TSC1 and *TSC2* Genotype in Tuberous Sclerosis Complex: Are Other Manifestations of this Multisystem Disease Affected by Genotype?

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Lymphagioleiomyomatosis (LAM) is a cystic lung disease that is associated with kidney (e.g., angiomyolipomas [AML]) and lymphatic (e.g., lymphangioleiomyomas, chylous effusions, adenopathy) involvement. LAM occurs sporadically (S-LAM) and in association with tuberous sclerosis complex (TSC), an autosomal-dominant disorder that is characterized by additional hamartomatous manifestations in multiple organs, including brain, skin, cardiac, and bone (1). TSC results from pathogenic variants in the *TSC1* or *TSC2* genes. LAM cells from patients with S-LAM also share genetic alterations of the *TSC1* and *TSC2*, but these mutations are not found throughout the body as in TSC, accounting for its sporadic occurrence and more restricted organ involvement.

The two proteins encoded by *TSC1* and *TSC2*, hamartin and tuberin, respectively, form a complex together with TBC1D7 (Tre2-Bub2-Cdc16 1 domain family member 7) (2). The TSC complex regulates mTORC1 (mechanistic target of rapamycin 1) via RHEB (Ras homolog enriched in brain), with tuberin acting as a RHEB GTPase that catalyzes the conversion of active RHEB-GTP to inactive RHEB-GDP. RHEB-GTP activates mTORC1, which serves as a nutrient, energy, and redox sensor that regulates cell growth and proliferation, by effects on anabolic processes such as protein synthesis and lipid biogenesis and by inhibiting autophagy. Pathogenic variants in *TSC1* or *TSC2* result in loss of tuberin GTPase activity of the heterotrimeric TSC protein complex, leading to increased RHEB-GTP and enhanced mTORC1 activity. mTORC1 is inhibited by the drug rapamycin (sirolimus), which is U.S. Food and Drug Administration approved for the treatment of LAM (1). In S-LAM, it appears that most of the variants are found in *TSC2*, perhaps because *TSC1* mutations appear not to have the same effects on the GTPase activity of the heterotrimeric TSC complex. In this regard, it may be that pathogenic variants of *TSC2* may be more relevant to TSC complex activity, as it is the *TSC2*-encoded protein tuberin that contains the catalytic GTPase site.

In the study reported in this issue of *AnnalsATS*, Tian and colleagues (pp. 815–819) report that in patients with TSC, those with *TSC2* pathogenic variants had more severe cystic lung disease than patients with *TSC1* pathogenic variants (3). Those results mirror the findings by Muzykewicz and colleagues (4), who reported similar observations in patients with TSC. However, importantly, the report by Tian and colleagues extends those findings by looking at other biomarkers of TSC lung disease and, particularly, of LAM cystic lung disease. In this regard, it has been shown that concentrations greater than 800 pg/ml of serum VEGF-D (vascular endothelial growth factor-D), a lymphangiogenic and angiogenic factor, are diagnostic of LAM and that patients with higher concentrations are more likely to respond to treatment with sirolimus (5). Tian and colleagues (3) report that serum VEGF-D concentrations were higher in patients with *TSC2* variants than those with *TSC1* variants.

Another finding characteristic of TSC-mediated lung disease is the presence of multifocal micronodular pneumocyte hyperplasia (MMPH), a hamartomatous proliferation of type II pneumocytes. Tian and colleagues looked at the difference in MMPH in patients with *TSC2* and *TSC1* pathogenic variants as well as in those patients with TSC with no mutation identified (NMI). As opposed to the cystic lung disease, semiquantitative assessment of MMPH did not show any differences in MMPH between patients with *TSC2* and *TSC1* variants (3). Further quantitative analysis of these lesions may determine whether there are age and genotype effects on the presence of MMPH or growth of the lesions.

The cohort reported by Tian and colleagues (3) had a relatively high percentage of individuals with NMI, accounting for 13/55 or 23% of the individuals. One possible explanation for the inability to identify a pathological variant in these individuals is the presence of mosaicism arising from a postzygotic somatic mutation in a TSC gene. Variant detection is hindered in these individuals because each is composed of a mixture of cells either with or without the variant, so methods of variant detection must detect a variant allele fraction typically below 20%. Indeed, one individual in the cohort was mosaic with a *TSC2* variant allele fraction of 1.2%. It is interesting that VEGF-D concentrations and the extent of cystic lung disease in those patients with NMI are similar to those with *TSC2* variants. Future studies may determine whether serum VEGF-D

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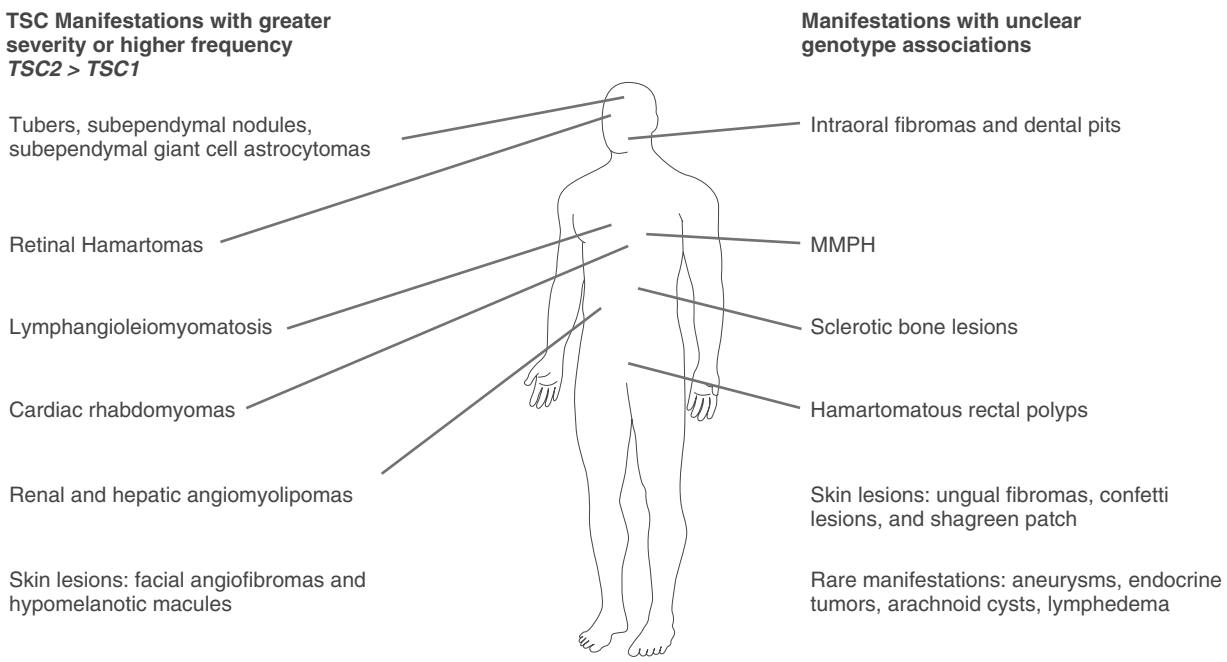

Figure 1. Frontiers of genotype–phenotype correlations in tuberous sclerosis complex. MMPH = multifocal micronodular pneumocyte hyperplasia; TSC = tuberous sclerosis complex.

concentrations and the cystic lung disease reflect *TSC2* mosaicism.

The age at TSC diagnosis in the adult cohort reported by Tian and colleagues (3) was 14 years, ranging from 0 to 35 years. TSC is typically diagnosed in the first few years of life, particularly in those presenting with neurological symptoms (1). However, there is a subgroup of TSC patients who present later in life, many of whom present with LAM or AMLs and who are less likely to have a history of seizures (6). These individuals with "mild" TSC early in life are nonetheless at risk for potentially life-threatening pulmonary and renal disease later in life. Those manifesting TSC later in life may be enriched with patients with mosaicism and fewer disease manifestations (7, 8). Mosaicism should be suspected in those who are NMI and in those exhibiting phenotypic markers for mosaicism, such as asymmetric distribution of facial angiofibromas or absence of brain lesions (9, 10). In individuals with possible mosaicism, the mosaic pathological variant may be identified using samples of TSC skin lesions as the source of DNA (7, 8).

Extrapulmonary manifestations of TSC, while not significantly affected by genotype in the report by Tian and colleagues, show differences in cohorts involving large numbers of patients or a different age population (reviewed in Reference 11). Regarding the neurological manifestations, in a cohort of 1,657 individuals with TSC, those with *TSC2* pathogenic variants were more likely to have seizures before age 2 years and more likely to have infantile spasms (12). In a study of infants with TSC, those with *TSC2* variants had more frequent and larger brain lesions, had earlier onset of seizures, and were more likely to have specific skin lesions (hypomelanotic macules and angiofibromas) (13). Patients with *TSC2* variants were more likely to have cyst-like cortical tubers on magnetic resonance imaging (14) and more likely to have cerebellar lesions (15) than those with *TSC1* variants. Other organs with evidence for a higher frequency of involvement in those with *TSC2* variants compared with those with *TSC1* variants include cardiac rhabdomyomas, renal AMLs, renal cysts, hepatic AMLs, and retinal lesions (11). Sclerotic bone lesions are observed in TSC more commonly in those with mutations identified than in those with negative mutational studies (16). Thus, for many pulmonary and extrapulmonary manifestations of TSC, those with variants in *TSC2* have more severe disease as a group than those with *TSC1*. Particular TSC manifestations tend to appear at different ages, so it will be interesting to study larger adult populations with TSC for correlations of genotype and phenotype.

The interesting studies by Tian and colleagues suggest that there are opportunities for additional studies with larger cohorts to see effects of genotype on phenotype of the multiple organ systems affected in patients with TSC (Figure 1). These types of studies can be applied to less common manifestations of TSC, which may associate even more strongly with one TSC gene. It will be interesting to explore phenotypic manifestations of those previously with NMI who are now known to be mosaic. Quantitative phenotype–genotype assessment of some of the other manifestations of TSC may demonstrate further differences based on *TSC1-TSC2* genotype and mosaicism. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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Accurately Measuring Preventable Ventilator-associated Pneumonia Deaths Using Observational Data: It's about Time

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Ventilator-associated pneumonia (VAP) is the most commonly diagnosed infection among critically ill patients, with associated all-cause mortality rates of 20–50% (1–3). Prevention of VAP represents a cornerstone of infection prevention efforts and is a benchmark for hospital performance reporting (4). Although absolute mortality associated with VAP diagnosis is substantial, the excess mortality directly conferred by VAP itself remains a matter of debate (5, 6). VAP is fundamentally a complication of critical illness; thus, the substantial mortality experienced by patients with VAP is in no small part a consequence of their underlying critical illness.

In this issue of *AnnalsATS*, Steen and colleagues (pp. 830–837) set out to identify rates of preventable mortality due to VAP (7). Prior studies have produced disparate

estimates of VAP-attributable mortality, in part because of heterogeneity in VAP definitions and therapeutic approaches as well as potential confounding by severity of illness and other underlying differences between those who do and those who do not develop VAP. As the authors note, identifying deaths attributable to VAP

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