Intraventricular hemorrhage (IVH) is a major cause of adverse outcome for very low birth weight (VLBW) preterm neonates. Ten to 20% of these infants have IVH, and nearly 75% of children who survive Grade 3 or 4 lesions, involving either acute distension of the cerebral ventricular system with blood (Grade 3) or parenchymal infarction (Grade 4), develop mental retardation and/or cerebral palsy. A familial susceptibility for IVH in VLBW preterm twins has been suggested, although no causative genes have been identified.

IVH has been attributed to alterations in cerebral blood flow to the immature germinal matrix microvasculature. COL4A1 is a gene that encodes type IV collagen α-chain 1. This is 1 of 6 α-chains that contribute to type IV collagen, the principal component of basement membranes. Along with COL4A2, COL4A1 forms a heterotrimer, [(α1(IV))2(α2(IV)), that is ubiquitously expressed during early mammalian development. Truncating mutations in mouse Col4a1 result in cerebral hemorrhage in both neonatal and adult mice. Missense mutations have been reported in human infants with congenital porencephaly and adults with cerebral small vessel disease, and recently a heterozygous missense mutation was found in 2 siblings with fetal hemorrhagic stroke.

We searched for COL4A1 variants in 41 preterm infants presenting with IVH and identified a rare heterozygous duplication (a genetic mutation resulting in the insertion of a string of nucleic acids on 1 of 2 alleles) within a highly conserved residue in COL4A1 in dizygotic twins affected with IVH.

**Case Reports**

Patients IVH-018 and IVH-019 were dizygotic twins, born at 24 weeks’ gestation. Delivery was complicated by chorioamnionitis and preterm labor to a 29-year-old G2 P0 AB1 female. The fetuses received antenatal steroids, and delivery was by spontaneous vaginal delivery. The parents had no related significant medical history.

Twin A, a girl, had a birth weight of 720 g; Apgar scores were 1 at 1 minute and 1 at 5 minutes. She was intubated in the delivery room and received surfactant and low-dose indomethacin. She was found to have a Grade 3 IVH on the second postnatal day (Figure, A). Her course was complicated by respiratory distress syndrome, hyperbilirubinemia, bronchopulmonary dysplasia, and retinopathy of prematurity.

Twin B, a boy, also weighed 720 g; his Apgar scores were 2 at 1 minute and 5 at 5 minutes. He was also intubated in the delivery room and was treated with surfactant and early low-dose indomethacin. Twin B was diagnosed with Grade 4 IVH on the second postnatal day (Figure, A), and his course was complicated by respiratory distress syndrome, late-onset coagulase-negative staphylococcal sepsis, patent ductus arteriosus, hyperbilirubinemia, and retinopathy of prematurity.

**Genetic Analysis**

This study was performed at Yale University School of Medicine and was approved by the institutional review board and Human Investigations Committee. Written parental permission was obtained for the study protocol.

Mutation screening of the COL4A1 gene in the twins was part of a project to resequence this gene in 41 preterm infants presenting with IVH. Primers for polymerase chain reaction (PCR) flanking each of the exons for COL4A1 (OMIM 120130; NM_001845) were designed using the program PRIMER3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). PCR amplicons were generated from patient-derived genomic DNA using standard procedures. Sequence data were analyzed using Sequencer software (Genecodes, Ann Arbor, Michigan).

A c.4582-4586dupCCCATG insertion in exon 4 was identified in both twins (Figure, B). This mutation results in a third proline-methionine repeat inserted into the highly conserved noncollagenous (NC1) domain.
other 39 infants with IVH had this mutation. The zygosity of twins IVH-018 and IVH-019 was confirmed using a GeneChip mapping 10 K array (Affymetrix Inc, Santa Clara, California) containing 11,555 single nucleotide polymorphism (SNP) markers for genotype analysis, according to the company’s protocols. Affymetrix Micro-Array Suite 5.0 software was used to obtain raw microarray feature intensities, the results of which were processed to derive SNP genotypes using the Affymetrix Genotyping Tools software package (GTYPE; Affymetrix, Santa Clara, California).

The mother and maternal grandmother of the twins were identified as heterozygous carriers of this mutation through

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Figure. COL4A1 mutation. A, Representative transcranial ultrasound images through the lateral ventricles of patient IVH-018 (left) and IVH-019 (right) are shown. Hyperechogeneity within the lateral ventricles and germinal matrix (arrows) can be seen representing grade III and IV hemorrhage, respectively. B, Direct sequencing of genomic DNA (left panel) reveals a suspected insertion mutation. Subsequent T/A cloning of the PCR amplicon demonstrates the c.4582-4587dupCCCATG mutation. C, Examples of TGCE chromatograms representing a double-peak (right), suggesting a heteroduplex, in both patients, as compared with the normal, homoduplex found in a representative control (left). D, Sequence alignment of the NC1 domain of COL4A1 shows high conservation across species. The site of the duplication is marked with a bar.
resembled by the mutated collagen. This vascular dynamic perforator branches of the cerebral vessels are probablyorrhages in mice and human subjects suggests that smalllike prematurity. Of note, the presence of basal ganglia hem-
tomatis, deletions, or copy number variations (CNVs) within
control chromosomes. These findings and the highly con-
served nature of the amino acids at the point of substitution
all strongly point to a role for rare COL4A1 mutations in
IVH. The finding of this variant in 1 set of twins of 41 cases
of IVH suggest that the condition is likely to be genetically
heterogeneous.

The COL4A1 mutation that we report results in the inser-
tion of 2 amino acids into the highly conserved NC1 domain (Figure, D). Mutations in the NC1 domain in type IV and
other collagens were shown to be detrimental to trimer and
hexamer formation and consequently to basement mem-
brane stability. Furthermore, there are no known inser-
tions, deletions, or copy number variations (CNVs) within
the NC1 domain of type IV collagen.

Mice and adult humans with mutations in COL4A1 have
cerebral hemorrhages within a wide spectrum of phenotypic
severity and variability. The Col4a1 mutations leading to
hemorrhage in newborn mice were thought to be in the con-
text of trauma (birth), although adult mice also had cerebral
hemorrhages. Adult humans with COL4A1 mutations have
hemorrhagic strokes and white matter abnormalities as
well as intracranial aneurysms. The context and severity of
the phenotype probably depends on other genetic interac-
tions, environmental factors, and constitutional stressors
like prematurity. Of note, the presence of basal ganglia hem-
orrhages in mice and human subjects suggests that small
perforator branches of the cerebral vessels are probably
weakened by the mutated collagen. This vascular dynamic
is similar to the germinal matrix of the premature infant.

Although the functional consequences of the c.4582-
4586dupCCCATG mutation remain to be elucidated, these
findings, along with the previous demonstration of hetero-
ygous mutations leading to a range of vascular phenotypes,
suggest that it is not likely to be an incidental finding. Both
functional studies and resequencing of larger groups of
patients are necessary to confirm these results. Nonetheless,
as survival among vulnerable VLBW infants continues to
improve, identifying contributory genetic factors and their
connected pathophysiologic mechanisms that increase risk
for IVH will lead researchers to better strategies for preven-
tion and treatment of an important morbidity.

The authors thank Drs Deborah Hirtz, Walter Allan, and Betty Vohr
for scientific advice and Drs Fateh Bayrakli, Mahamad Bydon, Charles
C. Duncan, and Matthew W. State for assistance in preparing the
manuscript.

Submitted for publication Dec 15, 2008; last revision received Mar 5, 2009;
accepted Apr 8, 2009.
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References

1. Neubauer AP, Voss W, Kattner E. Outcome of extremely low birth
weight survivors at school age: the influence of perinatal parameters
2. Sherlock RL, Synnes AR, Grunau RE, Holsti L, Hubber-Richard P,
Johannesen D, et al. Long term outcome after neonatal intraparenchymal
hemorrhages and porencephaly with porencephaly. Arch Dis Child Fetal
Familial and genetic susceptibility to major neonatal morbidities in pre-
4. Favor J, Gloeckner CJ, Janik D, Klement M, Neuhauer-Klaus A,
Precht W, et al. Type IV procollagen missense mutations associated
with defects of the eye, vascular stability, brain, kidney function and
embryonic or postnatal viability in the mouse, Mus musculus: an extension
of the COL4a1 allelic series and the identification of the first 2 COL4a2 mu-
ganization and expression of basement membrane collagen IV genes
and their roles in human disorders. J Biochem (Tokyo) 1998;123:
767–76.
et al. Mutations in COL4A1 cause perinatal cerebral hemorrhage and pore-
et al. Novel mutations in three families confirm a major role of
8. Plassier E, Girbouval O, Alamowitch S, Mougenot B, Prost C,
Verpont MC, et al. COL4A1 mutations and hereditary angiopathy, ne-
phropathy, aneurysms, and muscle cramps. N Engl J Med 2007;357:
2687–95.
9. De Vries LS, Koopman C, Gerenendaal F, Schooneveld MV,
Verheijen FW, Verbeek E, et al. COL4A1 mutation in two preterm sib-
lings with antenatal onset of parenchymal hemorrhage. Ann Neurol
10. Abelson JF, Kwan KY, O’Roak BJ, Baek DY, Stillman AA, Morgan TM,
et al. Sequence variants in SLITRK1 are associated with Tourette’s syn-
of COL10A1 in Schmid metaphyseal chondrodysplasia. Hum Mutat
NC1 domain of collagen IV encodes a novel network composed of the
alpha 1, alpha 2, alpha 3, and alpha 6 chains in smooth muscle basement
13. Poschl E, Schlote-Schreiber U, Brachvogel B, Saito K, Ninomiya Y,
Mayer U. Collagen IV is essential for basement membrane stability but
disposable for initiation of its assembly during early development.