Frontonasal Dysplasia: Towards an Understanding of Molecular and Developmental Aetiology

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Key Words

Craniofacial midline · Frontonasal dysplasias · Malformations

Abstract

The complex anatomy of the skull and face arises from the requirement to support multiple sensory and structural functions. During embryonic development, the diverse component elements of the neuro- and viscerocranium must be generated independently and subsequently united in a manner that sustains and promotes the growth of the brain and sensory organs, while achieving a level of structural integrity necessary for the individual to become a free-living organism. While each of these individual craniofacial components is essential, the cranial and facial midline lies at a structural nexus that unites these disparately derived elements, fusing them into a whole. Defects of the craniofacial midline can have a profound impact on both form and function, manifesting in a diverse array of phenotypes and clinical entities that can be broadly defined as frontonasal dysplasias (FNDs). Recent advances in the identification of the genetic basis of FNDs along with the analysis of developmental mechanisms impacted by these mutations have dramatically altered our understanding of this complex group of conditions.

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The diversity of the frontonasal dysplasia (FND) phenotypes and the overlap with related craniofacial and more complex syndromes can confound the identification of FND. Although frontonasal malformations and their associated comorbidities may need to be viewed in light of more recent molecular findings, the defining features of FND were established decades ago [Sedano et al., 1970; Sedano and Gorlin, 1988] and still serve as the foundation for classifying these conditions (table 1). The core characteristics of FND are listed in table 2 and while the presentation is highly variable, at least 2 of these features are required to make a diagnosis.

Developmental Origins of the Frontonasal Process

The rationale for association of the specific features defining FND is based on the developmental origin of the craniofacial midline. A brief introduction to the early stages of craniofacial development illustrates the common origins of the structures impacted in FND and aids in understanding why certain phenotypes occur together. The mid- and upper face is constructed from 3 major tissue blocks: the paired maxillary processes which produce the upper jaw, cheek bones, and lateral nasal structures, plus the frontonasal process (FNP) which produces the midline tis-

sue including the frontal bones, nasal bridge and nasal tip, and which are responsible for uniting the bilateral maxillary arch-derived structures (fig. 1). The maxillary and frontonasal processes are composed of an outer covering of ectoderm overlaying a small but highly proliferative neural crest derived mesenchyme responsible for the construction of the facial skeleton. However, while the maxillary process is enclosed on the prospective oral surface by an endodermally derived epithelium, FNP forms in direct contact with the anterior neural tube that will form the telencephalon and eventually the forebrain. Thus, FNP is subject to direct influence from the developing forebrain neuroepithelium, and this influence has profound implications for growth and patterning of the midface.

Table 1. Diagnostic criteria of FND

Anterior cranium bifidum occultum
V-shaped or widow's peak of frontal hairline
True ocular hypertelorism (IP distance >97th centile)
Broadening of nasal root
Lack of nasal tip formation
Median facial cleft (nose and/or upper lip and palate)
Unilateral or bilateral clefting of the alae nasi

At least 2 of these features are required to make a diagnosis.
Established from Sedano and Gorlin [1988].

Table 2. Features of defined FND syndromes

	Distinguishing craniofacial features	Integumentary	Musculoskeletal	CNS/cognition	Genitourinary	References
FND1 (<i>ALX3</i>)	long philtrum with periphiltral swelling, midline notch upper lip and alveolus, upper eyelid ptosis	midline dermoid cyst	–	normal cognition	–	Twigg et al., 2009
FND2 (<i>ALX4</i>)	large skull defect, blepharophimosis, microphthalmia	total alopecia/sparse scalp hair	–	midline intracranial lipoma, callosal anomaly, normal cognition to DD/ID	cryptorchidism, hypospadias	Kayserili et al., 2012; Kariminejad et al., 2014
FND3 (<i>ALX1</i>)	extreme microphthalmia, upper eyelid colobomata, bilateral facial cleft, complete cleft plate	sparse eyelashes, absence of eyebrows	–	–	–	Uz et al., 2010
Heterozygous <i>SIX2</i> deletion (FND4?)	high anterior hairline, frontal bossing large anterior fontanelle, ptosis, epicanthus inversus, parietal foramina, cranio-synostosis (metopic, sagittal)	–	–	normal cognition	–	Hufnagel et al., 2016
CFNS (<i>EFNB1</i>)	craniosynostosis (coronal), craniofacial asymmetry	longitudinal nail ridging, coarse thick hair	sloping shoulders with dysplastic clavicles (pseudoarthrosis), Sprengel deformity, chest deformity, duplicated/broad halluces, cutaneous syndactyly, congenital diaphragmatic hernia	callosal anomaly, normal cognition to mild DD/ID	–	van den Elzen et al., 2014
AFND (<i>ZSWIM6</i>)	parietal foramina (variable)	–	preaxial polydactyly, unilateral or bilateral hemimelia, hypoplastic patellae	PVNH, callosal anomaly, absent olfactory bulbs, hypopituitarism, DD/ID	cryptorchidism	Smith et al., 2014
OAFNS (unknown)	microtia, preauricular tags, facial asymmetry, mandibular hypoplasia, epibulbar dermoids (OAVS)	abnormal hairline and eyebrows	vertebral fusion, complex vertebral segmentation, rib anomalies	intracranial lipomas, encephalocoeles, callosal anomaly, calcification of the cerebral falx, DD/ID	–	Evans et al., 2013
AFFND (unknown)	retinal coloboma, optic nerve atrophy, ulcerated corneae, polar cataracts, iris atrophy, microphthalmia, S-shaped palpebral fissure	abnormal hairline/eyelashes/eyebrow, hypoplastic nails	short stature, scoliosis, polysyndactyly of fingers, camptobrachydactyly, (hypoplastic distal phalanges, short metacarpals), hip dislocation, hypoplastic fibulae, tarsal anomalies, and tibio-talar subluxation	mixed hearing loss, epilepsy, DD/ID	hypospadias, bifid/shawl scrotum (mainly in AFFND2)	Prontera et al., 2011; Richieri-Costa et al., 1989

AFFND = Acrofrontofacionasal dysplasia; DD/ID = developmental delay/intellectual disability; IP = interpupillary distance; PVNH = periventricular nodular heterotopia; – = not present.

Fig. 1. Embryological origins of the frontonasal process. The paired maxillary processes (blue) and FNP (yellow) have begun development by the end of the first month of gestation. Under normal circumstances, FNP has fused with the maxillary primordia by the end of the first trimester to form the midfacial structures. IMS = Intermaxillary segment; LNP = lateral nasal process; MNP = medial nasal process; Mx = maxillary process; NP = nasal pit.

Fig. 2. Origins and distribution of the frontonasal neural crest cells. **A** The frontonasal neural crest cell population (blue) emerges from the lateral margin of the neural folds prior to neural tube formation. **B, C** FNCs then migrate ventrally over the anterior neural folds (**B**) eventually reaching the frontonasal region of the neural tube soon after fusion of the neural folds (**C**). **D** FNCs are subsequently restricted to the prospective frontonasal region as the telencephalic vesicles rapidly expand. **E** The expansion of FNCs results in outgrowth of the midface and formation of the frontal bone over the forebrain. Data derived from Chai et al. [2000] and Jiang et al. [2002]. FNC = Frontonasal neural crest cell; FNP = frontonasal process; Fr = frontal bone; Mn = mandibular process; Mx = maxillary process; Pa = parietal bone; Tel = telencephalon.

The neural crest is a highly migratory and invasive population of mesenchymal cells derived from the lateral margins of the epithelial neural plate following a process known as epithelial to mesenchymal transformation [Le Douarin, 1982, 2012; Zhang et al., 2014]. The neural crest cells that contribute to FNP originate in the prospective caudal forebrain and rostral midbrain neural plate prior to cranial neural tube closure and migrate between the basal surface of the neural plate and overlying ectoderm (fig. 2) [Nichols, 1981; Chai et al., 2000; Jiang et al., 2002]. During this migratory phase, the frontonasal neural crest cells spread as a sheet-like population, passing over the prospective telencephalon and eye to take up residence in the most rostral aspect of the neural tube [Jiang et al., 2002]. As the telencephalon expands, this population becomes predominantly restricted to the prospective frontonasal region of the developing face.

The frontonasal neural crest cells are initially restricted in number as they exit the neural plate and must expand through rapid proliferation. Expansion begins during migration, but the neural crest cells encounter major mitogenic influences upon reaching their destinations within the frontonasal region and nasal processes. The coordinated expression of *Shh*, *Bmp4*, and *Fgf8* plays a profound role in establishing growth and patterning of the facial primordia, and disruption of this coordination results in FNP defects and facial clefting [Foppiano et al., 2007; Hu and Marcucio, 2009a; Hu et al., 2015]. The expression domains of *Fgf8* and *Shh* in the frontonasal ectoderm form a signalling centre known as the frontonasal ectodermal zone that dictates the morphogenetic outcome of FNP development [Hu et al., 2003, 2015; Hu and Marcucio, 2009a]. Bmp signalling, and Bmp4 in particular, is also a key factor in patterning and outgrowth of FNP [Ashique et al., 2002; Abzhanov et al., 2004; Wu et al., 2004]. In addition to promoting the growth of FNP, blockade of Bmp signalling results in downregulation of *Shh* in FNP [Foppiano et al., 2007] and compromises the ability of *Shh* to induce its own expression [Hu et al., 2015]. Similarly, Fgf signalling from the nasal pits is required for normal proliferation of FNP mesenchyme, and blocking this signalling also results in facial clefts [Szabo-Rogers et al., 2008]. The efficacy of these patterning systems requires tightly regulated spatial and temporal gene expression, and the distribution of these factors serve as markers of the individual facial primordia enabling a fine scale analysis of developmental defects (fig. 3). The patterning influence of the frontonasal ectodermal zone has been best characterised in the chicken embryo, where disruption of the tightly regulated distribution of *FGF8* and

Fig. 3. In situ hybridisation illustrating the expression of genes marking the components of the facial primordia. **A** *Twist1* expression marking the FNCs migrating ventrally towards the prospective FNP. **B** *Fgf8* expression marking the anterior neural ectoderm (prospective forebrain neural tube) and ectoderm covering the MNP and LNP. **C** *Msx2* expression marking the MNP, LNP, and MX prior to the completion of fusion. ANE = Anterior neural ectoderm; E = eye primordia; Md = mandibular process; NP = nasal placode. For further abbreviations see figure legends 1 and 2.

SHH results in failure of midface outgrowth [Hu et al., 2003; Hu and Marcucio, 2009a]. However, a similar signalling system has been demonstrated in mouse embryos, suggesting that the regulation of mid- and upper facial patterning by this SHH/FGF8 mechanism is broadly utilized [Hu and Marcucio, 2009b].

The individual facial processes expand and elongate as the mesenchymal populations proliferate and eventually adjacent processes make contact between opposing epithelial surfaces. The fusion process is best characterised in the formation of the secondary palate where individual epithelial cells make an initial contact through extension of cell surface protrusions known as filopodia [Taya et al., 1999]. Following this initial contact, opposing epithelial surfaces make direct contact, forming a transient bi-epithelial seam which is itself removed through a combination of programmed cell death and conversion of epithelial cells into mesenchyme via epithelial to mesenchymal transformation [Xu et al., 2006; Ke et al., 2015; Lan et al., 2015]. Following successful fusion, the previously separate mesenchymal populations become united as a single continuous population. The neural crest cells responsible for the production of skeletal elements within the mid- and upper face generate both bone and cartilage through direct transition from a multipotent mesenchymal state into terminally differentiated cell types [Bronner and LeDouarin, 2012]. The differentiation of neural crest-derived progenitors within the unified facial processes subsequently results in formation of a continuous facial skeleton. It is important to note that fusion of the facial processes precedes osteogenic differentiation of neural crest-derived progenitor cells. This implies that overt clefting and facial hypoplasia of otherwise intact skeletal elements may arise from defects in temporally separable developmental events. The challenge may therefore be to

determine if these defects in different individuals are attributable to the same or independent genetic or environmental insults.

Phenotypic Spectrum of the FNDs

The syndromes within the FND spectrum are diverse and genetically heterogeneous. There are currently 6 genes associated with FND spectrum disorders, and while these findings are invaluable in understanding the origins and presentation of each condition, they account for only a small fraction of cases with the majority of affected individuals yet to attain a molecular diagnosis (table 2). In addition to these genetic influences, environmental insults are also likely to contribute to a proportion of FND cases. Consideration of the clinical, molecular and developmental aspects of the molecularly defined FND spectrum disorders will assist in understanding the aetiology of FND still awaiting molecular definition.

Craniofrontonasal syndrome (CFNS; OMIM 304110) is a distinctive condition which presents in females as FND including severe hypertelorism, a bifid or hypoplastic nasal tip, coronal craniosynostosis, malformation of the clavicle, longitudinally grooved or split nails, and thick, wiry hair. CFNS is less distinct in males, often presenting as hypertelorism and occasionally cleft lip and/or palate. This mode of presentation strongly suggested an X-linked aetiology, and prior to identification of the causative genetic mutations, there was suspicion of complicated modifier gene scenarios or embryonic lethality in males [Cohen, 1979; Sax and Flannery, 1986; Grutzner and Gorlin, 1988; Devriendt et al., 1995]. However, the identification of *EFNB1* mutations in affected females resulted in the discovery of similar mutations in mildly af-

affected males indicating that, in contrast to the more typical presentation of X-linked conditions, females have a more severe phenotype following mutation of *EFNB1*. Further, the development of very sensitive approaches to identifying low-level mosaicism has resulted in the identification of *EFNB1* mutations in the majority of CFNS patients [Twigg et al., 2004; van den Elzen et al., 2014].

The mechanistic concept explaining the phenomenon of the female phenotypic bias in CFNS was first proposed as metabolic interference in which random X inactivation leads to a metabolically mixed population in females [Rollnick et al., 1981; Feldman et al., 1997]. More recent experimental investigation has demonstrated that at least in the case of *EFNB1*, this phenomenon is occurring at the cellular level rather than being strictly metabolic, prompting adoption of the term ‘cellular interference’ [Davy et al., 2006; Twigg et al., 2013]. The *EFNB1* gene encodes ephrin-B1, a membrane-bound ligand for the Eph receptor, involved in repulsive signalling between individual cells [Krull et al., 1997; Mellitzer et al., 1999]. There appears to be no skewing of X inactivation in CFNS individuals, and this results in a mixed population of cells in females in which either the mutant or wildtype allele is expressed [Twigg et al., 2004; Wieland et al., 2007]. This results in the formation of ectopic boundaries within the frontal bone precursors in which osteogenic differentiation is inhibited, producing the characteristic hypoplastic calvarial phenotype [Davy et al., 2006]. The cell sorting/boundary formation activities of ephrin-B1 appear to be redundant since males which only produce mutant protein are less susceptible to this defect. However, in an intriguing twist, males harbouring a mosaic mutation in *EPHNB1* replicate the mixed population phenomenon seen in CFNS females and may exhibit a more severe and distinct CFNS phenotype than males with mutations in all cells, confirming the origins of the apparently paradoxically more severe phenotype in females [Twigg et al., 2013].

Frontorhiny (FND1; OMIM 606014) was the first of the FND syndromes to be defined molecularly with the discovery of homozygous *ALX3* mutations in 7 families [Twigg et al., 2009]. In addition to the core features of FND including hypertelorism, widely spaced nasal bones and a bifid nasal tip, FND1 is characterised by features affecting the distal aspect of FNP such as a short medial nasal region with a broad columnella that attaches to the widely spaced nasal alae producing a distinctive concave shape to the nasal tip and a long philtrum with raised and fleshy lateral margins. Overt clefting does not appear to be a major feature of FND1, although the generally defi-

cient FNP can result in midline notching in the upper lip and alveolar bone.

FND2 (OMIM 605420) is a recessive condition characterised by hypertelorism, severely depressed nasal bridge, malar flattening, bifid nasal tip, cleft nasal alae, craniosynostosis, hypoplastic calvaria resulting in extensive foramina and cranium bifidum, total alopecia, and brain abnormalities including agenesis of the corpus callosum. FND2 results from homozygous *ALX4* mutations and has been documented in 3 consanguineous families [Kayserili et al., 2009; Kariminejad et al., 2014]. Interestingly, heterozygous mutations in *ALX4* cause parietal foramina 2 (OMIM 609597) which, in severe cases, may include hypertelorism and nasal abnormalities [Wuyts et al., 2000; Mavrogiannis et al., 2001; Kayserili et al., 2012; Bertola et al., 2013; Altunoglu et al., 2014]. Parietal foramina 1 (OMIM 168500) is caused by a heterozygous mutation in the *MSX2* gene [Wilkie et al., 2000; Spruijt et al., 2005] and parietal foramina 1 and 2 are clinically indistinguishable [Mavrogiannis et al., 2006]. Further, activating mutations in both *MSX2* and *ALX4* have been implicated in craniosynostosis [Jabs et al., 1993; Ma et al., 1996; Yagnik et al., 2012; Florisson et al., 2013]. This highlights the involvement of calvarial development defects in the pathogenic presentation of FND and also supports the contention that FND, parietal foramina and craniosynostosis may represent components of a phenotypic spectrum [Yagnik et al., 2012].

FND3 (OMIM 601527) is a severe form of FND involving a profound hypoplasia of the tissue derived from FNP resulting in midfacial clefting due to a complete failure of fusion between the frontonasal and maxillary arch-derived tissues [Uz et al., 2010]. In addition, the 4 individuals (from 2 families) reported with this condition had hypertelorism, a wide nasal base, isolated nasal alae, malformed orbits, microphthalmia, cleft primary and secondary palate as well as low-set, posteriorly rotated ears. FND3 results from a complete loss of *ALX1* either through homozygous genomic deletion of *ALX1* or functionally through intragenic mutation. A milder manifestation of FND3 has been reported due to a homozygous splice acceptor site mutation which may encode a mutant *ALX1* protein harbouring residual activity [Ullah et al., 2016].

A similarly severe FND phenotype has been reported in Burmese cats selected for a brachycephalic trait which has become popular in recent decades [Noden and Evans, 1986]. Approximately 25% of kittens born from matings between short-faced parents exhibit this FND phenotype, suggesting a recessive inheritance. Genetic analysis of this variant, known as the ‘Contemporary’ Burmese, identi-

fied a 12-bp deletion within the *ALX1* gene in affected kittens [Lyons et al., 2016]. Interestingly, the prized Contemporary Burmese harbours the same deletion in heterozygous form, while no *ALX1* deletion has been identified in the traditional long-faced Burmese, suggesting that the trait appears to be semidominant. This is strikingly similar to the impact of *ALX4* mutations in humans causing parietal foramina in a heterozygous form but FND in a homozygous state, illustrating the value of studying a range of animal models to understand the complex developmental biology of human congenital malformation.

The *Alx* genes are closely related homeodomain containing transcription factors that additionally possess a conserved Aristaless domain. In mouse embryos, *Alx1* (*Cart1*), *Alx3* and *Alx4* are each expressed in FNP mesenchyme spanning the stages at which cranial neural crest cells are migrating into the facial primordia in strongly overlapping domains [Beverdam and Meijlink, 2001]. Mutations in the *ALX* genes causing FND spectrum disorders appear to occur throughout the protein but have a bias towards the homeodomain. The homeodomain is a hallmark of this class of transcription factors, exhibiting very high levels of homology within related family members [D'Elia et al., 2001]. Mutations in a range of homeodomain class transcription factors are associated with congenital anomalies in humans and missense mutations within the homeodomain can cause both gain and loss of DNA-binding activity as has been observed for both *ALX4* and *MSX2* [Ma et al., 1996; Wilkie et al., 2000; Wuyts et al., 2000; Yagnik et al., 2012; Florisson et al., 2013]. The *ALX4* deletion resulting in the Contemporary Burmese FND phenotype also occurs within the homeodomain [Lyons et al., 2016].

In zebrafish, while *alx1*, *alx3* and *alx4* are each expressed in the facial mesenchyme, *alx1* alone is expressed in the recently emigrating neural crest at the earliest stages of migration from the neural tube [Dee et al., 2013]. Inhibition of *alx1* expression in zebrafish embryos results in a severe FND-like phenotype with microphthalmia, coloboma, and severely hypoplastic facial skeleton. Inhibition of *alx3* expression did not result in abnormalities of the facial skeleton [Dee et al., 2013], suggesting that while there is significant functional redundancy between *alx* genes, the early expression of *alx1* relative to other *alx* family members results in a more acute requirement for *alx1* expression during development of the zebrafish frontonasal structures. The observation of an acute, early requirement for *alx1* during neural crest migration is consistent with the severe FND phenotype associated

with loss of *ALX1* in humans and suggests that as with zebrafish, human embryos null for *ALX1* are likely to have a severely compromised frontonasal neural crest cell population. In mice, loss of *Alx1* results in a failure of midbrain neural tube closure and consequent loss of the skull vault [Zhao et al., 1996]. Interestingly, *Alx1* null mice appear to harbour a robust FNP-derived nasal skeleton despite the severe exencephalic phenotype, suggesting a less strict requirement for *Alx1* in early mouse cranial neural crest cell development than in either human or zebrafish.

Homozygous loss of *Alx4* in mice does not produce an FND phenotype but results in reduced parietal bones in neonates [Qu et al., 1997]. *Alx4* mutants predominantly die perinatally due to complications arising from ventral body wall defects, but those that survive to adulthood exhibit normal-sized parietal bones, suggesting that a loss of *Alx4* results in a delay rather than an overt defect in parietal bone development. *Alx3* is expressed in a pattern that overlaps substantially with that of *Alx4* within the frontonasal mesenchyme [Beverdam and Meijlink, 2001]. Mice harbouring a homozygous deletion of *Alx3* appear normal. However, compound *Alx3/Alx4* null mice have a severe FND phenotype with complete midline facial clefting resulting from elevated cell death within FNP and resulting hypoplasia [Beverdam et al., 2001]. This clearly demonstrates the redundant functions in the frontonasal development of *Alx3* and *Alx4* in mice but again highlights the differential sensitivity to *Alx* gene dosage between mice and humans. Despite these differences, the phenotypes of the *Alx4* and *Alx3/4* null mouse models are consistent with those seen in human FND and therefore provide opportunities to understand the developmental anomalies and molecular mechanisms resulting in failure of FNP development.

Acromelic frontonasal dysplasia (AFND) is a very rare clinically distinct condition exhibiting severe craniofacial anomalies including hypertelorism, median cleft face, bifid nasal tip, widely spaced nasal alae, parietal defects, and limb anomalies including polydactyly, tibial hypoplasia, and talipes equinovarus. In contrast to other conditions within the FND spectrum, AFND individuals typically display marked mental retardation and possess a range of brain malformations including hydrocephalus, agenesis of the corpus callosum, interhemispheric lipoma and periventricular nodular heterotopia [Richieri-Costa et al., 1992; Verloes et al., 1992; Slaney et al., 1999]. The identical heterozygous c.3487C>T mutation in the *ZSWIM6* gene has been detected in 8 unrelated individuals [Smith et al., 2014; Twigg et al., 2016]. This specific,

putative gain of function, mutation appears to be crucial in the aetiology of AFND since deletions spanning *ZSWIM6* are present in individuals with unrelated phenotypes [Smith et al., 2014]. There is currently no animal model of AFND.

The function of *ZSWIM6* is poorly characterised, but the SWIM domain has homology to sequences within a group of SWI2/SNF2 containing proteins and is predicted to form a zinc finger domain, suggesting a role in DNA binding, chromatin dynamics and transcriptional regulation [Muthuswami et al., 2000; Makarova et al., 2002; Rieger et al., 2012]. The details of *ZSWIM6* expression within early embryonic development are yet to emerge, but it appears to be most strongly expressed in the cranial neural tube in mouse (<http://www.informatics.jax.org/gxd>) and zebrafish [Smith et al., 2014] consistent with the severe mental retardation phenotype of AFND. The function of *ZSWIM6* in the development of the frontonasal structures and calvaria is presently unknown.

A mild form of FND has recently been attributed to a heterozygous deletion of the *SIX2* gene in a single family [Hufnagel et al., 2016]. The proband had frontal bossing with a large anterior fontanelle, hypertelorism, ptosis, a flat nasal bridge, broad nasal tip, bilateral parietal foramina, complete sagittal synostosis as well as subtle cranial base anomalies and macrocephaly at 22 months of age. This form of FND (FND4?) is unusual in the involvement of the skull base, a malformation of which is not typically associated with developmental defects of FNP. The anterior skull and facial skeleton are produced from direct ossification of neural crest progenitors, while the skull base is derived from mesodermal precursors through endochondral ossification. However, mouse models of *Six2* involvement in craniofacial development have demonstrated a link between these 2 independently derived structures.

The Brachyrrhine (*Br*) radiation-induced mutant mouse line exhibits a severe FND phenotype that appears to be linked to a reduction in *Six2* expression, most likely due to a mutation in a regulatory region [McBratney et al., 2003; Fogelgren et al., 2008]. *Six2* expression normally appears in the frontonasal mesenchyme from mid-gestation when the frontonasal neural crest cells have completed migration but is also expressed in the maxillary and mandibular primordia [Oliver et al., 1995]. Heterozygous *Br* mice are retrognathic and harbour skull base defects, while homozygotes have a complete failure of FNP growth and exhibit midfacial clefting [Fogelgren et al., 2008]. The skull vault appears compressed in the anterior-posterior axis due to reduced skull base growth

since no craniosynostosis in *Six2* null or *Br* mice was observed [Fogelgren et al., 2008; He et al., 2010]. Cranial skeletal defects in these mouse models include loss of the nasal septum, primary and secondary palate and presphenoid as well as a malformation of the basisphenoid. The skull base grows through a process of endochondral ossification, and the restricted growth of the skull base in *Six2* null mice has been shown to be due to premature ossification of cartilaginous growth plates [He et al., 2010]. Once again, there appears to be a differential sensitivity for *Six2* dosage between mouse and human, particularly in the mandibular and frontonasal process neural crest. However, these mouse models recapitulate a number of core features of *SIX2* associated FND in humans and provide mechanistic insights into the origins of this craniofacial malformation.

Oculoauriculofrontonasal syndrome (OAFNS; OMIM 601452) is characterised by features overlapping those of FND and the oculoauriculovertebral spectrum (OAVS). The features of OAVS that have been observed in patients with OAFNS include microtia, preauricular tags, hemifacial microsomia, and epibulbar dermoids. Additional features include midline intracranial lipomas, encephaloceles, callosal anomalies, and calcification of the cerebral falx. Cognitive impairment does not seem to be a common finding, but developmental delay and learning difficulties are reported [Gabbett et al., 2008; Guion-Almeida and Richieri-Costa, 2010]. As the molecular pathogenesis of these conditions is still unknown, it is unclear if OAFNS is a distinct entity or if it represents a subtype of FND or OAVS [Evans et al., 2013].

The acrofrontofacionasal syndromes types 1 and 2 are ultra-rare conditions characterised by features of FND with postaxial synpolydactyly; type 1 is also associated with cleft lip/palate, while type 2 is associated with genitourinary anomalies such as hypospadias and bifid scrotum. Reported individuals have been born to consanguineous parents, and sibling recurrence supports autosomal recessive inheritance, although gonadal mosaicism cannot be totally excluded [Richieri-Costa et al., 1989; Guion-Almeida and Richieri-Costa, 2003; Chaabouni et al., 2008; Prontera et al., 2011].

In addition to the FND spectrum disorders that have been clinically defined as distinct groups, there are many cases that meet the minimal diagnostic criteria for FND but that do not clearly exhibit additional clinical features that allow definition of a distinct syndrome subclass. In many cases, the mode of inheritance for these conditions is uncertain, and in the absence of a molecular diagnosis, it is only possible to speculate on the existence of de novo

or mosaic mutations or even environmental influences. The advent of massively parallel sequencing technology, in conjunction with careful clinical phenotyping, has provided major new insights into the aetiology of the FND spectrum disorders. Hopefully, in conjunction with an understanding of the developmental mechanisms resulting in defects of FNP, these new technologies will eventually lead to a molecular characterisation of this large undifferentiated FND group as well.

Conclusion

FND is a diverse collection of conditions sharing key features affecting the frontal cranial and midfacial skeleton. Ironically, while the core features of expanded skull vault and epicanthal distance combine to produce a relative increase in craniofacial volume, the underlying mechanisms driving the aberrant development of these structures are centred on loss and reduction of skeletal precursors and their derivatives. This phenomenon highlights the central role of the cranial and facial midline in uniting the complex architecture of the craniofacial skeleton. The FND spectrum disorders are fundamentally defects of cranial neural crest development and can therefore be considered as neurocristopathies. However, the diverse presentations of FND involve a range of other developmental systems, and in these respects, animal models have been invaluable in understanding the complex roles of specific FND genes in the development of FNP and other comorbid tissues.

The advent of genomic technology has resulted in a profound change in the way many, especially rare, genetic disorders are viewed by providing a molecular diagnosis and the mechanistic insights that follow on from gene identification. With the exception of *ZSWIM6*, which is yet to be functionally characterised, all the FND genes identified so far are bona fide, tissue-specific developmental regulators. FND is a defined group of disorders impacting on FNP. Is it really so surprising these disorders should be caused by tissue-specific factors? This contrasts with the growing collection of genes identified for mandibulofacial dysostoses which so far appear to be ubiquitously expressed and involved in core biochemical processes such as ribosome biogenesis and RNA splicing [Trainor and Andrews, 2013; Lehalle et al., 2015]. Mandibulofacial dysostoses, such as FND, are highly tissue-specific conditions, yet they are caused by mutations in genes that might be expected to produce very broad phenotypes. Both classes of craniofacial dysmorphology arise from defects in the cranial neural crest, albeit subtly different subpopulations. The implications for the majority of FND yet to be defined molecularly are unclear but highlight the need to be open to unexpected scenarios in the continuing search for the causes of craniofacial dysmorphology.

Disclosure Statement

The authors have no conflicts of interest to declare.

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