, USA
L Khare
G D Strizheva
E P Henske

Department of
Molecular Biology,
Russian State Medical
University, Moscow,
Russia
G D Strizheva

Department of
Psychiatry, University
of California School of
Medicine, Los Angeles,
CA, USA
J N Bailey
S L Smalley

Division of Medical
Genetics, Department
of Pediatrics, The
University of Texas
Medical School,
Houston, TX, USA
K-S Au
H Northrup

Department of
Pediatrics, University
of California, Irvine,
CA, USA
M Smith

Correspondence to: Dr
Henske,
EP_Henske@fccc.edu

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

Leena Khare, Galina D Strizheva, Julia N Bailey, Kit-Sing Au, Hope Northrup, Moyra Smith, Susan L Smalley, Elizabeth Petri Henske

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.¹ 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 9q34² 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.⁴ 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 3.⁴

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 family⁵ 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.⁶ The PCR products were run on MDE gels (AT Biochem). To maximise the detection of variant bands, 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

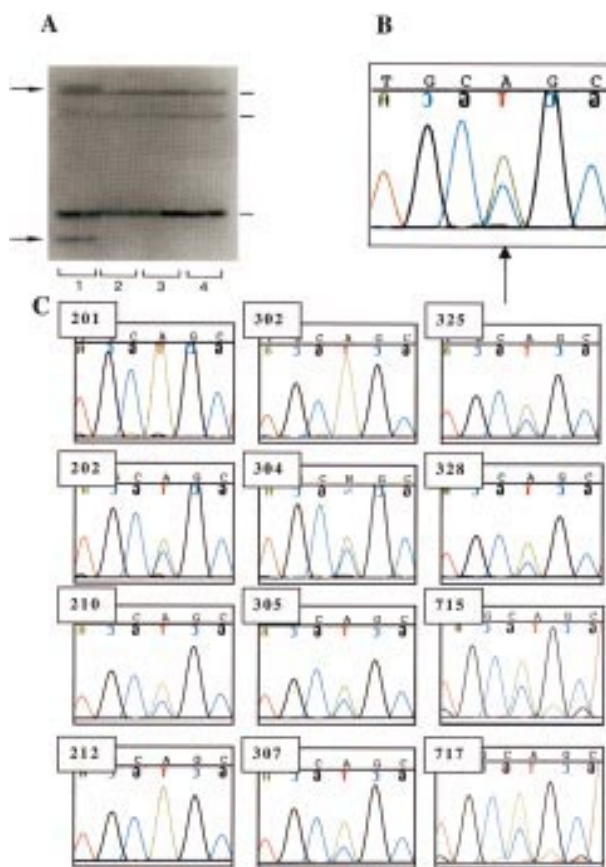


Figure 1 (A) Detection of variant bands in exon 34 using single strand conformation polymorphism analysis. The first two lanes (indicated below the figure with "1") contain DNA from patient 355. The other lanes (labelled 2, 3, and 4) contain DNA from three controls unrelated to this family. The arrows on the left indicate the variant bands and the lines on the right indicate the position of the wild type bands. (B) Sequencing of exon 34 from patient 355. The arrow indicates the double peak of A and C in germline DNA. (C) Sequencing of other affected and unaffected members of the families. Subjects 202, 210, 304, 305, 307, 325, and 328 are affected members of the first family. Subjects 715 and 717 are affected members of the second family. Subjects 201, 212, and 302 are unaffected members of the first family.

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.⁵ 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).³ Tuberlin has been shown to have GAP activity for both rap1⁷ and rab5.⁸ 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.⁴ 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.⁹ 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.¹⁰ Multiple genome wide screens have been performed,¹¹ two of which have identified



Figure 2 Evolutionary conservation of *TSC2* exon 34. Residues in red are identical to the human sequence, and those in green are similar. The arrow indicates the glutamine residue affected by the mutation.

potential autism susceptibility loci on chromosome 16p,^{12,13} 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 germline *TSC2* mutations and neuropsychiatric disease could elucidate the underlying biology of TSC and potentially facilitate studies aimed at prevention and/or early diagnosis.

This work was supported by the March of Dimes Birth Defects Foundation (6-FY99-181). We are grateful to Drs Warren Kruger and Rebecca Raftogianis for many helpful suggestions and comments, and to the patients and their families who participated in this research.

- 1 Bjornsson J, Short MP, Kwiatkowski DJ, Henske EP. Tuberous sclerosis-associated renal cell carcinoma: clinical, pathologic, and genetic features. *Am J Pathol* 1996;149:1201-8.
- 2 van Slegtenhorst M, de Hoogt R, Hermans C, Nellist M, Janssen B, Verhoef S, Lindhout D, van den Ouweland A, Halley D, Young J, Burley M, Jeremiah S, Woodward K, Nahmias J, Fox M, Ekong R, Osborne J, Wolfe J, Povey S,

- Snell RG, Cheadle JP, Jones AC, Tachataki M, Ravine D, Sampson JR, Reeve MP, Richardson P, Wilmer R, Munro C, Hawkins TL, Sepp T, Ali JBM, Ward S, Green AJ, Yates JRW, Kwiatkowska J, Henske EP, Short MP, Haines JH, Jozwiak S, Kwiatkowski DJ. Identification of the tuberous sclerosis gene *TSC1* on chromosome 9q34. *Science* 1997;277:805-8.
- 3 European Chromosome 16 Tuberous Sclerosis Consortium. Identification and characterization of the tuberous sclerosis gene on chromosome 16. *Cell* 1993;75:1305-15.
- 4 Smalley SL, Burger F, Smith M. Phenotypic variation of tuberous sclerosis in a single extended kindred. *J Med Genet* 1994;31:761-5.
- 5 Smith M, Smalley S, Cantor R, Pandolfo M, Gomez MI, Baumann R, Flodman P, Yoshiyama K, Nakamura Y, Julier C, Dumars K, Haines J, Trofatter J, Spence MA, Weeks D, Conneally M. Mapping of a gene determining tuberous sclerosis to human chromosome 11q14-11q23. *Genomics* 1990;6:105-14.
- 6 Au KS, Rodriguez JA, Finch JL, Volcik KA, Roach ES, Delgado MR, Rodriguez E, Northrup H. Germ-line mutational analysis of the *TSC2* gene in 90 tuberous sclerosis patients. *Am J Hum Genet* 1997;62:286-94.
- 7 Wienecke R, Konig A, DeClue JE. Identification of tuberlin, the tuberous sclerosis-2 product. Tuberlin possesses specific Rap1GAP activity. *J Biol Chem* 1995;270:16409-14.
- 8 Xiao GH, Shorinejad F, Jin F, Golemis EA, Yeung RS. The tuberous sclerosis-2 gene product, tuberlin, functions as a Rab5GAP in modulating endocytosis. *J Biol Chem* 1997;272:6097-100.
- 9 Hunt A. Psychiatric and psychologic aspects. In: Gomez M, Sampson J, Whittemore V, eds. *Tuberous sclerosis complex*. New York: Oxford University Press, 1999:47-62.
- 10 Smalley SL. Genetic influences in childhood-onset psychiatric disorders: autism and attention-deficit/hyperactivity disorder. *Am J Hum Genet* 1997;60:1276-82.
- 11 Risch N, Spiker D, Lotspeich L, Nouri N, Hinds D, Hallmayer J, Kalaydjieva L, McCague P, Dimiceli S, Pitts T, Nguyen L, Yang J, Harper C, Thorpe D, Vermeer S, Young H, Hebert J, Lin A, Ferguson J, Chiotti C, Wiese-Slater S, Rogers T, Salmon B, Nicholas P, Petersen PB, Pingree C, McMahon W, Wong DL, Cavalli-Sforza LL, Kraemer HC, Myers RM. A genomic screen of autism: evidence for a multilocus etiology. *Am J Hum Genet* 1999;65:493-507.
- 12 International Molecular Genetic Study of Autism Consortium. A full genome screen for autism with evidence for linkage to a region on chromosome 7q. *Hum Mol Genet* 1998;7:571-8.
- 13 Philippe A, Martinez M, Cuilloud-Bataill M, Gillberg C, Rastam M, Sponheim E, Coleman M, Zappella M, Aschauer H, van Malldergerme L, Penet C, Feingold J, Brice A, Leboyer M. Genome-wide scan for autism susceptibility genes. *Hum Mol Genet* 1999;8:805-12.

Interstitial deletion of 3p22.2-p24.2: the first reported case

H X Liu, P T S P Oei, E A Mitchell, J M McGaughran

J Med Genet
2001;38:349-351

Department of
Cytogenetics, Starship
Children's Hospital,
Auckland, New
Zealand
H X Liu
P T S P Oei

Department of
Paediatrics, University
of Auckland,
Auckland, New
Zealand
E A Mitchell

Northern Regional
Genetic Service,
Building 18, Auckland
Hospital, Grafton,
Auckland, New
Zealand
J M McGaughran

Correspondence to: Dr
McGaughran,
juliemc@ahsl.co.nz

Autosomal deletions or chromosomal haploinsufficiency syndromes are observed in 1 in 7000 live born infants¹ 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 syndrome² with breakpoints between 3p24 and 3p25 and proximal deletion 3p syndrome³ 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 it was felt he 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