

A Mutation in *COL4A2* Causes Autosomal Dominant Porencephaly With Cataracts

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Mutations in *COL4A1* are well described and result in brain abnormalities manifesting with severe neurological deficits including cerebral palsy, intellectual disability, and focal epilepsy. Families with mutations in *COL4A2* are now emerging with a similar phenotype. We describe a family with an autosomal dominant disorder comprising porencephaly, focal epilepsy, and lens opacities, which was negative for mutations in *COL4A1*. Using whole exome sequencing of three affected individuals from three generations, we identified a rare variant in *COL4A2*. This *COL4A2* (c.2399G>A, p.G800E, CCDS41907.1) variant was predicted to be damaging by multiple bioinformatics tools and affects an invariable glycine residue that is essential for the formation of collagen IV heterotrimers. The cataracts identified in this family expand the phenotypic spectrum associated with mutations in *COL4A2* and highlight the increasing overlap with phenotypes associated with *COL4A1* mutations.

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INTRODUCTION

Collagen IV (COL IV), an important component of the basal lamina, is comprised of six α -polypeptide chains encoded by six differentially expressed genes [Schmidt et al., 1993]. These genes are arranged in pairs on chromosomes 2, 13, and X, and each pair produces two genetically identical paralogous α -peptides through a bidirectional promoter [Haniel et al., 1995; Khoshnoodi et al., 2008]. The COL IV α -peptides assemble into heterotrimers with the following stoichiometries: $\alpha 1\alpha 1\alpha 2$, $\alpha 3\alpha 4\alpha 5$, and $\alpha 5\alpha 5\alpha 6$

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[Khoshnoodi et al., 2008]. Aberrations to COL IV heterotrimers can lead to genetic and acquired diseases [Lemmink et al., 1997] (Supplementary Table SI). *COL4A1* and *COL4A2* are the most abundantly and ubiquitously expressed COL IV peptides [Favor et al., 2007]. Mutations in *COL4A1* and *COL4A2* can affect multiple organs resulting in various cerebral, ocular, renal and muscular pathologies [Favor et al., 2007; Kuo et al., 2014].

Mutations in *COL4A1* result in well-recognized phenotypes (Supplementary Table SI). The current clinical picture of

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COL4A2 is limited to porencephaly type II (MIM 614483) and intracerebral haemorrhage (MIM 614519). While systemic and eye pathologies have been reported in isolated patients, these clinical features are less well recognized as part of the *COL4A2* spectrum (Supplementary Table SII) [Verbeek et al., 2012; Gunda et al., 2014].

Cerebral defects are prominent in all patients with *COL4A2* mutations and there is *in vivo* evidence from mice model studies that missense mutations in *Col4a2* can also induce ocular, myopathic, and systemic defects [Favor et al., 2007; Kuo et al., 2014]. We expand the clinical spectrum of *COL4A2* by identifying a novel missense mutation in a family with autosomal dominant inheritance of porencephaly, focal epilepsy and lens opacities.

MATERIALS AND METHODS

Informed written consent was obtained from all subjects or their parents or legal guardians in the case of minors. The study was approved by the New Zealand Health and Disability Ethics Committee and the Adelaide Women's and Children's Health Network Human Research Ethics Committee.

Exome Sequencing

Whole exome sequencing (Roche Nimblegen SeqCap v3) was performed on a HiSeq2500 (Illumina) by the South Australian Cancer Council Genomics Facility on three affected individuals (I-2, II-2, III-1; Fig. 1A). Reads were mapped to the human genome (hg19) using BWA-MEM [Li and Durbin, 2009] and mapping refined using Genome Analysis Toolkit (GATK) version 3.2-2 [DePristo et al., 2011]. Mapping achieved a minimum median target coverage depth of 46 reads/sample and covered 95% of intended targets with at least 10 reads (Supplementary Fig. S1). Single nucleotide variants and small insertions and deletions were called by the GATK haplotype caller version 3.2-2 [DePristo et al., 2011]. Larger copy number and structural variants were analysed by CoNIFER [Krumm et al., 2012]; however, no segregating, rare copy number variants were detected. All variants were annotated for allele frequency, clinical significance, locus identity, and likely pathogenicity using ANNOVAR [Wang et al., 2010]. Novel genotypes shared between affected individuals, but absent from a control set of 15 exomes, were separated using the *vcf-contrast* module from VCFtools [Danecek et al., 2011].

RESULTS

Clinical Reports

The proband (Fig. 1A, III-1) was a 16-year-old male with spastic quadriplegia and apparent intellectual disability who was non-verbal and fully dependent. First concerns arose at 11 weeks due to poor head control with seizure onset at 2 years. He developed intractable multiple types of focal seizures and status epilepticus. Non-progressive bilateral small posterior lens opacities were found at 3 years. EEGs showed left hemisphere slowing and right parietal epileptiform activity. MRI brain showed right frontal communicating porencephaly with dilated lateral ventricles (Fig. 1B, i) and destruction of the right caudate nucleus, lentiform nucleus, internal capsule, thalamus, and right hippocampus (Fig. 1B, ii).

The proband's 13-year-old brother (III-2) had developmental delay with walking at 18 months and first words at 2 years. Autism spectrum disorder was diagnosed at 5 years. He had apparent intellectual impairment and could read simple sentences and do basic addition. He had focal dyscognitive seizures from 3 to 11 years. At 14 months, he had non-progressive bilateral posterior subscapular lens opacities. His neurological examination was normal. EEG at three years was normal. MRI revealed a right frontal porencephalic cyst communicating with the right lateral ventricle (Fig. 1B, iii). There was partial destruction of the periventricular white matter and caudate body. A smaller cyst was present in the right external capsule. The corpus callosum had an unusual appearance with thickened genu and splenium and absence of the intervening body (Fig. 1B, iv). *COL4A1* sequencing was normal.

The 41-year-old mother (II-2) was asymptomatic and of apparent normal intellect. She had a mild left internuclear ophthalmoplegia. MRI showed a cluster of small cysts in the right frontal periventricular white matter and bilateral anterior caudate nuclei (Fig. 1B, v). There were numerous scattered T2/FLAIR hyperintense lesions in keeping with foci of gliosis, scattered throughout the white matter but prominent frontally (Fig. 1B, vi).

The 69-year-old maternal grandmother (I-2) had a mild right hemiplegia noted in the first year of life. She had learning difficulties at school but was of apparent normal intellect in adult life. She developed focal dyscognitive seizures in childhood and convulsive seizures at 47 years. She continued to have intractable focal seizures with episodes of status epilepticus. On examination, she has an obvious right hemiplegia. She refused neuroimaging and an EEG. She developed bilateral cataracts at 62 years of age.

Exome Analysis

There were 8,388 novel genotypes that were shared between the three affected individuals (Supplementary Table SIII). Of these, 409 variants passed our frequency and read quality criteria (Supplementary Table SIII). Only one out of 69 coding variants passed our filtering criteria and segregated with the clinical phenotype: *COL4A2* (c.2399G>A, p.G800E, CCDS41907.1; Supplementary Table SIV). Sanger sequencing confirmed the segregation of the variant in the proband, his brother, mother and grandmother. (Supplementary Fig. S2F). The *COL4A2* p.G800E variant was predicted as likely disease causing by multiple bioinformatics tools (Fig. 1C). The glycine at position p.800 is invariable, from primates through to fish (Fig. 1D).

DISCUSSION

Lens opacities have not been recorded in previous *COL4A2* reports but are well recognized in *COL4A1*-related disorders, together with other ocular defects [Coupry et al., 2010]. To date, only one *COL4A2* mutation has been associated with ocular defects that included myopia, amblyopia, and abnormal optic discs (Supplementary Table SII) [Verbeek et al., 2012]. Lens opacities in humans have not been described and are noted here in young children with the *COL4A2* mutation; in contrast, the grandmother's cataracts may be an unrelated incidental age-related finding.

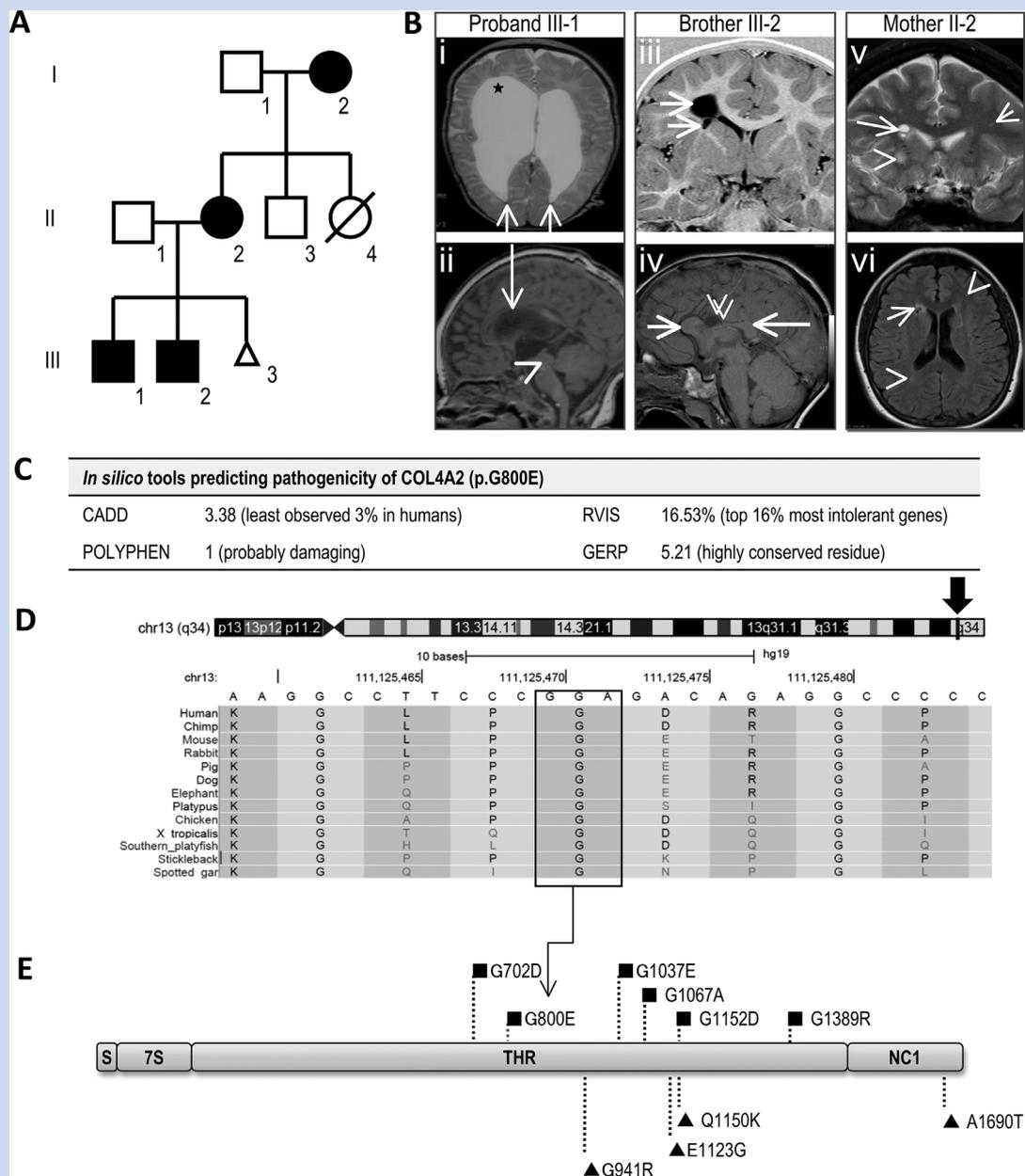


FIG. 1. Familial porencephaly caused by a *COL4A2* mutation. **A:** Pedigree. Affected individuals shown in black boxes (males) and circles (females). **B:** MRI brain scans. (i) Proband III-1: Axial T2 demonstrates right frontal porencephalic cyst (star) and hydrocephalus. Hemosiderin staining (T2 hypointensity) of the ependymal lining of the posterior horns of the lateral ventricles (arrows). (ii) Sagittal T1 weighted image demonstrates thinning of the corpus callosum (long arrow) and loss of visualization of the aqueduct of Sylvius (arrowhead). (iii) III-2: Coronal T1 IR sequence shows multiple right frontal porencephalic cysts (arrows), the largest extends into the superior frontal lobe. (iv) Sagittal T1 weighted image demonstrates a dysmorphic corpus callosum the thick rostrum (short arrow) and splenium (long arrow) missing the callosal body (double arrowheads). (v) II-2: Coronal T2 weighted image of the frontal lobes demonstrate multiple small cysts in the right frontal periventricular white matter (long arrow). Numerous scattered foci of T2 hyperintensity are in keeping with gliosis related to small vessel disease (short arrows). (vi) Axial FLAIR demonstrates two small cysts in the right frontal periventricular white matter with surrounding hyperintense gliosis (arrow). Foci of gliosis in the left frontal lobe and right posterior temporal periventricular white matter (arrowheads). **C:** Predicted pathogenicity of *COL4A2* c.2399G>A, p.G800E determined by four prediction algorithms. **D:** Ideogram of chromosome 13 and the *COL4A2* locus (arrow) at 13q34. The mutated amino acid G800 is highly conserved across different orthologs (boxed). **E:** Domain structure and mutations of *COL4A2*. *COL4A2* p.G800E (gray) is located within the triple helical region (THR). Published *COL4A2* mutations predisposing to porencephaly (■) and intracerebral haemorrhage (▲) are shown. 7S, N-terminal propeptide domain; CADD, combined annotation dependent depletion; GERP, genomic evolutionary rate profiling; NC1, non-collagenous domain; RVIS, residual variation intolerance score; S, signal peptide.

The phenotypes observed in human *COL4A2*-related disorders are also found in mice carrying heterozygous *Col4a2* mutations (Supplementary Table SII) [Favor et al., 2007; Kuo et al., 2014]. The severity and penetrance of eye, brain, and muscular defects varies markedly among inbred (C57B1/6J) mice with the same *Col4a2* mutations [Kuo et al., 2014]. In humans, dominant *COL4A2* mutations also exhibit incomplete penetrance (Supplementary Table SII) [Verbeek et al., 2012; Yoneda et al., 2012].

The majority of *COL4A1* and *COL4A2* mutations occur at highly conserved glycine residues within the triple helical region (THR; Fig. 1D) [Favor et al., 2007]. These THR glycine mutations likely destabilize COL IV heterotrimer formation leading to the accumulation of a misfolded protein and subsequent chronic stress on the endoplasmic reticulum [Yoneda et al., 2012; Kuo et al., 2014]. In both mice and humans, *COL4A2* THR glycine mutations usually lead to severe, early onset cerebral pathologies but with a better prognosis compared to similar mutations in *COL4A1* (Supplementary Table SII) [Favor et al., 2007]. It has been hypothesized that the difference in phenotypic severity of *COL4A1* versus *COL4A2* mutations is due to the stoichiometry of $\alpha 1:\alpha 1:\alpha 2$ per heterotrimer [Favor et al., 2007; Khoshnoodi et al., 2008; Jeanne et al., 2012]. A heterozygous *COL4A2* mutation will lead to 50% of *COL4A1/A2* trimers with a mutant peptide, which will be less than a heterozygous *COL4A1* mutation with up to 75% of *COL4A1/A2* trimers having a mutant peptide [Favor et al., 2007; Jeanne et al., 2012]. The location of the pathogenic variant relative to different functional domains of the *COL4A1/A2* peptides also influences the clinical outcome [Des Parkin et al., 2011]. Non-glycine mutations (NGMs) within the THR and also the non-collagenous domain are likely low penetrance, risk alleles for late onset intracranial haemorrhage with good survival; a distinctly different phenotype to that caused by glycine mutations (Supplementary Table SV) [Jeanne et al., 2012]. Our review of *COL4A2* variations showed that all three previously reported NGMs [Jeanne et al., 2012] are present in the ExAC database while pathogenic THR glycine mutations are absent (Fig. 1E, Supplementary Table SVI). Nonetheless, some NGMs decrease the ratio of extracellular to intracellular *COL4A2* relative to wild-type and also two of three variants induced stress in the endoplasmic reticulum in a similar way to THR glycine mutations, which supports their pathogenicity [Jeanne et al., 2012].

In conclusion, we identified a novel *COL4A2* (c.2399G>A, p.G800E, CCDS41907.1) mutation in an autosomal dominant family with porencephaly and ocular abnormalities. The mutation is predicted to be pathogenic based on the substitution of a highly conserved, critical THR-glycine residue and segregation with the phenotype. We extend the *COL4A2* phenotype spectrum, with the observation of lens opacities in some affected individuals, thus reducing the distinction between *COL4A1* and *COL4A2* mutations. This highlights the importance of simultaneous screening of both *COL4A1* and *COL4A2* genes for accurate diagnosis of early onset porencephaly and further ophthalmological management where indicated.

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