

Intrafamilial Phenotypic Variability in Tuberous Sclerosis Complex

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Clinical manifestations were retrospectively assessed in 5 families with tuberous sclerosis complex, including 1 pair of monozygotic twins. Interfamilial variation in tuber count was significantly larger than intrafamilial variation. Severity of epilepsy and cognitive profiles varied both between and within families, particularly between the monozygotic twins, and IQ was inversely related to tuber count. Cutaneous, renal, and cardiac findings did not appear to cluster within families. Although the monozygotic twins displayed similar physical manifestations of tuberous sclerosis complex (renal

and cardiac hamartomas), they differed markedly in neurocognitive profiles. Phenotypic variation within these families may be explained largely as a function of the randomness of second-hit events that cause hamartomas in tuberous sclerosis complex or by as-yet-unidentified genetic modifiers. Familial variation in tuberous sclerosis complex phenotype has important implications for genetic counseling.

Keywords: tuberous sclerosis complex; phenotypic variation; monozygotic twins

Tuberous sclerosis complex (TSC) is an autosomal dominant disorder characterized by hamartomas and hamartias in multiple organs, including the brain, skin, kidneys, heart, liver, eyes, and lungs.^{1,2} Approximately 85% of those with TSC have an identifiable mutation in 1 of 2 genes: *TSC1* (9q34) and *TSC2* (16p13.3).³⁻⁵ In general, *TSC2* mutations are associated with a more severe phenotype than *TSC1* mutations.³⁻⁵ Although phenotypic variation has been described in the TSC population,⁶ there is little literature on variation within families. We report 5 families with TSC that exhibit broad intrafamilial phenotypic variation, with some members severely affected and others displaying only minor symptoms.

Methods

We reviewed the medical records of 183 patients meeting the clinical criteria for TSC² who received medical care

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through the Carol and James Herscot Center for Tuberous Sclerosis Complex at Massachusetts General Hospital from January 2002 to May 2005. A total of 49 familial cases were identified in 27 families. Of these 27 families, 5 had multiple affected family members who had received comprehensive clinical evaluations including neuropsychological testing and neuroimaging, and these 5 families were selected for further study. In all, 4 of these families were multigenerational, and 1 had a pair of monozygotic twins. For these patients, we gathered reports from clinic visits, radiological studies, electroencephalograms, neuropsychological and psychiatric evaluations, and correspondence via telephone and e-mail. Family histories provided by these patients were also examined. Genetic testing reports from Athena Diagnostics, Inc (Worcester, Massachusetts) and mutations identified in the laboratory (D.J.K.) were reviewed and confirmed.

A neuropsychologist (M.B.P.) performed and subsequently reviewed the results of comprehensive neuropsychological test batteries administered to 15 patients in the 5 families, as appropriate for each patient's chronological age and level of function (referenced in Table 1). The tests assessed 7 domains, including tests of intelligence, expressive language, receptive language, visual-spatial perception, verbal memory, executive function, and emotion/behavior.

Two radiologists (D.Y.J. and P.E.G.) reviewed the most recent magnetic resonance images of 14 patients to count

Table 1. Neuropsychological Results

	Age ^a		Intelligence ^b	Receptive Language ^c	Expressive Language ^d	Visual-Spatial ^e	Working Memory ^f	Story Memory ^g	Executive Function ^h	Emotional-Behavioral ⁱ
	y	mo								
A-I-1	49	11	N	N	N	N	N	N	BNL	CS
A-II-1	19	7	N	N	N	BNL	N	N	N	CS
A-II-2	16	4	BNL	BNL	N	BNL	BNL	BNL	BNL	CS
B-II-1	5	6	N	N	NA	N	NA	BNL	NA	N
B-II-2	5	6	BNL	BNL	BNL	BNL	NA	NA	NA	CS
C-III-1	29	10	N	NA	NA	NA	N	NA	BNL	NA
C-III-4	25	4	BNL	NA	NA	NA	BNL	N	BNL	NA
C-III-5	22	6	BNL	N	N	BNL	N	N	BNL	N
D-III-2	5	10	N	N	N	BNL	NA	N	NA	N
D-III-3	1	3	N	NA	NA	NA	NA	NA	NA	NA
E-I-1	61	10	N	ANL	ANL	N	N	N	BNL	N
E-II-1	39	4	N	N	ANL	N	ANL	N	ANL	N
E-II-4	33	4	N	ANL	ANL	N	N	N	N	N
E-III-1	4	6	N	ANL	ANL	N	NA	NA	NA	N
E-III-2	2	3	ANL	NA	NA	NA	NA	NA	NA	N

Abbreviations: ANL, above normal limits (SS > 115 or > 84 percentile); BNL, below normal limits (SS < 85 or < 16 percentile); CS, clinically significant; N, normal (SS 85-115 or 16-84 percentile); NA, not available; SS, standard score; COWA, Controlled Oral Word Association F-A-S subtest; TMT, Trail Making Test.

^aAge at time of neuropsychological evaluation. Each remaining column shows results for 1 age-appropriate test listed in Table 1 except Executive Function (which shows composite result for COWA and TMT) and Emotional-Behavioral (which shows composite result for multiple tests listed in Table 1).

^b1-30 mo: Bayley Scales of Infant Development—2nd ed. (BSID-2)⁷; 2.5-4 y: Stanford-Binet Intelligence Scale—5th ed. (SBIS-5)⁸; 4-5 y: Wechsler Preschool and Primary Scale of Intelligence—3rd ed. (WPPSI-3)⁹; ≥6 y: Wechsler Abbreviated Scales of Intelligence (WASI).¹⁰

^c≥2 y: Peabody Picture Vocabulary Test—3rd ed. (PPVT-3).¹¹

^d≥2 y: Expressive One-Word Picture Vocabulary Test—3rd ed. (EOWPVT-3).¹²

^e<18 y: Developmental Test of Visual-Motor Integration (VMI)¹³; ≥18 y: Rey-Osterrieth Complex Figure Test (Rey-O)—copy.^{14,15}

^f<16 y: Wechsler Intelligence Scale for Children—4th ed. (WISC-4) digit span subtest¹⁶; ≥16 y: Wechsler Adult Intelligence Scale—3rd ed. (WAIS-3) digit span subtest.¹⁷

^g<16 years) Wechsler Memory Scales—3rd ed. (WMS-3), logical memory I subtest¹⁸; ≥16 y: Wide Range Assessment of Memory and Learning (WRAML)—story memory (or Stanford-Binet Sentence Memory if WRAML was not available).¹⁹

^h≥7 y: Controlled Oral Word Association (COWA) F-A-S subset^{15,20}; ≥7 y: Trail Making Test (TMT) subtest B.¹⁵

ⁱ2-21 y: Behavior Assessment System for Children (BASC) behavioral symptoms index²¹; ≥16 y: Symptom Checklist-90-Revised (SCL-90-R)²²; ≥18 y: Current Symptoms Scale (CSS)—Adult ADHD Checklist²³; 2-18 y: Child Behavior Checklist (CBCL) parent form^{24,25}; 4-14 y: Child Symptom Inventory—4th ed. (CSI-4).²⁶

cortical tubers and subependymal nodules. If 1.5T and 3T studies of an individual were available, lesions were counted on the study of the same magnet strength as studies of most other family members. Image sequences included sagittal T1, axial T2 fast spin echo, and axial fluid-attenuated inversion recovery. The radiologists were blinded to clinical features and neuropsychological test results.

Statistical tests were performed using SPSS v. 11.5 (Chicago, Illinois).

Results

Pedigrees of the 5 families are presented in Figure 1, and clinical profiles of the individuals in these families are given in Table 2. In family A, 3 individuals met clinical criteria for TSC; in family B, 2 (the monozygotic twins only); in family C, 8; in family D, 6; and in family E, 6. Large deletions (family B), frameshift (families C and D), missense (family A), and splice site (family E) were the mutations identified (Table 2).

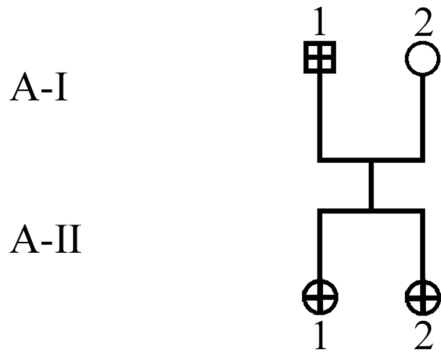
Neuroanatomic Features

Magnetic resonance imaging (MRI) studies were available for 14 individuals (Table 3). Familial differences in total tuber count were significant (Welch's test; $P = .006$). Although MRI reports had identified 7 individuals with lesions suspicious of subependymal giant-cell tumor, only 1 individual (E-II-1) was confirmed to have a subependymal giant-cell tumor, defined as a subependymal nodule >10 mm with the potential to cause obstruction based on its location in the foramen of Monro.²⁷

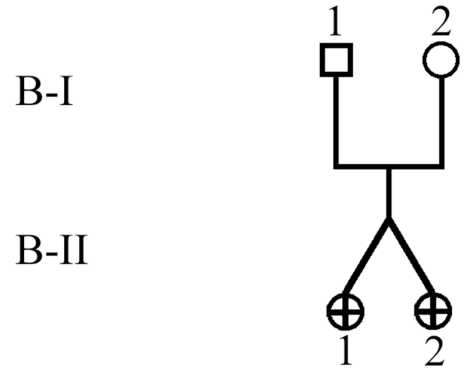
Seizure Disorders

At least 1 member of each family was affected with seizures, but the severity of seizure disorders ranged widely within each family. The broadest range of seizure disorders was seen in family A, in which 1 member (A-II-1) had only occasional seizures since age 21, whereas her sister (A-II-2) had an intractable seizure disorder since age 8. In families C and D, some individuals had relatively mild seizure disorders that responded well to antiepileptic drugs (AED), whereas others

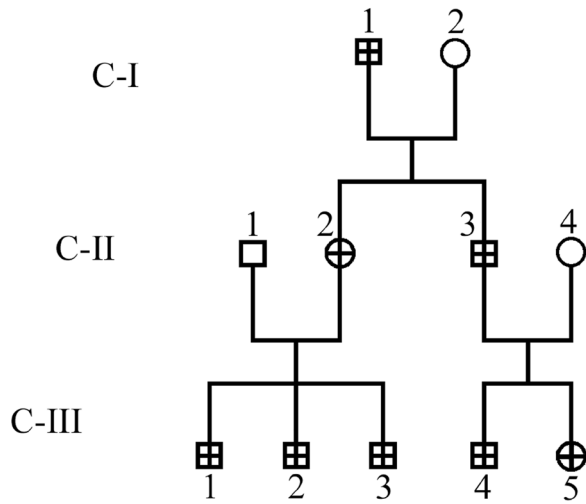
Family A



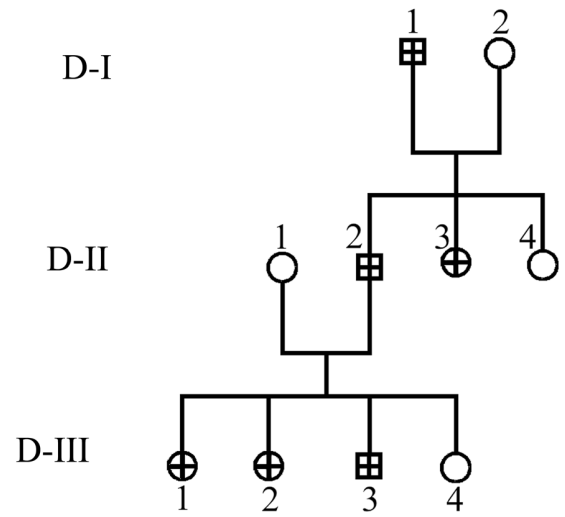
Family B



Family C



Family D



Family E

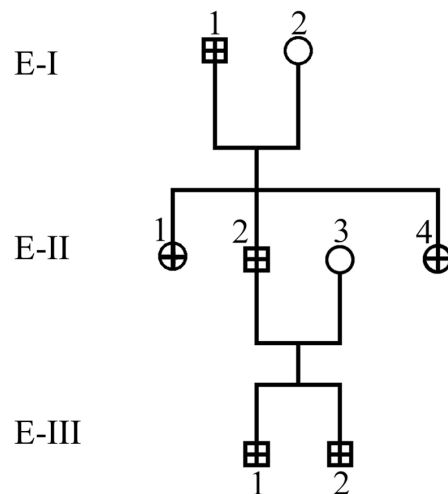


Figure 1. Pedigrees of the 5 families, A-E. Individuals with tuberous sclerosis complex are indicated with a cross.

Table 2. Clinical Profiles

	Age ^a	Mutation ⁵²	Current AEDs ^b	Seizures	IQ ^c	Neuroimaging	Cutaneous	Renal	Other
A-I-1	51	NA	1	Con	100	CT, SEN	AF, HPM, PUF	RCC	— ^d
A-II-1	21	TSC1 E04 c.403T>G p.L61R	0	Occ	101	CT, SEN	AF, HPM, PUF, SP	— ^e	— ^d
A-II-2	17	As above	3 + VNS	Int	72	CT, SEN	AF, HPM, PUF		
B-II-1	6	TSC2 g.-1065_IVS1 773del, E1 del. Total deletion of 1838bp.	0	Occ	85	CT, SEN	AF, HPM	AML, C	CR
B-II-2	6	As above	1	Int, IS	<42	CT, SEN	HPM, SP	AML, C	CR, s/p temporal lobectomy
C-I-1	88	NA	0	NA	NA	NA	AF, LP, PUF, SP	NA	RC
C-II-2	55	TSC1 E20 g.2790insG	0	NA	NA	NA	AF, HPM, PUF	NA	NA
C-II-3	52	NA	NA	NA	NA	NA	NA	NA	NA
C-III-1	31	TSC1 E20 c.2790insG	2	Int	87	CT, SEN, ?SGCT	AF, PUF	— ^e	— ^d
C-III-2	30	As above	0	Con	NA	CT, SEN, ?SGCT	HPM	— ^e	RH
C-III-3	28	As above	5	Int	NA; CI	NA	AF, HPM, PUF, SP	— ^e	— ^d
C-III-4	27	As above	3	Con	79	CT, SEN	HPM	AML	— ^d
C-III-5	23	As above	3	Int	73	CT, SEN	HPM	— ^e	RH
D-I-1	NA	NA	NA	Con	NA	NA	HPM	NA	NA
D-II-2	NA	NA	NA	Con	NA	NA	HPM	NA	NA
D-II-3	NA	NA	NA	Con	NA	NA	HPM	NA	NA
D-III-1	9	TSC2 E33 c.4422_4423delAG	0	Con, IS	NA	CT, SEN	AF, HPM	— ^e	CR
D-III-2	6	As above	2	Con, IS	85	CT, SEN	AF, HPM	— ^e	CR
D-III-3	3	As above	1	Con, IS	101	CT, SEN, cystic changes	Single HPM	AMLs	
E-I-1	62	NA	NA	NA	106	CT, SEN	NA	NA	NA
E-II-1	39	NA	NA	NA	115	CT, SGCT	NA	NA	NA
E-II-2	38	NA	NA	— ^f	NA	CT, ?SEN	AF	AML, C	HC
E-II-4	34	NA	NA	NA	105	CT	NA	NA	NA
E-III-1	5	TSC2 E35 g.4662G>A p.1554Q silent, probable splice mutation	0	Con	102	CT, SEN	AF, HPM, SP	— ^e	CR, RP
E-III-2	3	As above	0	— ^f	121	CT, SEN	AF, HPM	— ^e	— ^d

Abbreviations: A, adenine; AED, antiepileptic drugs; AF, angiofibromas; AML, angiomyolipoma; bp, base pairs; c, cDNA sequence; Con, controlled; C, cysts; CI, cognitive impairment; CL, confetti lesions; CR, cardiac rhabdomyoma; CT, cortical tuber(s); del, deletion; DP, dental pitting; E, exon; g, genomic DNA sequence; G, guanine; HC, hepatic cysts; HPM, hypopigmented macules; I, intron; Int, intractable; ins, insertion; IS, seizure phenotype included infantile spasms; IVS, intervening sequence within an intron; L, leucine; LP, labial papules; NA, not available; Occ, occasional (<5 seizures total); p, protein sequence; PUF, periungual fibromas; Q, glutamine; R, arginine; RC, retinal calcification; RCC, renal cell carcinoma; RH, retinal hamartoma; RP, atypical retinal pigmentary changes; SEN, subependymal nodule(s); ?SEN, probable SEN; SGCT, subependymal giant cell tumor; ?SGCT, probable SGCT; SP, shagreen patch; VNS, vagus nerve stimulator.

^aYears at the time of this study.

^bNumber of AEDs.

^cFull-scale intelligence quotient determined by an age-appropriate intelligence test (Table 1).

^dNormal exam or no findings.

^eNo involvement.

^fNone.

had intractable epilepsies. Likewise, one of the twins in family B had intractable seizures requiring surgical intervention, whereas the other had a history of just 3 febrile seizures. Family E is unusual in that only 1 member had seizures, and those were relatively easily controlled by medication (this family had a *TSC2* splice-site mutation). We observed both interfamilial and intrafamilial variations with respect to seizure type. Although statistical confirmation is impossible in a sample of this size, seizure phenotype appears to vary tremendously within families.

Neurocognition and Behavior

Cognitive profiles also varied widely (Table 1). In families A, B, and D, 1 member of each family had global cognition in the borderline or impaired range, whereas siblings and relatives were in the normal range. In family C, full-scale IQ ranged from impaired to low average. In family E, all members had full-scale IQ in the normal range.

Some of the variation in global intelligence may be accounted for by AED regimen and normal genetic variation.

Table 3. Neuroimaging

Patient	Age ^a	Magnet Strength (Tesla)	Tubers ^b	Mean Tubers by Family (mean size of largest, mm)	SENs ^c	Mean SENs by Family
A-I-1	47	1.5	14 (15)	14.7 (16.0)	5 S	6.0
A-II-1 ^d	11	1.5	12 (19)		3 S	
A-II-2	14	1.5	18 (14)		10 S	
B-II-1 ^d	5	1.5	65 (39)	62.5 (40.5)	5 L, 2 M, 11 S	13.5
B-II-2 ^d	5	1.5	60 (42)		2 L, 1 M, 6 S	
C-III-1 ^d	23	Per report on outside MRI and CT: multiple cortical and subcortical tubers, some calcified. Multiple calcified SENs.				
C-III-2 ^d	24	Per report on outside MRI: bilateral SENs. One at the left foramen of Monro showed slight enhancement following contrast.				
C-III-4	26	3	9 (11)	27.0 (20.5)	4 S	5.5
C-III-5	23	3	45 (30)		7 S	
D-III-1	6	1.5	39 (25)	44.0 (28.0)	2 M, 8 S	17.5
D-III-2 ^d	2	Per report on outside MRI: subcortical tubers in all lobes and multiple SENs.				
D-III-3	3	1.5	49 (31)		1 M; 24 S	
E-I-1	61	3	7 (18)	11.8 (20.4)	4 S	3.4
E-II-1	38	3	12 (22)		1 SGCT	
E-II-2 ^d	17	Per report on outside MRI: calcifications and CNS involvement of TSC.				
E-II-4	34	3	8 (19)		0	
E-III-1	5	1.5	14 (18)		4 S	
E-III-2	2	1.5	18 (25)		8 S	

Abbreviations: CNS, central nervous system; CT, cortical tuber; MRI, magnetic resonance imaging; SEN, subependymal nodule; SGCT, subependymal giant cell tumor; TSC, tuberous sclerosis complex.

^aYears at the time of imaging.

^bTotal number of cortical tubers and size of the largest tuber (mm), measured along the cortical surface.

^cNumber of subependymal nodules; S, <5 mm; M, 5-10 mm; L, >10 mm; SGCT, >10 mm and located in the foramen of Monro.

^dStudy performed outside of our institution.

Mean \pm SD of AEDs among individuals who underwent cognitive testing was 1.13 ± 1.25 , and the negative correlation between number of AEDs approached but did not reach significance (Pearson's correlation coefficient = -0.570 ; $P = .053$).

Full-scale IQ was inversely related to tuber count (Pearson's correlation coefficient = -0.754 ; $P = .012$) and to size of the largest tuber (Pearson's correlation coefficient = -0.655 ; $P = .040$). Discrepancies between verbal and nonverbal IQs were significant (≥ 1 SD) in 2 individuals: 1 individual (A-I-1) with a verbal IQ that was 23 points lower than his or her nonverbal IQ, and 1 individual (E-III-1) with a nonverbal IQ that was 16 points lower than his or her verbal IQ. Though the difference was not of large enough magnitude to be considered significant, 2 more individuals had nonverbal IQs that were 12 to 14 points lower than his or her verbal IQs.

Behavioral difficulties were also prevalent. In family A, A-I-1 had symptoms of anxiety, obsessive or compulsive behaviors, and generalized psychiatric distress; A-II-1 had symptoms of depression and somatic complaints; and A-II-2 met the criteria for attention-deficit hyperactivity disorder-inattentive type. Individuals in families B (B-II-2) and D (D-III-1) also endorsed symptoms indicating attentional and behavioral disorders.

Possible confounding factors were present in 3 individuals. One had suffered a closed-head trauma (A-I-1 at age 8). Two individuals had undergone neurosurgery (B-II-2 had a

left frontal tuberectomy at age 27 months and E-II-1 had a subependymal giant-cell tumor).

Nonneurologic Manifestations

Cutaneous findings were consistently extensive in some families (eg, family A), consistently minor in others (eg, family D), and quite variable in still others (Table 2). Renal manifestations (angiomyolipoma, cysts, or renal cell carcinoma) were present in 1 member each in families A, C, D, and E and in both members of family B. Cardiac rhabdomyoma was found in 1 member each in families D and E and in both members of family B, but many individuals may not have had echocardiograms during the neonatal period. No members of our study had lymphangioleiomyomatosis. In general, therefore, the presence or absence of cutaneous, renal, or cardiac symptoms in a given individual did not appear to be predicted by the presence or absence of such manifestations in an affected relative. The monozygotic twins (family B) were the exception in our study, with nearly identical physical findings (including rhabdomyomas and renal angiomyolipoma) despite radically different neurocognitive and seizure profiles.

Discussion

Our sample is typical of the TSC population with respect to frequency of most manifestations; for example, in our

study, 17/19 (90%) of individuals had a history of seizure of activity, which is consistent with the 80% to 90% rate generally reported.²⁸ However, this study demonstrates that clinical and anatomic findings are highly variable even within families sharing the same mutation.

Neurocognition and Behavior

Intrafamilial distributions of IQs in our study were concordant with the bimodal distribution of IQs generally reported in the literature, according to which about half probands with TSC have near-normal global cognition and about a third have grossly impaired cognition.^{29,30} That our sample reflects a somewhat greater-than-expected proportion with normal intelligence reflects the bias inherent in a study of familial cases, which may tend to have a milder phenotype than sporadic cases. Consistent with other studies, the proportion of *TSC1* mutations, which generally are associated with a less impaired neuropsychological phenotype, is also larger in our sample of familial cases than in the TSC population as a whole.³⁻⁵

Even among those with IQs in the normal range (85-115), many individuals with TSC exhibit significant, specific cognitive deficits, including 2 individuals in our study.^{31,32} The symptoms of anxiety and depression, problem behaviors, and deficiencies in executive function that were found in our cohort are also consistent with the literature on attentional and psychiatric disorders in TSC.³²⁻³⁴

Inheritance and Phenotype or Genotype Associations

Several inheritance studies have shown substantial phenotypic variation within families, but most antedate MRI technology and therefore cannot accurately reflect the range of manifestations of TSC.³⁵⁻³⁸ More recently, 3 studies have reported phenotypes in multigenerational families, but with unusually mild phenotypes that included several asymptomatic individuals, due to 3 different missense mutations in *TSC2*.³⁹⁻⁴¹ The cases reported here, in contrast, demonstrate a wide range of neurological, cognitive, and physical symptoms.

Studies have also reported a total of 7 pairs of monozygotic twins with TSC. In 4 pairs of twins reported in these studies, both twins suffered chronic seizures and mental retardation.⁴²⁻⁴⁵ In more recent studies by Gomez et al⁴⁶ and Humphrey et al,⁴⁷ 3 pairs of twins were reported, each pair included 1 twin with severe seizures and mental retardation and another with less severe manifestations and closer-to-normal cognition. Our twins (family B) are similar to those reported by Gomez et al⁴⁶ and Humphrey et al⁴⁷ insofar as they have sharply divergent seizure severity and cognitive/developmental profiles. Although Humphrey does not report nonneurologic symptoms, it is worth noting that both pairs of twins in the study of Gomez et al,⁴⁶ like the twins in family B, have

little heterogeneity within pairs on physical exam and imaging. The twins in family B have moderate cutaneous involvement, renal angiomyolipomas and cysts, cardiac rhabdomyomas, and similar findings on neuroimaging.

Explaining Phenotypic Variation

Variation in tuber location, size, and possibly histopathology may be important in explaining disparities in cognitive and neurological health among individuals within these families, especially so among the monozygotic twins. More subtle widespread morphologic and histologic abnormalities may also contribute to variation in central nervous system manifestations.⁴⁸ Unpredictable factors that likely contribute to variability in familial phenotype include genetic modifiers and number and timing of second-hit events leading to hamartoma formation. Although there is as yet no direct evidence for a 2-hit mechanism in the pathogenesis of TSC cortical tubers, much correlative data implicate this as a critical event in their pathogenesis.⁴⁹⁻⁵¹ A more thorough understanding of the mechanisms of dysgenesis in central nervous system hamartomas may contribute to explaining phenotypic variation in TSC.

Conclusions

The broad phenotypic range of TSC is well established, but phenotypic range within families with TSC is not well understood. In our study, most features of TSC showed great variability even within families, including seizure disorders, cutaneous features, renal manifestations, cardiac rhabdomyomas, and deficits in executive function. IQ appeared to show both interfamilial and intrafamilial variation, but a larger sample is needed to confirm this statistically. Only cortical tuber count showed significant interfamilial variation; certain psychiatric disorders (especially anxiety) may also cluster within families. The neurocognitive manifestations in the monozygotic twins are of particular interest because they highlight the unpredictability of TSC. Because the manifestations of TSC may be very subtle in some individuals, clinicians should have a low threshold for performing a complete diagnostic workup on individuals in whom TSC is suspected.² Mutational analysis is also particularly valuable when TSC is known or suspected in a family member.

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