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Tuberous Sclerosis Complex Genotypes and Developmental Phenotype

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Abstract

Background: Children with tuberous sclerosis complex (TSC), caused by pathogenic variants in *TSC1/TSC2*, are at risk for intellectual disability. *TSC2* pathogenic variants appear to increase the risk, compared with *TSC1*. However, the effect of *TSC2* pathogenic variants on early and specific

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Supplementary data

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domains of development hasn't been studied. Using an extensively phenotyped group, we aimed to characterize differences in early intellectual development between genotypes.

Methods: The study group (n = 92) included participants with TSC enrolled in a multicenter study involving genetic testing and detailed prospective phenotyping including the Mullen Scales of Early Learning, a validated measure of cognition, language, and motor development in babies and preschool children. Mean T-scores at 24 months for each Mullen Scales of Early Learning domain were calculated for children with, versus without, a *TSC2* pathogenic variant. Multivariable linear regression models were used to compare the groups, adjusting for seizures.

Results: T-scores on every Mullen Scales of Early Learning domain were significantly worse in the *TSC2* group. Below average composite scores were present in three-fourths of the *TSC2* group, compared with one-fourth of those without *TSC2*. Having a *TSC2* pathogenic variant was associated with lower composite Mullen Scales of Early Learning scores, even when corrected for seizures.

Conclusions: In a well-characterized patient population with standardized assessment of multiple aspects of development, we found that having a *TSC2* pathogenic variant was associated with significantly lower Mullen Scales of Early Learning scores at age 24 months, independent of seizures. These data suggest that a baby with a *TSC2* pathogenic variant is at high risk for significant developmental delays by 24 months.

Keywords

Tuberous sclerosis complex (TSC); Genotype; Phenotype; Developmental delay; Mullen scales of early learning (MSEL); Genotype-phenotype correlation; Cognition

Introduction

Tuberous sclerosis complex (TSC), which results from pathogenic variants in *TSC1* and *TSC2*, is a genetic disorder characterized by tumor formation throughout the body, most commonly in the skin, brain, kidneys, heart, and eyes. In addition to the tumors, patients with TSC are at risk for neurological issues including seizures, developmental delay, intellectual disability (ID), and autism.[1] There is a great deal of variability in terms of TSC-related phenotypes, including neurological symptoms. For example, some individuals present early in life with infantile spasms and extreme developmental delays, whereas others may go undiagnosed until a family member is identified. Because of the variability in outcomes, it is vital to identify at-risk individuals as early as possible to develop and apply appropriate intervention and counseling strategies. As TSC genotypes are often available early in life, leveraging this information to predict phenotype could be particularly powerful. This is especially true for individuals who are at risk of developmental delay, as intervention improves outcomes.

Pathogenic variants in *TSC1* and *TSC2* lead to improper regulation of the mammalian target of rapamycin signaling pathway and multiple downstream effects, including uncontrolled cell growth and proliferation.[2,3] A clinical diagnosis of definite TSC can be made if a person has two major features or one major feature and at least two minor features.[4] Notably, a pathogenic variant in *TSC1* or *TSC2* cannot be found in 10% to 15% of patients who meet a

clinical diagnosis of definite TSC. The cause of TSC in these patients is thought to most likely be mosaicism[5,6] or an intronic variant in *TSC1* or *TSC2*[7] that is undetectable by current clinical tests. The possibility of another unidentified TSC locus also remains.

Genotype-phenotype correlations have been performed for many genetic disorders to determine if genotype can help predict prognosis and assist with management.[8-10] Regarding learning, an important genotype-phenotype correlation for TSC is that *TSC2* pathogenic variants have been associated with increased risk of ID.[11-14] *TSC2* pathogenic variants are also associated with increased risk of seizures,[13] which have been associated with poorer developmental outcomes.[15] They have also been associated with increased tuber burden,[16] which has been associated with increased risk of seizures[11] and severe cerebral disease.[17] Despite this, important gaps exist in our understanding of TSC genotype-phenotype associations. For example, previous genotype-phenotype studies were based on self-reported or medical history of ID, not direct assessment. Furthermore, the effect of *TSC2* variants on early or specific domains of development has not been studied.

Leveraging data from a well-characterized cohort of patients with TSC who have undergone genotyping and testing with Mullen Scales of Early Learning (MSEL), we aimed to better characterize differences in early motor, language, visual reception (i.e., visual perceptual ability), and global development between different TSC genotypes. We hypothesized that patients with *TSC2* pathogenic variants would be more likely to have developmental delay at 24 months than those with *TSC1* pathogenic variants or those with no mutation identified (NMI).

Materials and methods

Study population

The study was performed using data from the TSC Autism Center of Excellence Research Network study (). The inclusion and exclusion criteria for the study have been previously described.[18] Briefly, the study enrolled children aged three to 12 months with a clinically, or genetically, confirmed diagnosis of TSC, who were tracked for up to age 36 months.

Ninety-two children who had been genotyped for variants in *TSC1* and *TSC2* and who had completed the MSEL assessment at 24 months were included in the present study. The 24-month time point was chosen to reflect early cognitive, language, and motor development at a stage when the divergence of abilities would likely start to be apparent. Patients tracked the presence of seizures and seizure types with a seizure diary. Patients also underwent serial electroencephalographies. Presence of seizures was determined by the medical personnel at their study site.

Genotyping

Sixty children were genotyped during routine clinical care. For the remaining 32 children, Sanger sequencing was performed on DNA from peripheral blood as part of the TSC Autism Center of Excellence Research Network trial to identify coding variants in *TSC1* and *TSC2*. If a pathogenic variant was not identified through Sanger sequencing, multiplex ligation-dependent probe amplification was performed to detect deletions or duplications. Variants

identified were classified using American College of Medical Genetics standards and guidelines.[19] A participant was classified as NMI when both Sanger sequencing and multiplex ligation-dependent probe amplification failed to identify a pathogenic, or likely pathogenic, variant. Variants of uncertain significance, likely benign, or benign were categorized as NMI. Detailed TSC disease genotypes of these patients have been reported previously.[20]

Mullen Scales of Early Learning

The MSEL is a validated measure of cognition, language, and motor development in babies and young children.[21] There are five scales: Gross Motor, Visual Reception, Fine Motor, Receptive Language, and Expressive Language, as well as an Early Learning Composite standard score. The five scales are measured using T-scores, with a mean of 50 and an S.D. of 10, whereas the composite score has a mean of 100 and an S.D. of 15. Scores on these five scales can be classified as very low (≤30), below average (31 to 39). average (40 to 60). above average (61 to 69), and very high (70 to 80).

Statistical analysis

The genotypes were categorized as (1) *TSC1* pathogenic variant, (2) *TSC2* pathogenic variant, and (3) NMI. Summary statistics describing the distributions of sex, ethnicity, gestational age, seizure status, and parental ages by genotype were computed. The mean T-score at 24 months for each MSEL domain was calculated for each category (*TSC1 TSC2,* and NMI). Because *TSC2* pathogenic variants have been associated with more severe phenotypes than *TSC1* or NMI,[1,22] children with, versus without, a *TSC2* pathogenic variant were also compared. Additional comparisons were made excluding patients (N = 3) with *TSC2* pathogenic variants known to convey a milder phenotype.[23,24] Chi-squared and Fisher's exact tests were used to describe differences in categorical variables, and the Kruskal-Wallis test was used to describe differences in the distributions of continuous variables. Multivariable linear regression models were used to assess the independent effects of *TSC2* pathogenic variants and seizures on MSEL scores, adjusting for maternal age. Mean scores (± standard errors) in each domain for *TSC2* and non-*TSC2* groups were plotted at ages nine through 36 months, with the exception of Gross Motor, which is only measured in children aged up to 33 months. For all tests, statistical significance was defined as $P < 0.05$. All statistical analyses were performed in R, version 3.5.0 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Sixty-three patients had a *TSC2* pathogenic variant, 13 had a *TSC1* pathogenic variant, and 16 had NMI. All variants identified were germline, and none appeared to be mosaic. *TSC1* pathogenic variants included frameshift (50%), nonsense (42%), and splice site (8%). *TSC2* pathogenic variants included frameshift (28%), nonsense (23%), missense (23%), splice site (14%), and deletion (12%). Three of the patients had *TSC2* missense variants (Arg622Trp and Arg1200Trp) that had been previously associated with a milder phenotype[23,24] and unless otherwise specified, were included in the *TSC2* group in analyses. There were no significant differences in sex, ethnicity, gestational age, and paternal age between the three

groups: *TSC1, TSC2,* and NMI (Table 1). As previously reported[13] there was a significant difference in seizure history between the three groups, with seizures being most common in patients with *TSC2* pathogenic variants. Maternal age at birth was highest in the NMI group.

The T-scores on every MSEL domain were significantly different among the three groups. The visual reception, fine motor, and expressive language domains were all significantly lower ($P < 0.001$) among those in the *TSC2* group. In addition, gross motor and receptive language scales were significantly lower in the *TSC2* group ($P = 0.001$ and $P = 0.007$, respectively) (Supplemental Table 1). The MSEL composite scores were also statistically different among the three groups. Table 2 demonstrates that 73% of the patients with *TSC2* pathogenic variants scored below average compared with 31% of the patients with *TSC1* or 23% of the patients with NMI. Notably. 57% of patients with *TSC2* pathogenic variants had "very low" scores. One of the two participants in the *TSC2* group who scored "very high" had a pathogenic variant previously associated with a mild phenotype (Arg1200Trp). Two of the 13 participants in the *TSC2* group who scored in the average range also had genotypes previously associated with mild phenotypes (Arg622Trp and Arg1200Trp).

In comparing the *TSC2* group with those without a *TSC2* pathogenic variant, patients with a pathogenic variant in *TSC2* scored significantly lower in all domains of the MSEL (Table 3). In multivariable linear regression models, having a *TSC2* pathogenic variant, as opposed to *TSC1* or NMI. was associated with five- to 10-point reduction in MSEL T-scores after adjustment for seizures and maternal age. An additional one- to three-point reduction occurred when the three participants with known mild *TSC2* variants were excluded from the *TSC2* group (Supplemental Table 2). Presence of seizures was correlated with reduction (9-23 points) in MSEL T-scores in all domains independent of genotype (Table 4).

Mean trajectories for each domain and the composite score from nine through 36 months were plotted for the *TSC1*/NMI group, the *TSC2* group, and the *TSC2* group excluding children with variants known to confer a mild phenotype. Mean scores for the *TSC2* group were below average (mean 50, S.D. = 10 for the individual domains; mean 100, S.D. = 15 for the composite scores) at all time points, in all categories (Fig). Mean scores for the *TSC1*/NMI group were consistently higher than those for the *TSC2* group in all domains and the composite score. Mean scores for the *TSC2* group excluding the three children with mild variants were consistently lower than those for the *TSC2* group with all pathogenic variants included, although the magnitude of this difference was small.

Discussion

Utilizing prospectively collected data from a study with standardized assessment of cognitive development by means of a validated tool, we found that patients with *TSC2* pathogenic variants are significantly more likely to have developmental delay at 24 months than patients with *TSC1* pathogenic variants or NMI. Although *TSC2* pathogenic variants are known to be associated with a higher risk of ID in older children and adults,[13,25] this study is the first to demonstrate the association of *TSC2* pathogenic variants and cognitive delay in children as young as 24 months. It is also the first genotype-phenotype study of TSC to prospectively use a validated diagnostic measure of developmental status. Using this

approach, we were also able to document that differences in developmental delay were global, present in every MSEL domain, along with the composite score. In addition, a greater proportion of patients with *TSC2* pathogenic variants had composite scores in the lowest range (Mullen composite: standard score less than 70). Fifty-seven percent of the *TSC2* group scored in the "very low" range for the composite score (compared with 15% and 6% in the *TSC1* and NMI groups, respectively), indicating that the majority of people with *TSC2* pathogenic variants are very delayed at 24 months. Trajectories suggest that the difference in development between patients with a *TSC2* pathogenic variant, and those without, is apparent at as early as nine months and present in every domain (Fig).

Genotype-phenotype correlation studies have provided valuable prognostic information in many genetic disorders.[8-10] Although they have also been helpful in TSC,[26] most correlations are general, and more specific information is always desired to give families the most accurate expectations. Prognostic information is not only useful for families who are trying to envision life with TSC but also can guide management and early intervention, thus optimizing the developmental outcome of the child. For example, it is well known that early identification and control of seizures, as well as early intervention with developmental therapies, improves developmental outcomes.[27] Identification of the most at-risk patients allows for closer monitoring and more rapid intervention. Owing to these recognized benefits, attempts to find correlations that may help provide prognostic information to families and physicians of patients with TSC are being undertaken across an array of specialties.[18,28] Use of genotype as a predictor can be extremely advantageous as the genotype can be determined before most clinical predictors, for example, *in utero*.

As noted, our findings are consistent with those of other studies in demonstrating that patients with *TSC2* pathogenic variants are more likely to have seizures [13,25] and that patients with seizures are more likely to have developmental delay or ID.[15] It is possible that the *TSC2* pathogenic variants that lead to increased risk for seizures, in turn, increase the risk for developmental delay. However, we found that the *TSC2* group had significantly lower MSEL composite scores, independent of seizures. The same was observed in the gross motor, visual reception, and expressive language domains. Delays in fine motor and receptive language appeared to be similar between *TSC2* and those without *TSC2,* when controlled for seizures. Prognostically, these correlations are important because patients with both a *TSC2* pathogenic variant and seizures have two risk factors for developmental delay. Therefore they need extremely close monitoring and proactive intervention.

Our findings were also consistent with those of previous studies that determined Arg622Trp [24] and Arg1200Trp [23] *TSC2* pathogenic missense variants to be associated with a milder phenotype. Although the sample size of three was too small for statistical analysis, two of the children (Arg622Trp and Arg1200Trp) had composite scores in the "average" range, whereas the third (Arg1200Trp) had a "very high" composite score. Excluding these patients from the *TSC2* group in the multivariable linear regression models led to a one- to three-point reduction in mean MSEL scores in the *TSC2* group (Supplemental Table 2). Trajectories (Fig) also demonstrate that excluding the *TSC2* mild variants from the *TSC2* group decreased the mean at all time points in all domains, along with the composite score.

Mechanistically, there are plausible explanations for why most *TSC2* pathogenic variants would confer a more severe neurological phenotype than *TSC1* pathogenic variants. The common consensus is that TSC1 plays a role specifically in stabilizing TSC2 and making the TSC2-GTPase-activating protein domain active, thereby inactivating Rheb-GTP, preventing Rheb-GTP from activating mammalian target of rapamycin.[29] We now know that there is at least one more protein. TBCD7, found with the TSC1-TSC2 complex, so there may be additional mechanisms present to stabilize TSC2 activities.[30-32] The ability of TSC2 to function in some ways independent of TSC1 would possibly explain why pathogenic variants in *TSC2* are associated with more severe effects. Thousands of pathogenic variants discovered throughout the exons of *TSC1* and *TSC2* argues for the need to deeply dissect and characterize every functional domain of TSC1 and TSC2. In addition, the more severe phenotype of patients with *TSC2* pathogenic variants could be due to a higher frequency of somatic *TSC2* pathogenic variants[16] given the larger size of the gene when compared with *TSC1.* Cortical tuber burden may also contribute as higher tuber burden has been associated with *TSC2* pathogenic variants[11] and severe cerebral disease,[17] although effect of tuber burden on ID does not appear to be independent of seizures.[33] Understanding the roles that TSC1 and TSC2 play in the cell could be helpful in identifying treatment of the nonhamartomatous symptoms of TSC, such as ID, autism, and epilepsy.

Except for a few exceptions,[23,24,34-38] most reported genotype-phenotype associations in TSC are between *TSC1* and *TSC2,* as well as in patients with NMI. Variant-specific genotype-phenotype correlations are less common due to the number of different variants in TSC, combined with smaller patient numbers per variant (http://chromium.lovd.nl/LOVD2/TSC). In our study, there were few repeats of variants in any single site, resulting in insufficient power to investigate specific pathogenic variants. Another limitation was that there were not adequate numbers to make comparisons between the *TSC1* and NMI groups. Future investigations with larger sample sizes could be performed to evaluate whether there is a difference in development between the *TSC1* and NMI groups. In addition, it would be useful to ascertain what percent of patients with TSC and developmental delay go on to have ID. Adjustments were made for seizure status in our study due to the well-described negative effect of seizures on development in patients with TSC.[27] Tuber burden was not included as a variable because of the less clear association, but may also be useful to include in future studies.

Conclusion

In this well-characterized patient population with standardized assessment of multiple aspects of development, we found that having a *TSC2* pathogenic variant was associated with significantly lower MSEL scores in all domains. It appears that the effect of *TSC2* pathogenic variants on learning occurs by 24 months and is associated with a global developmental delay. Although *TSC2* is associated with increased risk of seizures, which can also adversely affect development, the association between *TSC2* and lower MSEL composite scores was independent of seizures. These data suggest that a baby identified to have a *TSC2* pathogenic variant is at very high risk for significant global developmental delays early in development, e.g., by 24 months.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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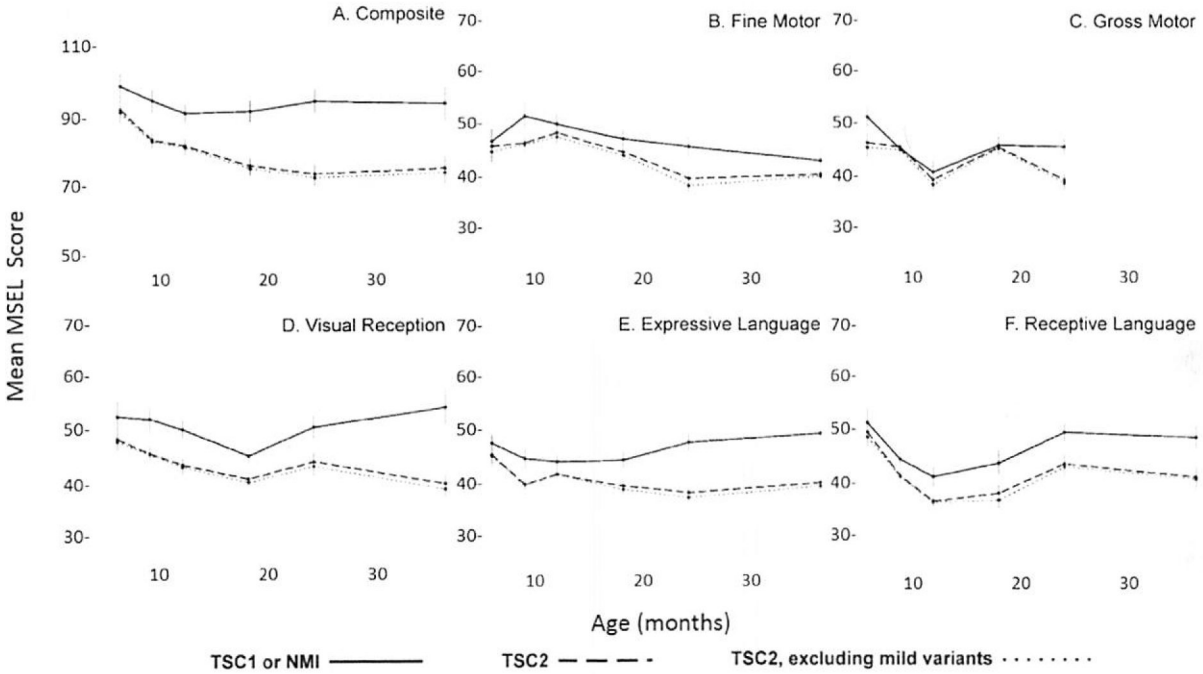

FIGURE.

Trajectories of mean (±standard error) MSEL domain scores from nine to 36 months for participants with *TSC1*/NMI, *TSC2,* and *TSC2* excluding those with pathogenic variants known to confer a mild phenotype. The composite scale (A) has a mean standard score of 100 with S.D. of 15. The other domains (B–F) have a mean T-score of 50 with S.D. of 10. Scores in the *T5C2* group are consistently lower than those of *TSC1*/NMI group in all domains, with the lowest scores belonging to the *TSC2* group, which excludes mild variants.

TABLE 1.

Child and Parental Characteristics by Genotype

	NMI (n = 16)	***TSC1* (n = 13)**	***TSC2* (n = 63)**	***P* Value ***
Child's sex. n (%)				0.14
Female	7 (43.3)	4 (30.8)	37 (58.7)	
Male	9 (56.2)	9 (69.2)	26 (41.3)	
Child ethnicity, n (%)				0.73
Hispanic	2 (12.5)	3 (23.1)	14 (22.2)	
Non-Hispanic	14 (87.5)	10 (76.9)	49 (77.8)	
Term Birth, n (%)				0.06
No	1 (6.2)	2 (15.4)	1 (1.6)	
Yes	15 (93.8)	11 (84.6)	62 (98.4)	
Seizures, n (%)				**<0.001**
No	5 (31.2)	10 (76.9)	9 (14.3)	
Yes	11 (68.8)	3 (23.1)	54 (85.7)	
Maternal age at birth, mean (S.D.) years	34.4 (4.3)	31.8 (6.1)	30.8 (5.1)	**0.02**
Paternal age at birth, mean (S.D.) years	36.8 (5.8)	34.5 (4.5)	33.0 (6.8)	0.06

Abbreviation:

NMI = No mutation identified.

Bold values indicate significance.

* For sex, the *P* value is derived from chi-squared test. For other categorical variables. *P* values were derived from Fisher's exact test, and for continuous variables *P* values were derived from the Kruskal-Wallis rank sum test. The *P* value is representative of the difference across the three groups.

TABLE 2.

Twenty-Four-Month Mullen Cutoff Scores by Genotype

	NMI n (%)	*TSC1* n (%)	*TSC2* n (%)	*P* Value*
Early Learning Composite T-scores				**<0.001**
Very high	0 (0.0)	0 (0.0)	2 (3.2)	
Above average	1 (6.2)	2 (15.4)	2 (3.2)	
Average	10 (62.5)	8 (61.5)	13 (20.6)	
Below average	4 (25.0)	1 (7.7)	10 (15.9)	
Very low	1 (6.2)	2 (15.4)	36 (57.1)	

Abbreviation:

NMI = No mutation identified.

Bold value indicate significance.

*The *P* value was derived from Fisher's exact test.

TABLE 3.

Comparison of 24-Month Mullen Scales T-Scores and Mullen Composite Score Patients With and Without a *TSC2* Pathogenic Variant

	TSC2 **Mean (S.D.)**	***TSC1*/NMI** **Mean (S.D.)**	***P*** **Value** [*]
Gross Motor T-Score[†]	36.2 (11.4)	44.9 (9.3)	**<0.001**
Visual Reception T-Score[†]	35.7 (14.7)	50.5 (12.0)	**<0.001**
Fine Motor T-Score[†]	33.2 (12.4)	44.0 (12.1)	**<0.001**
Receptive Language[†] T-Score	35.5 (15.6)	46.2 (12.4)	**0.002**
Expressive Language T-Score[†]	35.1 (11.8)	46.5 (10.6)	**<0.001**
Early Learning Composite Standard Score[‡]	74.3 (23.2)	94.5 (18.9)	**<0.001**

Abbreviations:

NMI = No mutation identified.

Bold values indicate significance.

[*]From the Kruskal-Wallis Rank Sum Test.

[†]T-scores have a mean of 50 and an S.D. of 10.

[‡]Early Learning Composite score has a mean of 100 and an S.D. of 15.

TABLE 4.

Multivariable Linear Regression Models for the Effect of *TSC2* Genotype and Presence of Seizures on 24 Month Mullen T-Scores

	TSC2, β (95% CI)[*,†]	*P* Value	Seizures, β (95% CI)[‡]	*P* Value
Gross Motor	−6.20 (−11.20, −1.20)	**0.016**	−9.50 (−14.68, −4.33)	<**0.001**
Visual Reception	−10.01 (−16.58, −3.44)	**0.003**	−13.83 (−20.63, −7.02)	<**0.001**
Fine Motor	−5.72 (−11.47, 0.02)	0.051	−13.27 (−19.21, −7.32)	<**0.001**
Receptive Language	−5.16 (−12.17, 1.84)	0.147	−14.05 (−21.30, −6.80)	<**0.001**
Expressive Language	−7.23 (−12.71, −1.74)	**0.010**	−10.65 (−16.33, −4.97)	<**0.001**
Early Learning Composite	−10.79 (−21.08, −0.49)	**0.040**	−23.25 (−33.91, −12.59)	<**0.001**

Abbreviations:

β = Unstandardized coefficient

Cl = Confidence interval

[*]Versus *TSC1* or NMI.

[‡]Adjusted for seizure status and maternal age.

[†]Adjusted for genotype (TSC vs not) and maternal age.