An emerging, recognisable facial phenotype in association with mutations in GLI-similar 3 (*GLIS3*)

Dimitri P[1*], De Franco E[2], Habeb AM[3], Gurbuz F[4], Moussa K[5], Taha D[6], Wales JKH[7], Hogue J[8], Slavotinek A[9], Shetty A[10], Balasubramanian M[11*]

[1]Department of Paediatric Endocrinology, Sheffield Children's NHS Foundation Trust, UK

[2]Institute of Biomedical and Clinical Science, University of Exeter Medical School, UK

[3]Paediatric Department, Prince Mohamed Bin Abdulaziz Hospital, NGHA, Al-Madinah, Kingdom of Saudi Arabia

[4]Ankara Pediatric Hematology Oncology Education and Training Hospital, Ankara, Turkey

[5] Paediatric Department, Maternity and Children Hospital, Jeddah, Kingdom of Saudi Arabia

[6] Division of Pediatric Endocrinology, Children's Hospital of Michigan, Wayne State University, Detroit, Michigan, USA

[7]Department of Paediatric Endocrinology and Diabetes, Lady Cilento Children's Hospital, South Brisbane, Queensland, Australia

[8]Department of Paediatrics, Madigan Army Medical Center, Tacoma, United States

[9]Institute for Human Genetics, University of California, San Francisco, United States

[10]Department of Paediatrics, Nevill Hall Hospital, Abergavenny, Wales, UK

[11]Sheffield Clinical Genetics Service, Sheffield Children's NHS Foundation Trust, UK

* Both authors contributed equally to this article

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Joint Corresponding Authors:

Professor Paul Dimitri, The Department of Paediatric Endocrinology, Sheffield Children's NHS Foundation Trust, Western Bank, Sheffield S10 2TH; Email- paul.dimitri@sch.nhs.uk; Tel- +44 114 271 7118

Dr M Balasubramanian, Consultant Clinical Geneticist, Sheffield Clinical Genetics Service, Sheffield Children's NHS Foundation Trust, Western Bank , Sheffield S10 2TH; E-mail: meena.balasubramanian@sch.nhs.uk; Tel- +44 114 2717025

ABSTRACT

Neonatal diabetes and hypothyroidism (NDH) syndrome was first described in 2003 in a consanguineous Saudi Arabian family where two out of four siblings were reported to have presented with proportionate IUGR, neonatal non-autoimmune diabetes mellitus, severe congenital hypothyroidism, cholestasis, congenital glaucoma, and polycystic kidneys. Liver disease progressed to hepatic fibrosis. The renal disease was characterised by enlarged kidneys and multiple small cysts with deficient cortico-medullary junction differentiation and normal kidney function. There was minor facial dysmorphism (depressed nasal bridge, large anterior fontanelle, long philtrum) reported but no facial photographs were published. Mutations in the transcription factor GLI-similar 3 (*GLIS3*) gene in the original family and two other families were subsequently reported in 2006. All affected individuals had neonatal diabetes, congenital hypothyroidism but glaucoma and liver and kidney involvement were less consistent features. Detailed descriptions of the facial dysmorphism have not been reported previously. In this report, we describe the common facial dysmorphism consisting of bilateral low-set ears, depressed nasal bridge with overhanging columella, elongated, upslanted palpebral fissures, persistent long philtrum with a thin vermilion border of the upper lip in a cohort of seven patients with *GLIS3* mutations and report the emergence of a distinct, probably recognisable facial gestalt in this group which evolves with age.

INTRODUCTION

Taha *et al*., in 2003 described the first consanguineous Saudi Arabian family in which 2 of 4 siblings had permanent neonatal diabetes associated with intrauterine growth retardation (IUGR), congenital hypothyroidism, facial anomalies, congenital glaucoma, hepatic fibrosis, and polycystic kidneys, described as NDH (Neonatal Diabetes and Hypothyroidism) syndrome [Taha *et al*., 2003]. Genome wide linkage analysis and sequencing of candidate genes performed on this family by Senee *et al*., in 2006 identified a homozygous frameshift mutation (c.1873dupC, previously reported as 2067insC) in *GLIS3* which is likely to result in transcript degradation by nonsense mediated decay (NMD). Both children with this mutation died in infancy. A child born subsequently in this family died of the same condition prior to confirmatory genetic testing [Habeb *et al*., 2012]. Senee *et al.,* 2006 described two further families with mutations in *GLIS3*. The first harboured a homozygous 426-kb deletion, which encompassed the *SLC1A1* gene and part of *GLIS3.* The affected offspring in the second family carried a homozygous 149-kb deletion that included a portion of *GLIS3* as well; the region common to both deletions mapped to the known start codon of *GLIS3.* Patients in both these families presented with a milder phenotype with the absence of renal or liver disease. Additional features in these and subsequent patients described include intrauterine growth retardation (IUGR), developmental delay and congenital glaucoma [Senee *et al*., 2006]. We recently published a large series of 12 patients worldwide with mutations in *GLIS3* thus expanding the clinical spectrum of abnormalities resulting from disruption of Glis3 function. These include exocrine pancreatic insufficiency, osteopenia, fractures with delayed fracture healing, craniosynostosis, hiatus hernia, congenital cardiac disease, splenic and pancreatic cysts and choanal atresia [Dimitri *et al*., 2015].

A facial phenotype has been cited in patients with mutations in *GLIS3* [Taha *et al*., 2003; Senee *et al*., 2006]. Facial dysmorphology previously described includes a large and squared shape of the face, with a thin curved nose and previous reports suggest that the facial features attenuate with growth. To date, the facial phenotype in patients with *GLIS3* mutations has not been described in detail and the degree of consistency in these features

between patients has not been reported. We thus reviewed the features in 7 patients with *GLIS3* mutations in whom written consent was obtained for publication of images to determine whether a consistent facial phenotype was present in patients with mutations in *GLIS3* and whether there was a recognisable facial gestalt.

MATERIALS AND METHODS

The study was conducted in accordance with the Declaration of Helsinki principles with informed parental consent given on behalf of children. Clinical information was provided by the referring clinicians, from clinical notes and subsequently using a questionnaire circulated to referring clinicians to gain further information. Consent was received from parents to publish photographs of facial features resulting from mutations in *GLIS3*.

Genetic analysis

GLIS3 gene mutations were identified by PCR amplification (primer sequences available on request) and sequence analysis of exons 1–11 by comparison with the reference sequence NM_001042413. Exon 1 is non-coding (the 5' UTR) and the start codon is located within exon 2. The effect of coding variants on the protein was investigated *in silico* using the bioinformatic tool ALAMUT (Interactive Biosoftware, Rouen, France). When failure of PCR amplification occurred, suggesting a homozygous deletion, parental samples were investigated by real-time quantitative PCR on an ABI 7900 (TaqMan assay with SYBR Green detection) and the copy number of exons 1–11 was determined by the $2^{-\Delta\Delta Ct}$ method.

Patient 4 was analysed for all the known neonatal diabetes genes using a targeted next generation assay [Ellard *et al*., 2013]. Mutations identified by this assay were confirmed by Sanger sequencing.

RESULTS

Table 1 describes the nucleotide and predicted protein changes of the *GLIS3* mutations identified in the patients studied. Table 2 describes the clinical features in the cohort reported here and the homozygous *GLIS3* mutation results (6 with deletions and one with a homozygous missense mutation). Patients 2 and 3 are siblings aged 7 and 2.4 years respectively. A consistent facial phenotype is seen at a younger age in patients *i.e.* bilateral low-set ears, depressed nasal bridge with upturned nose, prominent eyes, long philtrum with a thin vermilion border of the upper lip (Figure 1). With age, rather than being attenuated as described in previously published literature [Senee *et al.*, 2006], it appears to evolve into a more recognisable facial gestalt consisting of bilateral low-set ears, depressed nasal bridge with overhanging columella, elongated, upslanted palpebral fissures, persistent long philtrum with a thin vermilion border of the upper lip. This demonstrates the consistent facial phenotype in patients with *GLIS3* mutations. The oldest patient in this series is 7-years of age and appears to have a distinct facial gestalt (Patient 1).

Figure 2 demonstrates the progression of the facial phenotype with age in the same patient (Patient 5) from newborn, age 2 up to 4.3 years whilst Figure 3 demonstrates the striking resemblance of patients in this series to the images previously published by Senee *et al.*, 2006 (reproduced with relevant permission from Nature Publishing Group).

DISCUSSION

GLIS3 is a member of the GLI-similar zinc finger protein family encoding for a nuclear protein that maps to chromosome 9p24.3-p23 (OMIM *610192) [Kim *et al.*, 2003]. Mutations in *GLIS3* have been reported in association with Neonatal diabetes mellitus and hypothyroidism syndrome (NDH syndrome - OMIM #610199). *GLIS3* is expressed in early embryogenesis and plays a critical role as both a repressor and activator of transcription by interacting with a specific nucleotide sequence, known as the Gli response element (GLI-RE) in the promoter region of target genes [Kim *et al.*, 2003; Beak *et al.*, 2008]. Glis proteins contain a DNA binding domain consisting of five C_2H_2-type zinc finger motifs that are critical for nuclear localisation. Two major *GLIS3* transcripts from the 11 exon gene have previously

been described – 7.5 kb and smaller (0.8–2.0 kb); the 7.5-kb transcript is strongly expressed in pancreas, thyroid and kidney with smaller transcripts predominantly expressed in liver, kidney, eye, heart and skeletal muscle [Senee *et al*., 2006]. The cardinal feature of mutations in *GLIS3* is the concomitant presentation of neonatal diabetes and congenital hypothyroidism although recently a patient with a compound heterozygous mutation in *GLIS3* who did not develop hypothyroidism was reported [Dimitri et al., 2015].

Neonatal diabetes is likely to result from the disrupted interaction of *GLIS3* with key regulatory genes in pancreatic embryogenesis including *ONECUT1* and *NEUROGENIN3* (*NEUROG3*) [Kim *et al*., 2012; Poll *et al*., 2006; Kang *et al.,* 2009]. *GLIS3* expression persists beyond the embryonic period promoting beta cell proliferation and regulating insulin gene expression through binding to GLI-RE on the *INS* gene [Yang *et al*., 2013]. The proposed mechanism by which biallelic pathogenic variants in *GLIS3* causes a multi-system phenotype has been reported elsewhere [Senee *et al*., 2006; Dimitri *et al*., 2015].

GLIS3 is expressed during embryonic face development [Kim *et al*., 2003], which may help in part to explain the dysmorphic facial features observed in these patients. In studies of mouse embryos, whole mount *in situ* hybridisation from stage e6.5 to e14.5 was used to determine the temporal and spatial patterns of *GLIS3* expression during development. Facial expression of *GLIS3* was greatly increased form e11.5 and e12.5, and expression was mesenchymal in origin. *GLIS3* is also expressed in a dynamic pattern during eye development and at e8.75, *GLIS3* transcripts were evident in the region of the otic vesicles. Given the commonality of *GLIS3* expression with bone morphogenic proteins (BMPs) and other members of the TGFβ superfamily, interactions between BMPs and GLIS3 have been speculatively proposed as a possible underlying mechanism that is important for facial development. However, beyond this, there is no clear genetic interaction to explain the common facial dysmorphology observed in patients with mutations in *GLIS3*.

Table 2 provides clinical features in patients reported in this series demonstrating the multi-system phenotype associated with mutations in *GLIS3*. Patients 1-5 presented with mild

developmental delay with age at walking ranging between 13-21 months of age and first word between 15-18 months of age. Patients 6-7 did not have any evidence of developmental delay. As evident from the patient images, all the patients in this series appear to have a similar facial gestalt with bilateral low-set ears, prominent eyes with upslanted palpebral fissures, depressed nasal bridge, long philtrum and thin vermilion border of the upper lip (Figure 1). The facial features become more evident and established with advancing age (Figure 2). The patients reported in this series appear to have similar facial features as the patients reported by Senee *et al*., 2006. The two siblings from the third family in the reported series- NDH3-3 and NDH3-4, at age 6-months and 2 years respectively were said to have characteristic facial features (Figure 3). In our cohort, Patient 7 in particular appears to have a very similar facial dysmorphology to the patient at 6-months of age (NDH3-3- Senee *et al*., 2006) whilst Patient 1 in our series appears to share the same facial gestalt as the patient at 2-years of age (NDH3-4- Senee *et al*., 2006). Two of the patients in this series, patient 4 and 6, have mutations which affect only the *GLIS3* gene (an interstitial deletion and a missense mutation), supporting the hypothesis that the facial features observed result from the *GLIS3* defect alone and not from the deletion of contiguous genes. The patients not included in this series (including a 36-year old adult) that were previously reported have the same facial dysmorphism as described above but as we have been unable to obtain written consent from patient and their families, we have been unable to include this in this report [Dimitri et al., 2015].

In combination with the other features in NDH, it is important to recognise the facial gestalt in this group of patients in order to direct targeted genetic testing and provide genotype-phenotype correlation. This is particularly important as making an early diagnosis and instituting therapeutic intervention is crucial given the course of disease pathology in NDH syndrome. In conclusion, we report a common and emerging facial gestalt in patients with *GLIS3* mutations that are not attenuated with age. Further case reports of this nature are crucial in elaborating the common facial phenotype in NDH and attributing a consistent facial phenotype to this genetic condition.

FIGURE LEGENDS:

Figure 1: Patients 1-7 demonstrating common facial dysmorphism including bilateral low-set ears, depressed nasal bridge with upturned nose, long philtrum with thin vermilion border of the upper lip; with advancing age the facial phenotype becomes more distinctive with up-slanting, elongated palpebral fissures, long philtrum with thin vermilion border of the upper lip and more rounded appearance to the face.

Figure 2: Patient 5 showing evolving facial phenotype from newborn to age 4.3 years.

Figure 3: Images of Patient 7 and 1 in comparison to facial dysmorphism of patients published by Senee *et al*., 2006 [NDH3-3 aged 6-months and NDH3-4 aged 2 years; reproduced with relevant permission from Nature Publishing Group].

CONFLICTS OF INTEREST: J Hogue: The views expressed are those of the author and do not reflect the official policy of the Department of the Army, the Department of Defense or the U.S. Government. All others: The authors have no conflicts of interest relevant to this article to disclose.

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TABLES:

Table 1: Mutations and nucleotide changes associated relating to mutations in *GLIS3*

Patient number	Exon	Mutation	Nucleotide change	*In silico* prediction	Current age (years)
1	1-2	exons 1-2 del/exons 1-2 del	c.-?_388+?del/c.-?_388+?del	Pathogenic	7.1
2	1-4	exons 1-4 del/exons 1-4 del	c.-?_1710+?del/c.-?_1710+?del	Pathogenic	7.0
3	1-4	exons 1-4 del/exons 1-4 del	c.-?_1710+?del/c.-?_1710+?del	Pathogenic	2.4
4	5-9	exons 5-9 del/exons 5-9 del	c.1711-?_2473+?del/c.1711-?_2473+?del	Pathogenic	4
5	9-11	exons 9-11 del/exons 9-11 del	c.2298-?_2657+?del/c.2298-?_2657+?del	Pathogenic	Died at 6 years
6	4	p.His561Tyr/p.His561Tyr	c.1681C>T/c.1681C>T	Pathogenic	5.3
7	1-2	exons 1-2 del/exons 1-2 del	c.-?_388+?del/c.-?_388+?del	Pathogenic	5

Table 2: Clinical features presenting in patients with *GLIS3* mutations

Patient number	Current age (years)	Birth weight (g)	IUGR*	Gestation (weeks)	Ethnicity	Gender	Consanguineous	PND Onset~ (days)	Congenital Hypothyroidism	Liver disease	Kidney disease	Exocrine pancreatic disease	Congenital Glaucoma	Skeletal Disease	Developmental delay	Facial dysmorphism	Other features
1	7.1	1170	Yes	35	Bangladeshi	Female	Yes	3	Yes	Yes	Yes	Yes	No	Osteopenia, fractured ribs, scoliosis	Yes, mild	Yes	No
2	7.0	1430	Yes	35	Caucasian	Male	No	4	Yes	Yes	Yes	Yes	No	No	Yes, mild	Yes	BSNHL^ PDA Pancreatic cyst
3	2.4	2020	Yes	38	Caucasian	Male	No	2	Yes	Yes	Yes	Yes	No	No	Yes, mild	Yes	Pancreatic & Splenic cyst BSNHL
4	4	1750	Yes	34	Arab	Female	Yes	2	Yes	Yes	Yes	No	No	No	Yes, mild	Yes	No
5	Died at 6 years	1530	Yes	37	African-American	Female	Unknown	7	Yes	Yes	Yes	No	Yes	Sagittal craniosynostosis	Yes, mild	Yes	No
6	5.3	973	Yes	31	Kurdish	Male	Yes	31	Yes	Yes	Yes	No	Yes	No	No	Yes	PDA$
7	5	1730	Yes	39	Arab	Male	Yes	19	Yes	No	Yes	No	Yes	No	No	Yes	Ostium secundum ASD#

*Birth weight < 10th centile for gestational age; ~ND – Permanent neonatal diabetes; ^BSNHL- Bilateral sensori-neural hearing loss; $PDA- Patent ductus arteriosus; #ASD – Atrial Septal Defect