Supplemental Data

Clinical Features of Patients with GACI

Family A was an African-American family in which two of four children presented at birth with features of GACI. The proband, Patient 1 (II-3), is a male who with his fraternal twin brother was delivered at 32 weeks via Cesarean-section for fetal distress. His birth weight was 1.4 kg. Prenatal ultrasound at 18 weeks gestation had noted arterial calcification (Fig. 2A). His three-month neonatal intensive care unit stay was complicated by hyperbilirubinemia requiring two exchange transfusions, multifocal intracerebral hemorrhage, bilateral ischemic brain injury, and hypertension. Subsequently, he developed spastic quadriparesis with truncal hypotonia, poor vision, and severe encephalopathy as sequelae of brain injury. He has required multiple hospital admissions for cardiac failure and hypertensive crises. Physical examination at age 5 years was negative for angioid streaks or optic disc drusen as well as skin laxity or PXE-like skin lesions. His twin brother is healthy and clinically unaffected, and standars X-rays have failed to reveal any vascular calcifications.

Patient 2 in Family A (II-4) is a female who was born at full term with a birth weight of 3.1 kg. The child was discharged on day 2 of life, but was re-admitted at five days of life for evaluation of decreased appetite, reduced activity, and cold extremities. Ultrasound examination disclosed diffuse calcifications involving the renal, splenic, and common iliac arteries, in addition to arterial calcifications and echogenic thalami consistent with CNS involvement. The patient was treated with one dose of pamidronate (1mg/kg) and then begun on daily etidronate therapy (15 mg/kg/day). Two weeks after admission, the patient experienced an ischemic stroke involving the left parieto-occipital and posterior temporal lobes, splenium of the corpus callosum, right

parietal lobe, superior vermis and left lateral thalamus. She had no evidence of angioid streaks or optic disc drusen when tested at 18 months and 4 years of age, and there were no skin findings suggestive of PXE.

Family B, Patient 3 (II-1), a Caucasian female, was previously reported as a case of GACI with normal *ENPP1* sequence (Glatz *et al.*, 2006) and was subsequently reported to have mutations in the ABCC6 gene (Nitschke *et al.*, 2012). The patient was born after an unremarkable full term pregnancy, and subsequently presented for evaluation on day 33 of life with tachypnea, tachycardia, cool extremities, and poor peripheral pulses. Echocardiogram showed dyskinesia of the left ventricular free wall with a shortening fraction of 26%, left ventricular dilation, mild to moderate mitral valve regurgitation, and moderate tricuspid regurgitation. Brain MRI/MRA showed no structural or neurovascular abnormalities. On hospital day 13, the patient experienced a bradycardic and hypotensive episode. She was resuscitated, but echocardiogram documented worsening cardiac function, despite increasing ionotropic support. Her parents chose to withdraw support and the patient expired at 6 weeks of age. At autopsy, gross examination revealed a markedly enlarged heart. Microscopic examination disclosed extensive calcification of all major coronary arteries, aorta, main and branch pulmonary arteries, celiac, hepatic, suprarenal, pancreatic duodenal, splenic, mesenteric, renal and lumbar arteries. Involved arteries showed primary calcification of the internal elastic lamina with varying degrees of calcification of the external elastic lamina (Fig. 2B-D). Elastin stains revealed disintegration of elastic lamina in areas of heavy calcification. Intraparenchymal arterial calcifications were found in the spleen, thymus, trachea, diaphragm, salivary glands, pancreas, thyroid and larynx.

Family C had one affected child, Patient 4 (II-2), a Caucasian male born at full term after an unremarkable pregnancy. Soon after delivery, he was noted to have pallor of the lower extremities and poor femoral pulses with an otherwise normal physical examination. No cardiac abnormalities were noted on echocardiogram and the patient was hemodynamically stable. Computed tomography and magnetic resonance angiography revealed increased echogenicity in the walls of the abdominal aorta, right and left common iliac arteries, external iliac arteries, and common femoral arteries. The origin of the right renal artery, main hepatic artery, and superior mesenteric arteries also had increased echogenicity in the walls suggesting calcification. There were no calcifications in the brain, carotid arteries or coronary arteries. He had an older unaffected sister. The patient was treated with daily etidronate (18-20 mg/kg/day) for approximately 16 months; existing calcifications were unchanged and no new calcifications developed. Femoral and dorsalis pedis pulses gradually strengthened and returned to normal as the infant grew. The patient is currently 6 years old with normal growth and development.

Family D included three affected individuals. The proband, Patient 5 (II-4), was born at 30 weeks gestation by C-section because of hydrops fetalis. Based on the family history of GACI in two prior pregnancies, she was diagnosed *in utero* with GACI by prenatal ultrasound. One older male sibling, II-1, died of GACI at one month of age, while the other affected male (II-3) died *in utero* with GACI. At birth, the proband had hypertrophic cardiac ventricles and Doppler studies showed massive calcification of the renal arteries as well as internal and external carotid arteries. Head ultrasound showed abnormal signal at basal ganglia bilaterally. The patient was treated with pamidronate, 0.1 mg/kg/dose, by weekly IV infusions for three months without apparent effect.

Family E included a single affected Chinese female patient, Patient 6 (II-1), in whom a GACI was diagnosed around 20 weeks of gestation. The mother was discovered at the time to have primary hyperparathyroidism with serum calcium levels in the mid 13 mg/dL and 10-fold elevated levels of intact parathyroid hormone. During the early second trimester the mother underwent parathyroidectomy with removal of a single parathyroid adenoma with normal serum calcium and parathyroid hormone levels thereafter. Prenatal ultrasound of the fetus revealed portions of the proximal external carotid artery with calcification (Fig. 2E). Subsequent prenatal ultrasound showed some improvement in the GACI of the fetus, albeit diffuse echobrightness of pulmonary arteries and aorta were noted with supravalvular pulmonary stenosis and stenosis of a pulmonary artery branch. Brain and renal ultrasound examination was normal. At birth the patient had no evidence of cardiac failure, hypertension or renal problems. Subsequent echocardiograms have demonstrated progression of the supravalvular pulmonary stenosis.

Family F included a seven-week-old female, Patient 7, (II-3) who was diagnosed *in utero* with GACI on the basis of a prior sibling dying at 8 hours of age from GACI. Echocardiogram showed slightly enlarged left heart chamber, with normal function, associated with calcifications in the aortic bifurcation (Fig. 2F).

References

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