Novel GLI3 mutation in a Greek–Cypriot patient with Greig cephalopolysyndactyly syndrome

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Greig cephalopolysyndactyly syndrome (GCPS) is typically characterized by preaxial or mixed preaxial and postaxial polydactyly with or without syndactyly and craniofacial features including hypertelorism and macrocephaly. Although GLI3 shows considerable pleiotropy, it is the only gene known to cause this particular phenotype. We report on a patient with GCPS caused by a novel GLI3 mutation. In addition, the patient had asymmetry of the calf muscles, most likely secondary to chronic hypertrophic radiculopathy. The GLI3 mutation identified by targeted Sanger sequencing analysis in our patient is predicted to lead to premature termination of translation. This is the first report of a Cypriot patient with a GCPS because of a novel GLI3 mutation. The report provides additional evidence in support of the rich variability in phenotypic expression, the mutational heterogeneity and ethnic diversity associated with this rare condition. Clín Dysmorphol 24:102–105

Keywords: Cyprus, GLI3, Greig cephalopolysyndactyly syndrome

Introduction

Greig cephalopolysyndactyly syndrome (GCPS; MIM #175700) is an autosomal dominant disorder typically characterized by preaxial or mixed preaxial and postaxial polydactyly with or without syndactyly and craniofacial features including hypertelorism and macrocephaly (Greig, 1926; Biesecker, 1993).

This typically fully penetrant disorder is characterized by greater interfamilial rather than intrafamilial variability in phenotypic expression (Biesecker, 1993). It is caused by deleterious mutations, deletions or chromosomal rearrangements involving the GLI3 (GLI-Kruppel family member 3) gene on chromosome 7p14.1 (Vortkamp et al., 1991; Wild et al., 1997).

Whereas GCPS is caused by loss-of-function GLI3 mutations spread across the gene (Vortkamp et al., 1991; Kalff-Suske et al., 1999), Pallister–Hall syndrome is another autosomal dominant disorder caused by gain-of-function truncating mutations within the middle third of this gene (Kang et al., 1997). Pallister–Hall syndrome is typically characterized by insertionally polydactyly, bifid epiglottis, anal abnormalities and hypothalamic hamartoma. Other phenotypes associated with GLI3 mutations include the acrocallosal syndrome (Elson et al., 2002) and non-syndromic polydactyly (Radhakrishna et al., 1997, 1999).

We report on a Cypriot patient with GCPS and calf muscle asymmetry caused by a novel GLI3 mutation.

Patients and methods

The proband, a 32-year-old, right-handed man, presented to the neurology clinic with an 8-month history of right gastrocnemius muscle pain associated with cramps. The symptoms were present mostly in the evening and were gradually worsening. The patient also noted enlargement of his right calf muscle. He was prescribed oral magnesium, which resulted in improvement of the muscle cramps. As he reported a history of polysyndactyly, he was referred to our Clinical Genetics Department for evaluation.

Review of the family history indicated that the proband was one of two siblings born to nonconsanguineous parents. His mother had a history of non-Hodgkin’s lymphoma diagnosed at the age of 30 years and a history of papillary thyroid carcinoma diagnosed at the age of 54. The family history was otherwise noncontributory. Review of the medical history showed that the index case was born with tetramelic polydactyly (Fig. 1a–c). He reported a history of motor delay and he started walking independently at the age of 22 months. He also had a history of a right-sided ‘lazy eye’, bilateral pes planus and myopia. There was no history of intellectual disability, paroxysmal events nor seizures.

On general examination at the age of 33 years, he was macrocephalic, with an occipitofrontal circumference of 60 cm. His height was 171.5 cm and his weight was 110 kg. He had a ‘dished-out’ face with a prominent jaw.
His ears were relatively simple, low-set with over-folded helices. He had a broad-based nose. His palate was normal. His patellae were present. He had significant asymmetry of the gastrocnemii muscles with a 5 cm girth difference between the two sides (Fig. 1d), the right being larger and firmer. He was relatively hirsute. He had evidence of preaxial and postaxial polysyndactyly of the hands with surgically removed nubbins on the postaxial side (Fig. 1a and b). He had duplicated thumbs, which were also reconstructed. He had duplicated halluces, bilateral second to third toe syndactyly and what looked like insertional polydactyly just above the fourth and the fifth toes bilaterally (Fig. 1c). On neurological examination, the patient was alert and oriented, with a mini-mental state examination score of 30/30. Cranial nerve examination indicated abnormal conjugate eye movements. Fundoscopy showed posterior vitreous detachment bilaterally. Both optic discs and maculae were unremarkable. Pupils were symmetrical, reactive to light. There was no nystagmus nor diplopia, no dysarthria nor dysphonia. There were also no hearing nor taste impairments. There was no facial weakness and trigeminal sensation was normal. The tongue was at midline with normal motion of the palate. Sternocleidomastoid and trapezius muscles were normal bilaterally. Motor examination indicated normal muscle tone. There was no significant weakness in the upper and lower limbs. Deep tendon reflexes were symmetric and plantar responses were flexor bilaterally. Sensory examination showed decreased pinprick sensation on his thighs and knees bilaterally. Vibration sensation was decreased on his knees bilaterally as well as on the pelvis. Joint position sense and cerebellar examination were normal. Gait and posture were
normal and he could tandem walk and walk on his heels and toes. Ophthalmology evaluation indicated mild myopia bilaterally. Best-corrected visual acuity was 10/10 from both eyes. Intraocular pressure was 14 mmHg on the right eye and 15 mmHg on the left eye. Visual field testing showed a superior scotoma in the right eye, although the visual field in the left eye was within normal limits.

An abdominal ultrasound scan indicated fatty infiltration of the liver and normal kidneys. An MRI of the lumbar spine showed a moderate posterior L4–L5 disc prolapse. MRI scan of both legs indicated asymmetry in the appearances of the calf muscles, the right being thicker, with no evidence of fatty infiltration, atrophy nor oedema. Normal or negative investigations included a brain computed tomography, MRI of the hips and thighs, flash electretroentigraphy, duplex ultrasound scan of the lower limb veins, full blood count, biochemistry, liver and thyroid function tests, clotting times, complex karyotype, karyotype and array-comparative genomic hybridization analyses.

Nerve conduction studies and electromyography of the lower limbs showed moderate chronic denervation in bilateral L5 and S1 myotomes, with additional signs of irritability in the form of fasciculations only in the right medial gastrocnemius muscle. These findings were consistent with chronic radiculopathies at these levels, and in particular, hypertrophic right S1 radiculopathy (Chang et al., 2008), which may explain in part the hypertrophy of the right calf. This was in keeping with his lumbar spine MRI findings.

The differential included acrocallosal and Bardet–Biedl syndromes, mosaicism and GCPS. In the absence of callosal abnormalities, and as preaxial polydactyly is very rare in Bardet–Biedl syndrome patients, we proceeded with targeted GLI3 mutation analysis. On the basis of the clinical features, targeted analysis of the 14 coding exons of GLI3 by bidirectional fluorescent Sanger sequencing was carried out.

Results

GLI3 sequencing indicated heterozygosity for the c.4653delC mutation in exon 14 of this gene. This solitary nucleotide deletion is predicted to result in the replacement of methionine with a termination codon at residue 1552 (p.Met1552*), leading to premature termination of translation. Parental samples were unavailable to establish whether this mutation had occurred as a de novo event.

Discussion

On the basis of the clinical presentation, the location within the gene and the type of the GLI3 mutation identified, we believed that our patient’s phenotype best fitted with that of GCPS. The GLI3 gene encodes a 1580 amino acid zing-finger transcription factor that is unusual in that it possesses a bi-functional mode of transcriptional action both as an activator and as a repressor of downstream targets in the sonic hedgehog pathway during mammalian skeletogenesis (Ruiz i Altaba, 1999; Johnston et al., 2005; Biesecker, 2006). More specifically, SHH is a key mediator of the zone of polarizing activity, a mesodermal area that controls anteroposterior limb patterning and that, alongside with GLI3 (one of its mainstream targets), specifically regulates digit identity and number (Pearse and Tabin, 1998; Debever et al., 2007).

In addition to skeletal development, there is evidence to support the importance of GLI3 in embryonic myogenesis (Gustafsson et al., 2002), postnatal muscle regeneration and neovascularization (Renault et al., 2013) as well as in the differentiation of other embryonic tissues such as the retinal pigment epithelium (Perron et al., 2003). More specifically, Renault et al. (2013) reported that in mice GLI3 deficiency resulted in severely delayed ischaemia-induced myogenesis and that GLI3-regulated postnatal myogenesis is necessary for muscle repair-associated angiogenesis. It seems that GLI3 plays an important role in adult muscle regeneration following ischaemic injury (Renault et al., 2013).

It is not entirely clear whether the ‘pathological’ muscle in our patient was actually the larger one; however, the presence of localized symptoms on the ipsilateral side in conjunction with the nerve conduction and electromyographic studies makes this assumption more probable. Overall, we believe that the calf muscle asymmetry was most likely secondary to chronic hypertrophic radiculopathy. Of note is that abnormalities in fibre ratios (type I fibre predominance) as well as the presence of vacuoles with inclusion bodies confirmed by electron microscopy have only been reported in the muscle biopsy of a female GCPS patient with a 7p13 microdeletion involving GLI3 (Kroisel et al., 2001).

This is the first report of a molecularly confirmed GCPS patient of Cypriot descent. The observations presented in this report provide additional evidence for the variability in phenotypic expression, the mutational heterogeneity and the ethnic diversity associated with GLI3-related phenotypes.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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