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Mutations in the *ABCC6* Gene as a Cause of Generalized Arterial Calcification of Infancy – Genotypic Overlap with Pseudoxanthoma Elasticum

Qiaoli Li[1,*], **Jill L. Brodsky**[2,3,*], **Laura K. Conlin**[4], **Bruce Pawel**[4], **Andrew C. Glatz**[3,5], **Rachel I. Gafni**[6], **Leon Schurgers**[7], **Jouni Uitto**[1], **Hakon Hakonarson**[3,8], **Matthew A. Deardorff**[3,8], and **Michael A. Levine**[2,3]

[1]Departments of Dermatology and Cutaneous Biology, and Biochemistry and Molecular Biology, Thomas Jefferson University, Philadelphia, Pennsylvania, USA [2]Division of Endocrinology and Diabetes, The Children's Hospital of Philadelphia [3]Department of Pediatrics, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pennsylvania, USA [4]Department of Pathology and Laboratory Medicine, The Children's Hospital of Philadelphia [5]Division of Cardiology, The Children's Hospital of Philadelphia [6]National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, Maryland, USA [7]The Cardiovascular Research Institute, University of Maastricht, The Netherlands [8]Division of Genetics, The Children's Hospital of Philadelphia

Abstract

Generalized arterial calcification of infancy (GACI) is an autosomal recessive disorder characterized by congenital calcification of large and medium sized arteries, associated with early myocardial infarction, heart failure, and stroke, and premature death. Most cases of GACI are caused by mutations in the *ENPP1* gene. We first studied two siblings with GACI from a non-consanguineous family without mutations in the *ENPP1* gene. To search for disease-causing mutations, we identified genomic regions shared between the two affected siblings but not their unaffected parents or brother. The *ABCC6* gene, which is mutated in pseudoxanthoma elasticum (PXE), resided within a small region of homozygosity shared by the affected siblings. Sequence analysis of *ABCC6* revealed that the two affected siblings were homozygous for the missense mutation p.R1314W. Subsequently, *ABCC6* mutations were identified in five additional GACI families with normal *ENPP1* sequences. Genetic mutations in *ABCC6* in patients with PXE are associated with ectopic tissue mineralization in the skin and arterial blood vessels. Thus, our findings provide additional evidence that the *ABCC6* gene product inhibits calcification under physiologic conditions and confirm a second locus for GACI. In addition, our study emphasizes

Address for Correspondence: Dr. Michael A. Levine, The Children's Hospital of Philadelphia, Suite 30 Northwest Tower 11, 34th and Civic Center Boulevard, Philadelphia, PA 19104, Tel 215.590.3618, Fax 215.590.3053, levinem@chop.edu.

*These authors contributed equally to this work

Conflict of Interest

The authors state no conflict of interest.

the potential utility of shared homozygosity mapping to identify genetic causes of inherited disorders.

Introduction

Generalized arterial calcification of infancy (GACI, OMIM# 208000) is an autosomal recessive disorder that is characterized by calcification of the internal elastic lamina of large- and mediumsized arteries and stenosis due to fibroproliferation of the intima of muscular arteries. Radiographs show both arterial and periarticular soft tissue calcifications. GACI usually presents with congestive cardiac failure, hypertensive disease, and/or myocardial ischemia, and the majority of children die within the first 6 months of life (Maayan *et al.*, 1984), with only a few case reports of survivors into later childhood or adulthood (Ciana *et al.*, 2006; Patel *et al.*, 2004; Rutsch *et al.*, 2003; van der Sluis *et al.*, 2006).

Most, but not all, patients with GACI have recessive mutations in the *ENPP1* gene (OMIM# 173335) located on chromosome 6q22-q23 (Rutsch *et al.*, 2003), suggesting that GACI is genetically heterogeneous. *ENPP1* encodes the ecto-nucleotide pyrophosphatase phosphodiesterase 1 (ENPP1, EC 3.6.1.9, EC 3.1.4.1) enzyme that regulates soft tissue calcification and bone mineralization by generating inorganic pyrophosphate (PPi), an essential inhibitor of hydroxyapatite deposition. Hence, GACI patients with deficiency of ENPP1 are unable to synthesize sufficient PPi to inhibit ectopic mineralization and are, therefore, prone to arterial calcification. In addition to a deficiency of PPi, recent studies indicate that some children with GACI due to *ENPP1* mutations develop hypophosphatemia after the first year of life (Rutsch *et al.*, 2008). Hypophosphatemia is assumed to be mediated by elevated plasma levels of the phosphatonin FGF23, which inhibits expression and internalization of the renal sodium-phosphate cotransporters SLC34A1 and SLC34A3 and inhibits synthesis of 1.25-dihydroxyvitamin D. In patients with GACI, hypophosphatemia appears to ameliorate soft tissue calcification (Rutsch *et al.*, 2008). Remarkably, other patients with *ENPP1* mutations develop hypophosphatemic rickets rather than GACI, a paradox that at present lacks a biochemical explanation (Levy-Litan *et al.*, 2010; Lorenz-Depiereux *et al.*, 2010; Rutsch *et al.*, 2008).

Sequence analyses of *ENPP1* have failed to disclose disease-causing mutations in approximately 25% of subjects with GACI (Le Boulanger *et al.*, 2010), and linkage analyses have pointed to other chromosomal regions where other pathogenic genes may be located (Ruf *et al.*, 2005). Recently Nitschke et al. (Nitschke *et al.*, 2012) described a cohort of patients with GACI with mutations in *ABCC6*, which has previously been implicated in pseudoxanthoma elasticum (PXE) (Uitto *et al.*, 2010) and a variant of GACI (Le Boulanger *et al.*, 2010), suggesting that this gene plays a causative role in this disorder. Prior to these publications we investigated two siblings born to non-consanguineous parents with severe GACI and normal sequences for *ENPP1* (Li *et al.*, 2012a). We performed a genome-wide search for a genetic defect underlying GACI and searched for replication of the best candidates in a larger independent cohort of children with GACI. Here we describe additional patients with mutations in the *ABCC6* gene as the second locus for GACI, emphasizing the genotypic overlap between GACI and PXE.

Results

Clinical Findings

A total of seven patients in six families with clinical manifestations consistent with GACI were examined; these families are referred to as Families A–F. The nuclear pedigrees of these families are shown in Fig. 1, and the diagnostic clinical characteristics are shown in Fig. 2 and are detailed in Supplementary Material.

Identification of *ABCC6* as a candidate gene

We initially focused on Family A with two affected siblings with characteristic features of GACI (Fig. 2A). Sequencing of *ENPP1* as one of the candidate genes for mutations (Table 1), which is known to cause GACI, did not reveal the presence of pathogenic mutations. To facilitate the identification of the molecular basis of GACI in this family, we performed genome-wide (610K) SNP array analysis on all individuals. We analyzed the data from the three siblings and the parents to phase informative SNP markers and determine shared haplotype segments between the affected siblings that were not also shared with the unaffected sibling. As expected, this analysis confirmed the exclusion of *ENPP1* as a candidate gene, as well as the *MGP* and *ADIPOQ* genes, each considered as candidates based on known association to mineralization (Table 1). Further analysis of the genotypes within the region containing the *ABCC6* gene revealed that the shared haplotypes between the affected siblings reside in a relatively small, 799 kb region of homozygosity, thus identifying *ABCC6* as the primary candidate gene for mutations in this family.

We subsequently used Sanger sequencing to assess the *ABCC6* gene for mutations in affected members of this family and other unrelated individuals in additional families with GACI.

Mutations in the *ABCC6* gene underlie GACI

We used PCR to amplify and directly sequence all 31 exons and the flanking intronic sequences of the *ABCC6* gene in patients with GACI with normal *ENPP1* sequences, as well as their unaffected family members. Affected members of Family A (Patients 1 and 2), who shared a 799 kb region of homozygosity on chromosome 16, were found to have homozygous missense mutations (c.3940C>T, p.R1314W) in exon 28 of *ABCC6* (Fig. 3). This missense mutation replaces a conserved arginine residue with tryptophan in the second nucleotide binding fold of ABCC6. The proband in Family B (Patient 3) was found to have compound heterozygous mutations (c.2787+1G>T and c.3736-1G>A) in introns 21 and 26 of *ABCC6.* The proband in Family D (Patient 5) had compound heterozygous mutations c. 346-6G>A and p.R1141× in intron 3 and exon 24 of *ABCC6.* In Family F, the proband (Patient 7) was found to carry compound heterozygous mutations consisting of a large deletion (g.del 23–29) and a missense mutation (p.R760W). The arginine-to-tryptophan substitution affects the first nucleotide binding fold of ABCC6 protein. Only one *ABCC6* mutation has been identified for the proband of Family C (Patient 4; p.R391G) and the proband of Family E (Patient 6; c.3692insTT). The p.R391G mutation resides in the fourth intracellular loop corresponding to the second transmembrane domain of ABCC6 protein, while the c.3692insTT mutation causes a frameshift and predicts truncation of the protein as

a result of a premature termination codon 125 bp downstream of the mutation and likely leads to nonsense-mediated mRNA decay (Table 2). Multiplex ligation-dependent probe amplification (MLPA) failed to identify additional deletion mutations in Families C and E. All the identified mutations, with the exception of the mutation c.3692insTT, have previously been reported in subjects with PXE as biallelic mutations, and are predicted to result in reduction or loss of ABCC6 activity (Pfendner *et al.*, 2007; Uitto *et al.*, 2010).

Biochemical findings

Serum levels of calcium, phosphorus, and alkaline phosphatase were normal in all subjects (data not shown). In addition, because circulating FGF23 is elevated in patients with GACI due to *ENPP1* mutations, we measured plasma levels of FGF23 using an immunoassay that detects both the intact form and carboxy-terminal fragments of FGF23 protein. We found that circulating levels of FGF23 were normal in all affected subjects from whom serum could be obtained (Table 2).

Immunofluorescence findings in arterial blood vessels

To further characterize the consequences of the *ABCC6* mutations, we performed histopathologic examination of arteries in patients with GACI, investigating the activation status of matrix gla protein (MGP), a powerful, local anti-mineralization factor when activated by γ-glutamyl carboxylation and which has been implicated in the pathomechanism of PXE (Berkner, 2008; Uitto *et al.*, 2010). We also examined for the presence of fetuin-A, a systemic anti-mineralization factor on the lesional area of mineralized blood vessels (Brylka and Jahnen-Dechent, 2013). Histopathology of the affected vessels revealed extensive mineralization. Because MGP has been shown to participate in the arterial mineralization processes, the presence of both carboxylated (cMGP, active) and uncarboxylated (ucMGP, inactive) forms of this protein was investigated by immunofluorescence with conformation-specific monoclonal antibodies (Schurgers *et al.*, 2005). While the active form (cMGP) was detectable in association with the mineral deposits (Fig. 4, frame A), a similar, but stronger staining pattern was noted with the antibody recognizing ucMGP (Fig. 4, frame B), suggesting that MGP was predominantly in the inactive form. Immunostaining with an antibody recognizing fetuin-A revealed co-localization of the protein with the mineral deposits (Fig. 4, frame C). The controls without the primary antibody were negative (Fig. 4, frames D–F).

Discussion

In this study we used a strategy of shared haplotype mapping to identify potential candidate genes for GACI in a family with two affected siblings and one unaffected sibling, confirmed by Sanger sequencing. This mapping led to the identification of *ABCC6*, which has been previously shown to be mutated in patients with PXE (Pfendner *et al.*, 2007; Uitto *et al.*, 2010) or associated in heterozygous state with premature coronary artery disease (Köblös, 2010; Trip *et al.*, 2002), as the candidate gene. These studies indicate the usefulness of both shared haplotype and homozygosity analysis for identification of genetic causes of rare inherited disorders in patients even from apparently non-consanguineous families. Our results confirm the successful use of individuals from outbred populations in homozygosity

mapping (Hildebrandt *et al.*, 2009), which together with rapid next generation sequencing, should greatly accelerate gene discovery.

We subsequently used Sanger sequencing to confirm the association between *ABCC6* and GACI in five additional unrelated families where the clinically affected subjects had normal *ENPP1* gene sequences. We identified mutations in both alleles of *ABCC6* in three subjects, but we found only a single mutant allele in two patients with GACI. Our results are similar to those noted in other studies in which mutations in both *ABCC6* alleles were disclosed in only ~80% of patients with PXE (Pfendner *et al.*, 2007; Uitto *et al.*, 2010). Because GACI, similar to PXE, is an autosomal recessive disease, it is likely that individuals with a detectable mutation in only one allele harbor a second mutation in the other *ABCC6* allele *in trans*. In this context, it should be noted that the mutation detection strategy we used to analyze the *ABCC6* gene employs PCR amplification of all exons and flanking intronic sequences, combined with multiplex ligation-dependent probe amplification. This combined approach should detect most point mutations, insertion/deletion mutations and copy number variants, but would not detect small mutations in the promoter region or imbedded in the introns of *ABCC6* that might affect transcription or splicing. An alternate, less likely possibility is that the affected individuals in Families C and E demonstrate digenic inheritance due to one mutation in the *ABCC6* gene and a second heterozygous mutation in another, genetically distinct, yet functionally overlapping gene involved in ectopic tissue mineralization. This possibility is raised in light of our recent demonstration that heterozygosity for one mutation in the *ABCC6* gene and one mutation in the *GGCX* gene can result in PXE-like cutaneous findings (Li *et al.*, 2009a).

Our molecular results confirm and extend previous studies that have indicated a significant overlap between the clinical features of PXE and GACI (Nitschke and Rutsch, 2012). PXE predominantly affects the elastic tissues of the skin, eyes, and cardiovascular system (Neldner, 1988). In the skin, histopathology demonstrates accumulation of pleiomorphic elastic structures in the mid- and upper reticular dermis with profound, progressive mineralization. The eye manifestations characteristically consist of angioid streaks due to mineralization of an elastinrich Bruch's membrane behind the pigmented retina (Georgalas *et al.*, 2009). Mineralization of this membrane results in blood vessel rupture and subsequent neovascularization, leading to loss of visual acuity and occasionally to blindness. The cardiovascular manifestations include arterial calcification resulting in intermittent claudication, decreased peripheral pulses, hypertension, hemorrhage, and early myocardial infarcts. A recent report described two siblings with identical *ABCC6* gene mutations that led to classical PXE in the older brother and a phenotype similar to GACI in the younger brother (Le Boulanger *et al.*, 2010). The authors demonstrated that MGP and fetuin-A, involved in the mineralization process in PXE, were also expressed in the affected younger sibling with GACI. These proteins act physiologically as local and systemic inhibitors of mineralization. Additionally, a patient with GACI due to homozygous missense mutation in the *ENPP1* gene has recently been reported to have PXE-like cutaneous manifestations consisting of yellowish papules coalescing into plaques of inelastic skin (Li *et al.*, 2012b). Skin histopathology of this patient demonstrated accumulation of pleiomorphic elastotic structures with progressive mineralization, a diagnostic feature of PXE. Hence, GACI may

be an atypical and severe end of the vascular phenotype spectrum of PXE. A comparison of clinical features of PXE and GACI is provided in Table 3. It should be noted that all the *ABCC6* mutations discovered in this study in patients with GACI, with the exception of a mutation c.3692ins TT, have been previously reported in patients with PXE (Li *et al.*, 2009b; Pfendner *et al.*, 2007). The reasons for the development of such divergent phenotypes as a result of the same mutation in *ABCC6* remain unclear. One could postulate, however, that genetic modifier genes or epigenetic factors profoundly alter the phenotypic expression of the *ABCC6* mutations. This notion is supported by recent mouse studies indicating that a non-synonymous single nucleotide polymorphism in the *Abcc6* gene, which results in aberrant mRNA splicing, can result in highly different degrees of mineralization in four inbred mouse strains (Berndt *et al.*, 2013). Finally, diet, lifestyle variables and environmental factors can modify the phenotypic presentations of patients with PXE, possibly extending to GACI (Uitto *et al.*, 2013).

The basis for the ectopic mineralization in GACI is only partially understood. Loss of function mutations in *ENPP1* result in decreased extracellular levels of inorganic pyrophosphate (PPi), an inhibitor of tissue mineralization. As a result, ectopic deposition of hydroxyapatite occurs in large and medium-sized arteries leading to intimal proliferation, arterial stenosis, and visceral ischemia. Recently, biallelic loss of function mutations in *ENPP1* have also been identified as a cause of autosomal recessive hypophosphatemic rickets (Levy-Litan *et al.*, 2010; Lorenz-Depiereux *et al.*, 2010; Mehta *et al.*, 2012; Saito *et al.*, 2011). As in other forms of hypophosphatemic rickets, serum levels of the phosphatonin FGF23 are elevated in rickets patients with *ENPP1* mutations. Hence, we sought to determine whether loss of ABCC6 might also be associated with elevated serum concentrations of FGF23. Using an immunoassay we found that serum concentrations of FGF23 were essentially normal in all affected patients that we assessed. Thus, it is unlikely that FGF23 plays a significant role in the development of intraarterial calcification in patients with GACI.

The mechanism for vascular and soft tissue calcification in patients with GACI or PXE due to mutations in *ABCC6* is unknown (Uitto *et al.*, 2013). The mineral deposits in the affected tissues of patients with PXE have been shown by Alizarin Red and von Kossa staining to consist of calcium and phosphate, and this composition has been confirmed by energy dispersive X-ray analysis (Kavukcuoglu *et al.*). *ABCC6* encodes a putative transmembrane transporter protein, ABCC6, expressed primarily in the baso-lateral surface of hepatocytes, and previously shown by *in vitro* studies to serve as an efflux pump that transports anionic small molecular weight conjugates (Ilias *et al.*, 2002). Interestingly, *in vitro,* ABCC6 does not transport calcium or phosphate.

In conclusion, our findings confirm that loss of function mutations in the *ABCC6* gene provide the second genetic basis for GACI. In addition to *ABCC6* and *ENPP1*, a number of additional genes can harbor mutations causing heritable diseases manifesting with ectopic mineralization of the skin and/or vascular tissues due to mutations in different genes. These include familial tumoral calcinosis, the normophosphatemic type being due to mutations in the *SAMD9* gene, and the hyperphosphatemic types due to mutations in *FGF23*, *GALNT3*, and *KL* genes (Sprecher, 2010). In addition, CD73 deficiency due to mutations in the *NT5E*

gene manifests with vascular mineralization similar, but distinct from that in PXE (Markello *et al.*, 2011). At the same time, a number of different gene products, such as MGP, α-fetuin, and osteopontin can serve as powerful anti-mineralization factors. Thus, there is an intricate network of factors which either promote or antagonize mineralization processes, and a delicate balance between these factors is required under normal physiologic conditions to maintain tissue homeostasis and prevent ectopic mineralization (Li and Uitto, 2013; Rutsch *et al.*, 2011). Collectively, these observations underscore the role of aberrant expression of such regulatory factors in ectopic mineralization in humans, providing potential opportunities for pharmacologic intervention to prevent the development and progression of these disorders.

Materials and Methods

Patients

We studied seven affected individuals from six unrelated families with clinical features of GACI and normal sequences of the *ENPP1* gene (Fig. 1). In all patients the serum levels of calcium, phosphorus, alkaline phosphatase and vitamin D metabolites were normal. The clinical details of these patients are presented in the Supplementary Material.

All patients were enrolled with written informed consent or assent into an institutional review board-approved study at either The Children's Hospital of Philadelphia or the National Institutes of Health. All studies adhered to the Helsinki Guidelines.

Genome-wide arrays and analyses

DNA samples from Family A were analyzed using Illumina HapMap Quad610 arrays per manufacturer's protocol (Illumina, San Diego, CA), and copy number analysis was performed as described previously (Conlin *et al.*, 2010; Shaikh *et al.*, 2009). The Homozygosity/LOH Detector v.1.0.3 Auto-Bookmark plug-in within Illumina Beadstudio 3.1.3 Genotyping Module 3.3.4 (Illumina, San Diego, CA) was used for detection of regions of homozygosity. We excluded the X chromosome from our homozygosity analyses because the male and female siblings were similarly affected.

Shared haplotypes were determined using parental genotypes to phase parental alleles for each of the three siblings in Family A. Subsequently, inconsistent and uninformative genotypes were removed from the analysis and regions were identified where both maternal and paternal haplotypes were shared among the affected siblings but not with the unaffected sibling. Candidate genes were assessed regarding their exclusion or inclusion from approximate shared regions (Table 1).

Mutation analysis in the *ABCC6* and *ENPP1* genes

Genomic DNA was isolated from peripheral blood (QIAamp Blood Maxi kit; Qiagen Inc., Valencia, CA). PCR was performed using Taq polymerase (Qiagen) according to the manufacturers' instructions. The entire coding region and intron/exon boundaries of the *ABCC6* gene were amplified using PCR primers as described previously (Pfendner *et al.*, 2007). For the detection of the genomic deletion of exons 23–29, the primers previously

described (Le Saux *et al.*, 2001) were used. The PCR products were analyzed with direct sequencing using an Applied Biosystems 3730 Sequencer (Applied Biosystems, Foster City, CA). The +1 in the *ABCC6* gene corresponds to the A nucleotide in the ATG translation initiation codon (GenBank accession no. AF076622). We subjected DNA from patients in whom only a single *ABCC6* mutation was identified to direct sequencing analysis by multiplex ligation-dependent probe amplification (SALSA MLPA P092 ABCC6 probemix, MRC-Holland, Amsterdam) according to the manufacturer's recommendations. Sequencing of the *ENPP1* gene was performed as described previously (Nitschke *et al.*, 2012).

Immunofluorescence

Immunofluorescence of the human arterial sections was performed on tissues embedded in paraffin. Sections were stained with moAb-cMGP (4.5 mg/ml; monoclonal antibody towards the carboxylated forms of human MGP), moAb-ucMGP (4.5 mg/ml; monoclonal antibody towards the uncarboxylated forms of human MGP) (Schurgers *et al.*, 2005), and goat anti-human fetuin-A (10 Pg/ml; R&D Systems, Minneapolis, MN), respectively. The secondary antibody, Alexa Fluor 488 goat anti-mouse IgG (1:800) (Invitrogen, Carlsbad, CA) or Alexa Fluor 488 donkey anti-goat IgG (1:800) (Invitrogen, Carlsbad, CA), was applied. Controls for the immunoreactions were performed by incubating the sections with blocking buffer instead of the primary antibody.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

GACI	generalized arterial calcification of infancy
PXE	pseudoxanthoma elasticum

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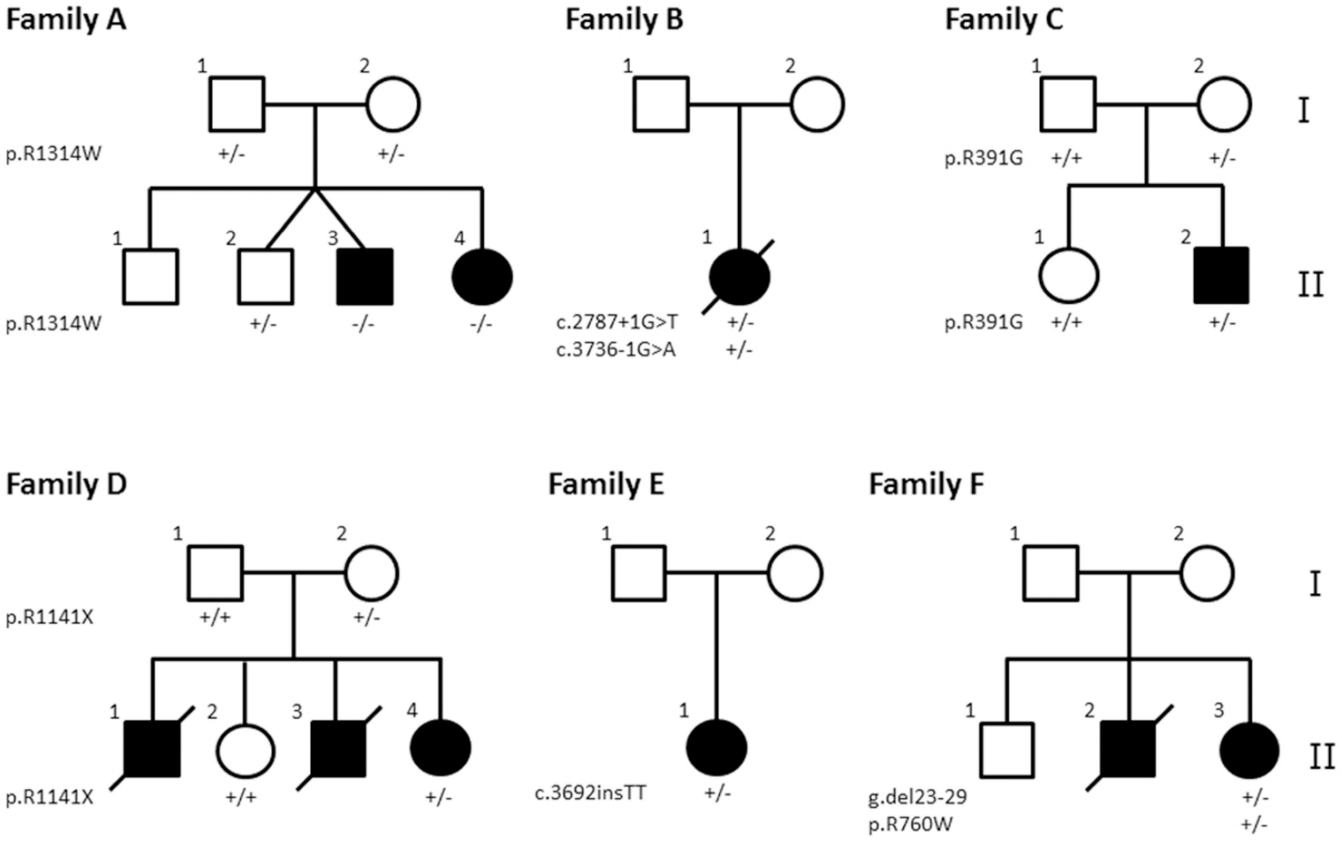

Figure 1. Nuclear pedigrees of Families A-F with GACI

The mutations discovered in the *ABCC6* gene in the individual family members are indicated below each individual: +/+, homozygous for the wild-type allele; +/−, heterozygous carrier; −/−, mutations in both alleles.

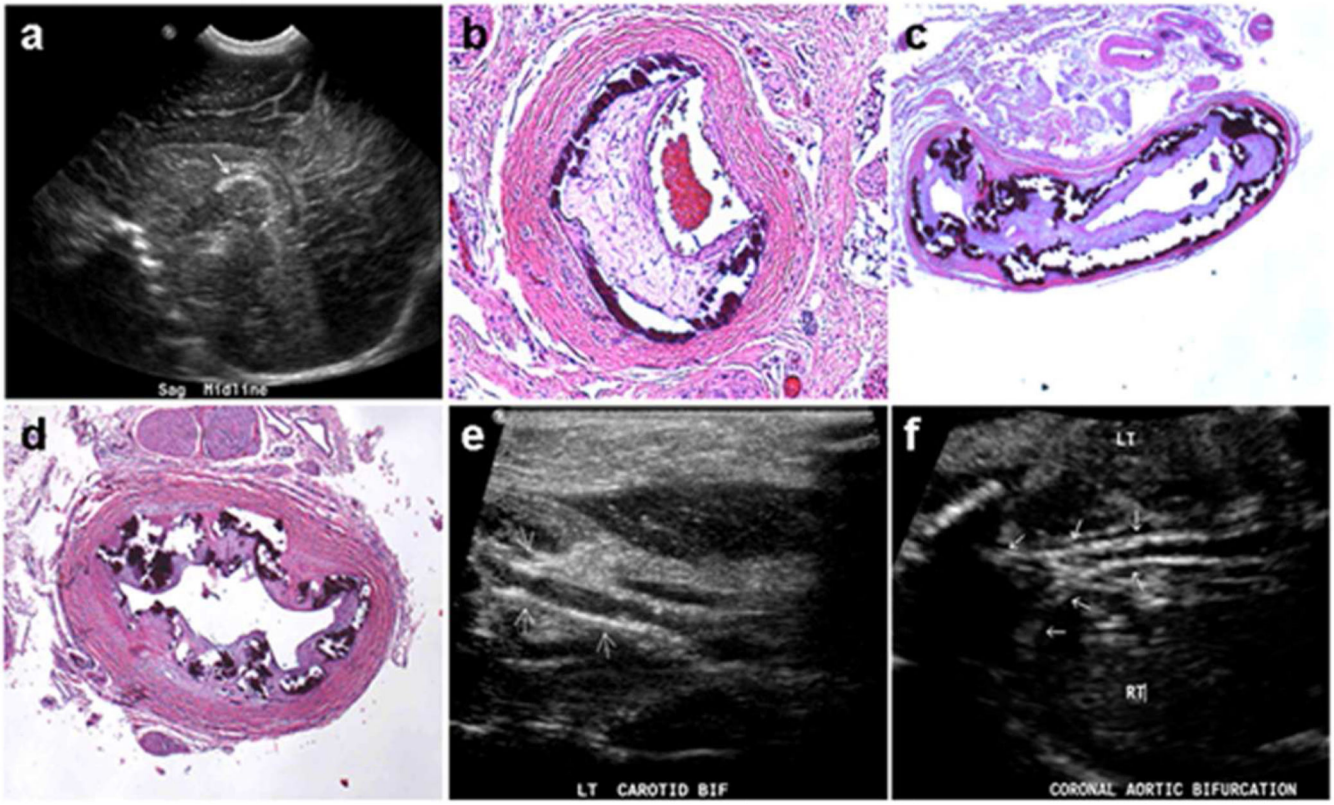

Figure 2. Ultrasound and histopathologic features in individuals with GACI

A: Ultrasound of Patient 1 in Family A (II-3) reveals, in lateral sagittal view of head, calcifications along the cores of lenticulostriate vessels (arrow). B-D: Histopathology of Patient 3 in Family B (II-1) shows calcification of mesenteric artery (B), abdominal artery (C), and left renal artery (D); Hematoxylin-eosin stain, original magnifications ×150. E: Patient 6 in Family E (II-1) demonstrates by ultrasound imaging portions of the proximal external carotid artery with thickened walls and irregular, speckled calcification (arrows). F: Patient 7 in Family F (II-3), prenatal ultrasound of aortic bifurcation demonstrates extensive calcification (arrows).

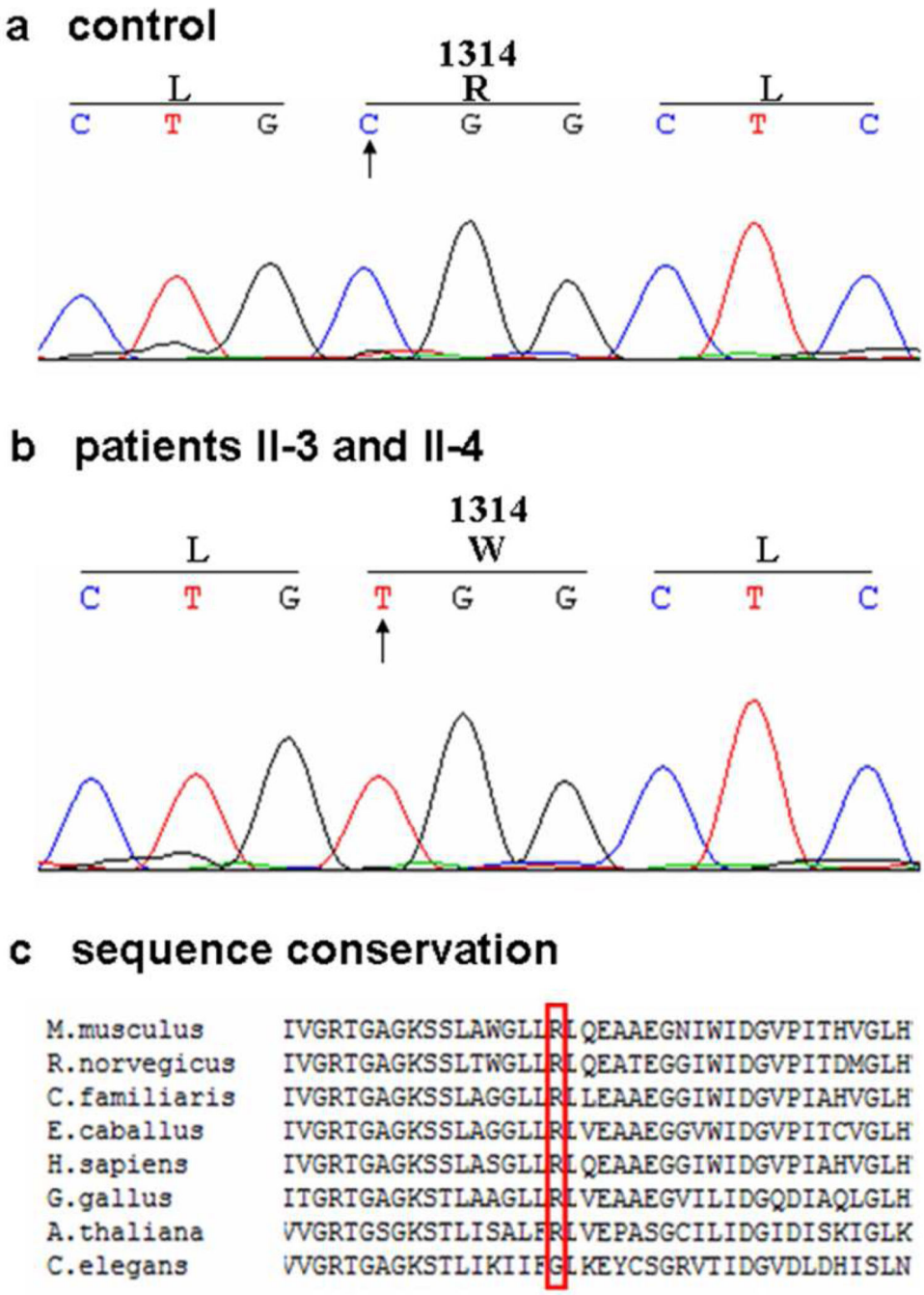

Figure 3. Mutation analysis of the *ABCC6* gene in Family A with *GACI*

Sequencing of the *ABCC6* gene in Patients 1 and 2 (II-3 and II-4 in Figure 1) revealed a homozygous nucleotide T substitution (B) replacing nucleotide C in the control DNA (A). This mutation results in substitution of arginine (R) by tryptophan (W) at amino acid position 1314. The arginine residue is well conserved through evolution (C).

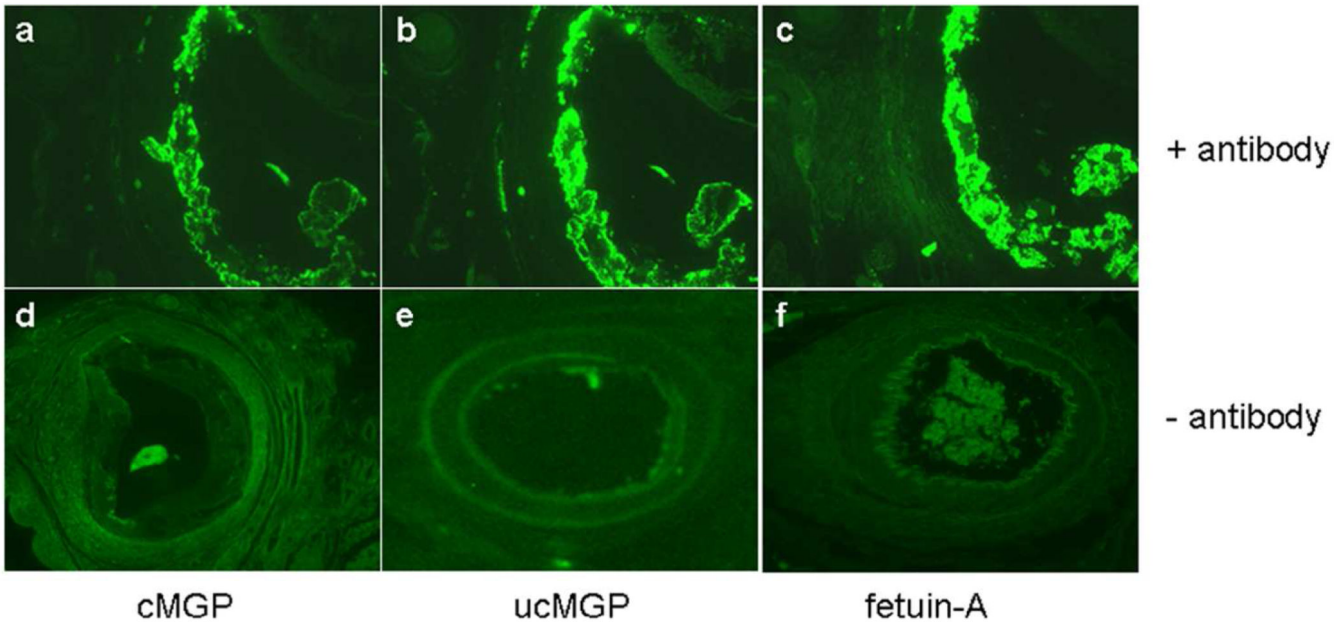

Figure 4. Immunofluorescence of the presence of carboxylated (active) and uncarboxylated (inactive) forms of MGP and fetuin-A in Patient 3 in Family B (II-1)

Immunofluorescence with antibodies recognizing carboxylated (A) and uncarboxylated (B) forms of MGP, and fetuin-A (C). Frames D, E and F represent the corresponding negative controls in which the primary antibodies were omitted.

Table 1

Candidate genes for GACI identified by shared homozygosity analysis in Family A with two affected patients with an unaffected sibling.

Gene	Reason assessed	Excluded from affected siblings	Excluded using affected and unaffected	Comments on haplotype analysis
ENPP1	Common cause of GACI	No	Yes	Same haplotypes in all three children
MGP	Anti-mineralization factor	Yes	Yes	Different in affected siblings
ADIPOQ	adiponectin involved in mineralization	Yes	Yes	Different in affected siblings
ADIPOR1	Adiponectin, type 1 receptor	No	No	Possible candidate gene
ABCC6	Common cause of PXE	No	No	All SNPs over a region of homozygosity

Table 2

Molecular and biochemical features of GACI patients

Patient	Family	Age	Mutation 1	Mutation 2	Mutation type[2]	Plasma [FGF23], RU/mL[3]
1	A	3 yrs	p.R1314W	p.R1314W	MS/MS	59
2	A	6 yrs	p.R1314W	p.R1314W	MS/MS	97
3	B	1 mo	c.2787+1G>T	c.3736−1G>A	SS/SS	NT[5]
4	C	5 yrs	p.R391G	ND[1]	MS/ND	83
5	D	1 mo	p.R1141*	c.346-6G>A	NS/SS[4]	NT
6	E	1 mo	c.3692 insTT	ND	FS/ND	374
7	F	1 mo	p.R760W	del23-29	MS/del	1430

[1] ND, not detected.

[2] MS, missense; SS, splice site; NS, nonsense; FS, frame shift

[3] FGF23 normal range is < 230 RU/mL for ages 3 months to 17 years. Values greater than 900 RU/mL are present in infants.

[4] The effects of the c.346-6G>A mutation on splicing have not been experimentally confirmed

[5] NT, not tested as serum sample was not available

Table 3

Genotypic and phenotypic overlaps of PXE and GACI

PXE	GACI
Vascular mineralization	Vascular mineralization
Skin and eye findings	Rare skin findings
Late onset	Pre- and perinatal diagnosis
Mostly normal life span	Demise usually <6 months
ABCC6 > ENPP1	*ENPP1 > ABCC6*