Mouse models of syndromic craniosynostosis

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Abstract

Craniosynostosis is a common craniofacial birth defect. It's clinical presentation, (genetic) diagnosis and treatment is discussed in detail elsewhere in this special issue of Molecular Syndromology. This review focuses on the advances that have been achieved through studying the pathogenesis of craniosynostosis using mouse models. Classic methods of gene targeting which generate individual gene knockout models have successfully identified numerous genes required for normal development of the skull bones and suture. However, the study of syndromic craniosynostosis has largely benefited from the production of knockin models that precisely mimic human mutations. These have allowed the detailed investigation of downstream events at the cellular and molecular level following otherwise unpredictable gain of function effects. This has greatly enhanced our understanding of the pathogenesis of this disease and has the potential to translate into improvement of the clinical management of this condition in the future.

Introduction

Craniosynostosis is a common feature of craniofacial birth defects, with a prevalence of 1:2,500 births (Cohen and Kreiborg, 1992). It is characterized by premature fusion of calvarial bones and can occur along single or multiple cranial sutures. Around 30% of craniosynostosis occurs within a characterised craniofacial syndrome ('syndromic craniosynostosis') with a genetic cause, whilst the majority of non-syndromic cases have a *de novo* cause ('non syndromic craniosynostosis') (Johnson and Wilkie, 2011). The molecular basis for craniosynostosis is complex: for example, a genetic cause such as a dominant mutation within one of the Fibroblast Growth Factor Receptor (FGFR) 1, 2 and 3 genes are well known, yet, environmental factors, most notably intra-uterine head constraint, has also been hypothesized as among the predisposing factors to this condition (Muenke et al., 1997, Muenke et al., 1994, Reardon et al., 1994, Johnson and Wilkie, 2011). The phenotypic consequence of craniosynostosis is skull shape distortion with secondary sensory-neurological deficits through an increase of intracranial pressure (Derderian and Seaward, 2012). Typically, FGFR mutations are responsible for the 'Crouzonoid' phenotype comprising of complex craniosynostosis, midfacial hypoplasia, strabismus and brachycephaly (Johnson and Wilkie, 2011). As a result of craniosynostosis, symptoms include optic atrophy, blindness and hearing deficits (Derderian and Seaward, 2012). There is currently no pharmacological treatment for craniosynostosis, with repeating surgical modalities the primary option to accommodate normal brain growth by correcting skull dysmorphology and reducing intracranial pressure, a procedure known as craniectomy (Johnson and Wilkie, 2011). Specifically, surgical interventions aim to re-open the suture ('distraction osteogenesis') with calvarial remodelling (Park and Yoon, 2012).

Historically, a mutation in the *MSX2* (Msh homeobox 2) gene was first to be associated with syndromic craniosynostosis, eliciting a clinical phenotype known as 'Boston-type craniosynostosis' (Jabs et al., 1993). Mutations in genes encoding FGFRs were identified later and are perhaps the most common genes involved in syndromic craniosynostosis (Wilkie, 2005). The most notable characteristics of craniofacial dysmorphology are often referred to as the 'Crouzonoid' phenotype, with coronal synostosis being the most common type of suture fusion (Wilkie and Morriss-Kay, 2001). This usually results from autosomal dominant mutations that constitutively activate the FGF receptor and as such can be thought of as 'gain-of-function' (GOF) mutations (Wilkie, 2005). A generalisation is that the craniofacial spectrum elicited by FGFRs signalling misregulation

will depend on the tissue specificity and precise allelic mutation within the receptor gene (Wilkie, 2005). Allelic mutations affecting the ligand binding domain (S252, P253, C278 and C342) account for 80% of all craniosynostosis cases. An interesting observation first mentioned by Wilkie (2005) is that identical substitutions across all FGFR paralogues are conserved at equivalent positions along the gene. For example, an amino acid change within the linker region of each receptor such as Proline 250 to Arginine (p.Pro250Arg), gives rise to either Pfeiffer (FGFR1), Apert (FGFR2) or Muenke (FGFR3) syndromes, with coronal synostosis being a common phenotype of all three (Wilkie, 2005). FGFR1, 2 and 3 are all expressed along the edges of the calvarial bones with FGFR2 predominant in the osteogenic front (Johnson et al., 2000, Iseki et al., 1999). However, the spatial localisation of the various splice forms is not well-characterised due to their high sequence homology. Instead, it is mainly through isoform specific knockouts in mouse models that their individual functions have been delineated (see below).

Undeniably, mouse models have offered a significant platform to study human disease progression, and generating models carrying specific knockin mutations can help to address questions concerning the phenotypic diversity caused by the various mutations identified in patients. Whilst a large body of research has focussed on the genomic landscape, the biochemical and transcriptomic consequences that influence cellular activity *in vivo* still remains to be fully elucidated. In order to advance translation to clinical practice, it will be critical to address the aberrant mechanisms that lead toward these craniofacial abnormalities. In this review, we will provide an overview of the currently available mouse models that have been associated with various forms of syndromic craniosynostosis (**Table 1**). Finally, we will draw conclusions about the work done so far and make suggestions where future research into this area is headed.

I FGF signalling related mouse models of human syndromic craniosynostosis

Fibroblast Growth Factor Receptors (FGFRs)

FGFR1

Mutations in FGFR1 have been reported in Kallman, Jackson-Weiss, Muenke and Pfeiffer Syndromes (Ornitz and Itoh, 2015). In addition to the common craniofacial abnormalities, severe Pfeiffer syndrome patients exhibit limb and digit abnormalities (Muenke et al., 1994). Specifically, these patients have varying degrees of

syndactyly, finger truncation, broad digits and short limbs (Muenke et al., 1994). The GOF p.P252R substitution responsible for Pfeiffer syndrome was originally identified in the early 1990s, affecting exon 5 of *FGFR1*, common to both splice forms (Muenke et al., 1994). The mutation was eventually reproduced in the mouse genome, creating a model for Pfeiffer syndrome (*Fgfr1*$^{P250R/+}$) with bi-coronal craniosynostosis along with enhanced expression of osteogenic genes (Zhou et al., 2000). Additionally, these mice have increased cell proliferation at postnatal day (P)5 in the sutures. On the other hand, loss of function (LOF) mutations (i.e. G237S, P722H and N724K) in this receptor are more usually associated with hormone dysregulation than with skeletal defects, and are related to Kallman syndrome (Pitteloud et al., 2006). *Fgfr1* is expressed prominently in the distal limb bud between E8.5-E12.5, and is required for its correct initiation and outgrowth (Verheyden et al., 2005, Li et al., 2005). Conditional inactivation of *Fgfr1*$^{-/-}$ in the limb bud mesenchyme (*T*^{Cre}) do result in long bone and digital defects during later stages of development, similar to those in Pfeiffer patients (Verheyden et al., 2005, Li et al., 2005). Therefore, the differential phenotype elicited by LOF mutations in humans and knockout mice suggest dosage sensitivity of FGFR1 signalling.

Complete abrogation of FGFR1 signalling is highly potent and embryonic lethality is consistently reported throughout the literature (Deng et al., 1994, Yamaguchi et al., 1994). Multiple strategies have been adopted to ameliorate this problem, including generation of hypomorphic models by reducing the expression of full length *FGFR1*, mutating binding sites for Frs2 on *Fgfr1*, or preventing Trk autophosphorylation (Partanen et al., 1998). Additionally, Partanen and colleagues (1998) have achieved isoform specific knockout to exons 8 (IIIb) and 9 (IIIc) by inserting a stop codon into these exons. *Fgfr1b* appears to be the major player in axial skeleton development, as *Fgfr1b*$^{-/-}$ mice display vertebrate column truncations and limb abnormalities, despite the craniofacial skeleton remains largely normal. Thus, the lack of a craniofacial phenotype exhibited by FGFR1 LOF implies its role in craniofacial development is minor. However, the *Fgfr1c* knockout mouse is embryonic lethal suggesting the importance of the mesodermal isoform in early embryogenesis (Partanen et al., 1998).

FGFR2

FGFR2 is a positive regulator for osteoblast differentiation and manipulating this signalling pathway has consequences for osteoblastogenesis. It is well characterised that *Runx2*, the master regulator for osteoblast

differentiation, is downstream of FGFR2 signalling (Miraoui et al., 2009). Therefore, a substantial proportion of clinical syndromes and bone diseases have been related to signalling misregulation caused by this receptor. The role of FGFR2 was first characterised using knockout models. Several groups have generated *Fgfr2* knockout lines with similar phenotypes, yielding a series of gastrulation, placental and osteogenesis defects (Yu et al., 2003, Arman et al., 1998, Arman et al., 1999, Xu et al., 1998). The first FGFR2 knockout was generated by targeted disruption to the kinase domain of the receptor, preventing autophosphorylation (Arman et al., 1998). Other *Fgfr2* knockouts followed, by disrupting immunoglobulin loops along the receptor gene (Xu et al., 1998). Xu et al., generated a knockout by removing exons encoding the IgIII loop responsible for ligand specificity. Despite homozygous lethality at E10.5, this study was the first to gain insights into the role of FGFR2 in limb development as these mutants fail to develop limb buds due to a loss of paracrine signalling that is responsible for tissue outgrowth (Xu et al., 1998). It was later discovered from the $Fgfr2b^{-/-}$ model that the IIIb isoform is critical for limb outgrowth, as these mice have a complete loss of the appendicular skeleton (De Moerlooze et al., 2000, Revest et al., 2001). Fgf10 is a likely binding partner for FGFR2b, as $Fgf10^{-/-}$ mice exhibit striking similarities to $Fgfr2b^{-/-}$ mice (Sekine et al., 1999, Min et al., 1998). On the other hand, $Fgfr2c^{-/-}$ mice illustrate that this isoform is required for normal craniofacial development as bi-coronal synostosis and under development of the auditory bulla were reported characteristics (Eswarakumar et al., 2002). Others have also generated conditional *Fgfr2* knockouts to study tissue specific effects: Conditional knockout in the mesenchyme using $Dermo1^{Cre}$ leads to defects in both axial and craniofacial skeleton (Yu et al., 2003). Specifically, these mice have decreased bone density, truncated femurs owing to insufficient chondrocyte and osteoblast proliferation, brachycephaly and dwarfism (Yu et al., 2003).

A large cohort of characterised craniofacial syndromes are commonly associated with *FGFR2* germline mutations (Wilkie, 2005). GOF mutations in the *FGFR2* gene are characteristic of Apert, Crouzon, and Beare-Stevenson syndromes (Wilkie, 2005) and establish that FGFR2 signalling is a key player in craniofacial development. Crouzon syndrome is most commonly caused by a substitution mutation in FGFR2c (FGFR2c-p.C342Y; at the DIII Ig loop) and is autosomal dominant (Reardon et al., 1994). The substitution of a cysteine to a tyrosine residue results in the stabilization of intermolecular disulphide bonds at the receptor extracellular domains, leading to constitutive activation (Eswarakumar et al., 2005). The phenotypes affecting the IIIc

isoform in Crouzon syndrome are mainly craniofacial, whilst the p.S252W mutation found in Apert syndrome is associated with additional limb phenotypes such as truncation and syndactyly, since the mutation affects both FGFR2 splice variants (Johnson and Wilkie, 2011). Mouse models are available for the most common FGFR2 craniofacial syndromes: *Fgfr2c*$^{C342Y/+}$ (Crouzon), *Fgfr2c*$^{W290R/+}$ (Crouzon) and *Fgfr2*$^{S250W/+}$ (Apert) (Wang et al., 2005, Eswarakumar et al., 2004, Mai et al., 2010, Chen et al., 2003). A common characteristic in these models are shortened midface, brachycephaly and coronal suture obliteration, which mimicks the human disease phenotype. Interestingly, none of these models, including Apert mice, display a limb phenotype. On a cellular level, these mutations affect FGFR2 function by altering osteoblast proliferation, differentiation and apoptosis in the suture. Eswaraskumar et al 2004 reported from around E13.5 an early increase in cellular activity at the osteogenic front that is responsible for suture obliteration in *Fgfr2c*$^{C342Y/+}$ (Eswarakumar et al., 2004). Chen et al., 2003, however, reported increased apoptosis as being a key player for coronal synostosis development in a separate mouse model for Apert syndrome (*Fgfr2*$^{S250W/+}$) (Chen et al., 2003).

It is not well understood how a separate allelic mutation, also affecting the transmembrane domain of FGFR2, gives rise to Beare-Stevenson syndrome (*Fgfr2*$^{Y394C/+}$) (Wang et al., 2012). Similar to the C342Y mutation, FGFR2-Y394C stabilises intermolecular bonds of unpaired cysteine residues leading towards constitutive activation. However, despite showing craniofacial similarities, Beare-Stevenson patients have additional skin abnormalities including cutis gyrate (thickened scalp) and acanthosis nigricans (hyper pigmentation) (Wang et al., 2012). A mouse model has been generated to study this mutation (*Fgfr2*$^{Y394C/+}$), but the pathogenic origin of the cutaneous phenotype still remains unclear (Wang et al., 2012). In addition to introducing GOF mutations, increasing gene dosage also allows the identification of novel phenotypes in animal models. For example, a detailed analysis of *Fgfr2c*$^{C342Y/C342Y}$ homoygotes identified exencephaly, overt cleft of the secondary palate and a series of segmentation defects along the axial skeleton (Peskett et al., 2017).

Due to a common craniofacial phenotype elicited in animal models for syndromic synostosis, they provide a robust platform to test novel therapeutic approaches and evaluate the safety of potential clinical treatments. For instance, Shukla et al., 2007 attempted to rescue the coronal suture in *Fgfr2*$^{S252W/+}$ mice through knockdown of the RAS-MAPK pathway with short-hairpin RNA (shRNA) or MEK-ERK inhibition using U0126 treatment. Despite some rescue of the suture, longitudinal evaluation of these mice revealed growth

restriction in a proportion of treated animals along with spontaneous unexplained death in others (Shukla et al., 2007). Indeed, the activating nature of FGFR2 has led to the assumption that attenuation of downstream signalling is sufficient to rescue craniofacial malformations (Shukla et al., 2007, Pfaff et al., 2016). Several studies were able to demonstrate this *in vivo*: Firstly, craniofacial morphology was rescued when a mutant *Frs2α* allele was introduced onto the *Fgfr2c*$^{C342Y/+}$ mouse, which prevented activation of the downstream RAS-MAPK pathway (Eswarakumar et al., 2006). Secondly, systematic MAPK knockdown using shRNA or U0126 treatment was also sufficient to rescue craniosynostosis in *Fgfr2*$^{S252W/+}$ (Shukla et al., 2007). However, the idea of simply dampening the signal as a potential therapy may well be over-simplistic given the complexity of pathogenic FGFR2 signalling. Snyder-Warwick et al 2010 examined the nature of FGF signalling output in the palates of the Crouzon mouse model (Snyder-Warwick et al., 2010). The authors found that *Spry2, Spry4, Etv5* and *Dusp6,* all direct targets of FGF signalling, were downregulated at multiple developmental stages (Snyder-Warwick et al., 2010). At the cellular level, these embryos had reduced cellular proliferation that resulted in a delay to palatal shelf elevation. Moreover, isoform specific knockout of *Fgfr2c* (*Fgfr2c*$^{-/-}$) was sufficient to phenocopy the effects of *Fgfr2c*$^{C342Y/+}$ with apparent coronal synostosis (Eswarakumar et al., 2002). The paradoxical nature of FGFR2c signalling remains to be elucidated, but it is generally accepted that an intricate balance of signalling activity is required for normal development.

FGFR3

FGFR3 is a negative regulator for bone formation (Deng et al., 1996). A number of *FGFR3* knockout lines using different targeting methodologies have been reported, all showing consistent bone overgrowth phenotypes (Deng et al., 1996, Colvin et al., 1996, Eswarakumar and Schlessinger, 2007). The most notable characteristic is that these mice are larger in size as a consequence of ectopic chondrogenesis (Deng et al., 1996, Colvin et al., 1996). Despite this, their bones have increased porosity, most likely due to a reduction in bone mineralisation. Analysis of isoform specific knockouts revealed that the *Fgfr3c* isoform is responsible for the hyperplastic phenotype (Eswarakumar and Schlessinger, 2007). In turn, an actived FGFR3 pathway through GOF mutations in *FGFR3* leads to increased negative regulation of endochondral bone formation and is associated with short-limbed dwarfism caused by skeletal dysplasias such as achondroplasia and thanatophoric dysplasia (Rousseau et al., 1994, Shiang et al., 1994, Bellus et al., 1995). The first missense

mutation for achondroplasia was identified as a glycine 380 to arginine substitution (FGFR3-p.G380R) within the transmembrane domain of FGFR3 (Shiang et al., 1994, Bellus et al., 1995). This mutation decreases receptor trafficking from the membrane, resulting in increased levels of phosphorylation during exposures to FGF ligands and signalling activation (Monsonego-Ornan et al., 2000). Histological analysis reveals a saturation of FGFR3 at mutant mouse growth plates, coincided with fewer chondrocytes in growth plates and hypertrophic zones (Monsonego-Ornan et al., 2000, Segev et al., 2000). Several mouse models have been made for achondroplasia such as *Fgfr3*$^{G374R/+}$ and *Fgfr3*$^{G369C/+}$, which also affect the transmembrane domain (Wang et al., 1999, Chen et al., 1999). Other models integrated a transgene containing the human FGFR3-p.G380R cDNA into the mouse genome, phenocopying the human disease (Segev et al., 2000, Lee et al., 2017). In addition to the dwarfism phenotype, these mice also display brachycephaly and brain distortion (Wang et al., 1999). Thanatophoric dysplasia is the most severe form of dwarfism (Tavormina et al., 1995). The genetic differences that separates acondroplasia and thanatophoric dysplasia can be explained by different modes of receptor activation. Mutations that result in achondroplasia are caused by ligand dependent receptor over-activation, whilst mutant FGFR3 for thanatophoric dysplasia are constitutively active (Ornitz and Itoh, 2015). Mutations responsible for thanatophoric dysplasia stabilises intramolecular bonds of FGFR3 at either the transmembrane domain (*Fgfr3*$^{S365C/+}$) or the ligand specific domain (*Fgfr3*$^{Y367C/+}$) (Chen et al., 2001, Pannier et al., 2009, Ornitz and Itoh, 2015). In humans, a further mutation affecting the TRK domain e.g. FGFR3-p.K650E was identified, but a mouse model is not yet available, perhaps owing to the potency of mutations affecting the kinase domain (Ornitz and Itoh, 2015). There have been reports linking craniosynostosis to achondroplasia and thanatophoric dysplasia in the literature but these links are not yet well established. For example, some patients with thanatophoric dysplasia exhibit a cloverleaf skull, suggestive of severe craniosynostosis (Tavormina et al., 1995), another mutation for achondroplasia-FGFR3-p.A391E, has been identified in Beare-Stevenson patients (Meyers et al., 1995) and similarly in the mouse, whilst an isolated study reports coronal synostosis in *Fgfr3*$^{G380R/+}$ (Lee et al., 2017).

The p.P250R mutation is the most common mutation identified in all three FGFR paralogues (Wilkie, 2005). In FGFR3, this leads to Muenke syndrome, where unilateral or bicoronal synostosis is an apparent characteristic (Muenke et al., 1994). The p.P250R mutation affects both FGFR3 isoforms, and results in

increased affinity for FGF ligands (Muenke et al., 1994, Wu et al., 2009). A knockin of this mutation in the mouse recapitulated Muenke syndrome (*Fgfr3*$^{P244R/+}$) (Twigg et al., 2009). However, the craniofacial phenotype was incompletely penetrant due to background differences between mouse strains. Despite the variability observed in the general craniofacial skeleton, it has been a useful mouse model for studying inner ear development (Mansour et al., 2009, Mansour et al., 2013). Muenke individuals were reported to have poor sensory reception towards the low end of the auditory spectrum (Mansour et al., 2009). Analysis of the *Fgfr3*$^{P244R/+}$ mouse identified multiple disruptions to the cochlear duct cytoarchitecture with alterations to the overall balance of support cells, with bias towards Dieter cell fate differentiation (Mansour et al., 2013, Mansour et al., 2009). This change of fate was largely due to the mutation causing the receptor losing ligand specificity, allowing ligands to bind promiscuously to both *Fgfr3b* and *Fgfr3c* isoforms (Mansour et al., 2013). Genetic rescue of the ear phenotype can be achieved by reducing expression of *Fgf10*, a ligand specific to both the FGFR3b isoform, in compound mutants (*Fgfr3*$^{P244R/+}$*; Fgf10*$^{+/-}$) (Mansour et al., 2013). Perhaps this study offers a useful insight into the reason why craniosynostosis does not develop in the Muenke mouse model, since in addition to background strain, available ligands in the coronal suture can act as limiting factors to the proposed phenotype.

FGF ligands

The embryonic coronal suture express a repertoire of FGF ligands (Hajihosseini and Heath, 2002). However, it is not known how FGF ligands coordinate craniofacial development or regulate suture patency. The question is complex largely due to FGF ligands having multiple affinities toward different FGF receptors (Zhang et al., 2006). Therefore, abrogation of FGF genes *in vivo* is likely to result in a redundant phenotype (Zhang et al., 2006, Barak et al., 2012, Wright and Mansour, 2003). In light of this, determining whether a phenotype is a consequence of a single FGF ligand or a combination working synergistically remains a challenge. FGF ligands are abundant during development and any genetic perturbation will most likely result in defects to organogenesis. One of the main purposes of FGF signalling in development is to mediate cross talks between the mesenchyme and epithelium (Ornitz and Itoh, 2015). The most common FGF ligands involved in this process include epithelially expressed FGF9 and FGF10 in the mesenchyme, each signalling reciprocally to their tissue specific FGFR isoforms (Ornitz and Itoh, 2015). An example of tissue specific

interaction can be seen in the developing limb bud, which institutes a positive feedback loop regulating its outgrowth (Li et al., 2007, Revest et al., 2001). Thus, genetically disrupting the tight coordination results in a series of skeletal dysplasias in the axial skeleton, largely affecting bone mass, densities and stunted growth (De Moerlooze et al., 2000, Revest et al., 2001, Eswarakumar et al., 2002, Eswarakumar et al., 2004). In addition to genetic approaches, surgical bead implantations soaked in different FGF ligands have been fundamental to understanding osteogenesis *in vivo* (Iseki et al., 1999). Explicitly, this classical embryology approach is well characterised to investigate the impact of specific ligands on the calvaria e.g. FGF2, to investigate ectopic osteogenesis (Iseki et al., 1999). All the FGF ligands are expressed in the coronal suture with the exception of FGF3 and FGF4 (Hajihosseini and Heath, 2002). To date there has not been a report of syndromic craniosynostosis associated with ligand function other than FGF9, although mutations in *FGF3*, *FGF8* and *FGF10* are sufficient to disrupt craniofacial development (Ornitz and Itoh, 2015).

Multiple synostoses syndrome 3 (SYNS3) is characterised by multiple fusion of synovial joints in the axial skeleton (Wu et al., 2009). It is autosomal dominant and is caused by a missense mutation in FGF9 (FGF9-p.S99N) (Wu et al., 2009). A phenotype closely resembling MSS in the mouse, with fusions of the elbow and knee, has been reported in the *Fgf9*$^{N143T/+}$ mouse (Harada et al., 2009). In addition to elbow-knee synostoses (EKS), these mice exhibit coronal synostosis. The consequence of the mutation has led to a loss-of-function for FGF9-heparin binding, which affects overall FGFR signalling potency. This protein interaction impairment resulted in hyper-diffusibility of the ligand in the extracellular matrix, encroaching into the joint and suture mesenchyme to induce synostosis. Additionally, mouse mutants carrying the missense mutation have ectopic expression of multiple osteoblast precursor markers such as *Runx2* and *Osteopontin* in the coronal suture mesenchyme at E16.5 (Harada et al., 2009). FGF9 has high affinity towards the IIIc isoforms, in particular to FGFR3c (Zhang et al., 2006). Thus, coronal synostosis for Muenke syndrome is indirectly linked to FGF9, as the FGFR3-p.P250R mutation causing Muenke syndrome affects the affinity of FGFR3c to FGF9 (Harada et al., 2009, Muenke et al., 1997).

Due to the promiscuity of ligand-receptor interaction, it is possible that ectopic expression of FGF ligands can also drive tissue specific phenotypes provided the receptors are expressed in the right tissue. In this scenario, Carlton and colleagues successfully phenocopied the Crouzon mouse model (*Fgfr2c*$^{C342Y/+}$) by disrupting the

intergenic regions of *Fgf3* and *Fgf4*, normally absent from the wild-type (WT) suture, with midfacial hypoplasia, brachycephaly and bi-coronal synostosis (Carlton et al., 1998). Phenotypic redundancy is a consequence of ligand compensation. For example, in respect to FGF9, a fully penetrant urogenital tract defect is only observed when both *Fgf9* and *Fgf20* are knocked out (Barak et al., 2012). This is similar during cardiovascular morphogenesis, with partial penetrance when single deletions to *Fgf3* and *Fgf10* occurred together (Urness et al., 2011).

In summary, the relatively small number of syndromic craniosynostosis cases associated with mutations in FGF ligands is likely due to ligand redundancies, especially given that the coronal suture expresses 20 of the 22 FGF ligands (Hajihosseini and Heath, 2002). The spatial-temporal dynamics of FGF signalling, along with the diversity of FGF ligands functioning synergistically, makes biological data difficult to interpret. As biological signals are conveyed through the receptors, targeting FGFRs appear to yield more substantial phenotypes to that observed in ligands while giving direct interpretation to its function.

II Other mouse models of human syndromic craniosynostosis

Twist1

The Twist gene family encodes two basic-helix-loop-helix transcription factors: *Twist1* and *Twist2* (also known as *Dermo1*) (Qin et al., 2012). Originally, *Twist1* was demonstrated to be required for neural tube morphogenesis while *Twist2*, involved in regulating cytokine gene expression (Chen and Behringer, 1995, Sosic et al., 2003). *Twist1* plays a variety of roles in mesoderm development focussing mainly in mesenchymal tissue and is expressed in the cranial mesenchyme (Bildsoe et al., 2013). *Twist1* is expressed prominently in the suture mesenchyme and its inactivation in mice results in coronal synostosis (Behr et al., 2011, Carver et al., 2002). This is due to *Twist1* acting as a negative regulator of bone formation where it prevents osteoblast differentiation through *Runx2* inhibition (Bialek et al., 2004). *Runx2* deficient mice display delayed osteogenic activity *in vivo*, but introducing a copy of the *Twist1* null allele into *Runx2*$^{+/-}$ (*Runx2*$^{+/-}$*; Twist1*$^{+/-}$) was sufficient to rescue a hypoplastic phenotype (Bialek et al., 2004, Komori et al., 1997). Conversely, *in vitro* analysis of *Twist1* knockdown revealed decreased *Runx2* expression with increased apoptosis (Yousfi et al., 2001, Maestro et al., 1999). Additionally, mutant *Twist1* drives increased expression of *FGFR2 in vitro* and *in vivo*, perhaps as a compensatory effect, to upregulate the sensitivity of FGF

signalling for cellular survival (Miraoui et al., 2010, Connerney et al., 2008). With regard to disease pathogenesis, autosomal inheritance of LOF mutations in *TWIST1* result in Saethre-Chotzen syndrome (Howard et al., 1997, el Ghouzzi et al., 1997). Saethre-Chotzen patients display complex suture abolishment most notably coronal, posterior frontal and lambdoid sutures with digit duplication (el Ghouzzi et al., 1997, Howard et al., 1997). The first *Twist1* mouse mutant constructed by substituting exon 1 with a neo-cassette was described in 1995 in a study of cranial neural tube closure (Chen and Behringer, 1995). However, these *Twist1* homozygotes were embryonic lethal by E11.5. *Twist1*$^{+/-}$ mice were viable with partial penetrance of limb and craniofacial phenotypes that replicated human disease according to the to genetic background of the animals (Bourgeois et al., 1998). In addition to *Twist1* being a negative regulator for osteogenesis, it also functions to inhibit chondrocyte differentiation. *Twist1*$^{+/-}$ demonstrates enhanced chondrocytic activity in the coronal suture mesenchyme with upregulation of chondrocyte markers such as *Sox9*, *Collagen II* and *Collagen X*, but its significance is currently elusive (Behr et al., 2011). Contact inhibition may also play an important role in mediating Saethre-Chotzen syndrome, as both the Notch ligand *Jagged1* (*Jag1*) and the ephrin receptor-*EphA4* are downstream of *Twist1*. Conditional knockout of either *Jag1* or *EphA4* on a *Twist1*$^{+/-}$ background augments the craniosynostosis phenotype, whilst removal of either *Jag1* or *EphA4* is not sufficient to drive this pathogenesis (Ting et al., 2009, Yen et al., 2010). Both of these studies stress the importance of contact inhibition in controlling osteoblast differentiation in the mesenchyme, since conditional removal leads to mis-specification, and loss of positional information in specifying the suture boundary. Additionally, LOF mutations in *JAG1* in humans are associated with Alagille syndrome, linking the pathogenesis of this syndrome to Saethre-Chotzen syndrome (Yen et al., 2010). bHLH proteins undergo a plethora of roles during development and tend to function as either hetero- or homo-dimers. bHLH proteins are classified by its tissue distribution, ability to dimerize and DNA binding specificities. Class I bHLH proteins are known as 'E proteins' and are abundant in multiple tissue types, whilst Class II e.g. Twist, have more restricted expression domains. Both Class I and Class II HLH proteins contain the basic domain and are DNA binding. Conversely, Class III proteins, which include Id, facilitate dimerization with E proteins and perturb formation of heterodimers between ClassI/II proteins (Massari and Murre, 2000). The degree of *Twist1* syndromic craniosynostosis is related to their binding partners and the dimers they form. For example, a severe craniosynostosis phenotype is caused by a frameshift in *Tcf12* in a *Twist1* background (*EIIa*$^{CRE/+}$*;*

Tcf12$^{flox/-}$*; Twist1*$^{+/+}$) (Sharma et al., 2013). *Tcf12* is an E protein, and the inability to form the Twist1-Tcf12 heterodimer results in a severe phenotype. The same scenario occurs to LOF of bHLH inhibitors *Id1* and *Id3* under a *Twist1* heterozygous background, with the former showing greater penetrance (*Id1*$^{+/-}$*; Twist1*$^{+/-}$) (Connerney et al., 2008).

Msx2

Mutations in *MSX2* are associated with Boston type craniosynostosis (Jabs et al., 1993). Substitution of a histidine 148 to a proline (p.His148Pro) increases the binding affinity of MSX2 to its target DNA sequence (Ma et al., 1996). *Msx2* is expressed in the sagittal and lambdoid sutures of the mouse, and its overexpression, which can be either of a WT allele or through a GOF mutation (*Msx2*$^{P7H/+}$), leads to narrowing of the sagittal suture and abnormal bone overgrowth, particularly to the parietal bone (Liu et al., 1995). In contrast, *MSX2* haploinsufficiency in humans leads to ectopic calvarial foramen and delayed suture closure, due to the loss of binding affinity to target DNA (Wilkie et al., 2000). A similar observation is reported upon knockout of *Msx1* and *Msx2 in vivo* (Roybal et al., 2010). The ectopic foramen was not a consequence of an embryonic patterning defect but rather, a mitotic decrease in the bone, likely from the ossification centre (Roybal et al., 2010, Ishii et al., 2003). *Msx2* and *Twist1* interact to coordinate cellular proliferation and differentiation: Firstly, haploinsufficiency of both genes lead to ectopic frontal foramen formation and secondly, knockout of both alleles result in increased phenotypic severity (Ishii et al., 2003). Analysis of embryos between E12.5 and E14.5 revealed that the pathogenesis is mainly a reduction of osteoblast differentiation beginning at E12.5. It is interesting to note that the transcripts of these genes do not display the same expression pattern embryonically, but do lead to the same phenotypic outcome. Therefore, this suggests these transcription factors may work synergistically as a co-factor to derive the same phenotype at the protein level (Ishii et al., 2003).

Hedgehog related genes

Gli3 LOF is associated with Greig cephalopolysyndactyly (GCP). GCP is an autosomal dominant disorder affecting both limb and craniofacial development (Hui and Joyner, 1993). The most notable characteristic includes supernumeric fingers (polydactyly), macrocephaly and a broad forehead with an additional phenotype being lambdoid synostosis (Rice et al., 2010). Gli3 is primarily a repressor of Hedgehog signalling,

which its LOF exacerbates, resulting in GCP. Specifically, GCP is caused by deletions to 7p13 on the human chromosome, and is also mapped to the same region in the mouse genome (Hui and Joyner, 1993). Johnson (1967) developed the first mouse model 'Xtra Toes', *Gli3*$^{Xt\text{-}J/Xt\text{-}J}$ (Xt-J), carrying an intragenic deletion of *Gli3* (Johnson, 1967, Hui and Joyner, 1993). Augmented Hedgehog signalling is related to Carpenter syndrome, through LOF to *RAB23* (Ras-related protein Rab23)- a separate negative regulator of Hedgehog signalling preventing signalling transduction through Gli2 repression (Jenkins et al., 2007, Eggenschwiler et al., 2006). Carpenter syndrome patients display pan synostosis, but *Rab23*$^{-/-}$ mice do not recapitulates its phenotype and is embryonically lethal (Eggenschwiler et al., 2001). Thus, the *Gli3*$^{Xt\text{-}J/Xt\text{-}J}$ was used to study Hedgehog misregulation in craniosynostosis instead (Rice et al., 2010). Further analysis of *Gli3*$^{Xt\text{-}J/Xt\text{-}J}$ reveals ectopic osteoblast differentiation in the lambdoid sutures, which leads to early suture abolishment. Lambdoid synostosis can be rescued through augmentation of FGF signalling, which upregulates expression of *Twist1* (Rice et al., 2010, Rice et al., 2000). Interestingly, missense mutations to human *MEGF8* (multiple EGF like domain 8) phenocopies features observed in Carpenter Syndrome (Twigg et al., 2012). Additionally left-right asymmetry and cardiac defects, were also reported in a mouse mutant (*Megf8*$^{C193R/+}$) generated as a result of a large-scale mutagenesis screen (Zhang et al., 2009, Aune et al., 2008). However, craniofacial or skeletal defects were not analysed in this paper. It is unclear as to the role of *Megf8* during development, but the close resemblance between *MEGF8* and Carpenter syndrome suggests the mechanism responsible is highly similar. One might speculate effects on early development, and given by the abnormalities in left right-asymmetry, it is likely to affect both Nodal and Hedgehog signalling. This is supported by the recently demonstration that *Megf8* dampens Hedgehog signalling in the primary cilia and that could explain the phenotypic similarities to Carpenter syndrome caused by *RAB23* mutation (Pusapati et al., 2018).

Masp1 **and** ***Colec11***

MASP1 (Mannose associated serine protease 1), *Colec10* and *Colec11* (Collectin subfamily 10 and 11) are components of the lectin pathway that is associated with inflammation. Mutations in these genes contribute to the pathogenesis of 3MC syndrome (Rooryck et al., 2011, Urquhart et al., 2016, Munye et al., 2017) which is the collective term for multiple related syndromes (Carnevale, Mingarelli, Malpuech and Michels syndromes) (Titomanlio et al., 2005). Common clinical features include craniosynostosis, cleft palate, hypertelorism,

hearing deficits, growth deficiencies and heart defects (Titomanlio et al., 2005), which are consequently suggested to be affected during development by abrogation to cytokine signalling (Newton and Dixit, 2012). Multiple amino acid changes in *MASP1* and *COLEC11* have been identified in human patients, predominantly resulting in a frameshift and LOF of the mature protein (Rooryck et al., 2011). There is no mouse model that describes 3MC at present. However, a double knockout of *Masp1* and *Masp3* (*Masp1/3*$^{+/-}$) is reported in the literature without describing any obvious role in craniofacial development (Takahashi et al., 2008).

Hdac4

Histone deacetylases (HDAC) modulate gene expression by altering chromatin structure and repressing transcription factors. Brachydactylyl mental retardation syndrome (BMRS) occurs as a consequence of the LOF of *HDAC4* (Williams et al., 2010), commonly due to deletion of Chr2q37 region that includes the *HDAC4* gene. Clinical presentation of BMRS includes craniofacial dysmorphology with craniosynostosis, developmental delay, obesity and mental deficit on the autistic spectrum (Williams et al., 2010). HDAC4 can bind to the *Runx2* promoter and as such is a regulator of the rate of ossification (Vega et al., 2004). *Hdac4*$^{-/-}$ mice show premature ossification of endochondral structures, whilst overexpression of *Hdac4* in the chondrocytic lineage (*Col2a1-Hdac4*) results in cartilage dysplasia (Vega et al., 2004). However, the authors did not report any craniofacial abnormalities in the *Hdac4* knockouts. The contribution of the role of (endochondral) cartilage in the process of suture abolishment remains elusive, despite a recent study showing that ectopic chondrocytes invading the suture mesenchyme lead to endochrondral ossification in a PDGFRα mutant (He and Soriano, 2017).

TGF signalling misregulation

TGFβ signalling misregulation is associated with Marfan syndrome, a rare disorder occurring in approximately 1: 5,000 individuals (Judge and Dietz, 2005). Marfan syndrome is a complex disease affecting multiple systems, including craniofacial and skeletal dysmorphology, cardiovascular abnormalities, tissue fibrosis, ocular, and mental deficits (Judge and Dietz, 2005). Loeys-Dietz disease and Shprintzen-Goldberg syndrome, have a varying phenotypic spectrum of the described 'Marfanoid phenotype' (MacCarrick et al., 2014, Carmignac et al., 2012, Loeys et al., 2005, Judge and Dietz, 2005). In particular, patients with Marfanoid phenotype and craniosynostosis are commonly referred to as 'Shprintzen-Goldberg syndrome'.

Missense mutations in the extracellular matrix protein Fibrillin 1 (*FBN1*) was first identified to be associated with Marfan syndrome, and has been implicated in craniosynostosis (Dietz et al., 1991, Sood et al., 1996). However, multiple knockin models have been described in the mouse without recapitulating craniosynostosis (Carta et al., 2006, Judge et al., 2004, Ng et al., 2004, Pereira et al., 1999). Mutations causing LOF of the TGF signalling repressor, *SKI*, are associated with Shprintzen-Goldberg syndrome indicating a potential role for this signalling pathway in craniosynostosis (Doyle et al., 2012, Carmignac et al., 2012). Mutations in *TGFBR1* and *TGFBR2* were identified as the cause of Loeys-Dietz disease (Loeys et al., 2005), a condition that typically presents with cardio-ventricular and outflow tract abnormalities, cleft palate, hypertelorism and occasionally craniosynostosis (Loeys et al., 2005). Substitution mutations in *TGFBR1* and *TGFBR2* augment TGF signalling activity, as shown by an increase of the pSMAD readout, and are therefore considered as GOF mutations (Loeys et al., 2005). Craniosynostosis of varying severity has been reported in mice with mutations in both of these receptors (*Tgfbr1*$^{M318R/+}$ and *Tgfbr2*$^{G357W/+}$) (Gallo et al., 2014). In particular, *Tgfbr1*$^{M318R/+}$ has partial coronal synostosis with additional kyphosis in the thoracic region (Gallo et al., 2014).

III Mouse models indirectly associated with human syndromic craniosynostosis

The previous sections described mouse mutants that model human mutations causing syndromic craniosynostosis. In addition, there are numerous mouse mutants that are not based on human genetic mutations (i.e. knockin mutants) that also exhibit craniosynostosis. A substantial number of these mutants are related to growth factor signalling misregulation. Further to *Fgfr2*$^{C342Y/+}$, isoform specific knockouts such as *Fgfr2b*$^{-/-}$ and *Fgfr2c*$^{-/-}$ also display a craniosynostosis phenotype (Eswarakumar et al., 2002, De Moerlooze et al., 2000). In particular, *Fgfr2c*$^{-/-}$ mice show coronal synostosis that phenocopies the *Fgfr2c*$^{C342Y/+}$ mutant (Eswarakumar et al., 2002). The main difference between both models lies within the disease progression, where the latter shows an accelerated phenotype during early embryogenesis (Eswarakumar et al., 2002, Eswarakumar et al., 2004). *Fgfr2b*$^{-/-}$ displays a subtle form of craniosynostosis, with fusion of the parietal and squamous temporal bones (De Moerlooze et al., 2000). It is well established that a hallmark of craniosyostosis is the upregulation of the MAPK/ERK pathway. Indeed, knockout of (*Dusp6*$^{-/-}$) a negative regulator of ERK, results in coronal synostosis (Li et al., 2007). Similarly, LOF mutations of the Ets2 repressor factor (*ERF*) are also implicated in compound craniosynostosis to the coronal and sagittal sutures (Twigg et al., 2013). ERF is

responsible for the export of active ERK from the nucleus in order to attenuate its transcriptional activation activities, and conditional knockout of ERF (*ERF*$^{flox/-}$) recapitulates the phenotype observed in patients with LOF mutations (Twigg et al., 2013).

Although not commonly associated with human craniosynostosis, Platelet derived growth factor (PDGF) signalling has also been related to craniofacial malformations and coronal synostosis (He and Soriano, 2017, Moenning et al., 2009, Soriano, 1997). This is most likely due to the conserved nature of growth factor signalling downstream of the receptor. Signalling misregulation in an isoform of the platelet derived growth factor receptor PDGFRα, is implicated in midline defects and a split face (*Pdgfrα*$^{-/-}$) (Soriano, 1997). Moreover, over-activation of PDGFRα (*Pdgfrα*$^{D842V/+}$) drives ectopic chondrogenesis, eliciting coronal and lambdoid synostosis through the P13K-AKT cascade (He and Soriano, 2017). A similar phenotype is replicated in a transgenic overexpression model located at the *Rosa26* locus (*R26R*$^{Pdgfrα\text{-}D842V/+}$) (Moenning et al., 2009).

In addition to growth factors, Wnt/β-catenin signalling is also a key player in regulating osteoblast proliferation and differentiation (Yu et al., 2005). In the absence of signalling activation, β-catenin is prevented from being translocated to the nucleus through degradation. Therefore, Wnt signalling modulators are critical to modulate signalling sensitivity. Axin serves as a scaffold for formation of a β-catenin degradation complex, and its degradation is therefore Axin dependent (Logan and Nusse, 2004). Augmentation of Wnt signalling by *Axin2*$^{-/-}$ results in coronal and interfrontal synostosis (Yu et al., 2005). Similarly, overexpression of Nel-like type 1 molecule (*NELL1*) elicits posterior frontal suture craniosynostosis, along with the partial closure of sagittal and coronal sutures (Zhang et al., 2002). *NELL1* was originally isolated from samples obtained from unilateral (non-syndromic) coronal synostosis patients, and is expressed in the mesenchyme and osteogenic fronts (Ting et al., 1999). *NELL1* expression is upregulated in sutures undergoing premature fusion, and is believed to augment Wnt/ β-catenin signalling, through interactions with β-integrins in osteoblasts (Ting et al., 1999, James et al., 2015).

There are a number of other craniosynostosis mutants reported in the literature. Growth differentiation factor 6 (*Gdf6*) is a secreted morphogen associated with the BMP signalling pathway (Ducy and Karsenty, 2000). Mutants lacking both alleles of *Gdf6* (*Gdf6*$^{-/-}$) display coronal synostosis and appendicular skeleton

abnormalities, with fusion of the tarsals and carpals (Settle et al., 2003). *Runx2* is indispensable for osteoblast differentiation, and overexpression of *Runx2* in the mesenchyme, driven under the endogenous promoter *Prx1* (*Prx1-Runx2*), elicits multiple synostosis, inclusive of multiple joint fusions in the appendicular skeleton (Maeno et al., 2011). Metopic suture synostosis is caused by LOF mutations to Fras1 related extracellular related gene 1 (*FREM1)*, and is a cause of trigonocephaly in humans (Vissers et al., 2011). *Frem1* encodes a protein secreted by mesenchymal cells that aids extracellular matrix remodelling remodelling (Smyth et al., 2004). In the mouse, *Frem1* is expressed along the periphery of the frontal bone, immediately adjacent to the interfrontal suture (metopic eqivalent) (Vissers et al., 2011). The orginal mouse model ENU by mutagenesis *Frem1*$^{Bat/+}$ is a hypomorph caused by exon skipping (Smyth et al., 2004) and an exon 2 knockout mouse is also available, *Frem1*$^{qbrick/+}$ (Kiyozumi et al., 2006, Vissers et al., 2011). Both models harbour interfrontal synostosis, with the latter mutant possessing a stronger phenotype (Vissers et al., 2011).

Conclusion/Future directions

Mouse models have been central to the study of human disease. Together with lineage tracing reporters, the phenotypes caused by knock in mutations have been critical to understand the pathways required for craniofacial development. Furthermore, mouse models provide a platform to test novel therapeutic strategies and management techniques (Wang et al., 2015, Perlyn et al., 2006, Maruyama et al., 2016). However, it is well understood in the murine research community that mouse models of human disease sometimes exhibit differences in phenotypic end points, which might simply be due to species differences. These can be the result of differing genetic redundancies and sensitivities. An example of this can be seen for a *RAB23* mutation responsible for human Carpenter syndrome (Eggenschwiler et al., 2001, Jenkins et al., 2007). LOF of the mammalian homologue of *RAB23* in the mouse results in exencephaly and early embryonic lethality implicating dosage dependency of the phenotype (Eggenschwiler et al., 2001). Other examples include Ets domain-containing transcription factor (*ERF*), where craniosynostosis is only observed in a mouse model harbouring a conditional allele (*Erf*$^{fl/-}$) reducing the expression level to about 30% in contrast to that of a heterozygote null allele (*Erf*$^{dl1/-}$) where the reduction would be 50% (Twigg et al., 2013, Papadaki et al., 2007). Similar can be said of those mouse models related to Marfan syndrome that do not recapitulate a craniofacial phenotype (Gallo et al., 2014, Judge et al., 2004, Ng et al., 2004, Pereira et al., 1999).

The ability to generate gene knockout models has been pivotal in our understanding of gene function, and this in turn is dependent upon successful and accurate targeting of the specific allele of interest. Therefore, it makes sense that the overall design of a targeting vector is vital to reproducibly of a consistent phenotype. However, there are instances in the literature where a wide phenotypic spectrum can be produced. Examples of this can be seen in the *Fgfr2* knockouts, as the receptor protein has multiple functionalities (Molotkov et al., 2017, Yu et al., 2003). A common strategy to generate an *Fgfr2* knockout is through the removal of the ligand-binding domain (exons 8-9, IgI loop3). However, this mutant does not result in the complete removal of the FGFR2, but instead yields a truncated, albeit non functional, receptor. On the other hand, removal of exon 5, common to both *Fgfr2b* and *Fgfr2c* isoforms, did not lead to the expression of a truncated receptor (Yu et al., 2003, Molotkov et al., 2017). In fact, there are slight phenotypic differences too between these knockouts, with the latter *Fgfr2c* knockout appearing less severe (Yu et al., 2003, Molotkov et al., 2017). Targeting constructs recombined between intragenic regions also have an effect on gene expression. Here, removal of exon 9 encoding *Fgfr2c* is able to cause a splice switch that results in ectopic *Fgfr2b* expression (Hajihosseini et al., 2001). LoxP sites were inserted into intergenic regions of *Fgfr2* exons 8-10, and conditional removal through recombination sems to have increased susceptibility to alternative splicing alterations (Hajihosseini et al., 2001). The upregulation of *Fgfr2b* subsequently shifted the phenotype from a Crouzon spectrum to that of Apert (Hajihosseini et al., 2001). Nonetheless, the successful targeting of specific genes has been vital to determine gene function contributing towards human disease.

The advancement of CRISPR-CAS9 will increase efficiency of generating novel mouse models for human disease (Singh et al., 2015). Conventional techniques generating mouse models with recombination technology has been slow and expensive, and the use of genome editing technologies could overcome these issues. This could be particular useful in phenotypic screens for mouse embryos (Adams et al., 2013). There is an international effort to delineate the phenotypes of knockout mice generated from the International Knockout Mouse Consortium (IKMC). The International Mouse Phenotyping Consortium (IMPC) (http://www.mousephenotype.org/), as it became known, aims to phenotypically screen 20,000 knockouts in 10 years (Adams et al., 2013). The importance of screening phenotypes allows the corroboration between genotype to phenotype, and especially in the case of embryonic lethal models that helps to pinpoint critical

pathways for development. There are several methods for analysis, with emphasis on 3D imaging such as μCT, Optical Projection Tomography (OPT) and High Resolution Episcopic Microscopy (HREM). The ultimate aim is to utilise these tools to generate data widely available to researchers. A simlar platform is also available specifically aimed for craniofacial development called 'FaceBase' (http://www.facebase.org), which has a wide range of curated datasets available online, both phenotypic and omics, for craniofacial research across multiple organisms. Several groups have contributed to this consortium by generating transcriptomic atlases of calvarial sutures, acquired using laser captured microdissection on several mouse models of craniosynostosis including Apert and Saethre-Choetzen. Despite the plethora of mouse models generated for craniofacial syndromes, few studies have characterised the downstream signalling misregulation that must contribute to the phenotype. Further disruption of downstream effectors will undoubtedly help to delineate the complex relationship between signal transduction and gene expression. This could be achieved by generating mouse models specifically targeting those signalling intermediates. A comprehensive review of these mouse models have been listed in a recent review by Dinsmore and Soriano (Dinsmore and Soriano, 2018). As growth factor signalling, such as FGF, is critical for craniofacial development, it would also be ideal to adopt mouse models that are designed to study cancer progression into birth defects research. This is because mouse models such as $Braf^{V600E/+}$ or the $Kras^{G12D/+}$, have mutations that specifically disrupt multiple levels of a signalling cascade (i.e. MAPK/ERK) (Tuveson et al., 2004, Mercer et al., 2005). Future studies will need to address the impact of the mutation on cascade activation at the level of the receptor *in vivo*. For example, it has been demonstrated by Miraoui et al., 2009 that the Apert mutation (FGFR2-p.S252W) preferentially activates the PLC-PKC cascade for osteogenesis *in vitro* (Miraoui et al., 2009). There have been attempts in the past to elucidate this by mutating binding domains on the catalytic domains of PDGFRs in the mouse (Klinghoffer et al., 2002, Klinghoffer et al., 2001, Fantauzzo and Soriano, 2016). As generating a mouse mutant for individual binding domain is time consuming, technologies such as CRISPR-CAS9 will no doubt assist with this endeavour.

In conclusion, mouse models offer a robust platform to study craniofacial birth defects. This is in particular to clinical syndromes, where a majority of knock in mice carrying a human mutation recapitulate the human disease phenotype. Together with conditional mouse models, significant progress has been made to dissect the

molecular basis for delineating craniofacial birth defects. With the dawn of genome editing technologies, there are exciting opportunities over the horizon, and it is doubtless that mouse models will continue to play a central part in biomedical science.

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References

ADAMS, D., BALDOCK, R., BHATTACHARYA, S., COPP, A. J., DICKINSON, M., GREENE, N. D., HENKELMAN, M., JUSTICE, M., MOHUN, T., MURRAY, S. A., PAUWS, E., RAESS, M., ROSSANT, J., WEAVER, T. & WEST, D. 2013. Bloomsbury report on mouse embryo phenotyping: recommendations from the IMPC workshop on embryonic lethal screening. *Dis Model Mech,* 6**,** 571-9.

ARMAN, E., HAFFNER-KRAUSZ, R., CHEN, Y., HEATH, J. K. & LONAI, P. 1998. Targeted disruption of fibroblast growth factor (FGF) receptor 2 suggests a role for FGF signaling in pregastrulation mammalian development. *Proc Natl Acad Sci U S A,* 95**,** 5082-7.

ARMAN, E., HAFFNER-KRAUSZ, R., GORIVODSKY, M. & LONAI, P. 1999. Fgfr2 is required for limb outgrowth and lung-branching morphogenesis. *Proc Natl Acad Sci U S A,* 96**,** 11895-9.

AUNE, C. N., CHATTERJEE, B., ZHAO, X. Q., FRANCIS, R., BRACERO, L., YU, Q., ROSENTHAL, J., LEATHERBURY, L. & LO, C. W. 2008. Mouse model of heterotaxy with single ventricle spectrum of cardiac anomalies. *Pediatr Res,* 63**,** 9-14.

BARAK, H., HUH, S.-H., CHEN, S., JEANPIERRE, C., MARTINOVIC, J., PARISOT, M., BOLE-FEYSOT, C., NITSCHKÉ, P., SALOMON, R., ANTIGNAC, C., ORNITZ, DAVID M. & KOPAN, R. 2012. FGF9 and FGF20 Maintain the Stemness of Nephron Progenitors in Mice and Man. *Developmental Cell,* 22**,** 1191-1207.

BEHR, B., LONGAKER, M. & QUARTO, N. 2011. Craniosynostosis of Coronal Suture in Twist1+/− Mice Occurs Through Endochondral Ossification Recapitulating the Physiological Closure of Posterior Frontal Suture. *Frontiers in Physiology,* 2.

BELLUS, G. A., HEFFERON, T. W., ORTIZ DE LUNA, R. I., HECHT, J. T., HORTON, W. A., MACHADO, M., KAITILA, I., MCINTOSH, I. & FRANCOMANO, C. A. 1995. Achondroplasia is defined by recurrent G380R mutations of FGFR3. *Am J Hum Genet,* 56**,** 368-73.

BIALEK, P., KERN, B., YANG, X., SCHROCK, M., SOSIC, D., HONG, N., WU, H., YU, K., ORNITZ, D. M., OLSON, E. N., JUSTICE, M. J. & KARSENTY, G. 2004. A twist code determines the onset of osteoblast differentiation. *Dev Cell,* 6**,** 423-35.

BILDSOE, H., LOEBEL, D. A., JONES, V. J., HOR, A. C., BRAITHWAITE, A. W., CHEN, Y. T., BEHRINGER, R. R. & TAM, P. P. 2013. The mesenchymal architecture of the cranial mesoderm of mouse embryos is disrupted by the loss of Twist1 function. *Dev Biol,* 374**,** 295-307.

BOURGEOIS, P., BOLCATO-BELLEMIN, A. L., DANSE, J. M., BLOCH-ZUPAN, A., YOSHIBA, K., STOETZEL, C. & PERRIN-SCHMITT, F. 1998. The variable expressivity and incomplete penetrance of the twist-null heterozygous mouse phenotype resemble those of human Saethre-Chotzen syndrome. *Hum Mol Genet,* 7**,** 945-57.

CARLTON, M. B., COLLEDGE, W. H. & EVANS, M. J. 1998. Crouzon-like craniofacial dysmorphology in the mouse is caused by an insertional mutation at the Fgf3/Fgf4 locus. *Dev Dyn,* 212**,** 242-9.

CARMIGNAC, V., THEVENON, J., ADES, L., CALLEWAERT, B., JULIA, S., THAUVIN-ROBINET, C., GUENEAU, L., COURCET, J. B., LOPEZ, E., HOLMAN, K., RENARD, M., PLAUCHU, H., PLESSIS, G., DE BACKER, J., CHILD, A., ARNO, G., DUPLOMB, L., CALLIER, P., ARAL, B., VABRES, P., GIGOT, N., ARBUSTINI, E., GRASSO, M., ROBINSON, P. N., GOIZET, C., BAUMANN, C., DI ROCCO, M., SANCHEZ DEL POZO, J., HUET, F., JONDEAU, G., COLLOD-BEROUD, G., BEROUD, C., AMIEL, J., CORMIER-DAIRE, V., RIVIERE, J. B., BOILEAU, C., DE PAEPE, A. & FAIVRE, L. 2012. In-frame mutations in exon 1 of SKI cause dominant Shprintzen-Goldberg syndrome. *Am J Hum Genet,* 91**,** 950-7.

CARTA, L., PEREIRA, L., ARTEAGA-SOLIS, E., LEE-ARTEAGA, S. Y., LENART, B., STARCHER, B., MERKEL, C. A., SUKOYAN, M., KERKIS, A., HAZEKI, N., KEENE, D. R., SAKAI, L. Y. & RAMIREZ, F. 2006. Fibrillins 1 and 2 perform partially overlapping functions during aortic development. *J Biol Chem,* 281**,** 8016-23.

CARVER, E. A., ORAM, K. F. & GRIDLEY, T. 2002. Craniosynostosis in Twist heterozygous mice: a model for Saethre-Chotzen syndrome. *Anat Rec,* 268**,** 90-2.

CHEN, L., ADAR, R., YANG, X., MONSONEGO, E. O., LI, C., HAUSCHKA, P. V., YAYON, A. & DENG, C. X. 1999. Gly369Cys mutation in mouse FGFR3 causes achondroplasia by affecting both chondrogenesis and osteogenesis. *J Clin Invest,* 104**,** 1517-25.

CHEN, L., LI, C., QIAO, W., XU, X. & DENG, C. 2001. A Ser(365)-->Cys mutation of fibroblast growth factor receptor 3 in mouse downregulates Ihh/PTHrP signals and causes severe achondroplasia. *Hum Mol Genet,* 10**,** 457-65.

CHEN, L., LI, D., LI, C., ENGEL, A. & DENG, C. X. 2003. A Ser252Trp [corrected] substitution in mouse fibroblast growth factor receptor 2 (Fgfr2) results in craniosynostosis. *Bone,* 33**,** 169-78.

CHEN, Z. F. & BEHRINGER, R. R. 1995. twist is required in head mesenchyme for cranial neural tube morphogenesis. *Genes Dev,* 9**,** 686-99.

COHEN, M. M. & KREIBORG, S. 1992. Birth prevalence studies of the Crouzon syndrome: comparison of direct and indirect methods. *Clinical Genetics,* 41**,** 12-15.

COLVIN, J. S., BOHNE, B. A., HARDING, G. W., MCEWEN, D. G. & ORNITZ, D. M. 1996. Skeletal overgrowth and deafness in mice lacking fibroblast growth factor receptor 3. *Nat Genet,* 12**,** 390-7.

CONNERNEY, J., ANDREEVA, V., LESHEM, Y., MERCADO, M. A., DOWELL, K., YANG, X., LINDNER, V., FRIESEL, R. E. & SPICER, D. B. 2008. Twist1 homodimers enhance FGF responsiveness of the cranial sutures and promote suture closure. *Dev Biol,* 318**,** 323-34.

DE MOERLOOZE, L., SPENCER-DENE, B., REVEST, J., HAJIHOSSEINI, M., ROSEWELL, I. & DICKSON, C. 2000. An important role for the IIIb isoform of fibroblast growth factor receptor 2 (FGFR2) in mesenchymal-epithelial signalling during mouse organogenesis. *Development,* 127**,** 483.

DENG, C., WYNSHAW-BORIS, A., ZHOU, F., KUO, A. & LEDER, P. 1996. Fibroblast growth factor receptor 3 is a negative regulator of bone growth. *Cell,* 84**,** 911-21.

DENG, C. X., WYNSHAW-BORIS, A., SHEN, M. M., DAUGHERTY, C., ORNITZ, D. M. & LEDER, P. 1994. Murine FGFR-1 is required for early postimplantation growth and axial organization. *Genes Dev,* 8**,** 3045-57.

DERDERIAN, C. & SEAWARD, J. 2012. Syndromic craniosynostosis. *Semin Plast Surg,* 26**,** 64-75.

DIETZ, H. C., CUTTING, G. R., PYERITZ, R. E., MASLEN, C. L., SAKAI, L. Y., CORSON, G. M., PUFFENBERGER, E. G., HAMOSH, A., NANTHAKUMAR, E. J., CURRISTIN, S. M. & ET AL. 1991. Marfan syndrome caused by a recurrent de novo missense mutation in the fibrillin gene. *Nature,* 352**,** 337-9.

DINSMORE, C. J. & SORIANO, P. 2018. MAPK and PI3K signaling: At the crossroads of neural crest development. *Dev Biol*.

DOYLE, A. J., DOYLE, J. J., BESSLING, S. L., MARAGH, S., LINDSAY, M. E., SCHEPERS, D., GILLIS, E., MORTIER, G., HOMFRAY, T., SAULS, K., NORRIS, R. A., HUSO, N. D., LEAHY, D., MOHR, D. W., CAULFIELD, M. J., SCOTT, A. F., DESTREE, A., HENNEKAM, R. C., ARN, P. H., CURRY, C. J., VAN LAER, L., MCCALLION, A. S., LOEYS, B. L. & DIETZ, H. C. 2012. Mutations in the TGF-beta repressor SKI cause Shprintzen-Goldberg syndrome with aortic aneurysm. *Nat Genet,* 44**,** 1249-54.

DUCY, P. & KARSENTY, G. 2000. The family of bone morphogenetic proteins. *Kidney Int,* 57**,** 2207-14.

EGGENSCHWILER, J. T., BULGAKOV, O. V., QIN, J., LI, T. & ANDERSON, K. V. 2006. Mouse Rab23 regulates hedgehog signaling from smoothened to Gli proteins. *Dev Biol,* 290**,** 1-12.

EGGENSCHWILER, J. T., ESPINOZA, E. & ANDERSON, K. V. 2001. Rab23 is an essential negative regulator of the mouse Sonic hedgehog signalling pathway. *Nature,* 412**,** 194-8.

EL GHOUZZI, V., LE MERRER, M., PERRIN-SCHMITT, F., LAJEUNIE, E., BENIT, P., RENIER, D., BOURGEOIS, P., BOLCATO-BELLEMIN, A. L., MUNNICH, A. & BONAVENTURE, J. 1997. Mutations of the TWIST gene in the Saethre-Chotzen syndrome. *Nat Genet,* 15**,** 42-6.

ESWARAKUMAR, V. P., HOROWITZ, M. C., LOCKLIN, R., MORRISS-KAY, G. M. & LONAI, P. 2004. A gain-of-function mutation of Fgfr2c demonstrates the roles of this receptor variant in osteogenesis. *Proceedings of the National Academy of Sciences of the United States of America,* 101**,** 12555-12560.

ESWARAKUMAR, V. P., LAX, I. & SCHLESSINGER, J. 2005. Cellular signaling by fibroblast growth factor receptors. *Cytokine & Growth Factor Reviews,* 16**,** 139-149.

ESWARAKUMAR, V. P., MONSONEGO-ORNAN, E., PINES, M., ANTONOPOULOU, I., MORRISS-KAY, G. M. & LONAI, P. 2002. The IIIc alternative of Fgfr2 is a positive regulator of bone formation. *Development,* 129**,** 3783-3793.

ESWARAKUMAR, V. P., ÖZCAN, F., LEW, E. D., BAE, J. H., TOMÉ, F., BOOTH, C. J., ADAMS, D. J., LAX, I. & SCHLESSINGER, J. 2006. Attenuation of signaling pathways stimulated by pathologically activated

FGF-receptor 2 mutants prevents craniosynostosis. *Proceedings of the National Academy of Sciences,* 103**,** 18603-18608.

ESWARAKUMAR, V. P. & SCHLESSINGER, J. 2007. Skeletal overgrowth is mediated by deficiency in a specific isoform of fibroblast growth factor receptor 3. *Proc Natl Acad Sci U S A,* 104**,** 3937-42.

FANTAUZZO, K. A. & SORIANO, P. 2016. PDGFRbeta regulates craniofacial development through homodimers and functional heterodimers with PDGFRalpha. *Genes Dev,* 30**,** 2443-2458.

GALLO, E. M., LOCH, D. C., HABASHI, J. P., CALDERON, J. F., CHEN, Y., BEDJA, D., VAN ERP, C., GERBER, E. E., PARKER, S. J., SAULS, K., JUDGE, D. P., COOKE, S. K., LINDSAY, M. E., ROUF, R., MYERS, L., AP RHYS, C. M., KENT, K. C., NORRIS, R. A., HUSO, D. L. & DIETZ, H. C. 2014. Angiotensin II-dependent TGF-beta signaling contributes to Loeys-Dietz syndrome vascular pathogenesis. *J Clin Invest,* 124**,** 448-60.

HAJIHOSSEINI, M. K. & HEATH, J. K. 2002. Expression patterns of fibroblast growth factors-18 and -20 in mouse embryos is suggestive of novel roles in calvarial and limb development. *Mechanisms of Development,* 113**,** 79-83.

HAJIHOSSEINI, M. K., WILSON, S., DE MOERLOOZE, L. & DICKSON, C. 2001. A splicing switch and gain-of-function mutation in FgfR2-IIIc hemizygotes causes Apert/Pfeiffer-syndrome-like phenotypes. *Proc Natl Acad Sci U S A,* 98**,** 3855-60.

HARADA, M., MURAKAMI, H., OKAWA, A., OKIMOTO, N., HIRAOKA, S., NAKAHARA, T., AKASAKA, R., SHIRAISHI, Y.-I., FUTATSUGI, N., MIZUTANI-KOSEKI, Y., KUROIWA, A., SHIROUZU, M., YOKOYAMA, S., TAIJI, M., ISEKI, S., ORNITZ, D. M. & KOSEKI, H. 2009. FGF9 monomer-dimer equilibrium regulates extracellular matrix affinity and tissue diffusion. *Nat Genet,* 41**,** 289-298.

HE, F. & SORIANO, P. 2017. Dysregulated PDGFRalpha signaling alters coronal suture morphogenesis and leads to craniosynostosis through endochondral ossification. *Development,* 144**,** 4026-4036.

HOWARD, T. D., PAZNEKAS WA FAU - GREEN, E. D., GREEN ED FAU - CHIANG, L. C., CHIANG LC FAU - MA, N., MA N FAU - ORTIZ DE LUNA, R. I., ORTIZ DE LUNA RI FAU - GARCIA DELGADO, C., GARCIA DELGADO C FAU - GONZALEZ-RAMOS, M., GONZALEZ-RAMOS M FAU - KLINE, A. D., KLINE AD FAU - JABS, E. W. & JABS, E. W. 1997. Mutations in TWIST, a basic helix-loop-helix transcription factor, in Saethre-Chotzen syndrome. *Nat Genet.,* 15**,** 36-41.

HUI, C. C. & JOYNER, A. L. 1993. A mouse model of greig cephalopolysyndactyly syndrome: the extra-toesJ mutation contains an intragenic deletion of the Gli3 gene. *Nat Genet,* 3**,** 241-6.

ISEKI, S., WILKIE, A. O. & MORRISS-KAY, G. M. 1999. Fgfr1 and Fgfr2 have distinct differentiation- and proliferation-related roles in the developing mouse skull vault. *Development,* 126**,** 5611-5620.

ISHII, M., MERRILL, A. E., CHAN, Y. S., GITELMAN, I., RICE, D. P., SUCOV, H. M. & MAXSON, R. E., JR. 2003. Msx2 and Twist cooperatively control the development of the neural crest-derived skeletogenic mesenchyme of the murine skull vault. *Development,* 130**,** 6131-42.

JABS, E. W., MÜLLER, U., LI, X., MA, L., LUO, W., HAWORTH, I. S., KLISAK, I., SPARKES, R., WARMAN, M. L., MULLIKEN, J. B., SNEAD, M. L. & MAXSON, R. 1993. A mutation in the homeodomain of the human MSX2 gene in a family affected with autosomal dominant craniosynostosis. *Cell,* 75**,** 443-450.

JAMES, A. W., SHEN, J., ZHANG, X., ASATRIAN, G., GOYAL, R., KWAK, J. H., JIANG, L., BENGS, B., CULIAT, C. T., TURNER, A. S., SEIM III, H. B., WU, B. M., LYONS, K., ADAMS, J. S., TING, K. & SOO, C. 2015. NELL-1 in the treatment of osteoporotic bone loss. *Nat Commun,* 6**,** 7362.

JENKINS, D., SEELOW, D., JEHEE, F. S., PERLYN, C. A., ALONSO, L. G., BUENO, D. F., DONNAI, D., JOSIFOVA, D., MATHIJSSEN, I. M., MORTON, J. E., ORSTAVIK, K. H., SWEENEY, E., WALL, S. A., MARSH, J. L., NURNBERG, P., PASSOS-BUENO, M. R. & WILKIE, A. O. 2007. RAB23 mutations in Carpenter syndrome imply an unexpected role for hedgehog signaling in cranial-suture development and obesity. *Am J Hum Genet,* 80**,** 1162-70.

JOHNSON, D., ISEKI, S., WILKIE, A. O. M. & MORRISS-KAY, G. M. 2000. Expression patterns of Twist and Fgfr1, -2 and -3 in the developing mouse coronal suture suggest a key role for Twist in suture initiation and biogenesis. *Mechanisms of Development,* 91**,** 341-345.

JOHNSON, D. & WILKIE, A. O. 2011. Craniosynostosis. *Eur J Hum Genet,* 19**,** 369-76.

JOHNSON, D. R. 1967. Extra-toes: a new mutant gene causing multiple abnormalities in the mouse. *Journal of Embryology and Experimental Morphology,* 17**,** 543.

JUDGE, D. P., BIERY, N. J., KEENE, D. R., GEUBTNER, J., MYERS, L., HUSO, D. L., SAKAI, L. Y. & DIETZ, H. C. 2004. Evidence for a critical contribution of haploinsufficiency in the complex pathogenesis of Marfan syndrome. *J Clin Invest,* 114**,** 172-81.

JUDGE, D. P. & DIETZ, H. C. 2005. Marfan's syndrome. *Lancet,* 366**,** 1965-76.

KIYOZUMI, D., SUGIMOTO, N. & SEKIGUCHI, K. 2006. Breakdown of the reciprocal stabilization of QBRICK/Frem1, Fras1, and Frem2 at the basement membrane provokes Fraser syndrome-like defects. *Proc Natl Acad Sci U S A,* 103**,** 11981-6.

KLINGHOFFER, R. A., HAMILTON, T. G., HOCH, R. & SORIANO, P. 2002. An allelic series at the PDGFalphaR locus indicates unequal contributions of distinct signaling pathways during development. *Dev Cell,* 2**,** 103-13.

KLINGHOFFER, R. A., MUETING-NELSEN, P. F., FAERMAN, A., SHANI, M. & SORIANO, P. 2001. The two PDGF receptors maintain conserved signaling in vivo despite divergent embryological functions. *Mol Cell,* 7**,** 343-54.

KOMORI, T., YAGI, H., NOMURA, S., YAMAGUCHI, A., SASAKI, K., DEGUCHI, K., SHIMIZU, Y., BRONSON, R. T., GAO, Y. H., INADA, M., SATO, M., OKAMOTO, R., KITAMURA, Y., YOSHIKI, S. & KISHIMOTO, T. 1997. Targeted Disruption of Cbfa1 Results in a Complete Lack of Bone Formation owing to Maturational Arrest of Osteoblasts. *Cell,* 89**,** 755-764.

LEE, Y. C., SONG, I. W., PAI, Y. J., CHEN, S. D. & CHEN, Y. T. 2017. Knock-in human FGFR3 achondroplasia mutation as a mouse model for human skeletal dysplasia. *Sci Rep,* 7**,** 43220.

LI, C., SCOTT, D. A., HATCH, E., TIAN, X. & MANSOUR, S. L. 2007. Dusp6 (Mkp3) is a negative feedback regulator of FGF-stimulated ERK signaling during mouse development. *Development,* 134**,** 167-176.

LI, C., XU, X., NELSON, D. K., WILLIAMS, T., KUEHN, M. R. & DENG, C.-X. 2005. FGFR1 function at the earliest stages of mouse limb development plays an indispensable role in subsequent autopod morphogenesis. *Development,* 132**,** 4755.

LIU, Y. H., KUNDU, R., WU, L., LUO, W., IGNELZI, M. A., SNEAD, M. L. & MAXSON, R. E. 1995. Premature suture closure and ectopic cranial bone in mice expressing Msx2 transgenes in the developing skull. *Proceedings of the National Academy of Sciences of the United States of America,* 92**,** 6137-6141.

LOEYS, B. L., CHEN, J., NEPTUNE, E. R., JUDGE, D. P., PODOWSKI, M., HOLM, T., MEYERS, J., LEITCH, C. C., KATSANIS, N., SHARIFI, N., XU, F. L., MYERS, L. A., SPEVAK, P. J., CAMERON, D. E., DE BACKER, J., HELLEMANS, J., CHEN, Y., DAVIS, E. C., WEBB, C. L., KRESS, W., COUCKE, P., RIFKIN, D. B., DE PAEPE, A. M. & DIETZ, H. C. 2005. A syndrome of altered cardiovascular, craniofacial, neurocognitive and skeletal development caused by mutations in TGFBR1 or TGFBR2. *Nat Genet,* 37**,** 275-81.

LOGAN, C. Y. & NUSSE, R. 2004. The Wnt signaling pathway in development and disease. *Annu Rev Cell Dev Biol,* 20**,** 781-810.

MA, L., GOLDEN, S., WU, L. & MAXSON, R. 1996. The molecular basis of Boston-type craniosynostosis: the Pro148-->His mutation in the N-terminal arm of the MSX2 homeodomain stabilizes DNA binding without altering nucleotide sequence preferences. *Hum Mol Genet,* 5**,** 1915-20.

MACCARRICK, G., BLACK, J. H., 3RD, BOWDIN, S., EL-HAMAMSY, I., FRISCHMEYER-GUERRERIO, P. A., GUERRERIO, A. L., SPONSELLER, P. D., LOEYS, B. & DIETZ, H. C., 3RD 2014. Loeys-Dietz syndrome: a primer for diagnosis and management. *Genet Med,* 16**,** 576-87.

MAENO, T., MORIISHI, T., YOSHIDA, C. A., KOMORI, H., KANATANI, N., IZUMI, S., TAKAOKA, K. & KOMORI, T. 2011. Early onset of Runx2 expression caused craniosynostosis, ectopic bone formation, and limb defects. *Bone,* 49**,** 673-82.

MAESTRO, R., DEI TOS, A. P., HAMAMORI, Y., KRASNOKUTSKY, S., SARTORELLI, V., KEDES, L., DOGLIONI, C., BEACH, D. H. & HANNON, G. J. 1999. Twist is a potential oncogene that inhibits apoptosis. *Genes Dev,* 13**,** 2207-17.

MAI, S., WEI, K., FLENNIKEN, A., ADAMSON, S. L., ROSSANT, J., AUBIN, J. E. & GONG, S. G. 2010. The missense mutation W290R in Fgfr2 causes developmental defects from aberrant IIIb and IIIc signaling. *Dev Dyn,* 239**,** 1888-900.

MANSOUR, S. L., LI, C. & URNESS, L. D. 2013. Genetic rescue of Muenke syndrome model hearing loss reveals prolonged FGF-dependent plasticity in cochlear supporting cell fates. *Genes Dev,* 27**,** 2320-31.

MANSOUR, S. L., TWIGG, S. R., FREELAND, R. M., WALL, S. A., LI, C. & WILKIE, A. O. 2009. Hearing loss in a mouse model of Muenke syndrome. *Hum Mol Genet,* 18**,** 43-50.

MARUYAMA, T., JEONG, J., SHEU, T. J. & HSU, W. 2016. Stem cells of the suture mesenchyme in craniofacial bone development, repair and regeneration. *Nat Commun,* 7**,** 10526.

MASSARI, M. E. & MURRE, C. 2000. Helix-loop-helix proteins: regulators of transcription in eucaryotic organisms. *Mol Cell Biol,* 20**,** 429-40.

MERCER, K., GIBLETT, S., GREEN, S., LLOYD, D., DAROCHA DIAS, S., PLUMB, M., MARAIS, R. & PRITCHARD, C. 2005. Expression of endogenous oncogenic V600EB-raf induces proliferation and developmental defects in mice and transformation of primary fibroblasts. *Cancer Res,* 65**,** 11493-500.

MEYERS, G. A., ORLOW, S. J., MUNRO, I. R., PRZYLEPA, K. A. & JABS, E. W. 1995. Fibroblast growth factor receptor 3 (FGFR3) transmembrane mutation in Crouzon syndrome with acanthosis nigricans. *Nat Genet,* 11**,** 462-4.

MIN, H., DANILENKO, D. M., SCULLY, S. A., BOLON, B., RING, B. D., TARPLEY, J. E., DEROSE, M. & SIMONET, W. S. 1998. Fgf-10 is required for both limb and lung development and exhibits striking functional similarity to Drosophila branchless. *Genes & Development,* 12**,** 3156-3161.

MIRAOUI, H., OUDINA, K., PETITE, H., TANIMOTO, Y., MORIYAMA, K. & MARIE, P. J. 2009. Fibroblast Growth Factor Receptor 2 Promotes Osteogenic Differentiation in Mesenchymal Cells via ERK1/2 and Protein Kinase C Signaling. *Journal of Biological Chemistry,* 284**,** 4897-4904.

MIRAOUI, H., SEVERE, N., VAUDIN, P., PAGÈS, J.-C. & MARIE, P. J. 2010. Molecular silencing of Twist1 enhances osteogenic differentiation of murine mesenchymal stem cells: Implication of FGFR2 signaling. *Journal of Cellular Biochemistry,* 110**,** 1147-1154.

MOENNING, A., JAGER, R., EGERT, A., KRESS, W., WARDELMANN, E. & SCHORLE, H. 2009. Sustained platelet-derived growth factor receptor alpha signaling in osteoblasts results in craniosynostosis by overactivating the phospholipase C-gamma pathway. *Mol Cell Biol,* 29**,** 881-91.

MOLOTKOV, A., MAZOT, P., BREWER, J. R., CINALLI, R. M. & SORIANO, P. 2017. Distinct Requirements for FGFR1 and FGFR2 in Primitive Endoderm Development and Exit from Pluripotency. *Dev Cell,* 41**,** 511-526 e4.

MONSONEGO-ORNAN, E., ADAR, R., FEFERMAN, T., SEGEV, O. & YAYON, A. 2000. The transmembrane mutation G380R in fibroblast growth factor receptor 3 uncouples ligand-mediated receptor activation from down-regulation. *Mol Cell Biol,* 20**,** 516-22.

MUENKE, M., GRIPP KW FAU - MCDONALD-MCGINN, D. M., MCDONALD-MCGINN DM FAU - GAUDENZ, K., GAUDENZ K FAU - WHITAKER, L. A., WHITAKER LA FAU - BARTLETT, S. P., BARTLETT SP FAU - MARKOWITZ, R. I., MARKOWITZ RI FAU - ROBIN, N. H., ROBIN NH FAU - NWOKORO, N., NWOKORO N FAU - MULVIHILL, J. J., MULVIHILL JJ FAU - LOSKEN, H. W., LOSKEN HW FAU - MULLIKEN, J. B., MULLIKEN JB FAU - GUTTMACHER, A. E., GUTTMACHER AE FAU - WILROY, R. S., WILROY RS FAU - CLARKE, L. A., CLARKE LA FAU - HOLLWAY, G., HOLLWAY G FAU - ADES, L. C., ADES LC FAU - HAAN, E. A., HAAN EA FAU - MULLEY, J. C., MULLEY JC FAU - COHEN, M. M., JR., COHEN MM JR FAU - BELLUS, G. A., BELLUS GA FAU - FRANCOMANO, C. A., FRANCOMANO CA FAU - MOLONEY, D. M., MOLONEY DM FAU - WALL, S. A., WALL SA FAU - WILKIE, A. O., WILKIE, A. O. & ET AL. 1997. A unique point mutation in the fibroblast growth factor receptor 3 gene (FGFR3) defines a new craniosynostosis syndrome. *Am J Hum Genet. ,* 60**,** 555-564.

MUENKE, M., SCHELL U FAU - HEHR, A., HEHR A FAU - ROBIN, N. H., ROBIN NH FAU - LOSKEN, H. W., LOSKEN HW FAU - SCHINZEL, A., SCHINZEL A FAU - PULLEYN, L. J., PULLEYN LJ FAU - RUTLAND, P., RUTLAND P FAU - REARDON, W., REARDON W FAU - MALCOLM, S., MALCOLM, S. & ET AL. 1994. A common mutation in the fibroblast growth factor receptor 1 gene in Pfeiffer syndrome. *Nat Genet.,* 8**,** 269-274.

MUNYE, M. M., DIAZ-FONT, A., OCAKA, L., HENRIKSEN, M. L., LEES, M., BRADY, A., JENKINS, D., MORTON, J., HANSEN, S. W., BACCHELLI, C., BEALES, P. L. & HERNANDEZ-HERNANDEZ, V. 2017. COLEC10 is mutated in 3MC patients and regulates early craniofacial development. *PLoS Genet,* 13**,** e1006679.

NEWTON, K. & DIXIT, V. M. 2012. Signaling in innate immunity and inflammation. *Cold Spring Harb Perspect Biol,* 4.

NG, C. M., CHENG, A., MYERS, L. A., MARTINEZ-MURILLO, F., JIE, C., BEDJA, D., GABRIELSON, K. L., HAUSLADEN, J. M., MECHAM, R. P., JUDGE, D. P. & DIETZ, H. C. 2004. TGF-beta-dependent pathogenesis of mitral valve prolapse in a mouse model of Marfan syndrome. *J Clin Invest,* 114**,** 1586-92.

ORNITZ, D. M. & ITOH, N. 2015. The Fibroblast Growth Factor signaling pathway. *Wiley Interdisciplinary Reviews: Developmental Biology,* 4**,** 215-266.

PANNIER, S., COULOIGNER, V., MESSADDEQ, N., ELMALEH-BERGES, M., MUNNICH, A., ROMAND, R. & LEGEAI-MALLET, L. 2009. Activating Fgfr3 Y367C mutation causes hearing loss and inner ear defect in a mouse model of chondrodysplasia. *Biochim Biophys Acta,* 1792**,** 140-7.

PAPADAKI, C., ALEXIOU, M., CECENA, G., VERYKOKAKIS, M., BILITOU, A., CROSS, J. C., OSHIMA, R. G. & MAVROTHALASSITIS, G. 2007. Transcriptional repressor erf determines extraembryonic ectoderm differentiation. *Mol Cell Biol,* 27**,** 5201-13.

PARK, D. H. & YOON, S. H. 2012. Craniofacial malformation treatment: craniosynostosis and positional plagiocephaly. *J Korean Med Assoc.,* 55**,** 878-886.

PARTANEN, J., SCHWARTZ, L. & ROSSANT, J. 1998. Opposite phenotypes of hypomorphic and Y766 phosphorylation site mutations reveal a function for Fgfr1 in anteroposterior patterning of mouse embryos. *Genes Dev,* 12**,** 2332-44.

PEREIRA, L., LEE, S. Y., GAYRAUD, B., ANDRIKOPOULOS, K., SHAPIRO, S. D., BUNTON, T., BIERY, N. J., DIETZ, H. C., SAKAI, L. Y. & RAMIREZ, F. 1999. Pathogenetic sequence for aneurysm revealed in mice underexpressing fibrillin-1. *Proc Natl Acad Sci U S A,* 96**,** 3819-23.

PERLYN, C. A., MORRISS-KAY, G., DARVANN, T., TENENBAUM, M. & ORNITZ, D. M. 2006. A model for the pharmacological treatment of crouzon syndrome. *Neurosurgery,* 59**,** 210-5; discussion 210-5.

PESKETT, E., KUMAR, S., BAIRD, W., JAISWAL, J., LI, M., PATEL, P., BRITTO, J. A. & PAUWS, E. 2017. Analysis of the Fgfr2C342Y mouse model shows condensation defects due to misregulation of Sox9 expression in prechondrocytic mesenchyme. *Biol Open,* 6**,** 223-231.

PFAFF, M. J., XUE, K., LI, L., HOROWITZ, M. C., STEINBACHER, D. M. & ESWARAKUMAR, J. V. P. 2016. FGFR2c-mediated ERK–MAPK activity regulates coronal suture development. *Developmental Biology,* 415**,** 242-250.

PITTELOUD, N., ACIERNO, J. S., MEYSING, A., ELISEENKOVA, A. V., MA, J., IBRAHIMI, O. A., METZGER, D. L., HAYES, F. J., DWYER, A. A., HUGHES, V. A., YIALAMAS, M., HALL, J. E., GRANT, E., MOHAMMADI, M. & CROWLEY, W. F. 2006. Mutations in fibroblast growth factor receptor 1 cause both Kallmann syndrome and normosmic idiopathic hypogonadotropic hypogonadism. *Proceedings of the National Academy of Sciences,* 103**,** 6281-6286.

PUSAPATI, G. V., KONG, J. H., PATEL, B. B., KRISHNAN, A., SAGNER, A., KINNEBREW, M., BRISCOE, J., ARAVIND, L. & ROHATGI, R. 2018. CRISPR Screens Uncover Genes that Regulate Target Cell Sensitivity to the Morphogen Sonic Hedgehog. *Dev Cell,* 44**,** 113-129 e8.

QIN, Q., XU, Y., HE, T., QIN, C. & XU, J. 2012. Normal and disease-related biological functions of Twist1 and underlying molecular mechanisms. *Cell Res,* 22**,** 90-106.

REARDON, W., WINTER RM FAU - RUTLAND, P., RUTLAND P FAU - PULLEYN, L. J., PULLEYN LJ FAU - JONES, B. M., JONES BM FAU - MALCOLM, S. & MALCOLM, S. 1994. Mutations in the fibroblast growth factor receptor 2 gene cause Crouzon syndrome. *Nat Genet.,* 8**,** 98-103.

REVEST, J.-M., SPENCER-DENE, B., KERR, K., DE MOERLOOZE, L., ROSEWELL, I. & DICKSON, C. 2001. Fibroblast Growth Factor Receptor 2-IIIb Acts Upstream of Shh and Fgf4 and Is Required for Limb Bud Maintenance but Not for the Induction of Fgf8, Fgf10, Msx1, or Bmp4. *Developmental Biology,* 231**,** 47-62.

RICE, D. P., ABERG, T., CHAN, Y., TANG, Z., KETTUNEN, P. J., PAKARINEN, L., MAXSON, R. E. & THESLEFF, I. 2000. Integration of FGF and TWIST in calvarial bone and suture development. *Development,* 127**,** 1845-55.

RICE, D. P., CONNOR, E. C., VELTMAAT, J. M., LANA-ELOLA, E., VEISTINEN, L., TANIMOTO, Y., BELLUSCI, S. & RICE, R. 2010. Gli3Xt-J/Xt-J mice exhibit lambdoid suture craniosynostosis which results from altered osteoprogenitor proliferation and differentiation. *Hum Mol Genet,* 19**,** 3457-67.

ROORYCK, C., DIAZ-FONT, A., OSBORN, D. P., CHABCHOUB, E., HERNANDEZ-HERNANDEZ, V., SHAMSELDIN, H., KENNY, J., WATERS, A., JENKINS, D., KAISSI, A. A., LEAL, G. F., DALLAPICCOLA, B., CARNEVALE, F., BITNER-GLINDZICZ, M., LEES, M., HENNEKAM, R., STANIER, P., BURNS, A. J., PEETERS, H., ALKURAYA, F. S. & BEALES, P. L. 2011. Mutations in lectin complement pathway genes COLEC11 and MASP1 cause 3MC syndrome. *Nat Genet,* 43**,** 197-203.

ROUSSEAU, F., BONAVENTURE, J., LEGEAI-MALLET, L., PELET, A., ROZET, J. M., MAROTEAUX, P., LE MERRER, M. & MUNNICH, A. 1994. Mutations in the gene encoding fibroblast growth factor receptor-3 in achondroplasia. *Nature,* 371**,** 252-4.

ROYBAL, P. G., WU, N. L., SUN, J., TING, M.-C., SCHAFER, C. A. & MAXSON, R. E. 2010. Inactivation of Msx1 and Msx2 in neural crest reveals an unexpected role in suppressing heterotopic bone formation in the head. *Developmental Biology,* 343**,** 28-39.

SEGEV, O., CHUMAKOV, I., NEVO, Z., GIVOL, D., MADAR-SHAPIRO, L., SHEININ, Y., WEINREB, M. & YAYON, A. 2000. Restrained chondrocyte proliferation and maturation with abnormal growth plate vascularization and ossification in human FGFR-3(G380R) transgenic mice. *Hum Mol Genet,* 9**,** 249-58.

SEKINE, K., OHUCHI, H., FUJIWARA, M., YAMASAKI, M., YOSHIZAWA, T., SATO, T., YAGISHITA, N., MATSUI, D., KOGA, Y., ITOH, N. & KATO, S. 1999. Fgf10 is essential for limb and lung formation. *Nat Genet,* 21**,** 138-41.

SETTLE, S. H., ROUNTREE, R. B., SINHA, A., THACKER, A., HIGGINS, K. & KINGSLEY, D. M. 2003. Multiple joint and skeletal patterning defects caused by single and double mutations in the mouse Gdf6 and Gdf5 genes. *Developmental Biology,* 254**,** 116-130.

SHARMA, V. P., FENWICK, A. L., BROCKOP, M. S., MCGOWAN, S. J., GOOS, J. A., HOOGEBOOM, A. J., BRADY, A. F., JEELANI, N. O., LYNCH, S. A., MULLIKEN, J. B., MURRAY, D. J., PHIPPS, J. M., SWEENEY, E., TOMKINS, S. E., WILSON, L. C., BENNETT, S., CORNALL, R. J., BROXHOLME, J., KANAPIN, A., WHOLE-GENOME SEQUENCES, C., JOHNSON, D., WALL, S. A., VAN DER SPEK, P. J., MATHIJSSEN, I. M., MAXSON, R. E., TWIGG, S. R. & WILKIE, A. O. 2013. Mutations in TCF12, encoding a basic helix-loop-helix partner of TWIST1, are a frequent cause of coronal craniosynostosis. *Nat Genet,* 45**,** 304-7.

SHIANG, R., THOMPSON, L. M., ZHU, Y. Z., CHURCH, D. M., FIELDER, T. J., BOCIAN, M., WINOKUR, S. T. & WASMUTH, J. J. 1994. Mutations in the transmembrane domain of FGFR3 cause the most common genetic form of dwarfism, achondroplasia. *Cell,* 78**,** 335-42.

SHUKLA, V., COUMOUL, X., WANG, R.-H., KIM, H.-S. & DENG, C.-X. 2007. RNA interference and inhibition of MEK-ERK signaling prevent abnormal skeletal phenotypes in a mouse model of craniosynostosis. *Nat Genet,* 39**,** 1145-1150.

SINGH, P., SCHIMENTI, J. C. & BOLCUN-FILAS, E. 2015. A mouse geneticist's practical guide to CRISPR applications. *Genetics,* 199**,** 1-15.

SMYTH, I., DU, X., TAYLOR, M. S., JUSTICE, M. J., BEUTLER, B. & JACKSON, I. J. 2004. The extracellular matrix gene Frem1 is essential for the normal adhesion of the embryonic epidermis. *Proc Natl Acad Sci U S A,* 101**,** 13560-5.

SNYDER-WARWICK, A. K., PERLYN, C. A., PAN, J., YU, K., ZHANG, L. & ORNITZ, D. M. 2010. Analysis of a gain-of-function FGFR2 Crouzon mutation provides evidence of loss of function activity in the etiology of cleft palate. *Proceedings of the National Academy of Sciences,* 107**,** 2515-2520.

SOOD, S., ELDADAH, Z. A., KRAUSE, W. L., MCINTOSH, I. & DIETZ, H. C. 1996. Mutation in fibrillin-1 and the Marfanoid-craniosynostosis (Shprintzen-Goldberg) syndrome. *Nat Genet,* 12**,** 209-11.

SORIANO, P. 1997. The PDGF alpha receptor is required for neural crest cell development and for normal patterning of the somites. *Development,* 124**,** 2691-700.

SOSIC, D., RICHARDSON, J. A., YU, K., ORNITZ, D. M. & OLSON, E. N. 2003. Twist regulates cytokine gene expression through a negative feedback loop that represses NF-kappaB activity. *Cell,* 112**,** 169-80.

TAKAHASHI, M., IWAKI, D., KANNO, K., ISHIDA, Y., XIONG, J., MATSUSHITA, M., ENDO, Y., MIURA, S., ISHII, N., SUGAMURA, K. & FUJITA, T. 2008. Mannose-binding lectin (MBL)-associated serine protease (MASP)-1 contributes to activation of the lectin complement pathway. *J Immunol,* 180**,** 6132-8.

TAVORMINA, P. L., SHIANG, R., THOMPSON, L. M., ZHU, Y. Z., WILKIN, D. J., LACHMAN, R. S., WILCOX, W. R., RIMOIN, D. L., COHN, D. H. & WASMUTH, J. J. 1995. Thanatophoric dysplasia (types I and II) caused by distinct mutations in fibroblast growth factor receptor 3. *Nat Genet,* 9**,** 321-8.

TING, K., VASTARDIS, H., MULLIKEN, J. B., SOO, C., TIEU, A., DO, H., KWONG, E., BERTOLAMI, C. N., KAWAMOTO, H., KURODA, S. & LONGAKER, M. T. 1999. Human NELL-1 expressed in unilateral coronal synostosis. *J Bone Miner Res,* 14**,** 80-9.

TING, M. C., WU, N. L., ROYBAL, P. G., SUN, J., LIU, L., YEN, Y. & MAXSON, R. E., JR. 2009. EphA4 as an effector of Twist1 in the guidance of osteogenic precursor cells during calvarial bone growth and in craniosynostosis. *Development,* 136**,** 855-64.

TITOMANLIO, L., BENNACEUR, S., BREMOND-GIGNAC, D., BAUMANN, C., DUPUY, O. & VERLOES, A. 2005. Michels syndrome, Carnevale syndrome, OSA syndrome, and Malpuech syndrome: variable expression of a single disorder (3MC syndrome)? *Am J Med Genet A,* 137A**,** 332-5.

TUVESON, D. A., SHAW, A. T., WILLIS, N. A., SILVER, D. P., JACKSON, E. L., CHANG, S., MERCER, K. L., GROCHOW, R., HOCK, H., CROWLEY, D., HINGORANI, S. R., ZAKS, T., KING, C., JACOBETZ, M. A., WANG, L., BRONSON, R. T., ORKIN, S. H., DEPINHO, R. A. & JACKS, T. 2004. Endogenous oncogenic K-rasG12D stimulates proliferation and widespread neoplastic and developmental defects. *Cancer Cell,* 5**,** 375-387.

TWIGG, S. R., HEALY, C., BABBS, C., SHARPE, J. A., WOOD, W. G., SHARPE, P. T., MORRISS-KAY, G. M. & WILKIE, A. O. 2009. Skeletal analysis of the Fgfr3(P244R) mouse, a genetic model for the Muenke craniosynostosis syndrome. *Dev Dyn,* 238**,** 331-42.

TWIGG, S. R., LLOYD, D., JENKINS, D., ELCIOGLU, N. E., COOPER, C. D., AL-SANNAA, N., ANNAGUR, A., GILLESSEN-KAESBACH, G., HUNING, I., KNIGHT, S. J., GOODSHIP, J. A., KEAVNEY, B. D., BEALES, P. L., GILEADI, O., MCGOWAN, S. J. & WILKIE, A. O. 2012. Mutations in multidomain protein MEGF8 identify a Carpenter syndrome subtype associated with defective lateralization. *Am J Hum Genet,* 91**,** 897-905.

TWIGG, S. R., VORGIA, E., MCGOWAN, S. J., PERAKI, I., FENWICK, A. L., SHARMA, V. P., ALLEGRA, M., ZARAGKOULIAS, A., SADIGHI AKHA, E., KNIGHT, S. J., LORD, H., LESTER, T., IZATT, L., LAMPE, A. K., MOHAMMED, S. N., STEWART, F. J., VERLOES, A., WILSON, L. C., HEALY, C., SHARPE, P. T., HAMMOND, P., HUGHES, J., TAYLOR, S., JOHNSON, D., WALL, S. A., MAVROTHALASSITIS, G. & WILKIE, A. O. 2013. Reduced dosage of ERF causes complex craniosynostosis in humans and mice and links ERK1/2 signaling to regulation of osteogenesis. *Nat Genet,* 45**,** 308-13.

URNESS, L. D., BLEYL, S. B., WRIGHT, T. J., MOON, A. M. & MANSOUR, S. L. 2011. Redundant and dosage sensitive requirements for Fgf3 and Fgf10 in cardiovascular development. *Developmental Biology,* 356**,** 383-397.

URQUHART, J., ROBERTS, R., DE SILVA, D., SHALEV, S., CHERVINSKY, E., NAMPOOTHIRI, S., SZNAJER, Y., REVENCU, N., GUNASEKERA, R., SURI, M., ELLINGFORD, J., WILLIAMS, S., BHASKAR, S. & CLAYTON-SMITH, J. 2016. Exploring the genetic basis of 3MC syndrome: Findings in 12 further families. *Am J Med Genet A,* 170a**,** 1216-24.

VEGA, R. B., MATSUDA, K., OH, J., BARBOSA, A. C., YANG, X., MEADOWS, E., MCANALLY, J., POMAJZL, C., SHELTON, J. M., RICHARDSON, J. A., KARSENTY, G. & OLSON, E. N. 2004. Histone deacetylase 4 controls chondrocyte hypertrophy during skeletogenesis. *Cell,* 119**,** 555-66.

VERHEYDEN, J. M., LEWANDOSKI, M., DENG, C., HARFE, B. D. & SUN, X. 2005. Conditional inactivation of Fgfr1 in mouse defines its role in limb bud establishment, outgrowth and digit patterning. *Development,* 132**,** 4235.

VISSERS, L. E., COX, T. C., MAGA, A. M., SHORT, K. M., WIRADJAJA, F., JANSSEN, I. M., JEHEE, F., BERTOLA, D., LIU, J., YAGNIK, G., SEKIGUCHI, K., KIYOZUMI, D., VAN BOKHOVEN, H., MARCELIS, C., CUNNINGHAM, M. L., ANDERSON, P. J., BOYADJIEV, S. A., PASSOS-BUENO, M. R., VELTMAN, J. A., SMYTH, I., BUCKLEY, M. F. & ROSCIOLI, T. 2011. Heterozygous mutations of FREM1 are associated with an increased risk of isolated metopic craniosynostosis in humans and mice. *PLoS Genet,* 7**,** e1002278.

WANG, E., NAM, H. K., LIU, J. & HATCH, N. E. 2015. The effects of tissue-non-specific alkaline phosphatase gene therapy on craniosynostosis and craniofacial morphology in the FGFR2C342Y/+ mouse model of Crouzon craniosynostosis. *Orthod Craniofac Res,* 18 Suppl 1**,** 196-206.

WANG, Y., SPATZ, M. K., KANNAN, K., HAYK, H., AVIVI, A., GORIVODSKY, M., PINES, M., YAYON, A., LONAI, P. & GIVOL, D. 1999. A mouse model for achondroplasia produced by targeting fibroblast growth factor receptor 3. *Proc Natl Acad Sci U S A,* 96**,** 4455-60.

WANG, Y., XIAO, R., YANG, F., KARIM, B. O., IACOVELLI, A. J., CAI, J., LERNER, C. P., RICHTSMEIER, J. T., LESZL, J. M., HILL, C. A., YU, K., ORNITZ, D. M., ELISSEEFF, J., HUSO, D. L. & JABS, E. W. 2005. Abnormalities in cartilage and bone development in the Apert syndrome FGFR2+/S252W mouse. *Development,* 132**,** 3537-3548.

WANG, Y., ZHOU, X., OBEROI, K., PHELPS, R., COUWENHOVEN, R., SUN, M., REZZA, A., HOLMES, G., PERCIVAL, C. J., FRIEDENTHAL, J., KREJCI, P., RICHTSMEIER, J. T., HUSO, D. L., RENDL, M. & JABS, E. W. 2012. p38 Inhibition ameliorates skin and skull abnormalities in Fgfr2 Beare-Stevenson mice. *J Clin Invest,* 122**,** 2153-64.

WILKIE, A. O. & MORRISS-KAY, G. M. 2001. Genetics of craniofacial development and malformation. *Nat Rev Genet,* 2**,** 458-68.

WILKIE, A. O., TANG, Z., ELANKO, N., WALSH, S., TWIGG, S. R., HURST, J. A., WALL, S. A., CHRZANOWSKA, K. H. & MAXSON, R. E., JR. 2000. Functional haploinsufficiency of the human homeobox gene MSX2 causes defects in skull ossification. *Nat Genet,* 24**,** 387-90.

WILKIE, A. O. M. 2005. Bad bones, absent smell, selfish testes: The pleiotropic consequences of human FGF receptor mutations. *Cytokine & Growth Factor Reviews,* 16**,** 187-203.

WILLIAMS, S. R., ALDRED, M. A., DER KALOUSTIAN, V. M., HALAL, F., GOWANS, G., MCLEOD, D. R., ZONDAG, S., TORIELLO, H. V., MAGENIS, R. E. & ELSEA, S. H. 2010. Haploinsufficiency of HDAC4 causes brachydactyly mental retardation syndrome, with brachydactyly type E, developmental delays, and behavioral problems. *Am J Hum Genet,* 87**,** 219-28.

WRIGHT, T. J. & MANSOUR, S. L. 2003. Fgf3 and Fgf10 are required for mouse otic placode induction. *Development,* 130**,** 3379-3390.

WU, X.-L., GU, M.-M., HUANG, L., LIU, X.-S., ZHANG, H.-X., DING, X.-Y., XU, J.-Q., CUI, B., WANG, L., LU, S.-Y., CHEN, X.-Y., ZHANG, H.-G., HUANG, W., YUAN, W.-T., YANG, J.-M., GU, Q., FEI, J., CHEN, Z., YUAN, Z.-M. & WANG, Z.-G. 2009. Multiple Synostoses Syndrome Is Due to a Missense Mutation in Exon 2 of FGF9 Gene. *The American Journal of Human Genetics,* 85**,** 53-63.

XU, X., WEINSTEIN, M., LI, C., NASKI, M., COHEN, R. I., ORNITZ, D. M., LEDER, P. & DENG, C. 1998. Fibroblast growth factor receptor 2 (FGFR2)-mediated reciprocal regulation loop between FGF8 and FGF10 is essential for limb induction. *Development,* 125**,** 753-65.

YAMAGUCHI, T. P., HARPAL, K., HENKEMEYER, M. & ROSSANT, J. 1994. fgfr-1 is required for embryonic growth and mesodermal patterning during mouse gastrulation. *Genes Dev,* 8**,** 3032-44.

YEN, H.-Y., TING, M.-C. & MAXSON, R. E. 2010. Jagged1 functions downstream of Twist1 in the specification of the coronal suture and the formation of a boundary between osteogenic and non-osteogenic cells. *Developmental Biology,* 347**,** 258-270.

YOUSFI, M., LASMOLES, F., LOMRI, A., DELANNOY, P. & MARIE, P. J. 2001. Increased bone formation and decreased osteocalcin expression induced by reduced Twist dosage in Saethre-Chotzen syndrome. *The Journal of Clinical Investigation,* 107**,** 1153-1161.

YU, H. M., JERCHOW, B., SHEU, T. J., LIU, B., COSTANTINI, F., PUZAS, J. E., BIRCHMEIER, W. & HSU, W. 2005. The role of Axin2 in calvarial morphogenesis and craniosynostosis. *Development,* 132**,** 1995-2005.

YU, K., XU, J., LIU, Z., SOSIC, D., SHAO, J., OLSON, E. N., TOWLER, D. A. & ORNITZ, D. M. 2003. Conditional inactivation of FGF receptor 2 reveals an essential role for FGF signaling in the regulation of osteoblast function and bone growth. *Development,* 130**,** 3063-74.

ZHANG, X., IBRAHIMI, O. A., OLSEN, S. K., UMEMORI, H., MOHAMMADI, M. & ORNITZ, D. M. 2006. Receptor Specificity of the Fibroblast Growth Factor Family: THE COMPLETE MAMMALIAN FGF FAMILY. *Journal of Biological Chemistry,* 281**,** 15694-15700.

ZHANG, X., KURODA, S. I., CARPENTER, D., NISHIMURA, I., SOO, C., MOATS, R., IIDA, K., WISNER, E., HU, F.-Y., MIAO, S., BEANES, S., DANG, C., VASTARDIS, H., LONGAKER, M., TANIZAWA, K., KANAYAMA, N., SAITO, N. & TING, K. 2002. Craniosynostosis in transgenic mice overexpressing Nell-1. *Journal of Clinical Investigation,* 110**,** 861-870.

ZHANG, Z., ALPERT, D., FRANCIS, R., CHATTERJEE, B., YU, Q., TANSEY, T., SABOL, S. L., CUI, C., BAI, Y., KORIABINE, M., YOSHINAGA, Y., CHENG, J. F., CHEN, F., MARTIN, J., SCHACKWITZ, W., GUNN, T. M., KRAMER, K. L., DE JONG, P. J., PENNACCHIO, L. A. & LO, C. W. 2009. Massively parallel sequencing identifies the gene Megf8 with ENU-induced mutation causing heterotaxy. *Proc Natl Acad Sci U S A,* 106**,** 3219-24.

ZHOU, Y.-X., XU, X., CHEN, L., LI, C., BRODIE, S. G. & DENG, C.-X. 2000. A Pro250Arg substitution in mouse Fgfr1 causes increased expression of Cbfa1 and premature fusion of calvarial sutures. *Human Molecular Genetics,* 9**,** 2001-2008.

Table 1 Overview of mouse models that display features of a syndromic craniosynostosis phenotype.

Gene	Mutation/Transgene allele	Human syndrome (OMIM#)	Affected sutures	Mechanism	Reference
Fgf9	N143T	SYNS3 (612961)	coronal	LOF	Harada et al 2009
Fgf3	mutagenesis	Crouzon (123500)	coronal	overexpression	Carlton et al 1998
Fgf4					
Fgfr1	P250R	Pfeiffer (101600)	coronal, interfrontal, sagittal	GOF	Zhou et al 2000
Fgfr2	C342Y	Crouzon (123500)	coronal	GOF	Eswarakumar et al 2004
	W290R	Crouzon (123500)			
	S252W	Apert (101200)	coronal, sagittal, lambdoid		Wang et al 2005
	S250W				Chen et al 2003
	Y394C	Beare-Stevenson (123790)	coronal		Wang et al 2012
Fgfr2c	*Fgfr2c*$^{-/-}$	N/A	coronal	LOF	Eswarakumar et al 2002
	Fgfr2c flox, Fgfr2c$^{flox/-}$				Hajihosseini et al 2001
Fgfr2b	*Fgfr2b*$^{-/-}$		squamous temporal-parietal	LOF	De Moerlooze et al 2000
Fgfr3	P244R	Muenke (602849)	coronal	GOF	Twigg et al 2009
Twist1	*Twist1*$^{+/-}$	Saethre-Chotzen (101400)	coronal, sagittal, lambdoid	LOF	Chen 1995, Behr 2011
Tcf12	*Tcf12*$^{flox/-}$*; Twist1*$^{+/-}$*; EIIa*$^{CRE/+}$				Sharma et al 2013
Id1	*Id1+/-; Twist1+/-*				Connerney et al 2008
Id3	*Id1+/-; Twist1+/-*				
Msx2	*Timp1-P7H*	Boston-type craniosynostosis (604757)	coronal, sagittal, lambdoid	GOF	Lin et al 1995
	CMV-P7H				
	CMV-WT			overexpression	
Jag1	*Jag1*$^{+/-}$	Alagille (118450)	no phenotype	LOF	Yen et al 2010
	Jag1$^{flox/-}$*; Twist1*$^{+/-}$*; Mesp1*$^{CRE/+}$		coronal, sagittal, lambdoid		

Gli3	*Gli3*$^{Xt\text{-}J/Xt\text{-}J}$	Carpenter (201000)	lambdoid	LOF	Hui 1993, Rice 2010
Rab23	*Rab23*$^{-/-}$		embryonic lethal		Eggenschwiler et al 2001
Megf8	C193R		sutures not analysed		Aune et al 2008
Masp1	*Masp1/3*$^{+/-}$ *(double KO)*	3MC (257920)			Takahashi et al 2008
Masp3					
Hdac4	*Hdac4*$^{-/-}$	Brachydactyly mental retardation (600430)			Vega et al 2004
Tgfbr1	M318R	Loeys-Dietz (613795)	coronal	GOF	Gallo et al 2014
Tgfbr2	G357W		no suture phenotype		
Nell1	WT (CMV)	N/A	coronal, sagittal, posterior frontal	overexpression	Zhang et al 2002
Axin2	*Axin2*$^{-/-}$		coronal, interfrontal	LOF	Yu et al 1995
Dusp6	exon 3 STOP, *Dusp6*$^{+/-}$		coronal		Li et al 2007
	Dusp6$^{-/-}$				
Gdf6	*Gdf*$^{-/-}$				Settle et al 2003
Pdgfrα	D846V (R26R)		coronal, interfrontal	overexpression	Moenning et al 2009
	Pdgfrα$^{D842V/+}$*; Meox2*$^{CRE/+}$		coronal	GOF	He and Soriano 2017
	Pdgfrα$^{D842V/+}$*; Mesp1*$^{CRE/+}$		coronal, lambdoid		
EphA4	*EphA4*$^{-/-}$		coronal	LOF	Ting et al 2009
	Twist1$^{+/-}$*; EphA4*$^{+/-}$				
Runx2	*Prx1-Runx2*		pan-synostosis	overexpression	Maeno et al 2011
Erf	*Erf*$^{-/-}$		no phenotype	LOF	Papadaki et al 2007
	Erf flox, *Erf*$^{flox/+}$				Twigg et al 2013
	Erf flox, *Erf*$^{flox/-}$		coronal, sagittal, lambdoid		
Frem1	Frameshift (T>C) at intron 25, *Frem1*$^{bat/+}$		posterior frontal	hypomorph	Smyth et al 2004
	exon 2 deletion, *Frem1*$^{QBrick/+}$			LOF	Vissers et al 2011

LOF = loss of function; GOF = gain of function; N/A = not associated (with a human syndrome)