A 7-year-old white girl presented with left hemiparesis and ischemic stroke secondary to moyamoya syndrome, a progressive cerebrovascular occlusive disorder of uncertain but likely multifactorial etiology. Past medical history revealed hearing loss and developmental delay/intellectual disability. Routine karyotype demonstrated extra chromosomal material on 6p. Single nucleotide polymorphism microarray revealed a previously unreported complex de novo genetic rearrangement involving subtelomeric segments on chromosomes 6p and 12q. The duplicated/deleted regions included several known OMIM-annotated genes. This novel phenotype and genotype provides information about a possible association of genomic copy number variation and moyamoya syndrome. Dosage-sensitive genes in the deleted and duplicated segments may be involved in aberrant vascular proliferation. Our case also emphasizes the importance of comprehensive evaluation of both developmental delay and congenital anomalies such as moyamoya. Pediatrics 2013;131:e1996–e2001

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KEY WORDS
moyamoya, 6p trisomy, 12q deletion

ABBREVIATIONS
ACA—anterior cerebral artery
FISH—fluorescence in situ hybridization
HLA—human leukocyte antigen
ICA—internal carotid artery
MCA—middle cerebral artery
OMIM—Online Mendelian Inheritance in Man
SNP—single nucleotide polymorphism

Dr Rosenberg guided conception and oversight of manuscript development and critically reviewed and revised manuscript for intellectual content; Dr Egan collected data, reviewed literature, and drafted the initial manuscript; Dr Rodgers collected and analyzed data; Dr Harter analyzed data and reviewed manuscript for intellectual content; Dr Burnside analyzed data, including statistical analysis, and critically reviewed manuscript for intellectual content; Dr Milla analyzed data; Dr Pappas analyzed data, guided conception and oversight of manuscript development, and critically reviewed manuscript for intellectual content; and all authors approved the final manuscript as submitted.

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Although pediatric ischemic stroke is an uncommon occurrence, moyamoya is responsible for 6% of cases.\textsuperscript{1–3} Moyamoya is a cerebrovascular occlusive disorder characterized by bilateral progressive stenosis and occlusion of the distal intracranial internal arteries (ICA) and the proximal anterior and middle cerebral arteries with development of compensatory collateral vessels. The latter appear radiographically as a “puff of smoke” (moyamoya in Japanese).\textsuperscript{4–6} In juvenile moyamoya, the most common initial presentation is extremity weakness or paralysis secondary to cerebral ischemia.\textsuperscript{4,7}

Moyamoya was first codified in Japan in 1969.\textsuperscript{6} Patients are characterized as having moyamoya syndrome if the vascular findings are associated with an underlying condition, including acquired disorders such as previous cephalic radiation therapy or infection and genetic disorders, such as Down syndrome, neurofibromatosis, sickle cell anemia, and Alagille syndrome.\textsuperscript{2,8}

Juvenile moyamoya incidence varies by ethnicity (0.1/100,000 in Caucasian children and 1/100,000 in Japan and Korea) and gender (female: male 2:1) with an overall higher prevalence in families (6%–12%).\textsuperscript{10} Genetic influences in moyamoya have been studied in twin, linkage, and identification analyses\textsuperscript{11,12} in various ethnic groups, but no consistent finding has emerged.\textsuperscript{10,12} This suggests both heterogeneity and epigenetic influences in moyamoya among different ethnic groups, with candidate mutations affecting human leukocyte antigen (HLA)-related genes (6p21),\textsuperscript{13} neurofibromatosis-linked genes (17q25),\textsuperscript{14} and metalloproteinase inhibitor genes involved in collagen vascular architecture (3p24).\textsuperscript{15} Early-onset, or juvenile, and late-onset moyamoya likely have different etiologies, as suggested by HLA-related research in Japan.\textsuperscript{16}

Within moyamoya-related syndromes, several are also associated with developmental delay. Developmental delay and intellectual disability affects 1% to 3% of US children.\textsuperscript{17} The American Academy of Neurology and American Academy of Pediatrics recommends evaluation, including genetic evaluation, of children and particularly those with isolated cognitive delay because of implications for prognosis, reproductive capability, and neurologic progress.\textsuperscript{17,18} Genetic etiologies, including chromosomal and subtelomeric duplicated or deleted sequences, can be identified in \textasciitilde 20% to 40% of cases.\textsuperscript{17,19} Previously, both 6p\textsuperscript{20} and 12q subtelomeric trisomies\textsuperscript{21} were reported among genetic rearrangements associated with developmental delay.

In this report, we describe a white child with moyamoya syndrome and mild developmental delay. High-resolution single nucleotide polymorphism (SNP) array, fluorescence in situ hybridization (FISH), and chromosome analysis identified a de novo complex genetic rearrangement: del 6p, dup 6p, and dup12q. This particular unclassified syndrome and genotype has not been previously reported in the literature to our knowledge.

### CASE PRESENTATION

A 7-year-old white girl with moderate bilateral conductive hearing loss and developmental delay presented to her pediatrician with a 1-day history of fever to 103°F, cough, and congestion. She progressed to persistent fever, chills, vomiting, and inability to tolerate oral liquids and was admitted to a community hospital for dehydration. The physical examination on admission was notable for a 3/6 systolic murmur with subsequent unremarkable echocardiogram.

On day 4 of illness, the patient developed acute left-sided hemiparesis and left-sided facial droop. Vital signs at that time were temperature 99.8°F, 122/55 blood pressure, heart rate 102 beats/minute, and respiratory rate 22 /minute. Her neurologic examination was otherwise unchanged without clinical seizures or change in mental status. A noncontrast head CT was unremarkable.

The patient was started on ceftriaxone for possible meningitis. White blood count was 3.4 K/ul with hemoglobin of 12.5 g/dL. Analysis of cerebrospinal fluid showed protein of 18 g/dL, glucose of 70 g/dL, 2 leukocytes, 0 red blood cells; Gram stain and culture were negative. Liver enzymes, total protein, albumin, partial thromboplastin time, prothrombin time, and international normalized ratio were all within normal limits; C-reactive protein was 9 mg/L, and erythrocyte sedimentation rate was 10 mm per hour. On day 5 of illness, an MRI of the brain without and with contrast was performed. Diffusion weighted imaging was consistent with bilateral acute watershed infarctions in the frontal lobe and white matter, with the right hemisphere more affected than left (Fig 1) and reduced diffusion within the splenium of the corpus callosum (not shown). Intracranial magnetic resonance angiogram (Fig 2) showed severe narrowing/occlusion of the right supraclinoid internal carotid artery (ICA) with no evidence of flow in the A1 segment of the right anterior cerebral artery (ACA). The M1 segment of the middle cerebral artery (MCA) had little to no flow; however, there was reconstitution of the M2 and distal segments of the MCA. MR angiogram of neck showed no abnormality. IV steroids were initiated for potential infectious vasculitis. Additional workup for vasculitis including ACA, anticiardiolipin...
antibodies, and anti-DNAse B were all unremarkable.

The patient was then transferred to our institution for pediatric neurosurgical evaluation and management. Neurologic examination demonstrated central left facial paresis and 0/5 motor strength in left upper and lower extremity with Babinski sign on the left but was otherwise unremarkable. Initial treatment consisted of volume expansion, 81 mg ASA daily and activity restriction.

To differentiate vasculitis from moyamoya and plan for potential intervention, the patient underwent conventional cerebral angiogram. Angiogram demonstrated bilateral ICA abnormalities, with narrowing and occlusion of the right suprachinoid ICA involving the right A1 segment of the anterior cerebral artery and M1 segment of the MCA (Fig 3), with similar but less severe left-sided involvement. Prominent tiny capsular and lenticulostriate collateral vessels suggested chronic vascular compensation consistent with moyamoya disease (Fig 3).

Clinical genetics was consulted to evaluate for a possible genetic syndrome. Findings included triangular face with a wide forehead, long nose with bulbous tip, short distal phalanges with short nails but no hepatosplenomegaly. Review of medical history revealed full-term, appropriate for gestational age infant (2.9 kg) of nonconsanguineous parents of German and Irish/Italian descent and neonatal hyperbilirubinemia requiring phototherapy. She had bilateral ossicular dysplasia causing moderate bilateral conductive hearing loss and mild developmental delay. Family history was significant for an older, brother with congenital intracerebral venous malformation who was otherwise healthy. On the basis of this information, physical examination and vascular abnormalities consistent with Alagille syndrome, patient and parent samples were sent for chromosome analysis, SNP microarray, and JAG1 gene sequencing (chromosome 20). Mutations in the JAG1 gene are associated with Alagille syndrome.

JAG-specific sequencing and deletion/duplication testing revealed no mutations and no variants of known significance.

Chromosome analysis was performed on peripheral blood lymphocytes by using standard protocols at the 500-band level and revealed additional unidentified chromosome material on distal 6p: 46XX, add(6) (p25). SNP microarray and FISH revealed a complex chromosome rearrangement involving chromosomal segments of 6p and 12q. SNP microarray analysis was performed by using the Cytoscan HD platform (Affymetrix, Santa Clara, CA). The gene chip contains 743 000 SNP probes and 1 953 000 NPCN probes with a median spacing of 0.88 kb; 250 ng of total genomic DNA extracted from lymphocytes was digested with Not I and then ligated to Not I adaptors, respectively, and amplified by using Titanium Taq with a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA). Polymerase chain reaction products were purified by using AMPure beads (Agencourt Biosciences, Beverly, MA) and quantified by using NanoDrop 8000 (Thermo Fisher, Wilmington, DE). Purified DNA was fragmented and biotin labeled and hybridized to the Affymetrix Cytoscan HD GeneChip. Data were analyzed by using Affymetrix Chromosome Analysis Suite v1.2. FISH was performed by using commercially available subtelomere probes from Abbott Molecular (ToTel
Mix 12, Abbott Molecular, Santa Clara, CA) according to standard protocols and counterstained with DAPI (Fig 4).

The exact rearrangement included a 402 Kb terminal deletion at 625.3; an inverted interstitial 14.2 Mb duplication of 6p25.3->p23; and a 7.6 Mb duplication of 12q24.32->qter localized to the terminal p arm of derivative chromosome 6, as confirmed by FISH analysis. The final karyotype of the patient is as follows: 46,XX,der(6)(12qter->12q24.32:6p23->6q25.3:6p25.3->6qter) with genomic positions of 6p25.3(94 649-402 748)x1, 6p25.3p23(430 545-14 587 883)x3, 12q24.32q 24.33(124 782 854-132 287 718)x3. The genomic coordinates are based on NCBI genome build 36 (Supplemental Tables and Figures). Both parents had normal high-resolution karyotype (550 band level). The duplication of 6p25.3->p23 and the duplication of 12q24.32->qter were both visible cytogenetically; because parental karyotypes were normal, these abnormalities are de novo.

During the remainder of hospital course the patient received extensive physical and occupational therapy including inpatient rehabilitation, with gradual improvement of left hemiparesis to preillness baseline. Three weeks after discharge, the patient underwent bilateral pial synangiosis, a form of indirect bypass commonly used in patients who are symptomatic but clinically stable or whose only initial presentation is headache or transient ischemic attack. The procedure was uncomplicated, and the patient is currently doing well.
without any neurologic deficits. She remains on daily aspirin therapy.

**DISCUSSION**

The acute and subacute management of pediatric ischemic stroke secondary to moyamoya is well documented. Additional assessment of risk and prognosis is related to both underlying neurovascular pathology and any associated syndromic comorbidities, such as in Down syndrome or neurofibromatosis, making clinical genetic evaluation helpful in discriminating between isolated/idiopathic and disease-associated moyamoya.2,4,16 Moyamoya in the presence of atypical facies and other medical and developmental issues in our patient was associated with a previously unreported unbalanced chromosome rearrangement including partial subtelomeric trisomies at both 6p25.3->p23 and 12q24.32->qter. Although this particular structural rearrangement has not been reported before, both 6p and 12q partial trisomies have been reported, although most duplications of 6p have been larger and involve imbalance of other chromosomes.20,22 The terminal 6p deletion of this patient includes a single Online Mendelian Inheritance in Man (OMIM) annotated gene, IRF4, which is not known to be dosage sensitive. The proximal duplication of interstitial 6p includes 51 OMIM annotated genes, including FOXC1, deletions and duplications of which have been associated with ocular abnormalities and Dandy-Walker malformation.23,24 Although 6p25.3 deletion is a known clinical entity, our patient does not share characteristic brain or facial features previously reported.25

The reported phenotype of partial trisomy 6p includes pointed chin, long philtrum, low set ears, hearing loss and developmental delay as in our patient but also typically involves moderate to severe ocular, cardiac and renal disease, and psychomotor delay.22,26,27 The relative sparing of stigmata in our patient with only mild developmental delay and hearing loss despite the large chromosome rearrangement is likely due to variability of expression frequently seen in chromosome duplication syndromes.

The 12q region includes 22 OMIM annotated genes, none of which have been associated with genetic disorders. Partial trisomy of distal 12q has also primarily been associated with imbalance of other chromosomes but generally includes dysmorphic features, developmental delay, and intellectual disability.26

Neither partial 6p25 trisomy nor partial 12q trisomy have been previously reported with moyamoya or any other cerebral vascular malformations. Genetic studies on juvenile moyamoya in Japanese and Korean pediatric cohorts correlate with specific HLA phenotypes associated with chromosome 6p. The HLA gene cluster is localized to 6p21.32, which is not included in the rearrangement in our patient, although sequence mutations cannot be ruled out.15,16 The de novo structural rearrangement we describe in this patient of western European descent may thus represent a true phenotypic association of partial trisomy 6p with moyamoya.

Genetic evaluation yielded valuable results on several levels. First, the chromosomal abnormality is somewhat reassuring because developmental regression has not occurred, as is the case with most of the patients with chromosome rearrangements. The presumed de novo nature of the cytogenetic abnormality is useful information for family planning for her parents and brother.29

Second, this case contributes to the small literature of juvenile moyamoya genetic association in proximity to HLA markers at chromosome 6p25. This previously unreported complex genetic rearrangement is associated with moyamoya, hearing loss, developmental delay, and Alagille-like phenotype in a white child. Duplications of this nature, known as copy number variants, often cause disease through a dosage-sensitive mechanism, which can, among other mechanisms, affect regulation of other gene expression.30 Further targeted and renewed research is warranted at these loci to examine the role of HLA alleles in potential aberrant vascular proliferation.1,2 The genetic causes of the abnormal cerebral vessel intimal thickening and disruption in moyamoya, regardless of associated disease, may offer clues to both pediatric stroke and atherosclerotic disease more broadly.10,11

Lastly, we confirmed the importance in primary care of a comprehensive genetic evaluation for developmental delay as well as complete genetic evaluation of patients presenting with moyamoya.17,18 In particular, the bilateral hearing loss, developmental delay, and atypical facies in our patient warranted further chromosomal analysis independent of moyamoya presentation.

**REFERENCES**

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