Case Report and Review of the Literature: Congenital Diaphragmatic Hernia and Craniosynostosis, a Coincidence or Common Cause?

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Congenital diaphragmatic hernia (CDH) is a life-threatening birth defect that presents either as an isolated diaphragm defect or as part of a complex disorder with a wide array of anomalies (complex CDH). Some patients with complex CDH display distinct craniofacial anomalies such as craniofrontonasal dysplasia or craniosynostosis, defined by the premature closure of cranial sutures. Using clinical whole exome sequencing (WES), we found a BCL11B missense variant in a patient with a left-sided congenital diaphragmatic hernia as well as sagittal suture craniosynostosis. We applied targeted sequencing of BCL11B in patients with craniosynostosis or with a combination of craniosynostosis and CDH. This resulted in three additional BCL11B missense mutations in patients with craniosynostosis. The phenotype of the patient with both CDH as well as craniosynostosis was similar to the phenotype of previously reported patients with BCL11B missense mutations. Although these findings imply that both craniosynostosis as well as CDH may be associated with BCL11B mutations, further studies are required to establish whether BCL11B variants are causative mutations for both conditions or if our finding was coincidental.

Keywords: case report, craniosynostosis, congenital diaphragmatic hernia (CDH), BCL11B, craniosynostosis syndromes

INTRODUCTION

The genetic etiology of congenital diaphragmatic hernia (CDH) is complex. Structural variants, small and large insertions or deletions and single-nucleotide variants (SNVs) in over a 100 genes have been associated with CDH (1). Only a few of these genes are mutated recurrently in multiple individuals and even then the phenotype can differ largely due to incomplete penetrance. CDH has a prevalence of 2.3–2.7 per 10,000 live births (2–4) and can present as isolated CDH or in association with additional congenital anomalies (non-isolated CDH or complex CDH), as seen in ~40 (5) to 49% (6) of the cases. Complex CDH may present as part of a recognizable genetic syndrome, chromosome abnormality, or a collection of major congenital malformations. One of the less common malformations in patients with CDH are craniofacial anomalies and in particular craniosynostosis.
Although less common in syndromes associated with complex CDH, several studies have described syndromes such as Apert’s syndrome and Craniofrontonasal syndrome, that include both CDH as well as craniosynostosis as cardinal or relatively common features (7–22). Craniosynostosis, a developmental disorder defined by the premature fusion of one or more sutures, has a prevalence of 7.2 per 10,000 live births (23). It can be divided into non-syndromic and syndromic craniosynostosis, with syndromic craniosynostosis being characterized by additional congenital anomalies, such as limb anomalies and neurodevelopmental delays (24, 25). As part of our standard clinical care, all patients diagnosed with craniosynostosis are offered targeted genetic analysis (26). In a female patient with both CDH as well as craniosynostosis, we observed a genetic variant of the B cell leukemia 11b gene (BCL11B; OMIM 606558) resulting in an amino acid change at position 667 [p.(Gly667Glu)].

Searching the literature for BCL11B mutations we found several case reports. First, a case report described a male patient with a heterozygous de novo missense BCL11B mutation (p.Asn441Lys), who presented with severe combined immunodeficiency as well as neurological, dermal and facial dysmorphisms including hypertelorism, short palpebral fissures and micrognathia (27). Second, a study reported on 13 patients with heterozygous germline mutations in BCL11B (28). Most of these patients presented with neurodevelopmental disorders and immunodeficiency with reduced type 2 innate lymphoid cell and were carriers of loss of function mutations in BCL11B. However, in the most severely affected patient (patient EII-I) a missense BCL11B mutation p.(Asn807Lys) was found. This patient had a similar phenotype as compared to the first patient described by Punwani et al. (27) including a myopathic facial appearance, hypertelorism and small palpebral fissures. Neither study reported on the presence of craniosynostosis or CDH although craniofacial anomalies were described for patient EII-I and are apparent for the patient described by Punwani et al. (27). However, we previously discovered a de novo BCL11B missense mutation in exon 1 which encodes for [p.(Arg3Ser)] in a male patient with unilateral coronal suture craniosynostosis (29). In addition, a de novo BCL11B missense mutation in exon 4 [p.(Arg350Cys)] was reported in a patient who presented with CDH, an abnormal optic nerve, increased intraocular pressure and scoliosis (30). These findings suggest that both craniosynostosis as well as CDH may be associated with BCL11B missense mutations.

For this report, we selected patients who had undergone genetic testing with craniosynostosis or combined CDH and craniosynostosis from the Erasmus MC- Sophia Children’s hospital. This search resulted in three additional BCL11B missense variants. In this report we present the clinical reports of these four new patients with BCL11B variants and a brief review of disorders that are characterized by both CDH as well as craniosynostosis. Informed consent to publish case descriptions and images was obtained from patients, and/or their parents if applicable. All genetic information is provided according to the HGNC guidelines (31).

CLINICAL REPORTS

Patient A
Patient A [NM_138576.3(BCL11B):c.2000G>A, p.(Gly667Glu)], a 30 year old female, is the third child of healthy non-consanguineous parents, born at 41 weeks of gestation with a birth weight of ∼2,200 g, following an uncomplicated pregnancy. Shortly after birth, the patient developed severe respiratory insufficiency, resulting in apnea and asystole. After resuscitation, the patient was transferred to our tertiary care center. She presented with left-sided CDH, resulting in respiratory insufficiency and pulmonary hypertension, for which she underwent surgery the second day after birth. She was weaned off respiratory support after 2 weeks. She showed several dysmorphic features including, slight ocular proptosis, hypertelorism, down slanting palpebral fissures, ptosis, arched eyebrows, and syndactyly of the second and third toes of both feet (Figures 1A–C). In addition, she suffered from feeding difficulties and gastroesophageal reflux due to pyloric stenosis, which was surgically corrected at the age of 2 months. She remained hypotonic and developed psychomotor delays during the first year of life. At the age of 11 months she was diagnosed with progressive sagittal suture craniosynostosis, for which she underwent surgery at the ages of 14 and 16 months. During surgery part of the calvarium looked abnormal and was sent for pathological investigation, revealing a capillary and cavernous hemangioma. She developed divergent strabismus, latent nystagmus, hypermetropia and astigmatism, and suffered from recurrent episodes of pneumonia, sinusitis and rhinitis. She showed no signs of severe cognitive impairment.

Patient B
Patient B [NM_138576.3(BCL11B): c.1744G>A, p.(Gly582Ser)], a 16 year old male, is the second child of unaffected non-consanguineous parents. He was born at 40 weeks of gestation, with a birth weight of 3,710 g. He presented with craniosynostosis of the sagittal suture and both lambdoid sutures, for which he underwent surgery at the age of 6 months. In addition, he suffered from an incomplete left-sided unilateral cleft lip including the alveolar arch, which was surgically corrected in three stages (at the ages of 6 months, 6, and 11 years). He showed several dysmorphic features, including frontal bossing, down-slanting palpebral fissures, hypotelorism, mild webbing of the neck, and hyperpigmentation on the left shoulder (Figures 1D–I). Magnetic Resonance Imaging (MRI) of the brain at the age of 8 years showed a Chiari I malformation. Ophthalmological assessment revealed astigmatism and myopia. He developed a proportionate short stature of −2.5 to 2 SD with delayed bone age, for which puberty was postponed using leuprorelin and letrozole. He had no neurodevelopmental delays. Motor and speech development were within normal range although he required speech therapy and physical therapy. He developed Kawasaki disease at the age of 7 years, which responded well to intravenous immunoglobulins. At the age of 16 years he underwent a second cranial vault surgery including biparietal expansion and occipital decompression. He did not suffer from frequent infections.
Patient C

Patient C [NM_138576.3(BCL11B):c.2018C>G, p.(Pro673Arg)], a 30 year old female, is the child of non-consanguineous phenotypically normal parents. A sibling and paternal half-sibling are both healthy. She presented with left-sided unicoronal craniosynostosis, for which she underwent surgery at the age of 14 months. She developed no neurodevelopmental delays and did not suffer from recurrent infections to our knowledge, although the cranial vault operation was postponed twice due to upper airway infections. She required glasses from the age of 8 years onwards. She did not display any evident dysmorphic features with the exception of mild vertical orbital dystopia (Figures 1J–L).

Patient D

Patient D [NM_138576.3(BCL11B):c.1265C>T, p.(Pro422Leu)], a 2 year old male, is the second child of unaffected non-consanguineous parents. In addition, the patient has two unaffected paternal half-siblings. He was born with a birth weight of 4,230 g at 41.3 weeks of gestation. He was born with the umbilical cord wrapped around his neck but recovered well after stimulation and did not require oxygen support. The pregnancy was otherwise uncomplicated. He presented with craniosynostosis of the sagittal suture at the outpatient clinic at the age of 3 months for which he underwent spring-assisted surgery at the age of 6 months. He had no neurodevelopmental delays and did not suffer from recurrent infections. Dysmorphic features were mild and included thick alae nasi and mild retrognathia with an overbite (Figures 1M–R).

OVERVIEW OF PATIENTS WITH BCL11B MISSENSE MUTATIONS AND VARIANTS

An overview of all patients described above, including genetic and phenotypic information of each patient can be found in Table 1. In addition, it shows the previously described patients with BCL11B missense mutations. Phenotypical features vary heavily among patients. The patient, reported by Goos et al. (29), displayed a similar phenotype to our Patient C, with both patients presenting with coronal suture synostosis without neurodevelopmental delays or other severe associated features. Patient A and patient B, are more similar to patients reported by Punwani et al. (27) and Lessel et al. (28). They have a more severe phenotype, presenting with a combination of multi-sutural craniosynostosis, CDH and cleft. In an additional study, one patient with a de novo BCL11B missense mutation [p.(Arg350Cys)] was reported to have presented with complex...
### Case descriptions of patients with BCL11B missense variants: genetic and phenotypical features.

| Patient | Nucleotide change* | Protein change | Exon | CADD score‡ | Type of variant | Mode of inheritance | Phenotype | **Gastro-Intestinal** | **Cognition, behavior, and motor development** | **Dysmorphic features** |
|---------|--------------------|----------------|------|-------------|-----------------|---------------------|-----------|-----------------------|---------------------------------------------|------------------------|
| Patient A | c.2000G>A | p.(Gly667Glu) | 4   | 18.89       | Missense**      | Maternal            | Left-sided | –                     | –                             | +, complex            |
| Patient B | c.1744G>A | p.(Gly582Ser) | 4   | 3.944       | Missense**      | Paternal           | –                     | –                             | – | NR                   |
| Patient C | c.2018C>T | p.(Pro673Arg) | 4   | 22.7        | Missense Missense | Maternal Maternal | –                     | –                             | NR                   |
| Patient D | c.1265C>T | p.(Pro422Leu) | 4   | 22.6        | Missense Missense | Maternal Maternal | De novo De novo | –                     | –                             | NR                   |
| Goos et al. (29) | c.7C>A | p.(Arg3Ser) | 1   | 24.5        | Missense Missense | De novo De novo | –                     | –                             | NR                   |
| Lessel et al. (28) | c.2421C>G | p.(Asn807Lys) | 4   | 25.5        | Missense Missense | De novo De novo | –                     | –                             | NR                   |
| Punwani et al. (27) | c.1323T>G | p.(Asn441Lys) | 4   | 24.8        | Missense Missense | De novo De novo | –                     | –                             | NR                   |
| Longoni et al. (30) (T45) | c.1048T | p.(Arg350Cys) | 4   | 31         | Missense Missense | De novo De novo | –                     | –                             | NR                   |

| Nucleotide change* | Protein change | Exon | CADD score‡ | Type of variant | Mode of inheritance | Phenotype | **Gastro-Intestinal** | **Cognition, behavior, and motor development** | **Dysmorphic features** |
|-------------------|---------------|------|-------------|-----------------|---------------------|-----------|-----------------------|---------------------------------------------|------------------------|
| Patient A          | c.2000G>A     | 4     | 18.89       | Missense**      | Maternal            | Left-sided | –                     | –                             | +, complex            |
| Patient B          | c.1744G>A     | 4     | 3.944       | Missense**      | Paternal           | –                     | –                             | – | NR                   |
| Patient C          | c.2018C>T     | 4     | 22.7        | Missense Missense | Maternal Maternal | –                     | –                             | NR                   |
| Patient D          | c.1265C>T     | 4     | 22.6        | Missense Missense | Maternal Maternal | De novo De novo | –                     | –                             | NR                   |
| Goos et al. (29)   | c.7C>A        | 1     | 24.5        | Missense Missense | De novo De novo | –                     | –                             | NR                   |
| Lessel et al. (28) | c.2421C>G     | 4     | 25.5        | Missense Missense | De novo De novo | –                     | –                             | NR                   |
| Punwani et al. (27) | c.1323T>G    | 4     | 24.8        | Missense Missense | De novo De novo | –                     | –                             | NR                   |
| Longoni et al. (30) (T45) | c.1048T | p.(Arg350Cys) | 4     | 31         | Missense Missense | De novo De novo | – | NR                   |

(Continued)
|          | Patient A | Patient B | Patient C | Patient D | Goos et al. (29) (E:II-1) | Lessel et al. (28) (E:II-4) | Punwani et al. (27) | Longoni et al. (30) (T45) |
|----------|-----------|-----------|-----------|-----------|--------------------------|--------------------------|-----------------------|--------------------------|
| Ptosis   | +         | +         | –         | –         | –                        | NR                       | NR                    | NR                       |
| Downslant of the eyes | +         | +         | –, upslant | –         | –                        | NR                       | NR                    | NR                       |
| Eversion of the lower eyelids | +         | +         | –         | –         | –                        | NR                       | NR                    | NR                       |
| Vertical orbital dystopia | –         | –         | +         | –         | +                        | NR                       | NR                    | NR                       |
| Retrognathia | –         | +, mild   | +, mild   | +, mild   | –                        | Micrognathia             | Micrognathia           | NR                       |
| **Dysmorphic features** |          |           |           |           |                          |                          |                       |                          |
| Dermatological anomalies | Eczematous skin | Hirsutism | –         | –         | –                        | Severe congenital erosive dermatitis | Erythematous psoriasisform dermatitis hirsutism | NR                       |
| Ear anomalies | Low-set ears | Low-set ears, fleshy upturned earlobes | –         | –         | –                        | Posteriorly rotated ears | Ear tag               | NR                       |
| Other | Deep-set eyes | Deep-set eyes | Mild asymmetry of the eyes; deep-set eyes; periorbital fullness | Deep-set eyes | -                        | Bitemoral hollowing, hypoplastic midface | Abnormal nasal creases, loose skin folds | NR                       |
| **Extremities** |          |           |           |           |                          |                          |                       |                          |
| Anomalies of the hands | Brachydactyly (bilateral) | Short fifth digits (bilateral) | –         | –         | –                        | Non-congenital syndactyly | NR                    | NR                       |
| Anomalies of the feet | Syndactyly of the second and third toes (bilateral) | Syndactyly of the second and third toes (bilateral) | –         | –         | –                        | Non-congenital syndactyly | NR                    | NR                       |
| **Neurological** |          |           |           |           |                          |                          |                       |                          |
| Hypotonia | +         | –         | –         | –         | –                        | –                        | +                     | +                        | NR                       |
| Unstable gait | +         | –         | –         | –         | –                        | –                        | +                     | NR                       |
| **Ophthalmological** |          |           |           |           |                          |                          |                       |                          |
| Refractive error | Hypermetropia, astigmatism | Myopia, astigmatism | + (type unknown) | Not tested | Hyperopia | –                        | NR                    | NR                       |
| Strabismus | –         | –         | –         | –         | Not tested | –                        | NR                    | NR                       |
| Other | Nystagmus | –         | –         | –         | –                        | –                        | NR                    | NR                       |
| **Dental** |          |           |           |           |                          |                          |                       |                          |
| Dental anomalies | –         | –         | –         | Overbite | –                        | Atypical teeth           | Neonatal teeth        | NR                       |
| **Immune system function** |          |           |           |           |                          |                          |                       |                          |
| Frequent infection | +         | –         | –         | –         | –                        | Low TREC at birth        | No TREC at birth       | NR                       |

(Continued)
CDH (30). All cases summarized above have missense variants in exon 4 with the exception of the patient reported by Goos et al. (29), who had a \textit{de novo} missense mutation of exon 1.

\section*{DISCUSSION}

In this study, we present four new patients with missense variants in \textit{BCL11B} (Patient A: \textit{p.Gly667Glu}); Patient B: \textit{p.Gly582Ser}); Patient C: \textit{p.Pro673Arg}); Patient D: \textit{p.Pro422Leu}). Patient A displayed both CDH as well as craniosynostosis of the sagittal suture. The other three patients were diagnosed with craniosynostosis without a diaphragm defect. To our knowledge, this is the second study to report on craniosynostosis in patients with \textit{BCL11B} mutations and the first to report on the co-occurrence of CDH and craniosynostosis in a patient with a missense \textit{BCL11B} variant. Based on our current cohort of patients with \textit{BCL11B} missense variants, the phenotype associated with \textit{BCL11B} mutations is highly variable. Clinical features range from isolated craniosynostosis to CDH, severe immunological deficiencies and neurodevelopmental delays (Table 1).

\textit{BCL11B} has a key function in fetal development and is involved in a multitude of systems and pathways (35). In line with this, many patients with \textit{BCL11B} missense mutations are reported to suffer from a wide array of clinical anomalies (27, 28). In addition to altered craniofacial development, mutations, mostly loss of function mutations in \textit{BCL11B}, have been reported to affect neurodevelopment as well as the development of the immune system, skin and the teeth (27–29, 36–49). In mice models, \textit{BCL11B} is key in regulating suture patency, with a single disrupted allele causing synostosis (36). Goos et al. further investigated \textit{BCL11B} variants in relation to craniosynostosis (29). A mouse model confirmed that the \textit{de novo} \textit{BCL11B} missense mutation (\textit{p.Arg3Ser}) in their patient could have a causative effect on the development of craniosynostosis. Furthermore, they found several rare variants in a British cohort of craniosynostosis patients. However, these variants were dismissed as polymorphisms because of low impact or because they were inherited from a healthy parent. The authors suggest that only a specific subset of \textit{BCL11B} mutations may cause craniosynostosis.

Our patients inherited the \textit{BCL11B} variants from phenotypically normal parents. This could suggest incomplete penetrance, which may be similar to other craniosynostosis syndromes such as TCF12 and SMAD 6-related craniosynostosis and could potentially complicate genetic counseling (32, 50–52). Notably, patients reported in previous \textit{BCL11B} studies mainly were carriers of \textit{de novo} mutations. Although Combined Annotation Dependent Depletion (CADD) scores in our patients vary, patient A, C and D are in the same range as CADD scores of variants previously reported (27–29, 33, 34). Although a low CADD score could potentially indicate a benign variant, it is remarkable that four patients have now presented with craniosynostosis bearing a missense variant in exon 4 of \textit{BCL11B}. Expression of this missense variant in blood excluded non-sense mediated RNA decay in patients A and B. Hence a dominant
| Clinical disorders with autosomal dominant inheritance pattern | Gene (MIM number) | Phenotype MIM number | Chromosome | CS* | CDH‡ | Key clinical features | Authors reporting on CDH and/or CS |
|---|---|---|---|---|---|---|---|
| Apert syndrome | FGFR2 (176943) | 101200 | 10q26.13 | Key feature, multisutural, progressive | Rare (six cases of CDH and one case of diaphragm agenesis) | – Symmetric syndactyly of hands and feet – Midface hypoplasia | Kaur 2019 Dap 2019 Kosinski 2016 Sobaih 2015 Builamante 2011 Wallis-Crespo 2004 Witters 2000 |
| Kabuki syndrome (focus on type 1) | KMT2D (602113) | 147920 | 12q13.12 | Occasional | Relatively common | – Characteristic facial features: long palpebral fissures, everted lower eyelids, ptosis, arched eyebrows, blue sclera, cupped ears, micrognathia – Short stature, microcephaly – Intellectual disability (mild to moderate) – High/cleft palate and dental anomalies – Brachydactyly, clinodactyly, persistent fetal pads – Cardiac anomalies | Scott 2021 Topa 2017 Martinez-Lopez 2010 David 2004 Geneviève 2004 Van Haelst 2000 |
| CEBALID** syndrome (MN1 C-terminal truncation syndrome) | MN1 (156100) | 618774 | 22q12.1 | Reported in three patients out of 25 identified patients identified to date | Reported in two patients out of 25 identified patients identified to date | – Characteristic facial features: midface hypoplasia, downslanting palpebral fissures, hypertelorism, exophthalmia, low-set ears, a short upturned nose – Intellectual disability, hypotonia, delay in motor development – Hearing loss – Structural brain anomalies | Mak 2020 |
| Chromosome 22q11.2 deletion syndrome | - | 145410; 188400; 192430; 600594; 601279; 601755; 602054; 609000 | 22q11.2 | Rare feature (may include CDC45 pathogenic variant in remaining allele) | Rare feature | – Highly variable phenotype (ranging from minor abnormalities to major structural defects) – Cardiovascular anomalies – Cleft palate – Cognitive impairment – Short stature – Characteristic facial features: hypoplastic nasal alae, wide nasal bridge, short palpebral fissures, low-set, small ears – Nasal speech | Unolt 2020, 2017 McDonal-McGinn 2005 |
| SPECC1L- related syndromes | SPECC1L (614140) | 145410 145420 600251 | 22q11.23 | Occasional | Occasional occurrence | – Characteristic facial features: hypertelorism, a wide, short nose, ptosis and retrorhaphia – Cleft lip/palate – Clinical features include branchial fistulas, omphalocele, genitourinary anomalies | Wild 2020 Bhoj 2019 Kruszka 2015 Robin 1995 |
| Gene (MIM number) | Phenotype MIM number | Chromosome | CS* | CDH‡ | Key clinical features | Authors reporting on CDH and/or CS |
|-------------------|----------------------|------------|-----|------|-----------------------|----------------------------------|
| 7q11.23 Duplicationsyndrome | 609757 | 7q11.23 | Rare | Rare | - Variable expression, with incomplete penetrance  
- Characteristic facial features: prominent forehead, hypertelorism, high and broad nose, straight eyebrows, and thin lips  
- Cognitive impairment and intellectual disability  
- Epilepsy | Morris 2015  
Van der Aa 2009  
Torniero 2008  
Kriek 2006 |
| X-Linked Craniofrontonasal syndrome (XLD) | EFNB1 (300035) | Xq13.1 | Common feature, often either unilateral or bilateral coronal CS | Relatively common/occasional | - More severe phenotype in females  
- Characteristic facial features: hypertelorism, craniofacial asymmetry, webbed neck, bifid tip of the nose, a broad nasal bridge  
- Clinodactyly of ≥ 1 digit  
- Longitudinal splitting/ridging of nails | Hogue 2010  
Kawamoto 2007  
Vasudevan 2006  
Twigg 2004 & 2006  
Brooks 2002  
McGaughran 2002  
Hurst 1988  
Morris 1987 |
| Cornelia de Lange syndrome | NIPBL (608667, AD)  
SMC1A (300040, XLD) | 122470  
300590 | 5p13.2  
Xp11.22 | Described for NIPBL variant; and for SMCA1 | Key feature  
- Characteristic facial features: thick, arched eyebrows or synophrys, long/smooth philtrum, short nose, thin upper vermillion  
- Limb defects  
- Intellectual disability  
- Growth retardation  
- Hirsutism | Desai 2021  
Xu 2018  
Gupta 2020 |
| Simpson-Golabi-Behmel syndrome, Type 1 (XLR) | GPC3 (300037) | 312870 | Xq26.2 | 3 case reports | Occasional | Schirwani 2019  
Villarreal 2013  
Li 2009 |

This table presents an overview of clinical disorders in which both craniosynostosis and congenital diaphragmatic hernia have been reported more than once. *CS, craniosynostosis; ‡CDH, congenital diaphragmatic hernia; **CEBALID, craniofacial defects, dysmorphic ears, structural brain abnormalities, expressive language delay, and impaired intellectual development; XLD, X-linked dominant; XLR, X-linked recessive.
| Gene | Phenotype | Chromosome | CS* | CDH‡ | Key clinical features | Authors reporting on CDH and/or CS |
|------|-----------|------------|-----|------|-----------------------|----------------------------------|
| TWIST1 (601622) | 7p21.1 | Key feature, often bicoronal CS | One case, unclear if co-occurrence of CDH is coincidental. Mouse models suggest a possible role for TWIST1 in development of the diaphragm. | – Characteristic facial features: ptosis, downward slanting palpebral fissure, depressed nasal bridge, facial asymmetry | Piard 2012 |
| 9p deletion syndrome | 9p | Key feature: metopic CS | One case described for 9p deletion syndrome | – Characteristic facial features: hypotelorism, upslanting palpebral fissures, low-set ears, malformed ears, long philtrum | Alfl 1973 |
| 15q24 deletion syndrome | 15q24.2 | 1 case report | Four reports | – Characteristic facial features: high forehead, facial asymmetry, downslanting of eyes, hypertelorism, and a long smooth philtrum, ear malformations | Ng 2011, Van Esch 2009, Sharp 2007, Bettelheim 1998 |
| DPF2-related Coffin–Siris syndrome | DPF2 (601671) | 11q12.1 | At least two out of a total of 10 reported patients (one patient was stated to have trigonocephaly but no x-ray was performed) | One patient described out of a total of 10 reported patients. | Knapp 2019, Vasileiou 2018 |
| - | DSC2 (125645) | - | One report of a patient with multisutural CS and CDH | One report of a patient with multisutural CS and CDH | Das 2019 |
| TGFBR1 (190181) TGFBR2 (190182) | 6q22.33 3p24.1 | Multiple cases reported | One report | – Aortic and arterial aneurysms – Characteristic facial features: hypertelorism, downslant of the eyes – Cleft palate, bifid uvula – Pectus anomalies – Arachnodactyly | Lobaton 2021, Loeys 2005 |
| Gain of function of RARB | RARB | One report of a patient with CS | Multiple patients with diaphragmatic hernia | Thirteen cases have been reported in total. Clinical features include microphthalmia and anophthalmia, sclerocornea, and coloboma, as well as cardiac anomalies, and malrotation of the bowel | Srour 2016 |

*CS, craniosynostosis; ‡CDH, congenital diaphragmatic hernia.
negative effect or an altered function of the mutated protein could be an explanation of the more severe phenotype.

To our knowledge, only one previous study reported on a patient with a CDH with a BCL11B missense mutation [p.(Arg350Cys); CADD score: 31] (30). We now report on a second patient with a BCL11B missense variant in exon 4 with CDH. Although further studies remain necessary to assess if BCL11B is a causative factor in this CDH phenotype the fact that BCL11B is involved in pathways that overlap with pathways that have been previously linked to CDH support this observation. BCL11B likely interacts with a CDH causative gene (NR2F2) as well as with other CDH candidate genes (CREBBP, EP300, CHD4) (1, 53). The genetic etiologies of both CDH and craniosynostosis are highly complex and definitive genetic pathways remain to be further elucidated. Although the combination of CDH and craniosynostosis is rare, the fact that multiple syndromes are associated with both craniosynostosis and CDH suggests a possible overlap between craniosynostosis and CDH pathways. For instance, NR2F2, a causative CDH gene with which BCL11B has been shown to interact (54, 55), appears to have a function in mesenchymal cell differentiation in embryogenesis including a regulatory function in myogenesis, chondrogenesis, and osteogenesis (56). These processes are disturbed in CDH and craniosynostosis. NR2F2 also regulates Runx2, which has been reported to be overexpressed in some types of craniosynostosis, and has a function in the retinoic acid signaling pathway regulation, which is key in the development of CDH (1, 56–61). Further studies are needed to establish definitive CDH and craniosynostosis pathways and to assess if these pathways are interlinked.

The phenotype of our patients is similar to previously reported clinical phenotypes of patients with BCL11B missense mutations, which included craniosynostosis and CDH (6, 27, 28). Therefore, it is implied that both CDH and craniosynostosis may be features associated with BCL11B missense mutations. Further functional studies are required to assess if these variants are coincidental findings or if they indeed have a causative effect on craniosynostosis and CDH.

Patients with missense mutations in BCL11B appear to be affected more severely than patients with loss of function or other types of BCL11B mutations (28). In a previous study, the most severely affected patient carried a missense mutation in a zinc-finger domain in exon 4 (patient EII-I, Table 1) (28). BCL11B encodes for a zing finger protein transcription factor and function both as a transcriptional activator as well as a repressor (35, 62). Lessel et al. hypothesized that these missense mutations may not only lead to a loss of DNA binding but also to novel DNA binding sites in other genes (28). Similar observations were made in a previous study, which demonstrated that a mutation p.(Asn441Lys) led to both decreased binding of BCL11B to original target DNA sites as well as to the promotion of novel target DNA binding sites (27). We hypothesize that also these BCL11B missense variants may either have a dominant negative effect or induce new target genes, thereby causing a more severe phenotype in patients with these missense variants /mutations as compared to eg loss of function type of mutations. Future studies should further examine the in vivo effect of these specific BCL11B mutations in animal models to establish if the described variants are indeed disease causing mutants or coincidental findings, as shown by Goos et al. for the mutation in their patient (29).

Although we are the first to report on the co-occurrence of CDH and craniosynostosis in a patient with a BCL11B missense mutation, the co-occurrence of CDH and craniosynostosis has been described previously for several syndromes, such as Aper's syndrome and craniofrontonasal syndrome (7–22). Craniosynostosis syndromes comprise ~30% of all craniosynostosis cases (63). Syndromic craniosynostosis is highly heterogeneous and is often associated with extracranial anomalies, including neurologic, limb, ophthalmologic and cardiac anomalies. Most craniosynostosis syndromes have an autosomal dominant inheritance pattern, although many cases arise from de novo mutations (32, 64). We conducted a literature search to identify which craniosynostosis syndromes are associated with CDH. We found nine syndromes to include both CDH as well as craniosynostosis as an associated feature, based on searches in OMIM, Medline, Science Direct and a gray literature search in Google Scholar. Table 2A summarizes the genetic anomalies as well as the main features of each syndrome reported to include both craniosynostosis as well as CDH. In addition, we found seven isolated case reports that describe patients presenting with both craniosynostosis as well as CDH, which are shown in Table 2B. The supplemental information includes Table 2 with a full reference list. Although CDH appears to be a rare feature in craniosynostosis syndromes, craniosynostosis is often treatable with surgical intervention and has high survival rates. In contrast, CDH is a potentially lethal disorder and is associated strongly with poor long-term clinical outcome (66–68). Awareness of this rare feature in craniosynostosis syndromes therefore is key.

CONCLUSION

This report implies that both CDH as well as craniosynostosis are features of BCL11B missense mutations. However, further studies are required to establish if BCL11B missense mutations are indeed a causative factor or if our finding was coincidental.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was provided by the participants’ legal guardian/next of kin. Written informed consent was obtained.
from the individual(s), and minor(s)’ legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

LG, AK, IM, and MD contributed to the study’s conception, wrote the manuscript, and provided critical revisions. LG, AG, and MD assessed the clinical and phenotypical features of the patients. FM and FJ assisted with data collection and provided genetic information. AG, QB, DV, and EB provided critical revision. All authors approved the article for submission.

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SUPPLEMENTARY MATERIAL

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