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Invited Review

INVITED REVIEW

New insights into craniofacial malformations

Stephen R.F. Twigg* and Andrew O.M. Wilkie

Clinical Genetics Group, Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Headington, Oxford OX3 9DS, UK

*To whom correspondence should be addressed. Tel: +44 1865222353; Fax: +44 1865222500; Email: stephen.twigg@imm.ox.ac.uk

Abstract

Development of the human skull and face is a highly orchestrated and complex three-dimensional morphogenetic process, involving hundreds of genes controlling the coordinated patterning, proliferation and differentiation of tissues having multiple embryological origins. Craniofacial malformations that occur because of abnormal development (including cleft lip and/or palate, craniosynostosis and facial dysostoses), comprise over one-third of all congenital birth defects. High-throughput sequencing has recently led to the identification of many new causative disease genes and functional studies have clarified their mechanisms of action. We present recent findings in craniofacial genetics and discuss how this information together with developmental studies in animal models is helping to increase understanding of normal craniofacial development.

Introduction

The head is the most complex structure of the body. The skull, including bones that enclose and protect the brain and sensory organs, also acts as a scaffold for the face to support the functions of feeding and breathing in combination with connective tissue, musculature, vasculature and associated innervation. Collectively these tissues are derived from endoderm, mesoderm, ectoderm and cranial neural crest cells (CNCCs) and their derivatives (1). Signalling between these cellular components and to the craniofacial mesenchyme (formed primarily by CNCCs with a mesodermal contribution) provides positional cues and regulates growth and differentiation (Fig. 1). These dynamic spatio-temporal processes are highly complex and susceptible to dysregulation as evidenced by the high proportion of congenital defects that involve the skull and face (2). We will summarise recent molecular insights into development of the skull and face, then discuss the latest discoveries in the genetic basis of human craniofacial malformations including craniosynostosis, facial exostoses and cleft lip and/or palate. Newly identified disease genes underlying these pathologies are listed in Table 1.

New Insights into Craniofacial Development

The mammalian skull is formed from both mesoderm and neural crest (NC)-derived mesenchyme (22,23). Following induction of the NC at the lateral edges of the neural plate, CNCCs undergo an epithelial-to-mesenchymal transition and migrate to different destinations depending on their position along the anteroposterior axis of the neural tube (Fig. 1).

The cranial sutures that separate the flat bones of the skull perform a vital role in coordinating growth with rapid brain expansion. Sutures contain mesenchymal stem cells (MSCs), progeny of which divide and mature to osteoblasts at the adjacent bone fronts (24). In the mouse the MSC precursors of the coronal suture originate from cephalic paraxial mesoderm arising at the mesencephalon/diencephalon boundary at embryonic day (E)7.5, in response to sonic hedgehog (SHH) signalling from the adjacent notochord (25) (Fig. 1). Subsequently, the MSCs migrate to an organizing centre located above the developing eye at the base of the future coronal suture (supra-orbital regulatory centre, SRC) (25). Here they lie between cephalic mesodermal cells (parietal bone-forming) and CNCCs (frontal bone-forming). Crucially this arrangement is maintained during growth so that, as the coronal suture develops, it is populated purely by mesodermal derivatives, whereas NC-derived cells do not cross from the frontal bone territory (25). Using conditional labelling with a *Gli1* driver, a population of MSC within the suture was recently demonstrated postnatally; these cells are critical for skull growth as their ablation leads to suture fusion (26).

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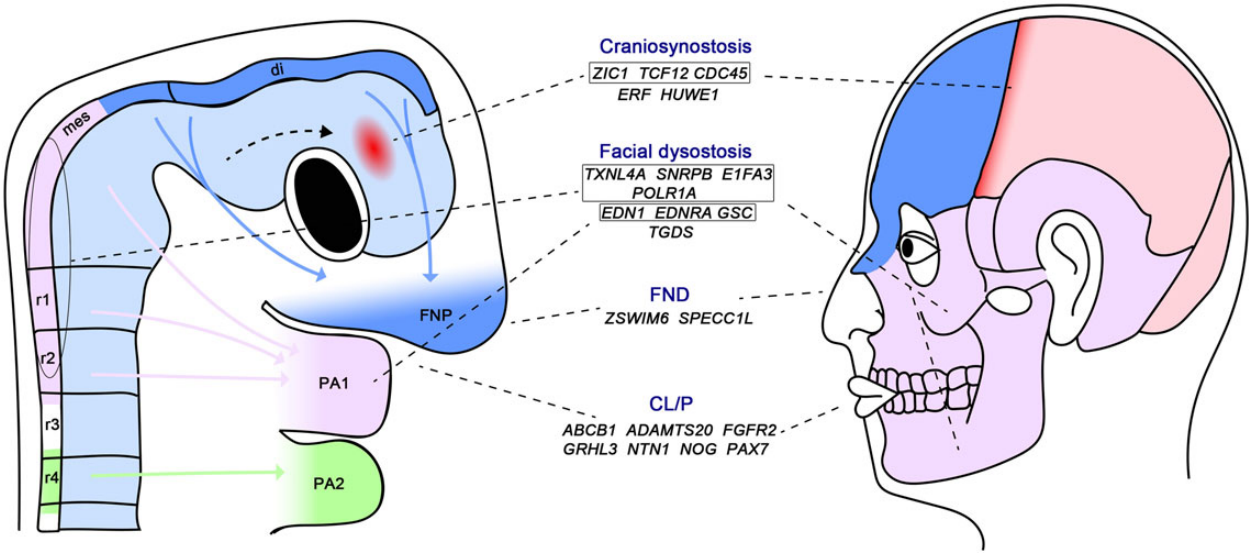

Figure 1. Development of the head and face and recently identified craniofacial genes. An embryo is shown on the left, the arrows represent the patterns of migration of diencephalic (di), anterior and posterior mesencephalic (mes), and rhombencephalic (r1–4) NCCs into the FNP and pharyngeal arches 1 and 2 (PA1, PA2). The dashed arrow indicates movement of mesoderm-derived MSCs to the supra-orbital regulatory centre above the eye. Neural crest-derived craniofacial regions formed from the FNP and PA1 are indicated with the same colours on the head (right). The parietal and occipital bones are mesoderm-derived (pink). The coronal suture contains purely mesoderm-derived cells (red) that have expanded apically from the supra-orbital region. Centrally, the genes discussed in this review are shown, with the embryological site underlying pathogenesis (if known—boxed genes), and the structures affected by mutation, indicated.

The facial prominences arise from proliferation of CNCCs that have migrated from the diencephalon and anterior mesencephalon to the frontonasal and periocular regions, and from the posterior mesencephalon and rhombomeres 1 and 2 of the hindbrain to the first pharyngeal arch (PA1) [Fig. 1; reviewed in (27)]. The face forms by integrated fusion of the five facial primordia, the frontonasal prominence and the paired maxillary and mandibular processes, which surround the oral cavity. Invagination of the nasal placodes forms the medial and lateral nasal prominences (28). Correct fusion of the facial prominences and secondary palate requires either cell death or epithelial-mesenchymal transformation of the epithelial cells at the adjacent processes (29). A key role for IRF6 was first reported in 2002 (30), when mutations were demonstrated in Van der Woude syndrome (VWS [MIM 119300]) families (31) with cleft lip with or without cleft palate (CL/P) or cleft palate (CP) alone. Mutations lead to haploinsufficiency, which can also be caused by disruption of a 600 bp *IRF6* enhancer element (MCS9.7) located ~10 kb upstream (32). IRF6 is regulated by phosphorylation and the kinase responsible has recently been pinpointed as RIPK4 (33,34), with its expression likely controlled by IRF6 (35). Recessive mutations of RIPK4 cause Bartsocas-Papas syndrome (MIM 263650) (36,37). Recently, enhanced SHH activity (caused by disruption of *Ptch1*) was found to lead to reduced WNT-p63-IRF6 signalling at the lambdoidal junction (where the maxillary, medial nasal and lateral nasal processes fuse) with increased proliferation and decreased cell death, resulting in epithelial seam persistence and cleft lip (38). Furthermore, correct SHH signalling is required for expression of the NCC marker *Tfap2a* in the frontonasal process (FNP), associated with CL/P either directly by mutation [branchio-oculo-facial syndrome (BOFS [MIM 113620] (39))] or by variation in an AP-2$\boldsymbol{\alpha}$ binding site in the *IRF6* MCS9.7 enhancer (40). The periderm, a layer of flattened cells that cover developing epithelia, has a key role in adhesion of the opposing medial edge epithelia (MEE). Its importance is highlighted by the development of abnormal inter-epithelial adhesions in the facial complex in loss-of-function mouse mutants including *Irf6* (41). The discovery of mutations in Grainyhead-like 3 (*GRHL3*) in syndromic clefting [(9) discussed below] provides a further link, since expression of *Grhl3* is regulated by *Irf6*, and both are required for development of the periderm (42). Figure 2 summarises the relationships between some of these signalling molecules. A TGFß-mediated IRF6 pathway is also important in palatal development; *Smad4* and *Irf6* genetically interact in the control of MEE disappearance during fusion (43), possibly downstream of TGFß3 (44).

The jaws are derived from the maxillary and mandibular processes of PA1 and patterning of this structure is reliant on signals from the foregut endoderm (45). Developmental abnormalities are thought primarily to arise because of a defect either in the generation and migration of NCCs to populate these tissues, or in their subsequent patterning and differentiation. A number of signalling pathways are involved in directing post-migratory CNCCs to form the jaws (46) with a pivotal role played by endothelin. In PA1 endothelin 1 (encoded by *Edn1*) is expressed in the ectodermal epithelium of the mandibular process and signalling is via endothelin receptor type A (encoded by *Ednra*), expressed in the underlying cephalic NC-derived ectomesenchyme (47,48). Migration of NCC into the pharyngeal arches appears to be unaffected in *Ednra* null embryos whereas expression of a number of genes within NC-derived ectomesenchymal cells is disrupted and accompanied by increased apoptosis (49). Recent discoveries of human mutations within different components of the endothelin signalling pathway are discussed below.

The identification of *TCOF1* mutations as the most common cause of Treacher Collins syndrome (50) led to the unexpected insight that several disorders of facial development are caused by ribosomopathies (51,52). Most recently mutation of *POLR1A* has been found to lead to mandibulofacial dysostosis (17). Analysis of *polr1a* in zebrafish demonstrated a requirement for the generation of NCCs, but not for the survival of migrating NC, that was linked to abnormal ribosome biogenesis and subsequent p53-dependent cell death (17), mirroring previous findings in *Tcof1*

Table 1. New disease genes in craniofacial malformations

Gene	Locus	Clinical disorder	OMIM # gene/ disorder	Inheritance pattern	Major phenotypic features	Mechanism/pathway/comments	References
Craniosynostosis							
CDC45	22q11.21	–	603 465/–	AR	Coronal suture synostosis, thin eyebrows, small ears, variable short stature	Peturbation of DNA replication, probable that some function remains	(3)
ERF	19q13.2	ERF-related craniosynostosis	611 888/600 775	AD	Mainly multisuture synostosis with postnatal onset, exorbitsm, midface hypoplasia, Chiari type I malformation, behavioural or learning difficulties	Haploinsufficiency; FGFR/ERK signalling	(4)
HUWE1	Xp11.22	–	300 697/–	XLD (*n*)	Multisuture or metopic craniosynostosis, learning difficulties	Unknown	(3)
TCF12	15q21.3	TCF12-related craniosynsotosis	600 480/615 314	AD	Coronal synostosis. May resemble Saethre-Chotzen syndrome with dysmorphic face, ears and minor limb anomalies. Other cases nonsyndromic, ~50% clinical nonpenetrance	Haploinsufficiency; heterodimerization with TWIST1; RUNX2, BMP and FGFR signalling	(5)
ZIC1	3q24	ZIC1-related craniosynostosis	600 470/–	AD (*n*)	Severe bicoronal synostosis with learning difficulties	WNT signalling	(3)
Selected CL/P candidates							
ABCB1	7q21.12	–	171 050/–	Complex	Non-syndromic orofacial cleft	Control of foetal exposure to foreign chemical substances	(6)
ADAMTS20	12q12	–	611 681/–	AR	CL/P and syndactyly	Uknown—Extracellular matrix processing?	(7)
FGFR2	10q26.13	–	176 943/–	Complex	NSCL/P	254 kb downstream of *FGFR2*; risk allele disrupts NC enhancer activity	(8)
GRHL3	1p36.11	Van der Woude syndrome 2	608 317/606 713	AD	CL/P, lip pits	Periderm development	(9)
NTN1	17p13.1	–	601 614/–	Complex	NSCL/P	Expressed in palatal shelves	(8)
NOG	17q22	–	602 991/–	Complex	NSCL/P	Risk allele shows significantly decreased enhancer activity	(8)
PAX7	1p36	–	167 410/–	Complex	NSCL/P	Involved in NC induction and specification of NC derivatives	(8,10)
FND							
ZSWIM6	5q12.1	AFND	615 951/603 671	AD (*n*)	Frontonasal dysplasia with median cleft face and hypertelorism, agenesis of the corpus callosum, neurocognitive and motor delay, tibial hemimelia, preaxial polydactyly of feet	Gain-of-function; pathogenetic mechanism is unknown	(11)

Table 1. Continued

Gene	Locus	Clinical disorder	OMIM # gene/ disorder	Inheritance pattern	Major phenotypic features	Mechanism/pathway/comments	References
SPECC1L	22q11.2	Opitz G/BBB	614 140/145 410	AD	Variable midline defects, hypertelorism, widow's peak, broad nasal bridge, CLP, and congenital heart defects and hypospadias	Haploinsufficiency; *SPECC1L* has role in cell adhesion and migration	(12)
Facial dysostoses							
EIF4A3	17q25.3	RCPS	608 546/268 305	AR	Micrognathia with median cleft of the mandible, small mouth, CP, radial and tibial hypoplasia, learning and language difficulties	Reduced expression; component of exon junction complex; directly interacts with spliceosome components	(13)
EDN1	6p24.1	ACS; IQME	131 240/615 706 (ACS) & 612 798 (IQME)	AR (ACS)/AD (IQME)	ACS—Micrognathia, anomalies of temporomandibular joint and condyle, small mouth, prominent cheeks and question-mark ears IQME—question-mark ears	Loss-of-function; likely that residual function remains with AR mutations	(14)
EDNRA	4q31.22	Mandibulofacial dysostosis with alopecia	131 243/616 367	AD (*n*)	Micrognathia, dysplastic zygomatic arch, thickened malar bones, absent or hypoplastic lateral margin of the orbits, CP, short nose with broad nasal tip, dysplastic ears, hearing loss, sparse eyelashes and hypoplasia of the eyelids, alopecia	Possible gain-of-function	(15)
GSC	14q31.23	Short stature, auditory canal atresia, mandibular hypoplasia, skeletal abnormalities (SAMS)	138 890/602 471	AR	Short stature, micrognathia, rhizomelic skeletal abnormalities including humeroscapular synostosis and abnormal pelvic ossification, hearing loss	Loss-of-function	(16)
POLR1A	2p11.2	Acrofacial dysostosis, Cincinnati type	616 404/–	AD	Variable mandibulofacial dysostosis, limb anomalies	Haploinsufficiency; ribosome biogenesis defect	(17)
SNRPB	20p13	Cerebrocostomandibular syndrome	182 282/117 650	AD (*n*)	Micrognathia, cleft soft palate, glossoptosis, posterior rib gaps	Mutations affect the recognition and inclusion of the premature termination codon-containing alternative exon; spliceosomal component	(18,19)
TGDS	13q32.1	Catel-Manzke syndrome	616 146/616 145	AR	Micrognathia, CP, bilateral hyperphalangy leading to index finger clinodactyly	Loss-of-function; likely that residual function remains; pathogenetic mechanism unknown	(20)
TXNL4A	18q23	Burn-McKeown syndrome	611 595/608 572	AR	CL/P, choanal atresia, sensorineural deafness, short palpebral fissures, lower eyelid coloboma, prominent nose with high nasal bridge	Loss-of-function; likely that residual function remains—combination of null and hypomorphic allele	(21)

AD, autosomal dominant; AR, autosomal recessive; XLD, X-linked dominant; (*n*), usually arises by new mutation.

mutants (53). How such a specific phenotype is caused by disruption of ribosome function is not fully understood, but may relate to the increased proliferative demands of the neuroepithelium and NC. Interestingly, activation of p53 may connect the pathophysiology of several human developmental defects including CHARGE syndrome (54).

Craniosynostosis

As discussed above, a key early event in skull vault development is establishment of the SRC (25). The earliest marker of this region is *En1* (encoding Engrailed1), which is expressed with other transcription factors involved in commitment to an osteogenic fate including Msx2 and Twist1. Recently, truncating mutations of *ZIC1* were reported in patients with severe coronal synostosis and learning difficulties (3). Given the known epistatic relationship of Zic proteins upstream of Engrailed in both *Drosophila (odd-paired homologue)* and Xenopus, these findings raise the possibility that misregulation of *EN1* expression in the organising centre disrupts early coronal suture patterning.

A key gene involved in preserving the undifferentiated state is *TWIST1*, a transcription factor expressed in the mid-sutural mesenchyme (55) that prevents RUNX2-mediated osteogenesis (56), and haploinsufficiency of which causes Saethre-Chotzen syndrome (MIM 101400) (57,58). Mutations in the partner protein TCF12 were discovered through exome sequencing of patients with coronal craniosynostosis (5). Combined dosage of TWIST1-TCF12 heterodimers is crucial for suture patency as, in mice, mutations in the two genes interact epistatically such that compound heterozygous *Twist1-Tcf12* mutant mice have severe coronal synostosis (5).

Precise control of proliferation-differentiation balance is crucial for maintaining cranial suture patency. This is illustrated by the fact that craniosynostosis can be caused by mutations of genes with important roles in cell division, a recently discovered example is *CDC45* (3), highlighting a requirement for rapid proliferation of cells within the sutures. Interestingly, Myc has been found to function upstream of Cdc45 in DNA replication initiation (59) providing a possible explanation for how mutations of the ubiquitin ligase *HUWE1* (3) can lead to craniosynostosis, as deletion of *Huwe1* leads to accumulation of N-Myc (60,61).

The importance of fibroblast growth factor/receptor signalling through the RAS-ERK pathway in suture maintenance is well established (62,63). Except for the receptors themselves (*FGFR1*, *FGFR2*, and *FGFR3*) (64), the only gene in this signalling pathway consistently associated with craniosynostosis is *ERF*, which encodes a transcription factor that is a monitor of ERK activity. Reduced dosage of ERF causes multisuture craniosynostosis with postnatal onset, in the mouse leading to paradoxically delayed expression of osteogenic markers indicating a deviation of the proliferation-differentiation balance over time (4). An analysis of the *FGFR2* mutations that cause bent bone dysplasia revealed a nucleolar role for FGFR2 in promoting osteoprogenitor differentiation while decreasing proliferation, providing an alternative mechanism for alterations in this balance (65).

Cleft Lip and/or Palate

Suspected heterogeneity in VWS (66) was recently confirmed with the identification of mutations within *GRHL3* (encoding Grainyhead-like 3) (9), a gene involved in the barrier function of the epidermis. Orthologous zebrafish and mouse mutants exhibited abnormal periderm development, occasionally associated with CP in mouse embryos (9).

Although less is known about the aetiology of non-syndromic CL/P (NSCL/P), extrapolation from syndromic causes has proved helpful. Progress has been expedited through genome-wide association studies (GWAS) on large numbers of samples, which have identified multiple regions/genomic loci associated with NSCL/P (67–71). However, the underlying causative variants from these studies, as well as from genome-wide linkage analyses, remain largely unknown. To address this, Leslie *et al*. resequenced selected regions and identified 123 *de novo* variants, including one encoding a missense substitution within the homeodomain of PAX7 (8). This is the first *de novo* mutation identified in this gene in a NSCL/P case; *PAX7* had previously been highlighted as a risk factor through GWAS and linkage studies (71,72). The missense substitution reduced both DNA binding and expression of a luciferase reporter, suggesting that causative variants in the GWAS signal are likely to affect *PAX7* expression (8). Interestingly, Pax3 and Pax7 regulate craniofacial development through interaction with Aryl hydrocarbon receptor (AHR) signalling that controls CNCC cell cycle exit; *Pax3/Pax7* homozygous mutant mice have a reduction in the facial prominences and clefting (10). As AHR is the receptor for dioxin, these findings provide an insight into environmental aetiologies for clefting (10). A study using case-parent trios provides further evidence that variants of *ABCB1*, encoding a transporter protein controlling foetal exposure to xenobiotics early in gestation, could be a risk factor in NSCL/P and CP (6).

Amongst the non-coding *de novo* variants identified in the resequencing study (8), a variant downstream of *FGFR2* was analysed further, as deletions and rare coding variants had been found in NSCL/P (73,74). This mutation was within a putative NC enhancer and functional assays demonstrated reduced activity (8). Two GWAS signals on chromosome 17 were refined using the resequencing data. Multiple CL/P-associated SNPs clustered near the transcription start site of *NTN1* and immunostaining showed expression of the encoded Netrin-1 within the palatal shelves. The most strongly associated SNP at 17q22 was within a putative regulatory region 105 kb downstream of *NOG* (encoding noggin, an antagonist of bone morphogenetic protein signalling); the risk allele was associated with reduced expression in a reporter assay, particularly when partnered with a second putative enhancer from the same linkage disequilibrium block (8).

Recent studies have validated previously associated genes and loci in NSCL/P, including *FOXE1* (75), *GREM1* (76), *CDH1* (77), 13q31.1 (78) and others that confirm the many different CL/P risk genes (72,79–84). The key NSCL/P susceptibility locus 8q24 harbours a medionasal enhancer that regulates expression of *Myc* acting in *cis* over more than 1 Mb (85). Deletions within this region affect growth of the medionasal process through downregulation of *Myc*, and misexpression of genes involved in ribosome assembly and translation (85). Potential new loci include 16p13.3, between *CREBBP* and *ADCY9* (86). GWAS in dogs has recently highlighted two candidate loci for CP and CL/P respectively, *DLX6* (and co-expressed *DLX5*) (89) and *ADAMTS20* (7).

Other facial clefts

Acromelic frontonasal dysostosis (AFND) is characterised by frontonasal malformation including severe facial clefts combined with limb anomalies (88,89). In four families the same heterozygous missense substitution (p.Arg1163Trp) was present within the highly conserved C-terminal domain of a protein containing a zinc finger SWIM domain and encoded by ZSWIM6 (11). *Zswim6* is expressed ubiquitously with highest

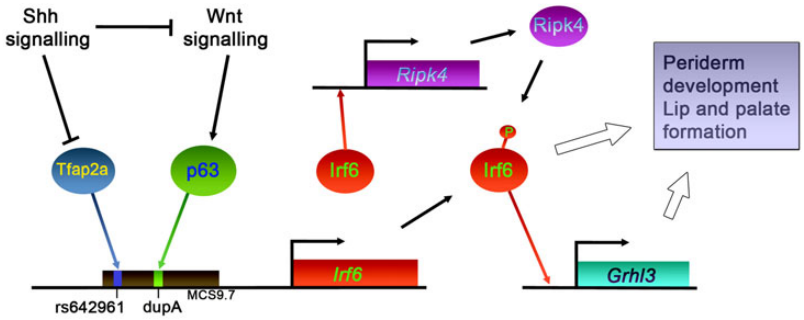

Figure 2. Irf6 regulation and pathways. Interactions involving Irf6 discussed in this review are shown. Sonic hedgehog has a role in correct fusion at the lambdoidal junction through modulation of Wnt signalling and Tfap2a upstream of *Irf6* expression (38). A CLP associated SNP (rs642961) and mutation (dupA) within the *Irf6* regulatory element MCS9.7 affect expression of *Irf6* by disrupting binding of AP-2α (40) and p63 (32), respectively. The dupA mutation also creates a Tcf4/Lef1 binding site. Irf6 regulates expression of both Ripk4 (35), a kinase that can phosphorylate Irf6 (33,34), and *Grhl3* (42) in periderm development (9,41).

levels in the brain and retina, but little is known about its function. Opitz G/BBB syndrome, characterised by midline defects, is genetically heterogeneous with mutations found in *MID1* (90) and recently in *SPECC1L* (12). Specc1l interacts with the actin cytoskeleton and microtubules, has a role in the migration and adhesion of the facial processes (91), and mutations have previously been identified in oblique facial clefts (92).

Disorders of Pharyngeal Arches 1 and 2

Facial dysostoses

The recent identification of mutation of multiple spliceosome components in facial dysostoses, suggests a shared underlying sensitivity of PA development with the ribosomopathies (see above). In 2014 mutations in three genes, *TXNL4A*, *EIF4A3* and *SNRPB*, all encoding factors involved in splicing were identified in Burn-McKeown syndrome (21), Richieri-Costa-Pereira syndrome (RCPS) (13) and cerebro–costo–mandibular syndrome (18,19), respectively. Spliceopathies associated with facial dysostoses also include mandibulofacial dysostosis, Guion-Almeida type (*EFTUD*) (93) and Nager syndrome (*SF3B4*) (94) and all are discussed in a recent review (95). A similar pathogenic mechanism involving NC cell death may underlie Catel-Manzke syndrome, caused by recessive mutations in *TGDS* encoding (dTDP-D-glucose 4,6-dehydrogenase) (20).

Endothelin signalling

Recent discoveries of mutations affecting human endothelin signalling emphasize its key role in development of post-migratory NC-derivatives within PA1/2. *EDN1* mutations were identified in auriculocondylar syndrome (ACS) and isolated question-mark ears (IQME) (14). PA1/2 derivatives are affected in ACS with the mandibular defect thought to represent a homeotic transformation to a maxillary identity [as also seen with mutations of *PLCB4* or *GNAI3* (96,97)]. The ACS *EDN1* mutations were homozygous, probably hypomorphic and predicted to interfere with maturation of active EDN1 (14). In contrast, mutations in IQME were heterozygous loss-of-function changes suggesting that development of the ear is more sensitive to EDN1 dosage than the mandible. *EDNRA* mutations cause a more severe phenotype consistent with a partial homeotic transformation of the upper jaw into a mandible-like structure (the inverse of the switch in ACS), accompanied by CP, hearing loss and alopecia (15). The underlying mechanism is likely gain-of-function with an increase of affinity for an alternative non-preferred ligand, EDN3, which is expressed throughout PA1. This is supported by the observation that misexpression of *Edn1* in the maxillary prominence leads to a similar conversion of the upper jaw (48).

In *Ednra* null embryos expression of *goosecoid* is lost in the PAs, although retained in the limb buds, implicating this transcription factor in downstream pathways (47). Goosecoid is expressed in PA1/2, and the mouse knockout has multiple craniofacial defects, including hypoplasia of the maxillary and mandibular bones (98,99). In humans, loss-of-function mutations lead to a multiple congenital anomaly syndrome termed SAMS (16).

Future Directions

One of the most exciting areas of craniofacial research is the investigation of the role of non-coding sequences in regulating the spatial, temporal and level of expression of genes in normal development. By determining the regions bound by the enhancer-binding protein p300 in embryonic facial tissue, Attanasio *et al.* (100) identified large numbers of candidate loci for craniofacial development. Similarly, investigation of H3K27Ac marked elements from maxillary arch tissue, has generated a dataset of potential upper jaw enhancers, including multiple novel regulatory elements near genes involved in palatal anomalies (101). These and future studies will help in the identification of the regulatory elements that underlie both normal and pathological variation in craniofacial development.

A second potentially fruitful area is the analysis of facial variation and investigation into whether faces can be successfully classified from images and predicted from genotype. Recognition of craniofacial features by clinical geneticists greatly aids genetic diagnosis and efforts are being made to improve classification accuracy from 2D images (102). Furthermore, phenotypic information extracted from clinical photographs can cluster patients by phenotype and/or connect them with the most likely diagnoses (103). For very rare disorders grouping of cases would considerably assist in causative gene discovery. 3D facial images have been utilized to assess the effect of gene dosage, demonstrating that reciprocal deletions and duplications have remarkably complementary effects on face shape (104). Methods for predicting the effect of genetic variation on face shape are being developed (105,106), unfortunately leading to hyperbole in claims for predictive power. The highly complex relationship of genotype to phenotype (107) is emphasized by the analysis of simpler continuous traits, such as height (108).

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