American Journal of Human Genetics, Volume *93*

Supplemental Data

TM4SF20 Ancestral Deletion and Susceptibility to a Pediatric Disorder of Early Language Delay and Cerebral White Matter Hyperintensities

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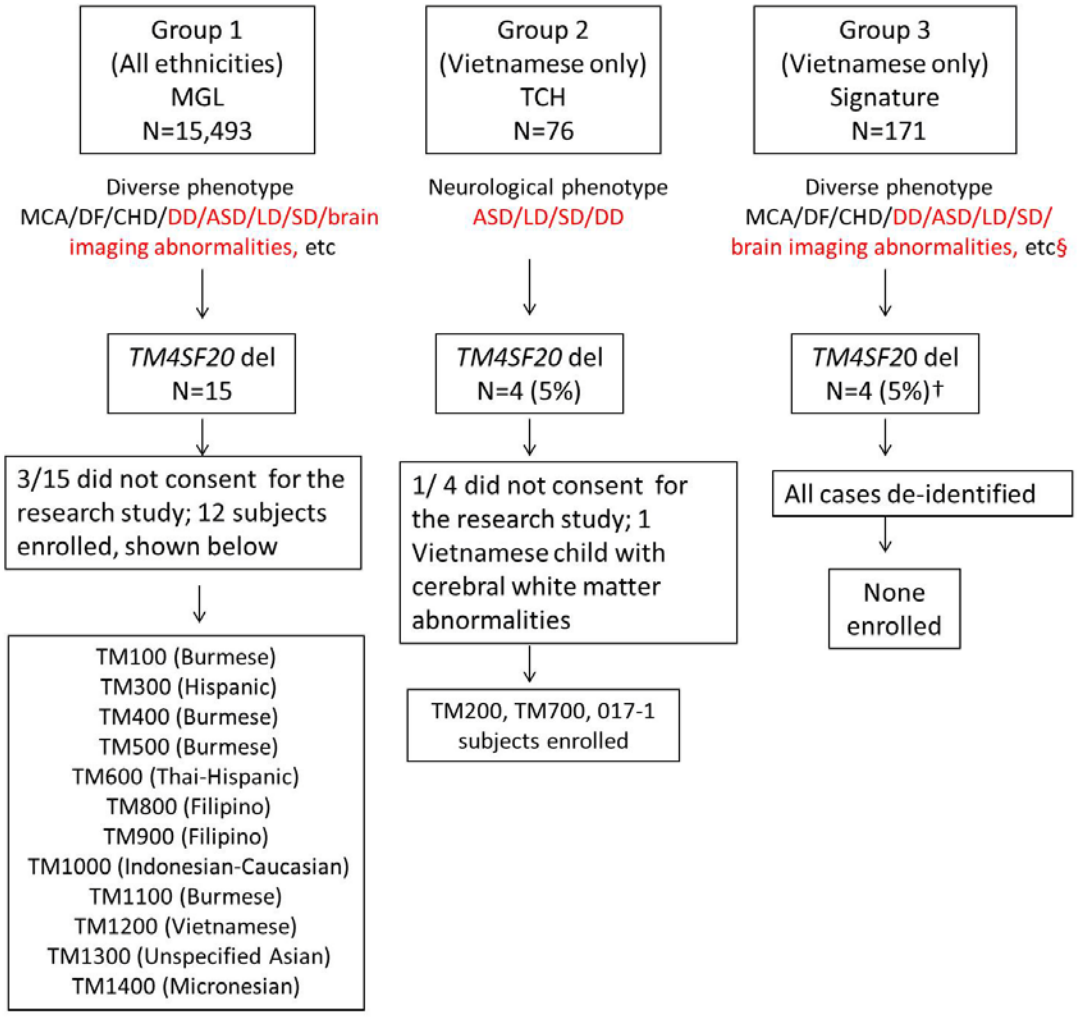


Figure S1. Study design showing evaluation of three major groups for the *TM4SF20* complex deletion

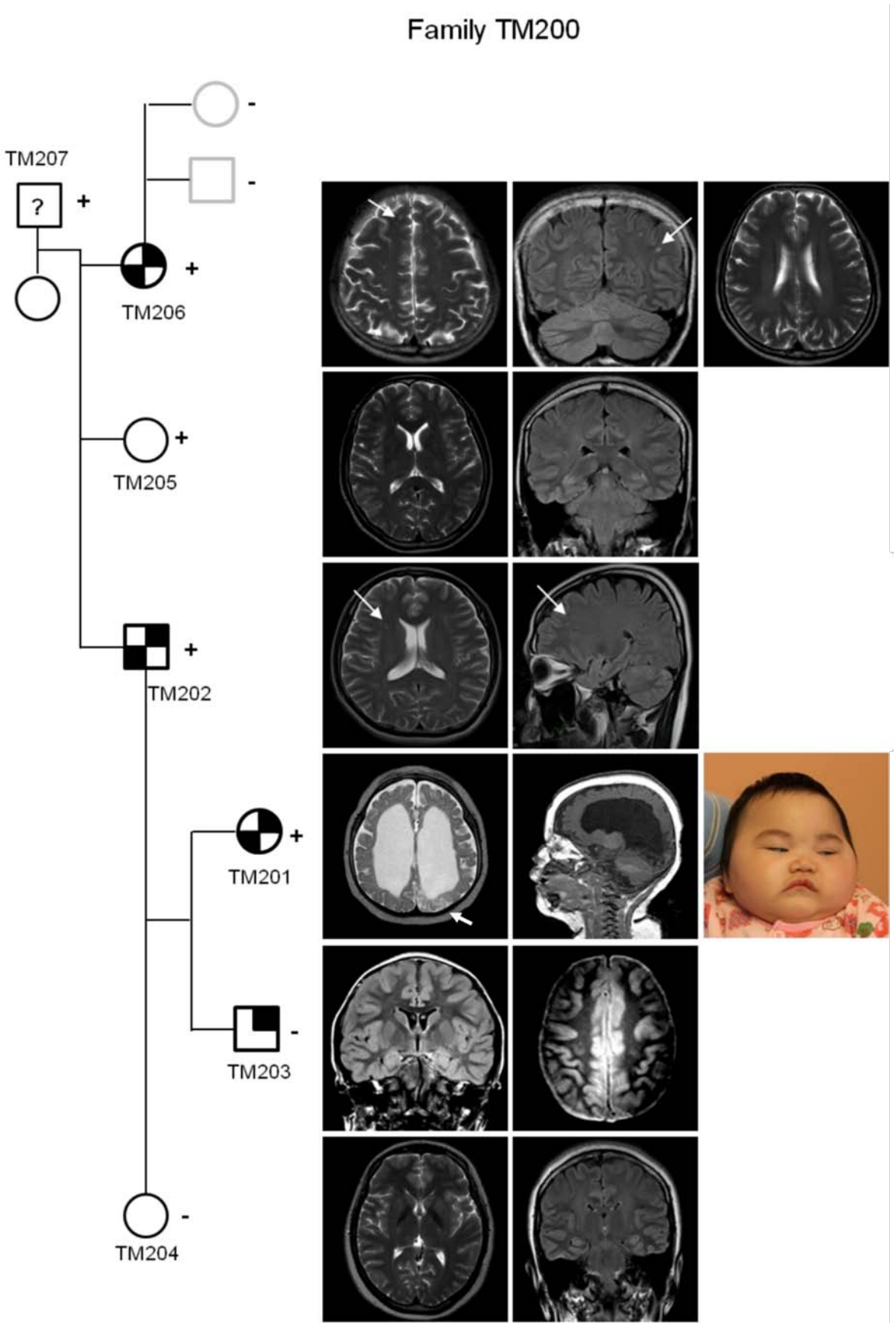
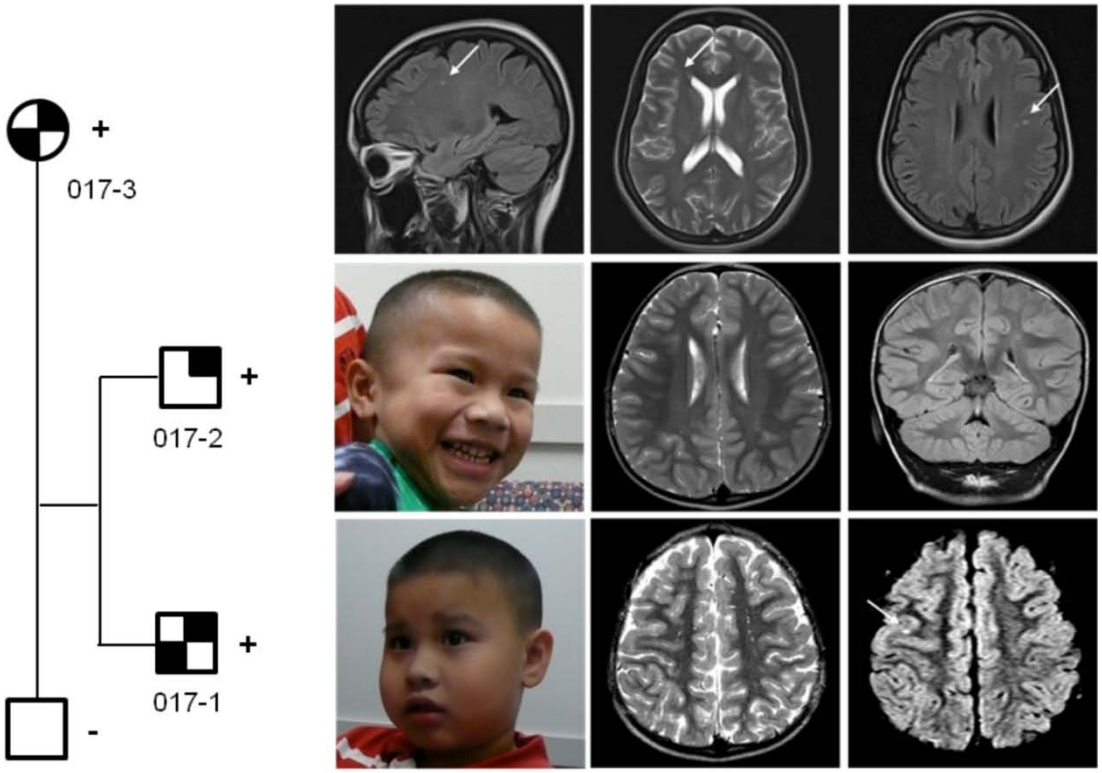

Family TM200
TM207
?
+
-
-
TM206
+
TM205
+
TM202
+
TM201
-
TM203
-
TM204

Figure S2. Extended pedigree analysis of the Vietnamese family, TM200, using brain imaging studies. The proband TM201 and her 37-year-old asymptomatic father, TM202, were ascertained with the 4-kb deletion. Subject TM201, born prematurely has gross enlargement of the third and lateral ventricles with near complete loss of periventricular white matter. There is additional T2 hyperintensity in a left PCA (posterior cerebral artery) pattern of distribution, consistent with an additional ischemic event involving a medium size vessel (marked by a broader white arrow). The father, TM202 has multi-focal punctate T2 hyperintensities, returned from the fronto-parietal white matter of both hemispheres. The deletion, present in the paternal grandfather, was also observed in proband's two asymptomatic paternal aunts, TM205 and TM206. The 40-year old paternal aunt, TM206 with no known vascular risk factors, has multi-focal T2 hyperintensities in the subcortical and periventricular white matter, very similar in distribution to the other carrier families. Her two children with normal speech acquisition were negative for the deletion. TM205 had a normal brain imaging study. The non-carrier mother, TM204, and the non-carrier brother had essentially normal brain MRI studies.


+
017-3
+
017-2
+
017-1
-

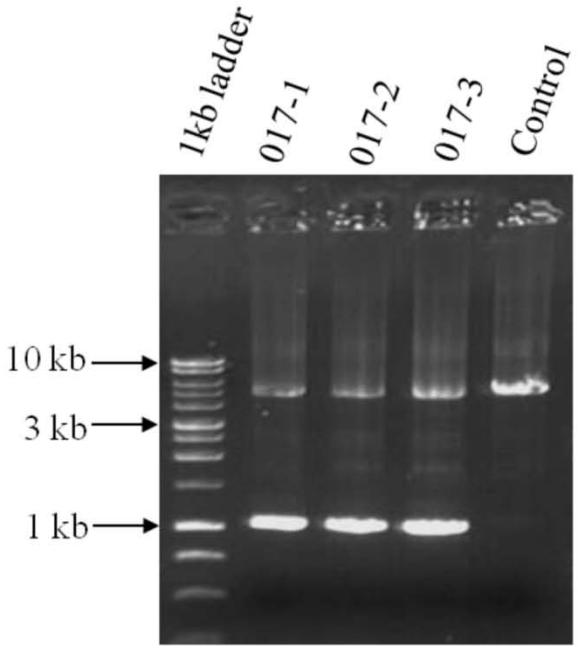

1kb ladder
017-1
017-2
017-3
Control
10 kb
3 kb
1 kb

Figure S3. *TM4SF20* deletion in the Vietnamese patient, 017-1 and his 5-year-old brother with significant language impairment. Family studies showing the 1-kb junction fragment in the two siblings with severe language delay and their 37-year-old mother. Note the multi-focal T2 hyperintensities in the periventricular and deep white matter (highlighted by arrows) in the apparently healthy mother with no known cerebrovascular risk factors.

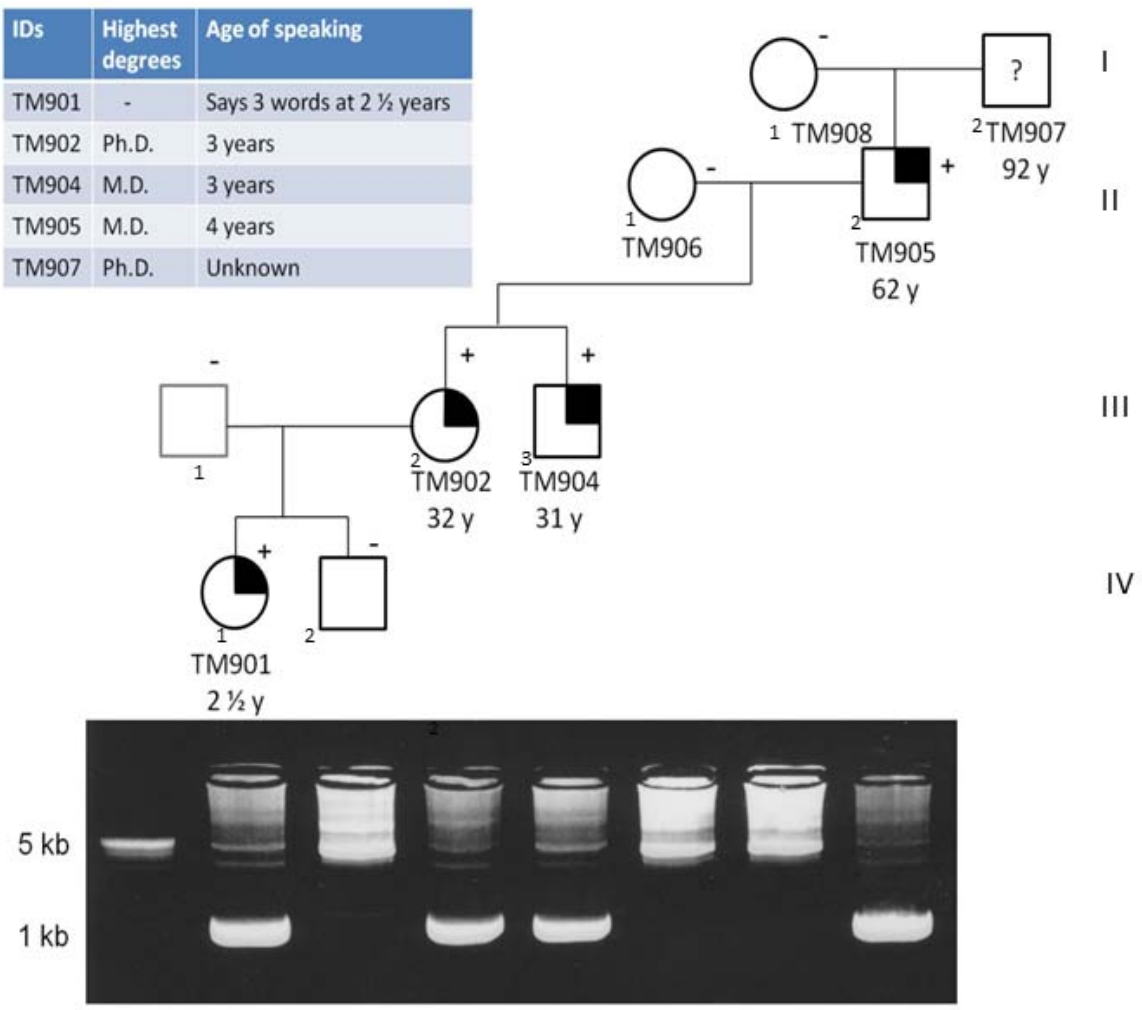
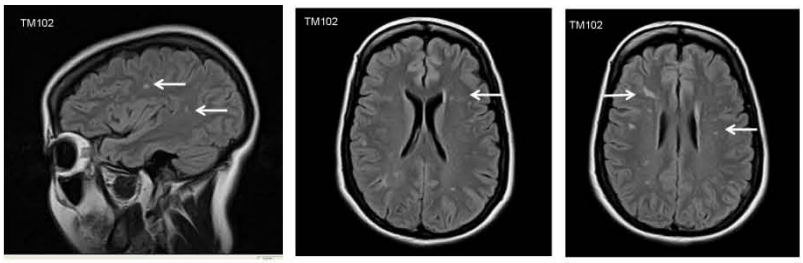


Figure S4. Extended analysis of family TM900 from Philippines, using long range PCR studies. The family was self-referred due to a strong history of early language delay in multiple family members, all demonstrating high educational achievement. PCR studies showed the deletion CNV segregating with early language delay in this family.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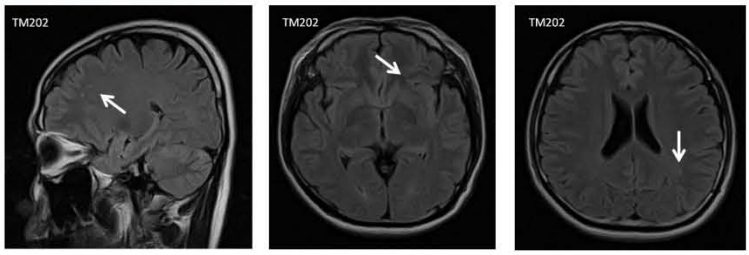
TM102
TM102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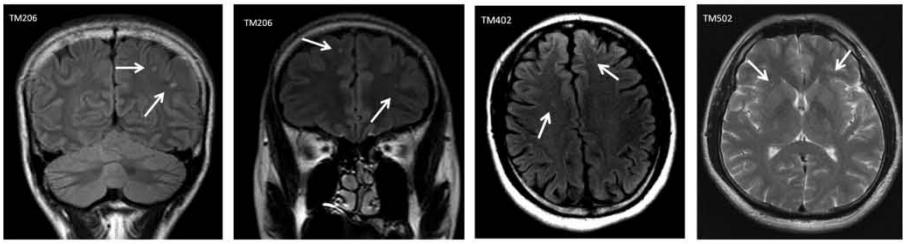
TM202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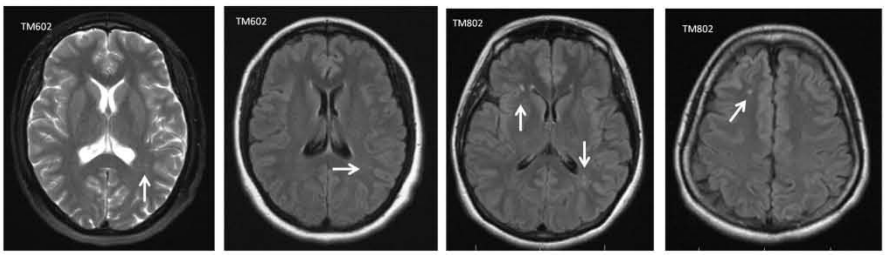
TM402
TM502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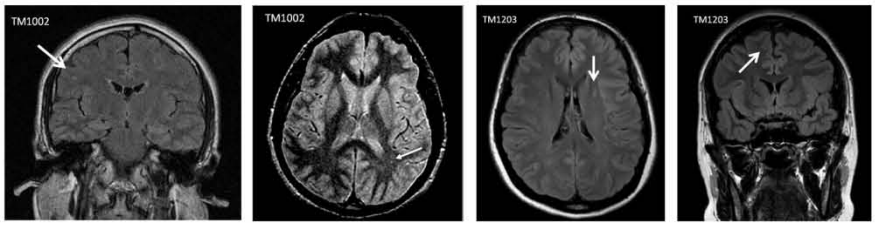
TM802
TM802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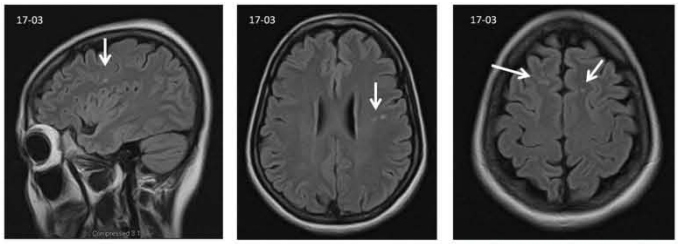
TM1002
TM1203
TM1203


17-03
17-03
17-03

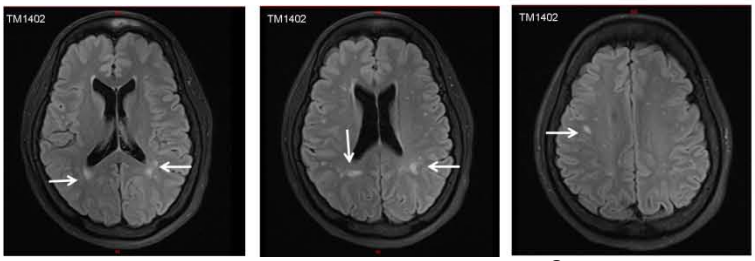

TM1402
TM1402
TM1402

Figure S5. WMH in adults with *TM4SF20* deletion. T2 weighted and fluid-attenuated inversion recovery (FLAIR) sequence magnetic resonance imaging (MRI) scans of adults with *TM4SF20* deletion are presented, showing varying degree of white matter disease ranging from punctate T2 hyperintensities in the subcortical white matter to multi-focal T2 hyperintensities in the periventricular and deep white matter. Note in particular, the brain imaging studies of TM1203, a high-functioning parent with *TM4SF20* homozygous deletion. There are punctate foci of abnormal T2 hyperintensity returned from the subcortical white matter in the right insular region. Her neuroradiological abnormalities are similar to those seen in most heterozygous carrier parents.

Figure S6. Geographical view of the Southeast Asian region, represented by the ancestry of individuals with the *TM4SF20* founder deletion mutation. Vietnam, Thailand, Burma, Philippines, and Indonesia are designated in red symbols. We ascertained one additional family from Micronesia (Map source: Nations Online Project).

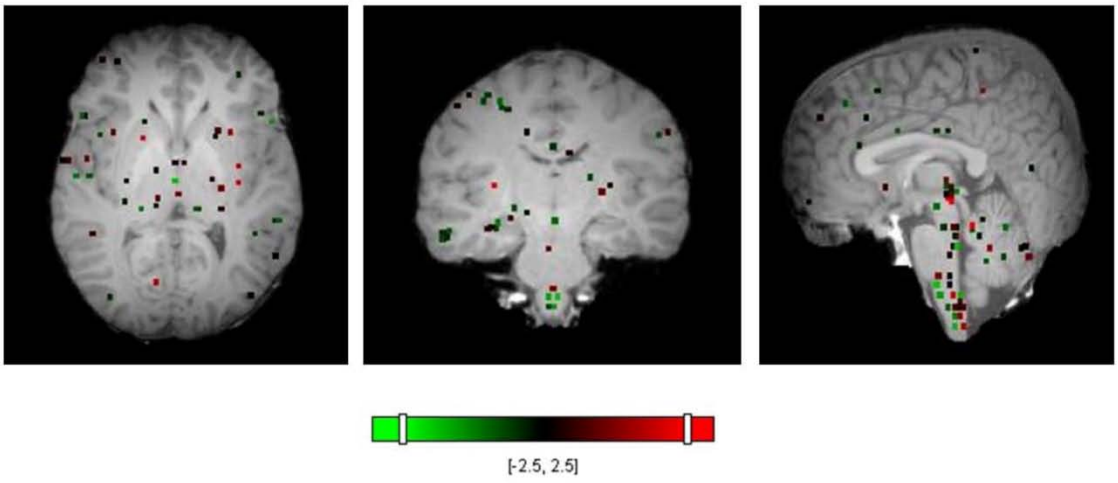


Figure S7. *TM4SF20* expression in adult human brain. Images were obtained from the Allen Institute for Brain Science

(http://human.brain-map.org/mri_viewer?donor=9861&probes=1038873). Note the relatively high expression of the gene (marked in red) in the parietal lobe, hippocampus, pons, white matter, and cerebellum.

Table S1

Clinical indication and *TM4SF20* deletion, observed in 15,493 children in group 1 (all ethnicities). Note that the 4-kb deletion is only seen in children referred for disorders of communication, developmental delay, and brain imaging abnormalities ($p = 1.7 \times 10^{-6}$).

Phenotype	Number of cases	*TM4SF20* deletion
Developmental delay	**4343**	**5**
Indication not specified	2906	0
Autism spectrum disorder	**1464**	**1**
Dysmorphic features	1336	0
Seizures	874	0
Multiple congenital anomalies	806	0
Congenital heart defects	701	0
Failure to thrive	452	0
Speech delay	**383**	**8**
Neuromuscular problems	368	0
Skeletal problems	344	0
ADHD	233	0
Suspected chromosomal abnormality	216	0
Brain imaging abnormalities	**200**	**1**
Pertinent family history	146	0
Urogenital abnormalities	129	0
Hearing loss	89	0
Behavioral problems	85	0
Cleft lip/palate	77	0
Endocrinopathy	66	0
Eye abnormalities	58	0
Neuropsychiatric disorder	58	0
Respiratory problems	45	0
Skin problems	31	0
Metabolic disease	30	0
Congenital Diaphragmatic Hernia	15	0
Hydrops	15	0
Tumor	13	0
Immune defect	10	0

Table S2: WMH frequency in *TM4SF20* deletion compared to other studies of pediatric WMH

References	Number of Individuals with WMH/ Total number of subjects studied with brain MRI	Frequency (95% CI)	Pediatric phenotype	Compared to children with *TM4SF20* deletion *p*-value
Specific genetic causes and WMH				
***TM4SF20* deletion**	**10/14**	**71.4% (41.9%, 91.6%)**	**Language delay**	**-**
Hyman *et al.*[1]	68/76	89.4% (80.3%, 95.3%)	Children with Neurofibromatosis type 1	0.986
Eichler *et al.*[2]	33/34	97% (84.6%, 99.9%)	Metachromatic leukodystrophy	0.999
Unpublished data (Texas Children's Hospital, Texas)	4/51	7.8% (2.1%, 18.8%)	16p11.2 deletion/duplication carriers with language delay, autism, seizures, or developmental delay	**1.406×10^{-5}**
Non-specific genetic/environmental causes and WMH				
Kim *et al.*	3/225	1.3% (0.3%, 3.9%)	Healthy children, 1 month to 18 years	**2.589×10^{-11}**
Kalnin *et al.*	8/281	2.8% (1.2%, 5.5%)	Unprovoked first seizure	**3.924×10^{-10}**
Gupta and Belay	30/666	4.5% (3.1%, 6.4%)	Migraines, seizures, developmental delay or attention deficit hyperactivity disorder	**1.603×10^{-9}**
Decobert *et al.*	17/100	17.0% (10.2%, 25.8%)	Unexplained intellectual impairment (IQ < 70)	**1.966×10^{-4}**
Kieslich *et al.*	15/75	20.0% (11.6%, 30.8%)	Celiac disease	**7.643×10^{-4}**
Verbruggen *et al.*	21/80	26.3% (17.0%, 37.3%)	Abnormal neurological signs, abnormal head circumference or other specific reasons for MRI	**3.786×10^{-3}**
Widjaja *et al.*	23/90	25.6% (16.9%, 35.8%)	Idiopathic developmental delay	**2.922×10^{-3}**

Table S3: WMH and 76 pediatric TCH Vietnamese cases with speech/developmental delay/autism spectrum disorder (group 2). The *TM4SF20* deletion is observed in 3/19 (~16%) children with WMH in this study. The frequency of WMH in deletion vs non-deletion cases is statistically significant (p = 0.0461, Fisher's exact test).

	WMH present	WMH absent	Total
Deletion present	3	1	4
Deletion absent	16	56	72
Total	19	57	76

Table S4. *TM4SF20* deletion observed in 415 Vietnamese children in the three study groups. Note that the deletion is only observed in Vietnamese children with disorders of communication and brain imaging abnormalities ($p = 5.4 \times 10^{-4}$).

Phenotype	Vietnamese Cases (Groups 1 and 2) N=244	Vietnamese Cases (Group 3) N=171	Total n=415	*TM4SF20* deletion present
Dysmorphic features/Multiple congenital anomalies/Congenital heart defects	60	67	127	0
Developmental delay	39	45	84	3
Autism spectrum disorder	40	23	63	1
Endocrinopathy/Urogenital abnormalities/Failure to thrive/skeletal problems	27	7	34	0
Seizures/neuromuscular problems	25	8	33	0
Speech/language delay	**24**	**3**	**27**	**3**
Indication not specified	13	6	19	0
Brain imaging abnormality	**7**	**8**	**15**	**3**
†Other	9	4	13	0

DF/MCA/congenital heart defects/endocrinopathy/urogenital abnormalities/FTT/skeletal problems-(127+34+33+13) **0/207**

DD/ASD/speech delay/brain imaging abnormalities (84+63+27+15) **10/189**

†Includes immune defects, metabolic abnormalities, tumor, non-immune hydrops

Table S5. Clinical characteristics of all studied children and adults with *TM4SF20* deletion (pediatric patients are highlighted in green)

	Family	IDs	Affected	Ethnicity	Ages	Language delay	MRI done	WMH	Family Structure in the United States
1	TM100	TM101	Proband	Burmese	4 y	+	+	+	Nuclear –Burmese refugees
2	TM100	TM102	Mother	Burmese	36 y	U	+	+	
3	TM200	TM201	Proband	Vietnamese	1 ½ y	+	+	+	Extended pedigree
4	TM200	TM202	Father	Vietnamese	35 y	+	+	+	
5	TM200	TM205	Pat aunt	Vietnamese	36 y	U	+	-	
6	TM200	TM206	Pat aunt	Vietnamese	40 y	+	+	+	
7	TM300	TM301	Proband	Hispanic	3 ½ y	+	+	+	Unknown-adopted
8	TM400	TM401	Proband	Burmese	7 ½ y	+	+	-	Nuclear-Burmese refugees
9	TM400	TM402	Father	Burmese	52 y	U	+	+	
10	TM500	TM501	Proband	Burmese	2 ½ y	+	+	+	Nuclear
11	TM500	TM502	Father	Burmese	39 y	U	+	+	
12	TM600	TM601	Proband	Thai/ Hispanic	4 ½ y	+	+	+	Nuclear-Thai mother adopted
13	TM600	TM602	Mother	Thai	35 y	U	+	+	
14	TM700	TM701	Proband	Vietnamese	4 y	+	+	-	Extended-declined further studies
15	TM700	TM702	Mother	Vietnamese	29 y	U	+	-	
16	TM800	TM801	Proband	Filipino/ Pakistani	3 y	+	+	+	Nuclear-Filipino mother adopted
17	TM800	TM802	Mother	Filipino	36 y	U	+	+	
18	TM900	TM901	Proband	Filipino	2 y	+	+	-	Extended-Studied
19	TM900	TM902	Mother	Filipino	32 y	+	+	-	
20	TM900	TM905	Mat GF	Filipino	61 y	+	ND	U	
21	TM900	TM904	Mat uncle	Filipino	31 y	+	ND	U	
22	017	017-1	Proband	Vietnamese	7 y	+	+	+	Nuclear
23	017	017-3	Mother	Vietnamese	39 y	U	+	+	
24	017	017-2	Sibling	Vietnamese	5 y	+	+	-	
25	TM1000	TM1001	Proband	Indonesian/ European	1 ½ y	+	+	-	Nuclear
26	TM1000	TM1002	Father	Indonesian	30 y	+	+	+	
27	TM1000	TM1003	Sibling	Indonesian/ European	2 y	+	ND	U	
28	TM1100	TM1101	Proband	Burmese	1 ½ y	+	+	+	Nuclear-Burmese refugees
29	TM1100	TM1102	Father	Burmese	42 y	U	+	-	
30	TM1100	TM1103	Sibling	Burmese	5 y	+	+	-	Nuclear-Burmese refugees
31	TM1100	TM1104	Sibling	Burmese	3 y	+	+	+	
32	TM1200	TM1201	Proband	Vietnamese/ European	4 y	+	ND	U	Extended
33	TM1200	TM1202	Deceased sibling	Vietnamese/ European	Still-birth	U	ND	U	
34	TM1200	TM1203	Parent	Vietnamese	36 y	+	+	+	
35	TM1300	TM1301	Proband	Unknown	1 ½ y	+	+	+	Unknown-adopted
36	TM1400	TM1401	Proband	Micronesian/ European	13 y	+	+	+	Extended
37	TM1400	TM1402	Parent	Micronesian	50 y	U	+	+	

ND-Not done

U-Unknown

Table S6. Neurocognitive profile of individual adult carriers in the study

	TM102		TM202		17-03		TM902	
	Raw Score	**Standard Score**	**Raw Score**	**Standard Score**	**Raw Score**	**Standard Score**	**Raw Score**	**Standard Score**
Orientation								
MMSE (max 30)	25		30		27		29	
Attention								
Digit Span Total (scaled score)	17	10	21	13	16	9	20	12
Forward	7		7		8		9	
Backward	5		7		3		6	
Spatial Span Total (scaled score)	15	10	11	6	13	8	17	10
Forward	8		4		5		6	
Backward	7		4		5		6	
Symbol Digit (z score)								
Written	35	-0.05	51	0.06	27	-3.05	60	0.25
Oral	45	-1.45	57	-0.27	34	-2.56	70	0.37
Visuospatial								
Clock-Command	7		7		4		10	
Clock-Copy	9		10		9		10	
JLO (%ile)	17	4	21	22	15	2	24	40
VMI (Standard Score)	28	98	28	98	21	57	27	92
Memory								
HVLT								
Total 1-3 (z score)	29	0.05	16	-2.00	19	-2.30	28	0.37
Delayed (z score)	11	0.41	6	-1.99	7	-1.50	8	0.67
Recognition (z score)	11	0.18	11	-0.10	12	0.06	11	0.50
WMS-III VR								
VRI Total (scaled score)	89	11	88	11	80	7	95	12
VRII Total (scaled score)	57	9	85	13	55	9	38	7
Language								
Verbal Fluency (T-score)	23	36	43	46	21	41	44	50
Semantic Fluency (T-score)	17	45	17	44	13	43	27	59
Executive Functioning								
WCST-64								
Categories (%ile)	2	11-16	3	>16	2	11-16	5	>16
Total Errors (T-score)	29	30	22	37	34	34	10	48
Perseverative Errors (T- score)	14	38	9	41	26	33	4	66
Mood								
GDS	1		2		19		1	

MMSE=Mini Mental Status Examination; Digit Span=Wechsler Memory Scale – III Digit Span; Spatial Span= Wechsler Memory Scale – III Spatial Span; Symbol Digit=Symbol Digit Modalities Test Oral and Written; JLO=Judgment of Line Orientation; VMI=Beery Visual Motor Integration Test; HVLT=Hopkins Verbal Learning Test; WMS-III VR= Wechsler Memory Scale – III Visual Reproduction I and II; WCST-64= Wisconsin Card Sorting Test-64 card version; GDS=Geriatric Depression Inventory

Scaled score: Mean=10; SD=3; Standard Score: Mean=100; SD=10; T-score: Mean=50; SD=10; z-score: Mean=0; SD=1; %ile: Mean=50; 1 SD range from 16-84%ile

Supplemental References

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