The American Journal of Human Genetics, Volume *103*

Supplemental Data

Missense Mutations of the Pro65 Residue of PCGF2 Cause a Recognizable Syndrome Associated with Craniofacial, Neurological, Cardiovascular, and Skeletal Features

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Figure S1. Additional images of individuals (1-5) with pathogenic *PCGF2* mutations. Images are clustered for each individual. Each cluster is marked with the number which corresponds to the one used in the manuscript. The images show: individual 1 (male) at 12 years (a-d); individual 2 (female) at 4.5 years (e), 8.5 years (f) and 13 years (g); individuals 3 and 4 (female) at 2 years (h); individual 3 at 4 years (i) and 8 years (j-m); individual 4 at 4 years (n,o) and 8 years (p-s); individual 5 (female) at 4 years (t-v) and 7 years (w). Consistent facial features include a high, broad forehead; long face; malar hypoplasia; small mouth and palpebral fissures; a prominent nose (particularly in older indiduals); and dysplastic, low-set ears. Individual 1, the oldest in the group, had multiple pigmented macules on his trunk (c). Similar macules were seen on the scalp of individual 3 (white arrow, l) and the neck of individual 4 (r).

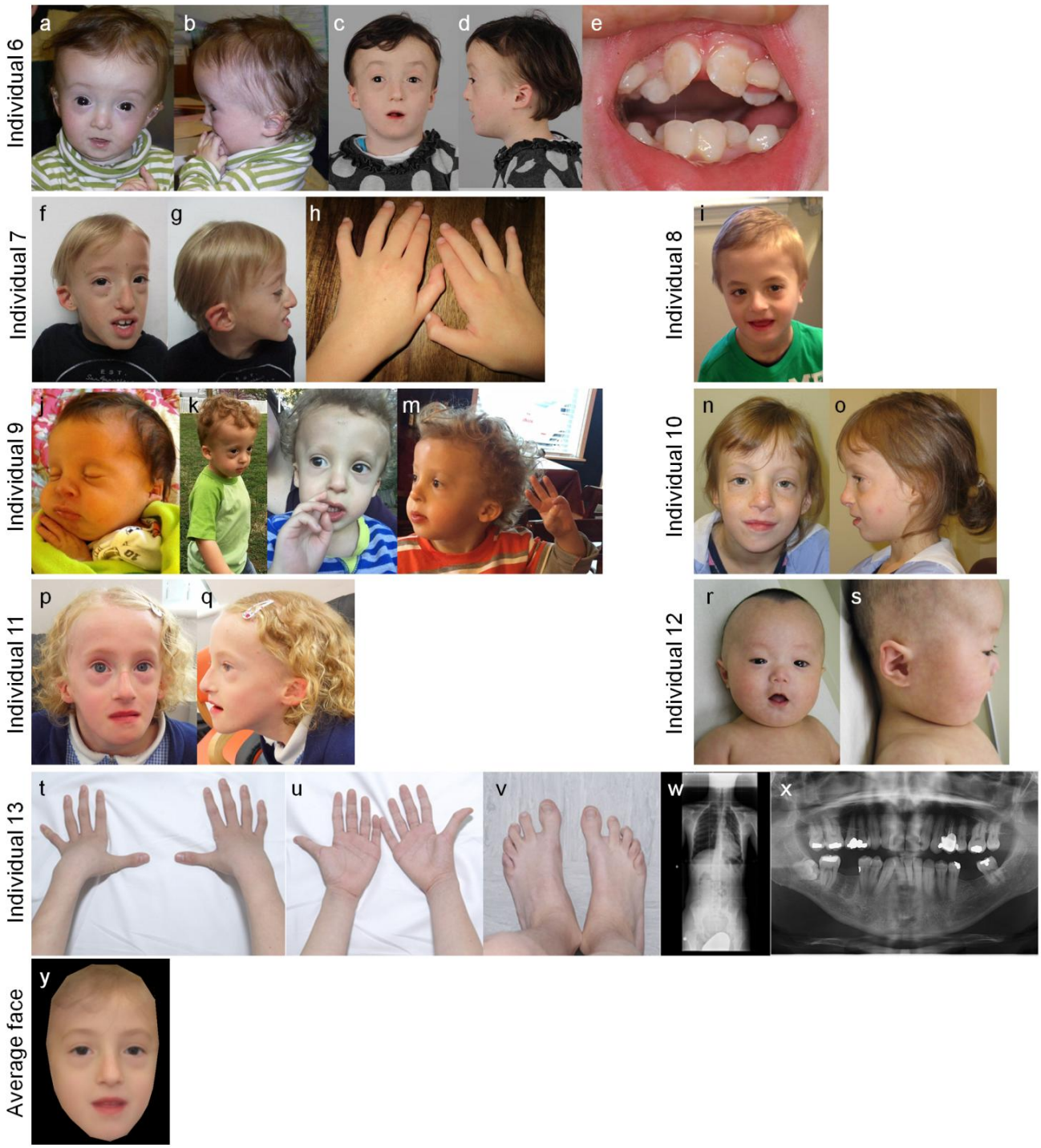

Figure S2. Additional images of individuals (6-12) with pathogenic *PCGF2* mutations. Images are clustered for each individual. Each cluster is marked with the number which corresponds to the one used in the manuscript. The images show: individual 6 (male) at 1 year (a,b) and 9 years (c-e); individual 7 (male) at 7 years (f-h); individual 8 (male) at 6 years (i); individual 9 (male) at 1 month (j), 2 years (k,l) and 3 years (m); individual 10 (female) at 9 years (n,o); individual 11 (female) at 8 years (p,q); individual 12 (male) at 3 months (r,s); individual 13 (male) at 11 years (t-v). Radiological images from individual 13 show a long narrow thorax with mild scoliosis (w) and radicular resorption of secondary teeth (x). Minor digital anomalies with mild 5th finger clinodactyly and tapering fingers are shown (h,t,u).The composite average face (y) was generated from images of nine unrelated individuals (1-3, 5-8, 10 and 11).

Supplemental Note: Case Reports

Individual 1

This 21-year-old male is the eldest of 3 siblings of unrelated white British parents. He was born at term weighing 2900g (9^{th} centile). His mother had taken mefloquine anti-malarial prophylaxis in the first 6 weeks of pregnancy. Postnatally he fed poorly and was hypotonic. Dysmorphic features were noted and his occipitofrontal circumference (OFC) was 90^{th} centile by the age of 1 year. He sat at 12 months and walked at 4 years. He developed conductive hearing impairment and chronic, severe constipation which improved slowly during childhood. He drooled constantly and underwent successful submandibular duct realignment surgery. Over time he remained small (growth centile at age 11 years were: height 1^{st} and weight 5^{th}) but with relative macrocephaly (OFC ~97^{th} centile). He was last evaluated at 21 years of age. His speech remains unintelligible, although his receptive language has improved and he demonstrates an eagerness to learn. He has a flat facial profile, thin hair, satyr ears, a narrow nose, and a number of pigmented nevi on his torso. A brain MRI at 16 months showed dilatation of the 3^{rd} and 4^{th} ventricles and thinning of the corpus callosum. There was a generalised reduction in the white matter but myelination was normal. A skeletal survey at 2.5 years revealed a gibbus deformity due to hypoplasia of L1 vertebra, delayed epiphyseal ossification, particularly of the carpal bones, and pseudo-epiphyses of many metacarpals. A recent echocardiogram showed dilation of the aorta at the sinus of Valsalva (diameter 4.7cm, Z score ~7.7). Extensive metabolic and genetic testing was normal. Microarray comparative genomic hybridization (array-CGH) analysis identified duplications of 0.4Mb at Xp21.2 and 0.5Mb at Xp21.1-p11.4, both inherited from his unaffected maternal grandfather. The DDD Study also identified maternally inherited

variants of uncertain significance in the *USP9X* and *PHF8* genes (classified using the ACMG guidelines).

Individual 2

This 15-year-old female is the elder of two sisters. She was born at 42 weeks gestation after an uneventful pregnancy, birth weight 3340g (25^{th}-50^{th} centile). She fed poorly and failed to thrive. At 6 months her growth centiles were: weight 0.4^{th}, length 25-50^{th}, OFC 25^{th}. Her feeding slowly improved and by late childhood her growth centiles were: weight 9^{th}, height 50-75^{th}, OFC 0.4^{th}. Developmentally, she rolled at 9 months, sat and crawled at 12 months, and walked at 2 years, at which time she demonstrated speech delay with a vocabulary of approximately 15 single words. Initially in mainstream school, she was moved to a unit with learning support, where she has made some progress. Severe chronic constipation was eventually managed successfully by an antegrade colonic enema procedure performed at 12 years of age. Individual 2 was last evaluated at 15 years of age. She has moderate intellectual disability with specific speech and language problems, and mild conductive hearing loss which required grommets. Clinical examination revealed frontal bossing with malar hypoplasia, a prominent nasal tip, small mouth, and short sternum with widely spaced nipples. She has bitemporal balding with generally sparse hair, but normal skin, nails and teeth. Her palpebral fissures are short and down-slanting, and she has a long face with a highly arched palate and prognathism, which has gradually become more prominent with age. She has long narrow hands and fingers. Cardiac MRI showed a normal aortic root at the sinus of Valsalva (2.5cm, Z score 0.29), mild aortic valve regurgitation, and mild dilatation of the left ventricle. This examination incidentally identified a Morgagni hernia of the diaphragm. Array-CGH, cranial MRI and EEG, were all reported to be normal.

Individual 3

This 8-year-old female is twin 1 of monozygotic twins (see Individual 4). They were the first live-born children to non-consanguineous parents. The pregnancy was complicated by polyhydramnios. Birth was at term, weight 2050g (<0.4th centile), length 45cm (1st centile), Apgar scores good. She suffered a grade 2 intraventricular/periventricular haemorrhage and a patient ductus arteriosus (PDA) was surgically closed at 2 months of age. At 21 months her length and weight were on the 5th centile, OFC 5th-10th centile. Both twins were noted to have speech delay, dysmorphic features, increased limb tone with axial hypotonia, and mild choreoathetotic movements of the hands. This twin sat at 1 year and walked at 3 years. At age 5 years 3 months her height was on the 25-50th centile. She was noted to be hyperactive and her language, motor skills, and understanding, were poor but improving. She had large adenoids and severe obstructive sleep apnea. Following adenoidectomy she continued open-mouth breathing. She had frequent, recurrent respiratory infections. Most recently evaluated at 8.5 years of age, she had poor expressive speech, coordination difficulties, constipation, mild diplegia, and features of attention deficit hyperactivity disorder. She had no history of seizures and an EEG was normal. Brain MRI at 21 months of age showed relatively symmetric, multifocal T2 hyperintensities within the deep white matter, most prominent around atria of the lateral ventricles where the changes were confluent. The lateral ventricles were slightly enlarged and some perivascular spaces were mildly prominent. There was extensive, bilateral polymicrogyria affecting the perisylvian regions, inferior frontal, parietal, temporal and superior occipital lobes. MR angiogram (MRA) showed tortuosity of the internal carotid arteries. Routine karyotype, subtelomeric MLPA and array-CGH were normal.

Individual 4

This 8-year-old female is twin 2 of monozygotic twins (see Individual 3). As for twin 1, her Apgar scores were good, and birth weight was 2090g (<0.4th centile), length 44cm (1st centile). She also suffered a grade 2 intraventricular/periventricular haemorrhage, and a PDA closed spontaneously. At 21 months length and weight were on the 5th centile, OFC 5th-10th centile. This twin sat at 1 year and walked at 2.5 years. At 3 years she was noted to have a small left-sided diaphragmatic Morgagni hernia, found incidentally following a foreign body aspiration, and surgically corrected. She had gastroesophageal reflux which improved following the hernia repair. At age 5 years 3 months her height was between the 75th and 91st centile. She was noted to be hyperactive and her language, motor skills, and understanding, were still poor but improving. She had large adenoids and severe obstructive sleep apnea. Following adenoidectomy she continued open-mouth breathing. She had frequent, recurrent respiratory infections. Most recently evaluated at 8.5 years of age, she had poor expressive speech, coordination difficulties, constipation, mild diplegia, and features of attention deficit hyperactivity disorder. She had no history of seizures and an EEG was normal. Brain MRI at 21 months of age showed relatively symmetrical, multifocal T2 hyperintensities within the deep white matter, with some early confluence. The lateral ventricles were slightly enlarged with some prominence of perivascular spaces, and extensive, bilateral polymicrogyria affected the perisylvian, inferior frontal, parietal and temporal regions. She did not have an MRA. Routine karyotype, subtelomeric MLPA and array-CGH were normal.

Individual 5

This 7-year-old female is the child of unrelated parents. Growth delay was noted after 20 weeks gestation and polyhydramnios developed at 28 weeks. Antenatal karyotype, array-CGH, and TORCH screen of amniotic fluid, were normal. Birth was induced at 38+2 weeks gestation, birth weight 2200g (~2nd centile), length 42 cm (<0.4th centile). Linear growth

caught up by 6 months of age but she remained underweight. At 3-4 months a blue coloured subcutaneous swelling was noted on the left side of her nose, diagnosed as a subcutaneous haemangioma or epidermal cyst. She initially bottle fed satisfactorily but suddenly stopped sucking at 8-9 months, was fed by nasogastric tube, and noted to be very hypotonic with clenched fists and adducted thumbs. Examination at 1.5 years of age revealed plagiocephaly with a flat occiput and large fontanelle (4cm x 4cm), sparse hair, triangular face with flat profile, downslanting eyebrows with synophrys and medial flare, and long eyelashes. She had upslanting palpebral fissures, epicanthic folds, a broad nasal tip, small dysplastic ears with triangular conchae, small lobes, narrow ear canals, and absent crus on the smaller left ear. She has a small mouth, smooth philtrum, high-arched palate and bifid uvula. She had a 5mm nevus at the left knee and diffuse hypertrichosis of the lumbar area. Developmentally, she smiled at 11 months, walked from 2 years, and spoke her first words at 3 years, following which she made progress and can use full sentences at 7 years of age. Moderate conductive hearing impairment, partly due to otitis media, was treated with grommets and hearing aids, which improved her speech. At 3.6 years a SON-R intelligence test showed a performance IQ of 75, and although the verbal scale IQ was 101, her speech is slow compared to her peers. She used to wake frequently at night and pulled the hair of other children. At 7 years of age she has progressed in her motor and speech development but tonal dysregulation and some coordination problems remain. She has chronic constipation, her hair remains sparse, she has a convergent squint, hypermetropia and astigmatism, and small, widely spaced teeth; her height is normal but weight low. Brain MRI at age 3 years 4 months showed relatively symmetric, multifocal T2 hyperintensities within the deep white matter, most prominent around atria of lateral ventricles, where changes showed early confluence. There were prominent perivascular spaces. The lateral and 4^{th} ventricles were slightly enlarged, but no evidence of obstructive hydrocephalus. There was a tiny focus of haemosiderin in the right

caudo-thalamic groove. An MRA showed tortuosity of internal carotid arteries. Echocardiography showed a small atrial septum defect (ASD) with left-right shunt, mild dilatation of the ascending aorta which later resolved, mitral and tricuspid valve prolapse, and a PDA, which was surgically closed. Renal and liver ultrasound scans were normal. Spinal X-rays showed the body of her T3 vertebra was small. Postnatal array-CGH found a *de novo* duplication of 15q11.2 (238kb) with no genes in the region.

Individual 6

This 15-year-old female is the eldest of three children born to unrelated parents. Intrauterine growth restriction (IUGR) was noted at 20 weeks gestation and she was born at 37+3 weeks, weight 1652g (<0.4th centile). In infancy she had central hypotonia but was peripherally hypertonic, which later resolved. Developmental milestones were globally delayed and she first walked on her 3rd birthday. She had severe gastroesophageal reflux and constipation. She remained very small but has responded to growth hormone, commenced age 7 years. She had conductive hearing impairment treated with grommets. Significant obstructive sleep apnea was diagnosed aged 10 years, not resolved by adenotonsillectomy. Menarche occurred at 11 years. At age 12 years she attended both mainstream and special schools, and had an anxiety disorder with some autistic behaviours. She has mild sensorineural hearing impairment, but compliance with aids is poor. Her vision is normal. She has persistent constipation and both bladder and bowel incontinence of uncertain cause. She has metatarsus adductus, planovalgus feet, and prominent interphalangeal joints. A skeletal survey showed tall vertebrae with mild scoliosis and thoracic kyphosis, slender bones, coxa valga, and a truncated sacrum with flexion deformity at S4 and only three sacral segments. Brain MRI at age 5 years 4 months showed relatively symmetric, multifocal T2 hyperintensities within the deep and subcortical white matter bilaterally, with early confluence in some deeper regions.

Prominent perivascular spaces, together with a cavum septum pellucidum and verge were observed. There was subtle bilateral perisylvian polymicrogyria, more prominent on the left, and a left cerebral parietal developmental venous anomaly. Compared to MR imaging four years previously, appearances were stable. Echocardiogram found an ASD which closed spontaneously. Metabolic and array-CGH testing were normal.

Individual 7

This 10-year-old male was born at 38 weeks gestation after a pregnancy complicated by polyhydramnios, mild fetal hydronephrosis, and IUGR, birth weight 2380g (1st centile). He fed poorly in the neonatal period requiring an NG tube. He had hypotonia, plagiocephaly, transient corneal opacities, and dysmorphic features including low-set ears, pectus excavatum and torticollis. He was treated for gastroesophageal reflux. He suffered frequent recurrent respiratory infections and febrile episodes in his preschool years, undergoing adenoidectomy and grommet insertion aged 2 years. This helped his feeding but mild hearing impairment remained. Ophthalmic examination revealed anisometropia, mild tortuosity of the retinal vasculature, and concave optic discs. Occasional nocturnal seizures started prior to age 2 years but responded to lamotrigine treatment. He developed a sleep disorder which responded to melatonin and clonazepam. He had global developmental delay, uttering his first words by 2 years, and started walking at 5 years with splints. At 7 years of age he had hypotonia, failure to thrive (height and weight <3rd centile), and could only walk and climb stairs unsteadily with splints. He is dysarthric but has a good vocabulary and is sociable. His skin is fair and hair sparse. The plagiocephaly persists and his face is flat with prognathism. He has mild blepharophimosis, dysplastic ear helices, a high palate, pectus excavatum, and normal hands, feet, and genitalia (the left testicle is small). Echocardiogram found a large ASD and small PDA, which were both closed by percutaneous catheterization at 1 year.

Renal ultrasound showed transient, moderate bilateral hydronephrosis. Brain MRI at age 10 months showed a few scattered deep white matter T2 hyperintensities, together with mild prominence of the lateral ventricles. Array-CGH was normal.

Individual 8

This 9-year-old male was born at 40+3 weeks gestation after a pregnancy complicated by polyhydramnios, birth weight 3200g (25^{th}-50^{th} centile). At birth he was noted to have small, low-set, crumpled ears, and torticollis. In infancy he had hypotonia and delayed developmental milestones, and sat independently at 11 months. He had significant gastroesophageal reflux exacerbated by multiple food allergies, oral tactile sensory sensitivity, and chronic constipation. He was diagnosed with exotropia at 13 months which ultimately required bilateral surgical repair. He has global developmental delay and autistic spectrum disorder with stereotypical movements, occasional self-injurious stimulatory behaviours, and disrupted sleep. He is nonverbal and communicates with signs, gestures, and tablet applications. At 6.5 years he was found to have a right thoracolumbar neuromuscular scoliosis (but normal spine MRI) that has improved without intervention. He has had several episodes of metabolic acidosis and hypoglycaemia with mild dehydration, cause unknown. Sequential brain MRI has shown stable non-specific patchy areas of T2 prolongation in the periventricular white matter. Brain MRI at age 3.1 years showed relatively symmetric, multifocal T2 hyperintensities within the deep white matter bilaterally, most marked around atria of lateral ventricles. There were prominent perivascular spaces, including within the corpus callosum. An MRA showed tortuous vessels, most prominently the carotid and vertebral arteries. Echocardiogram at 13 months of age demonstrated mild-moderate dilatation of the aortic root and ascending aorta, which has persisted. Further surveillance has shown stable dolichoectasia of the carotid, vertebra-basilar, posterior cerebral, and anterior

cerebral arteries. Routine karyotype, array-CGH and a range of genetic investigations were normal.

Individual 9

This 5-year-old male was born at 39+4 weeks gestation following an uncomplicated pregnancy and delivery, birth weight 2812g (>9th<25th centile). He had hypotonia in the neonatal period but a reasonable suck. He had global developmental delay from infancy, particularly in speech and language, and sat independently from 15 months. He underwent unilateral orchidopexy for an undescended testis. He has mild relative macrocephaly and dysmorphic facial features including a triangular appearance to his lower face, a high-arched palate, abnormal pinnae with truncated upper helices, telecanthus with shortened palpebral fissures, infra-orbital hypoplasia, and a significantly recessed mid-face. He had a prominent pectus carinatum. Brain MRI revealed enlarged extra-axial spaces, irregular gyral pattern and mild cerebellar vermis hypoplasia. Ultrasound imaging of the heart, kidneys, and hips were normal. Routine karyotype and array-CGH were normal.

Individual 10

This 8-year-old female was born at 39 weeks gestation following an uncomplicated pregnancy. birth weight 1928g (<0.4th centile). She had hypotonia in the neonatal period and dysmorphic features were noted, including posteriorly-rotated ears, hypertelorism, a flat nasal bridge, and pectus excavatum. She had persistent growth problems, with height and weight consistently less than 3rd centile, and relative macrocephaly (OFC ~75th centile). She had recurrent respiratory syncytial virus infections and multiple episodes of otitis media, requiring myringotomy and adenoidectomy. She walked at 18-24 months, began talking

around 2.5 years with significant articulation problems. She had hypotonia, global developmental delay, educational difficulties, and achieved toilet training at 6 years. She had a single seizure associated with a urinary tract infection, and an episode of supraventricular tachycardia associated with a near syncopal event. Echocardiogram showed a mildly dilated ascending aorta (Z score 2.97). Brain MRI at age 13 months showed scattered, multifocal deep white matter T2 hyperintensities and a thin corpus callosum. There was mild lateral ventriculomegaly, with coarctation of the frontal horns. Renal ultrasound revealed a duplex collecting system requiring surgical repair. Her karyotype and array-CGH were normal.

Individual 11

This 9-year-old female was the second child of unrelated parents. The pregnancy was complicated by intrauterine growth restriction, increased nuchal translucency and echogenic bowel. Amniocentesis showed a normal karyotype. She was delivered by caesarean section at 38 weeks gestation, birth weight 2440g (~5th centile). She required mechanical ventilation from which she was slow to wean due to episodes of hypoventilation. She had a suspected seizure in the newborn period but there was no recurrence. She sat independently at 13 months. She had significant feeding difficulties and severe constipation for the first two years. Initially tube-fed, she progressed to the bottle, then developed severe gastroesophageal reflux, and consequently poor weight gain. At 21 months growth centiles were: length 9th centile, weight 2nd centile, OFC 50th centile. The feeding problems slowly improved through childhood and the constipation improved with medical management. She has astigmatism and delayed language skills, including phonics, but is conversational. Her hearing is satisfactory. She attends a mainstream school with one-to-one support. At school she performs approximately 3 years behind her chronological age. She is sociable but lacks stranger awareness, has a short attention span, and is becoming stubborn. Her height has

varied between the 9th and 25th centiles, with OFC consistently >50th centile. Her hair is thin and slow growing, especially in the temporal regions. Her dysmorphic features include plagiocephaly, low set dysplastic ears, long face, asymmetric cry, prominent nose, protruding columella, thin upper lip, and small teeth in her lower jaw. She appeared to have bilateral adducted thumbs when young with in-curled 3rd and 4th toes. MRI brain at 11 weeks was reported to be normal. She had a normal echocardiogram. Routine karyotype, subtelomeric screen, array-CGH, myotonic dystrophy, *SMN1* and *SHOC2* testing, were all normal. Her mother, who was intellectually normal and not dysmorphic but had slightly sparse scalp hair, was mosaic for the *PCGF2* mutation (approximately 21% in blood DNA).

Individual 12

This 3-year-old male is the child of non-consanguineous parents. The pregnancy was uneventful and he was a normal birth at 39 weeks gestation following induction of labour weight 2820g (10th centile). His length was 46cm (3rd centile) and OFC 35.0cm (50th centile). He had an apneic spell at 5 minutes post-delivery and was subsequently noted to have central hypotonia, feeding difficulties and cryptorchidism, for which he later underwent bilateral orchidopexy. Significant feeding and vomiting persisted in early infancy but he was otherwise healthy, and growth parameters were maintained. He had dysmorphic features including redundant neck skin, small satyr ears, small upturned nose, brachycephaly and a flat facial profile. At 9 months he was globally delayed with persistent hypotonia, and weight fell to the 3rd centile. He walked unsteadily at 29 months, with some improvement by 36 months. At 30 months his OFC was 51cm (50th centile). At 3 years he is still non-verbal but showing progress with social interactions, though anxious. Feeding problems persist but he is managed orally. Brain MRI at age 2.5 years showed relatively symmetric, multifocal T2 hyperintensities within the deep white matter bilaterally, most marked around atria of lateral

ventricles, features which were not evident on initial imaging at 10 months. Bilateral perisylvian polymicrogyria was present. A cavum septum pellucidum was noted, with absence of the midsection of the septum. Array-CGH identified a paternally inherited 1.22Mb duplication at 1q44. Extensive metabolic investigations, and tests for Prader-Willi syndrome and myotonic dystrophy, were normal.

Individual 13

This 18-year-old male is the first child of unrelated parents. He was born at 40 weeks gestation following an uncomplicated pregnancy, birth weight 3250g (25^{th} centile). Developmental delay was noted from 8 months of age and at 9 months his growth centiles were: weight 1^{st}, length 3^{rd}, OFC 25^{th}-50^{th}. He had difficulty feeding and frequent vomiting in the first 18 months of life, and first walked at 26 months. He had learning and social skill difficulties, including difficulties in writing and language skills, but a good visual memory. He repeated a school year at age 7 years. He had facial hypotonia and drooled at the age of 12 years. At 13 years 5 months his growth centiles were: weight 9^{th}-25^{th}, height 75^{th}, OFC 50^{th}-75^{th}. At 16 years his growth centiles were: weight 9^{th}-25^{th}, height 25^{th}-50^{th}. He continues to have difficulties with pronunciation and is hard to understand. He smiles normally but it is difficult to interpret his emotions. He is impulsive and avoids contact with sand or grass when barefoot. He has fine and gross motor clumsiness but no history of seizures. On examination he has sparse hair, small ear lobes, wide forehead, triangular face, prominent nose, facial hypotonia with dribbling, and a high arched palate. He has long, narrow fingers and toes, bilateral 2^{nd} to 5^{th} finger camptodactyly, and mild pes cavus. He has a long, narrow thorax with kyphoscoliosis. He has dental malposition with radicular resorption of secondary teeth. Brain MRI at age 8 years was reported to be normal. A bone age at 5 years 7 months was delayed. Echocardiography has identified slowly progressive

dilation of the aorta, currently treated with beta blockers. Routine karyotype, array-CGH, metabolic testing, *FBN1* and *DMPK* testing, and a NGS panel of 35 genes related to aortic syndromes, were all normal. Permission to publish clinical photographs was not granted.