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Bad bones, absent smell, selfish testes: The pleiotropic consequences of human FGF receptor mutations

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Abstract

The discovery in 1994 that highly specific mutations of fibroblast growth factor (FGF) receptor 3 caused the most common form of human short-limbed dwarfism, achondroplasia, heralded a new era in FGF receptor (FGFR) biology. A decade later, the purpose of this review is to survey how the study of humans with FGFR mutations continues to provide insights into FGFR function in health and disease, and the clinical applications of these findings. Amongst the most interesting recent discoveries have been the description of novel phenotypes associated with *FGFR1* and *FGFR3* mutations; identification of fundamental differences in the cellular mechanisms of mutant FGFR2 and FGFR3 action; and the direct identification of *FGFR2* and *FGFR3* mutations in sperm. These clinical observations illustrate the pleiotropism of FGFR action and fuel ongoing efforts to understand the rich biology and pathophysiology of the FGF signalling system.

Keywords: FGFR; Genetics; Craniosynostosis; Bone; Sperm

1. Introduction

In 1994, two groups independently described the identification of specific heterozygous nucleotide substitutions (1138G > A or C, both encoding Gly380Arg) of the *FGFR3* gene in 39 unrelated individuals with achondroplasia, accounting for all cases studied [1,2]. This remarkable discovery presaged a burst of activity that led to the identification of germline mutations in the *FGFR1* and *FGFR2* genes within just a few months [3–5]. Striking parallels in the early findings between the different fibroblast growth factor receptors (FGFRs) heralded several emergent themes that have continued to dominate the agenda of human genetics research in this field. The mutations identified were dominantly acting, encoded a relatively limited repertoire of specific missense substitutions that caused congenital skeletal abnormalities (either short-limbed bone dysplasia or craniosynostosis, the premature fusion of the cranial sutures), and many occurred recurrently with remarkably high rates of new mutation. It soon became apparent, at least for *FGFR2* and *FGFR3*, that different mutations within each gene were associated with distinct phenotypes, constituting allelic series [6,7]. This in turn implied quantitative or qualitative differences in the function of mutant proteins, spawning efforts to study these differences using biochemical and mouse genetic approaches. Subsequent human genetic discoveries of particular importance have been the description of a common, hitherto unrecognised craniosynostosis syndrome caused by a specific FGFR3 P250R mutation [8,9] and the more recent findings that haploinsufficiency and constitutive gain-of-function mutations of *FGFR1*, and a putative dominant negative mutation of *FGFR3*, cause strikingly different phenotypes from those associated with the earlier described mutations [10–12].

Another challenge has been to explain the very high rates of specific FGFR mutations, some of which appear elevated ~500-fold above background levels. Several of the most frequent mutations have been shown to originate exclusively from the unaffected father and are associated with increased paternal age, relative to the population average, at the time of conception. These observations have led to efforts to identify these specific mutations in sperm, which have recently been successful [13–15]. Evidence for the paradoxical selective

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advantage of an FGFR2 mutation in the testis [14] provides an unexpected example of the pleiotropism of mutant FGFR action.

Many reviews on different aspects of the human genetics of FGFR mutation have been published [16–25]. Approaches such as biochemical dissection and structural analysis are addressed elsewhere in this issue [26–28]. The scope of this article will be to collate and update information gleaned from the clinical study of humans with germline FGFR mutations, focusing particularly on discoveries of the past five years. Somatically arising FGFR mutations associated with cancer are reviewed in an accompanying article [29].

2. Spectrum of germline FGFR mutations

The full spectrum of FGFR mutations in human genetic disorders, including the relative prevalence of different mutations, was comprehensively surveyed by Passos-Bueno et al. [18] and Muenke and Wilkie [20]. Table 1 itemises additional FGFR mutations [10–12,22,30–50] that were not included in those reviews. In this article I shall refer to the FGFR domains in which mutations occur, from N to C terminus, as immunoglobulin-like I, II, IIIa and IIIc (IgI, IgII, IgIIIa, IgIIIc), juxtamembrane-extracellular (JM-E), transmembrane (TM), juxtamembrane-intracellular (JM-I) and tyrosine kinase 1 and 2 (TK1, TK2). Table 2 summarizes the general categories of mutations that are associated with different human syndromes, with suggested further reading about the individual phenotypes. The distribution of mutations along the FGFR1, -2 and -3 molecules, with an indication of their relative prevalence, is shown in Fig. 1. No germline mutations have been identified in *FGFR4*.

The frequency spectrum of FGFR mutations illustrates several points. First, allelic mutations differ strikingly in their relative prevalence (Fig. 1). In an unbiased series of FGFR2 mutations, for example, substitutions at just four residues in the protein (S252, P253, C278, C342) accounted for ~81% of all unrelated cases [31]. Second, identical amino acid substitutions are frequently observed at the equivalent positions of paralogous proteins. Most notable is the IgII-IgIIIa linker proline → arginine mutation, which causes mild Pfeiffer, Apert and Muenke syndromes when the mutation occurs in FGFR1 [5], FGFR2 [6] and FGFR3 [8], respectively (Fig. 1, Table 2). These linker mutations all enhance the affinity of ligand binding [21,28,61,62]. Two other identical substitutions have been described in all three paralogues, asparagine → isoleucine in IgIIIc and tyrosine → cysteine in JM-E [11]. Although there are many additional examples of equivalent mutations between FGFR1 and FGFR2, or FGFR2 and FGFR3, it would be over-simplistic to assume that the molecular consequences of these mutations are necessarily equivalent in the different paralogues. The most striking (and currently unexplained) examples are that the FGFR1 mutations Y99C and C277Y identified in Kallmann syndrome, a putative haploinsufficiency [10] are equivalent to the FGFR2 mutations Y105C and C278Y associated with Crouzon syndrome (putative constitutive gain-of-function) [31,33,63,64]. Differences in transcriptional regulation and/or the processing of mutant proteins (see Section 4) may explain the rather striking differences in mutation spectra between the FGFRs (for example IgIIIa/IgIIIc mutations are abundant in FGFR2, but rare in FGFR3; conversely, TM and TK mutations are abundant in FGFR3, but occasional in FGFR2). Although it might be suggested that the germline mutation spectra illustrated in Fig. 1 simply reflect our current ignorance of the full range of human phenotypes associated with FGFR mutations, countering this is the fact that the spectra of FGFR2 and (particularly) FGFR3 missense mutations observed in the very different biological context of tumorigenesis closely reflect the germline spectra [29,65,66].

There is an interesting group of FGFR2 mutations that affect splicing. These fall into two classes and locate exclusively around the alternatively spliced IIIc exon (Fig. 1): this exon may be particularly susceptible to splicing defects owing to the reduced intrinsic strength of its splice sites, allowing choice in different cell types between the IIIb and IIIc exons, which encode receptors with different ligand binding repertoires [67]. Mutations of the exon IIIc acceptor splice site drive illegitimate expression of the IIIb exon in patient fibroblasts [68,69], which correlates with a greater severity of limb abnormalities [21,22,68]. Mutations either of the exon IIIc donor splice site, or of a cryptic site within the IIIc exon, cause switching to preferential use of the cryptic donor site, encoding a shortened form of exon IIIc that lacks 17 amino acids [70,71]. This might be the primary mechanism of action of the apparent missense substitution 1084G > T (“A362S”), which locates in the last base of exon IIIc, however effects on splicing have not been tested [40].

Perhaps the most surprising recent discovery has been that heterozygous, putative loss-of-function mutations in FGFR1 are associated with autosomal dominant Kallmann syndrome (KS), a disorder defined by the combination of anosmia (deficiency in the sense of smell) and hypogonadotrophic hypogonadism [10]. Underlying these phenotypes (which show no overlap with the previously described FGFR1-associated Pfeiffer syndrome) is absence or hypoplasia of the olfactory bulbs and tracts, indicating that development of these structures is particularly sensitive to FGFR1 dosage. Some heterozygous individuals (generally female) are non-penetrant, whilst others have additional phenotypes, most notably cleft palate (Table 1). These observations suggest that FGFR1 interacts, directly or indirectly, with anosmin-1, the product of the *KAL1* gene mutated in X-linked KS, and identifies several processes that require precise FGFR1 dosage for normal development. This highlights the general issue of FGFR dosage sensitivity, one that has previously been rather neglected in the general focus on pinpointing gain-of-function mechanisms of pathogen-

Table 1
Recently identified germline mutations in FGF receptors[a]

Gene	Nucleotide substitution	Amino acid substitution	Domain[b]	Phenotype[c]	Reference
FGFR1	290G > A	G97D	IgI	KS	[10]
	296A > G	Y99C	IgI	KS	[10]
	303_304insCC	V102PfsX2	IgI	KS	[10]
	320C > A	S107X	IgI	KS	[30]
	499G > T	A167S[d]	IgII	KS, cleft palate, agenesis of corpus callosum	[10]
	830G > A	C277Y	IgIIIa	KS	[10]
	936G > A	K312K[e]	IgIIIa	KS, dental agenesis	[10][f]
	989A > T	N330I	IgIIIc	OD	[11][f]
	1121A > G	Y374C	JM-E	OD	[11][f]
	1141T > C	C381R	TM	OD	[11]
	1819G > A	V607M	TK2	KS, bimanual synkinesis	[10]
	1864C > T	R622X	TK2	KS, cleft lip/palate	[10]
	1970_1971delCA	T657NfsX17	TK2	KS	[10]
	1996T > A	W666R	TK2	KS, cleft palate	[10]
	2048 + 1G > A		TK2	KS	[10]
	2156T > G	M719R	TK2	KS	[10]
	2233C > T	P745S	TK2	KS	[30]
	2314C>T	P772S	TK2	KS, cleft palate, coloboma	[10]
FGFR2	514_515GC > TT	A172F	IgII	PS	[31]
	755_756CG > TC	S252F	IgII-IgIII linker	AS	Unpubl[g]
	755C > T; 943G > T	S252L; A315S	IgII-IgIII linker; IgIIIc	syndactyly	[22]
	758C > T	P253L	IgIIIa	CS	Unpubl[g]
	760C > T	H254Y	IgIIIa	CS	[32]
	788C > T	P263L	IgIIIa	CS	[33]
	818_820del	D273del	IgIIIa	PS	[34]
	823_824ins12	V274_E275ins4	IgIIIa	CS	[35]
	833G > A	C278Y	IgIIIa	CS	[33]
	842A > G	Y281C	IgIIIa	CS	[36]
	863T > G	I288S	IgIIIa	CS	[33]
	923A > G	Y308C	IgIIIa	CS	[37][h]
	943G > T	A315S	IgIIIc	CR/normal	[38]
	958_959delAC	T320GfsX5	IgIIIc	JWS	[39]
	1009G > A	A337T	IgIIIc	CR/normal	Unpubl[g]
	1019A > C	Y340S	IgIIIc	CS	[37][h]
	1061C > A	S354Y	IgIIIc	CS	[33][f]
	1061C > T	S354F	IgIIIc	CS	[32]
	1084G > T	A362S[e]	IgIIIc	CS/normal	[40]
	1084 + 3A > C		IgIIIc	CS	[33]
	1576A > G	K526E	TK1	CS/normal	[41]
	1645A > C	N549H	TK1	CS	[31]
	1646A > C	N549T	TK1	PS	Unpubl[g]
	1694A > G	E565G	TK1	PS	[31]
	1694A > C	E565A	TK1	PS	[42]
	1922A > G	K641R	TK2	PS	[31]
	1977G > T	K659N	TK2	CR	[31]
	1988G > A	G663E	TK2	PS	[31]
	2032A > G	R678G	TK2	CS	[31]
FGFR3	598C > T	R200C	IgII	HCH	[43]
	749C > T	P250L	IgII-IgIII linker	CR	[44]
	784A > C	N262H	IgIIIa	HCH	[43]
	802G > T	G268C	IgIIIa	HCH	[43]
	983A > T	N328I	IgIIIc	HCH	[45][f]
	1037G > A	G346E	IgIIIc	ACH	[46]
	1142T > A	V381E	TM	HCH	[47]
	1346C > T	P449L	JM-I	HCH	[48]
	1619A > G	N540S	TK1	HCH	[49]
	1862G > A	R621H	TK2	See text	[12]
	1948A > C	K650Q	TK2	HCH	[50]
	1950G > C	K650N	TK2	HCH	[50]

Table 1 (*Continued*)

Gene	Nucleotide substitution	Amino acid substitution	Domain[b]	Phenotype[c]	Reference
	1950G > T	K650N	TK2	HCH	[50]
	2005C > G	R669G	TK2	HCH	Unpubl[g]

[a] Updated from [20] (*FGFR1*, *FGFR2*) and [18] (*FGFR3*). Note that the *FGFR2* mutation 940-3_946del10insACC was incorrectly listed as 940_946del7insACC in [20].
[b] See Section 2 of main text for domain abbreviations.
[c] KS, Kallmann syndrome; OD, osteoglophonic dysplasia; PS, Pfeiffer syndrome; AS, Apert syndrome; CS, Crouzon syndrome; JWS, Jackson–Weiss syndrome; CR, non-syndromic craniosynostosis; HCH, hypochondroplasia, ACH, achondroplasia.
[d] Homozygous mutation.
[e] Mutations located in exons that are suspected or proven to disrupt normal splicing.
[f] The nucleotide substitution was incorrectly assigned in the original reference.
[g] Unpublished cases from the author's laboratory.
[h] J. Bonaventure, personal communication.

esis. It is not currently known whether hemizygosity of any of the other FGFRs is associated with haploinsufficiency phenotypes. A further layer of complexity in the genotype-phenotype correlation of FGFR1 mutations has recently been added by the discovery of three heterozygous mutations (Table 1) causing osteoglophonic dysplasia, a disorder comprising both short-limbed bone dysplasia and craniosynostosis [11]. Interestingly, several of these patients were hypophosphatemic due to renal phosphate loss; this may be causally related to elevated FGF23 production from associated radiolucent bone lesions [11].

The further analysis of skeletal disorders already known to be associated with FGFR mutations has also yielded several surprises. A cluster of missense mutations newly identified in the TK1 and TK2 domains of FGFR2 in Crouzon and Pfeiffer syndromes has important implications for molecular genetic diagnostics, considerably increasing the number of exons of *FGFR2* that need to be screened for comprehensive coverage (Table 1). Although these FGFR2 mutations are comparatively rare, paradoxically they encompass a broader range of TK domain residues [31] than has been described for the much more frequent FGFR3 mutations; these include the residues equivalent to the FGFR3 mutation hotspots at N540 and K650 (Fig. 1).

A specific mutation that illustrates the value of the human gene pool as a natural resource of precise gene targeting experiments is the double mutation S252L; A315S in FGFR2 (both substitutions present on the same allele) [22]. Heterozygotes for each single mutation, as well as the double mutation, have been identified: people with either single mutation have normal extremities [38,72], but remarkably, the double mutation is associated with severe syndactyly, resembling Apert syndrome [22]. The absence of the A315S substitution from the alternative FGFR2-IIIb spliceform provides strong genetic support for the proposal [21] that the syndactyly of Apert syndrome (see Table 2) arises through illegitimate FGFR2-IIIc mediated binding of an FGF7/10-type ligand. Both the presence of a highly conserved serine at the 315 position in the IIIb exon [22] and structural data [73] suggest that the A315S substitution confers FGFR2-IIIb-like binding properties to the FGFR2-IIIc molecule, with the S252L substitution presumably playing a synergistic role. Confirming this hypothesis, binding by FGF10 to the double mutant was recently demonstrated utilizing surface plasmon resonance analysis [74].

Finally, a heterozygous R621H mutation in FGFR3 was recently found to segregate with a novel phenotype comprising sensorineural hearing loss, camptodactyly, tall stature, kyphoscoliosis, microcephaly and developmental delay: very different from the usual FGFR3-associated short-limbed bone dysplasias, but with similarities to the murine *Fgfr3*$^{-/-}$ phenotype [12]. For this reason it was suggested that the mutation, which is located within a highly conserved residue in the catalytic loop, acts in a dominant negative fashion. Although this explanation is attractive, experimental support is not available at the time of writing.

3. Prevalence of de novo FGFR mutations

The estimation of disease prevalence is important, when considering both the overall clinical burden of FGFR mutations, and for evaluating data on mutation levels in sperm (Section 6). Much of the epidemiological work on disease prevalence was undertaken in the pre-molecular era, so that some data are likely to be confounded by unrecognised genetic heterogeneity. Table 3 attempts to synthesize the most reliable studies, and proposes overall birth prevalence figures for de novo *FGFR2* and *FGFR3* mutations. Amongst visible phenotypes, *FGFR3* mutations seem to be about twice as common overall as *FGFR2* mutations: a new mutation in one of these two genes is estimated to occur in nearly 1 in 10,000 births [14,31,75–80]. Indeed the transition 1138G > A in *FGFR3*, which occurs at a CpG dinucleotide and causes 97% of cases of achondroplasia, may be the single most frequently occurring germline nucleotide substitution in the entire human genome.

No figures are currently available for the frequency of *FGFR1* mutations, but current data suggest that they may be an order of magnitude less common than *FGFR2* or *FGFR3* mutations.

Table 2
Phenotypic groups associated with FGFR mutations

Gene	Characteristic mutations/location	Name	Key clinical features	References
FGFR1	Widespread (haploinsufficiency)	Kallmann syndrome	Anosmia, hypogonadotrophic hypogonadism	[10]
	P252R (100%)	Pfeiffer syndrome (mild)	Craniosynostosis, cutaneous syndactyly	[51,52]
	N330I, Y374C, C381R	Osteoglophonic dysplasia	Short stature, craniosynostosis, bone demineralisation	[11]
FGFR2	S252W (66%), P253R (32%)	Apert syndrome	Craniosynostosis, crouzonoid facies[a], bony syndactyly	[21,25,53]
	IgIIIc (71%), IgIIIa (23%), TK, IgI	Crouzon/Pfeiffer/"Jackson–Weiss"/"	Craniosynostosis, crouzonoid facies, graded severity of	[22,25,31]
		Antley–Bixler" syndromes	limb anomalies (nil, broad first digits, elbow fusion)	
	Y375C (>85%), S372C	Beare Stevenson syndrome	Severe craniosynostosis, cutis gyrata	[54]
FGFR3	P250R (>99%)	Muenke syndrome	Coronal craniosynostosis, clinically not diagnostic	[9]
	HCH: N540K (42%). ACH: G380R (99%).	Hypochondroplasia (HCH)/achondroplasia	Short-limbed bone dysplasia of graded severity from	[19,55–59]
	TDI: R248C (56%), Y373C (22%), stop codon (12%).	(ACH)/SADDAN[b]/thanatophoric dysplasia I	mild short stature to neonatal lethality; acanthosis	
	TDII: K650E (100%). SADDAN: K650M (100%)	and II (TDI, TDII)	nigricans in severely affected survivors; craniosynostosis in TDII and some TDI	
	A391E (100%)	Crouzon syndrome with acanthosis nigricans	Craniosynostosis, crouzonoid facies, acanthosis nigricans	[60]
	R621H	New syndrome	Sensorineural hearing loss, camptodactyly, tall stature, kyphoscoliosis, microcephaly, developmental delay	[12]

[a] A "crouzonoid" appearance refers to a combination of prominent eyes, nose and jaw with underdeveloped midface that reflect growth abnormalities of the facial bones and skull base.
[b] SADDAN, severe achondroplasia with developmental delay and acanthosis nigricans.

4. In vivo consequences of FGFR mutations studied in humans

4.1. Introductory remarks

An important challenge is to understand how FGFR mutations lead to a diversity of specific phenotypes in humans; the phenotypes of greatest interest include long bone dysplasia, craniosynostosis, syndactyly, acanthosis nigricans and abnormalities of the central nervous system. To date, human studies have focused on the origins of long bone dysplasia [81–87] (Table 4) and craniosynostosis [88–104] (Table 5), using combinations of RNA in situ hybridization, histochemistry, cell culture and gene expression studies. Overall, work on FGFR3-associated bone dysplasia has generated a fairly consistent body of data, whereas that for FGFR2-associated craniosynostosis is confusing and sometimes contradictory. It is instructive to explore the reasons for this difference.

Clinicopathological investigation necessarily depends on access to the appropriate clinical material. This may comprise either autopsy specimens, usually sourced from termination of an affected pregnancy, or samples removed at operation. Whereas thanatophoric dysplasia (TD) is readily detectable on fetal ultrasound at mid-gestation, other FGFR-associated disorders are less so (Section 7): not surprisingly, the largest clinicopathological series are from TD-affected fetuses (Table 4).

Although per-operative samples are fairly readily available from infants during cranial surgery for craniosynostosis, their usefulness remains unproven for several reasons. By a few months of infancy, the abnormal developmental process that has led to the craniosynostosis is in an advanced or terminal phase. The affected cranial suture is likely to have been obliterated, making it difficult to define the developmental relationship between the bone fragments or cells obtained for culture during the operation, and the primary pathological process under investigation. Defects in signalling that cause abnormal morphogenesis occur in a complex three-dimensional architecture of different cell types: the isolation of specific cells (osteoblasts, fibroblasts, etc.) in culture destroys these relationships. Evidence that gain-of-function FGFR2 mutations are subject to feedback transcriptional downregulation (Section 4.3), suggests that abnormal developmental processes may arise during narrow time/tissue windows during which downregulatory feedback is less precisely tuned. A corollary is that the output measured from an artificial system, such as cell culture, will depend on how the delicate balance between the gain-of-function conferred by the mutation and compensatory dampening of the response, play out in particular culture conditions. In an experimental situation it may be difficult to control for all the factors in this balance, leading to measured outputs that are compromised by excessive biological noise.

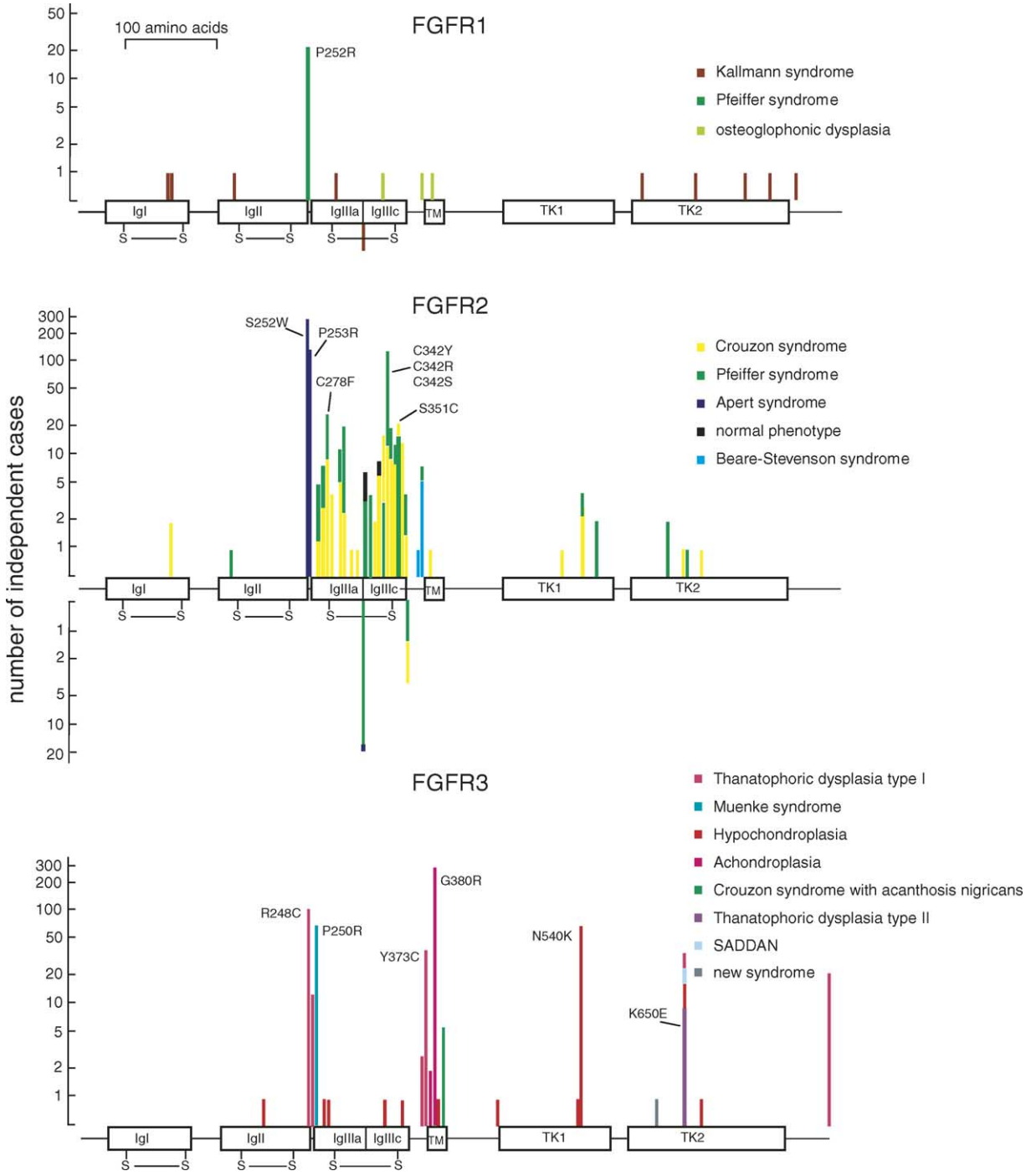

Fig. 1. Distribution of germline mutations in FGFR1, FGFR2 and FGFR3 causing missense substitutions (above protein) or probable splicing abnormalities (below protein). The mutation prevalence is indicated on a logarithmic scale and different phenotypes are color-coded. Where multiple phenotypes occur with mutation at a single position, the column colors are partitioned proportionately using a linear scale. Substitutions recorded in more than 20 independent subjects are identified individually. For clarity, insertions, deletions and nonsense mutations (none of which has occurred as a recurrent mutation) are omitted; ''Antley–Bixler'' and ''Jackson–Weiss'' phenotypes are lumped with Pfeiffer syndrome. Data for *FGFR1* and *FGFR2* are from the author's unpublished database; data for *FGFR3* are from [18], supplemented with additional mutations described more recently (Table 1). Abbreviations for domains are defined in Section 2. In each case the alternatively spliced IIIc spliceform is shown; no mutations have been described in any of the IIIb exons.

An inescapable conclusion is that well designed animal models are a prerequisite to understand the pathophysiology of FGFR mutations, and that the role of the human studies should be to provide corroborative evidence of their relevance to actual disease processes. Animal models are described elsewhere [23,26]: this section highlights those human genetic studies that have yielded valuable benchmark information against which such models should be compared.

Table 3
Estimates of birth prevalence of de novo FGFR mutations

Disorder	Birth prevalence (per million)
Apert syndrome	13.5[a]
Non-Apert *FGFR2* mutations	12.5[b,c]
Total *FGFR2* mutations	26.0
Achondroplasia	20.9[d]
Thanatophoric dysplasia	23.7[d]
Muenke craniosynostosis	7.6[c,e]
Total *FGFR3* mutations	52.2

[a] From combined epidemiological data [75–77]. Apert syndrome is easy to diagnose at birth so this figure should be fairly accurate.
[b] Based on the relative proportions of Apert and non-Apert de novo *FGFR2* mutations in [31]. Diagnostic difficulties and genetic heterogeneity undermine estimates based on clinically diagnosed cases.
[c] These figures are based on de novo mutations that are *phenotypically apparent*. Some *FGFR2* mutations might be inapparent. For example, measurements on sperm suggest that the 755C > T mutation (encoding S252L) has a birth prevalence of $\sim 5.6 \times 10^{-6}$ [14], but most cases are non-penetrant [72]. Similarly, non-penetrance occurs in an unknown proportion of Muenke craniosynostosis [78].
[d] From [79]. These cases were not molecularly confirmed, however diagnostic accuracy is probably good. The figure for thanatophoric dysplasia is based on combined live- and stillbirths; some cases were probably not ascertained owing to earlier prenatal diagnosis and termination of pregnancy.
[e] Reliable diagnosis is only possible by molecular testing of the child and both parents. This figure is based on data from the author's laboratory [80].

4.2. FGFR3 mutations and bone dysplasia

Impressive series of pathological fetal material have been obtained by American and French groups (Table 4). In general the FGFR3-mutant epiphyseal plate is characterized by short, irregular chondrocyte columns with small, scanty hypertrophic zone cells expressing low levels of collagen type X (a specific marker of this zone) in the matrix. Bone-perichondrial ring thickness is increased, the epiphyseal plate is disrupted by inward growths of fibrous tissue, and metaphyseal bone trabeculae are short and widened [55,86]. Interestingly, varying clinical severity (based on radiological appearance and chondro-osseous morphology) was shown to correlate with the electron microscopic appearance of chondrocytes [85]. A milder clinical variant compared to TDI, termed ''platyspondylic lethal skeletal dysplasia, San Diego type'' (PLSD-SD), was characterized by large cytoplasmic inclusions that stained positively for an antibody to FGFR3; these inclusions tended to be smaller or absent in classical TDI, even when the identical *FGFR3* mutation was present [85]. In independent studies, the intensity of in situ hybridization using an *FGFR3* antisense riboprobe did not differ between mutant and normal growth plate sections, but FGFR3 antibody staining was increased in both the proliferative and the hypertrophic zones of TDI fetuses [83]. Examination of cultured chondrocytes showed a perinuclear distribution of antibody staining specifically from TDI fetuses that was absent in normal controls [84]. The FGFR3 staining co-localized with nucleoporin, a nuclear membrane marker, rather than markers of lysosomes or late endosomes, providing evidence that the protein was not being targeted for degradation [84]. Recent experiments using cell transfection assays have corroborated these findings. Mutant TDII (K650E) and SADDAN (K650M) protein localized within the endoplasmic reticulum as incompletely glycosylated forms [105,106]; the stability of the mutant protein might be attributable to deficient lysosomal targeting by ubiquitination [107]. Collectively these observations provide strong evidence that variable processing of mutant FGFR3 protein, apparently modified by genetic background, is an important determinant of disease severity.

There is also good evidence that FGFR3 mutations cause abnormal activation of STAT1, STAT5 and p21/CIP1, which show strong nuclear immunoreactivity specifically in hypertrophic cartilage cells and in cultured chondrocytes from achondroplasia, TDI and TDII fetuses [82,84,87]. The

Table 4
Studies of tissue pathophysiology in patients with identified mutations in *FGFR3* associated with bone dysplasia

Mutation	Stage[a]	RNA in situ/tissue histochemistry	Primary cell culture: cell type	Reference
R248C/S371C (TDI)	NA (×4)		Fibroblast	[81]
G380R (ACH)	NA (×7)		Fibroblast	[81]
K650E (TDII)	NA (×4)		Fibroblast	[81]
K650E (TDII)	F (×2)	+		[82]
TDI (7 different mutations)	F (×14) 16–27 weeks	+		[83]
TDI (4 different mutations)	F (×14) 17–38 weeks	+	Chondrocyte	[84]
TDI (5 different mutations)	F (×72)	+		[85]
PLSD-SD[b] (4 different mutations)	F (×17)	+		[85]
G380R (ACH)	F (×9) 12–38 weeks	+		[86]
TDI (5 different mutations)	F (×21) 16–27 weeks	+		[86]
K650E (TDII)	F (×8) 22–23 weeks	+		[86]
G380R (ACH)	F (×14) 13–35 weeks	+	Chondrocyte	[87]
TDI (3 different mutations)	F (×19) 17–38 weeks	+	Chondrocyte	[87]
K650E (TDII)	F (×7) 17–25 weeks	+	Chondrocyte	[87]

[a] NA, information not available; F, fetal (with number of cases studied and range of fetal gestational age in weeks).
[b] Platyspondylic lethal skeletal dysplasia, San Diego type. For other disease abbreviations, see Table 2.

Table 5
Studies of tissue pathophysiology in patients with identified craniosynostosis mutations in *FGFR2* or *FGFR3*

Gene	Mutation	Stage[a]	RNA in situ/tissue histochemistry	Primary cell culture: cell type	Immortalized cells	Global expression	Reference
(*FGFR2*)[b]	Apert	P		Pericranial fibroblast			[88,94]
FGFR2	S252W (Apert)	F (19, 25, 27 weeks)	+	Calvarial osteoblast	+		[89]
FGFR2	S252F (Apert)	F (28 week)	+	Calvarial osteoblast			[89]
FGFR2	S252W (Apert)	P		Calvarial osteoblast			[89]
(*FGFR2*)[b]	Apert	P	+				[90]
(*FGFR2*)[b]	Apert	P		Pericranial fibroblast			[91,94]
FGFR2	P253R (Apert)	P		Calvarial osteoblast			[92]
FGFR2	C342R (Pfeiffer)	P		Calvarial osteoblast			[92]
(*FGFR2*)[b]	Apert	P		Calvarial osteoblast			[93,94]
FGFR2	S252W (Apert)	F (26, 27 weeks)	+	Calvarial osteoblast	+	+	[95–98,103]
FGFR2	P253R (Apert)	F (14 week)	+				[99]
FGFR2	C278F (Pfeiffer)	F (27 week)	+				[99]
FGFR2	S252W/P253R (Apert)	P (×5)		Calvarial osteoblast			[100]
FGFR2	C278F (Crouzon)	P (×2)		Calvarial osteoblast			[100]
FGFR3	P250R (Muenke)	P		Calvarial osteoblast			[100]
FGFR2	C342Y (Crouzon)	P (×3)		Pericranial fibroblast			[101]
FGFR2	P253R (Apert)	P		Pericranial fibroblast		+	[102]
FGFR2	G338R (Crouzon)	P		Pericranial fibroblast		+	[102]
FGFR2	S252W (Apert)	P (×2)		Digital osteoblast			[104]

[a] P, postnatal (operative specimen); F, fetal (gestational age in weeks).
[b] The precise mutation was not identified in these publications, but the ease of making a clinical diagnosis of Apert syndrome, combined with its narrow genotypic spectrum, makes it very likely that an *FGFR2* mutation was present in these cases. The same specificity cannot be assumed for other diagnoses such as Crouzon and Pfeiffer syndromes; these cases are only included if the mutation was identified.

simultaneous detection of high cytoplasmic FGFR3 immunoreactivity in the same cells suggests a direct causal link with STAT pathway activation [87]. Primary cultured TDI chondrocytes demonstrated constitutive FGFR3 tyrosine phosphorylation [87] in the absence of extracellular signal-related kinase (ERK) pathway activation, although higher and more sustained ERK phosphorylation in response to FGF9 stimulation was observed in mutant compared to control cells [84]. No difference was found for FGF2, -9 or -18 stimulated proliferation of cultured chondrocytes, nor for proliferating cell nuclear antigen (PCNA) staining between normal and TD fetuses [84,87]. Consistent differences in mRNA expression of Indian hedgehog (*IHH*) and parathyroid hormone-related peptide receptor 1 (*PTHR1*) were also not observed [86]. Legeai-Mallet et al. [87] summarise these data by proposing that p21/CIP1 overexpression at the junction between the proliferative and prehypertrophic zones in achondroplasia and TD fetuses causes premature exit of proliferative cells from the cell cycle, leading to accelerated chondrocyte differentiation. Several markers of apoptosis (cells labelled by the terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labelling [TUNEL] assay; increased and decreased levels of BAX and BCL-2, respectively) were also increased in FGFR3 mutant cultured chondrocytes [84].

4.3. *FGFR2* mutations and craniosynostosis

Elegant studies by Mathijssen et al. [108] traced the developmental timing of craniosynostosis by measuring the separation of the frontal and parietal bone centres in cadaveric skulls from normal and Apert syndrome fetuses and infants. A normal fetal growth curve was derived: it was then shown that the fronto-parietal bone centre separation for two neonatal Apert syndrome skulls was extremely small, corresponding to the normal value at 16 weeks' gestation, the time at which the definitive coronal suture is first established. This demonstrated that these Apert syndrome coronal sutures had failed to function since their initiation. In this situation, the rapid growth of the brain has to be accommodated by continuing patency of the midline (metopic and sagittal) sutures, which is reflected as a wide undifferentiated zone in the midline that often persists in infancy and through which the brain may herniate [109]. In typical Crouzon syndrome, by contrast, it seems likely that definitive cranial sutures are established at their normal positions but that later, accelerated closure occurs; this tends to affect the coronal and midline sutures with a similar timecourse, giving rise to less distortion of skull shape but a greater risk of raised intracranial pressure compared to Apert syndrome [110,111]. In established *FGFR2*-related craniosynostosis there is often a generalized abnormality of the membranous bones of the skull vault [112,113]. Clinically this is usually attributed to the secondary effects of raised intracranial pressure, but it may also reflect a primary abnormality of periosteal remodelling.

Two groups have risen to the challenge of attempting to correlate the appearance of the fetal skull, in cases with defined *FGFR2* mutations, with relevant RNA in situ hybridization and histochemical data. Lomri et al. [89] studied calvarial bone from four Apert syndrome fetuses, including two using histochemistry (Table 5). They found

that the total amount of subperiosteal calcified bone matrix was much higher in Apert fetuses than in age-matched controls; staining for alkaline phosphatase was stronger and more numerous in cells from the subperiostal regions of the Apert fetuses, indicating a more differentiated phenotype (by contrast, the osteoid and osteoblast surfaces were nearly normal at a distance from the periosteum). In further work, these changes were found to correlate with higher immunostaining of several markers of osteoblast differentiation (type I collagen, osteocalcin, osteopontin, N-cadherin and E-cadherin, but not osteonectin or N-CAM) [95,97]. Importantly, FGFR2 immunostaining was markedly reduced, whereas FGFR1 and FGFR3 changed little [95]. Osteoblast proliferative activity, assayed using PCNA staining, was normal in Apert fetal bone [95], but a higher proportion of cells were apoptotic, as measured by TUNEL staining [96]. Britto et al. [99] studied the calvariae of a 14-week Apert and a 27-week Pfeiffer fetus. Interpretation of the results from the Pfeiffer fetus was hampered by lack of an age-matched control. In the case of the Apert fetus, the expression of FGFR2 in the base of the future parietal bones was markedly reduced, based both on RNA in situ hybridization and immunohistochemistry, compared to a control; FGFR1 and FGFR3 were unaffected. This observation confirms the data in [95] (although that report suggested, based on cell culture studies, that *FGFR2* mRNA levels were unaltered) and is further corroborated by a study of postnatal Apert calvariae [90]. The implications of such down-regulation were discussed earlier (Section 4.1).

Investigations of cells from patients with defined mutations are summarized in Table 5. As previously argued (Section 4.1), these studies are probably more applicable for the corroboration and dissection of the cell biological processes described directly from histology and histochemistry, rather than to describe primary phenomena in craniosynostosis. Investigations of fetal calvarial cells are likely to be of greatest relevance, but these have only been described by one group [89,95–98,103]. Overall, these data have supported the in vivo observations well in terms of the relative contribution of cell division, differentiation and death to the Apert calvarial phenotype. By use of phosphorylation assays, specific chemical inhibitors and antibodies, it has been proposed that phospholipase Cγ-mediated activation of the protein kinase Cα (PKCα) and interleukin-1 (IL1) pathways occurs in Apert mutant cells and mediates cellular apoptosis, whereas ERK activation was not detected [95–97]. Evidence for downregulation of FGFR2 S252W protein as a consequence of c-CBL mediated ubiquitination has been presented [103].

The application of global approaches to the analysis of gene expression has been reported in two pilot studies [98,102], but its full power has yet to be realised. It will, for example, be interesting to discover whether different craniosynostosis-associated mutations are correlated with specific gene expression signatures. Although differences in cellular behaviour and gene expression between cells with Apert and Crouzon/Pfeiffer-type FGFR2 mutations have been reported [92,94,102], these studies have investigated too few samples to give confidence that the observed differences are attributable to the background genetic mutation rather than other, uncontrolled aspects of the experimental protocol.

In summary, the most secure conclusions from the work summarized in Table 5 are that the FGFR2 Apert mutation accelerates subperiosteal osteogenic differentiation and that this is associated with FGFR2 downregulation. Increased osteoblast apoptosis is also observed, but it is not clear to what extent this is a cause or consequence of the elevated osteogenesis.

4.4. Summary

Comparison of the work on FGFR3 mutations in the chondrocyte and FGFR2 mutations in calvarial osteoblast lineages reveals both similarities and differences. In both chondrocytes and pre-osteoblasts, the major consequence of these mutations is the premature differentiation into mature osteoblastic cells, associated with increased apoptosis. Effects on proliferation are not reproducibly observed. A striking difference between the two systems is that in the case of FGFR3 mutations, there is clear evidence of cytoplasmic accumulation of mutant protein; whereas in the cranial suture/periosteum, FGFR2 protein is reduced. This contrast is likely to reflect differences in both feedback control of mRNA synthesis and in receptor processing and may partly account for the predominant activation of different pathways downstream of the receptors. In the case of FGFR3 mutations causing bone dysplasias, there is strong evidence that activation of STAT1/STAT5 and p21/CIP1 is critical to pathogenesis; for FGFR2 mutations, PKCα activation may be more important although the evidence is less compelling. The contribution of the ERK pathway to pathogenesis remains an open question; although the data from human studies have largely been negative, evidence from mice indicates a role both in long bones [114] and in cranial sutures [115].

Little is known about the pathogenesis of other organ abnormalities from human studies, although some information is available in abstract form. In a 24-week fetus with TDI, expression of FGFR3 in the brain was higher in ependymal periventricular cells compared with an age matched control, but was absent in neurons of the cortical plate and deep brain nuclei [116]; various abnormalities of brain anatomy have been documented in TD [117]. In keratinocytes, acanthosis nigricans associated with pathogenic FGFR3 mutations was reported to be associated with stimulation of the anti-apoptotic protein BCL-2 via the phosphatidyinositol 3-kinase/AKT kinase pathway [118]. Although mouse models may often provide a more amenable entry point for the dissection of pathogenesis, a cautionary note is that neither of two existing models of Apert syndrome [119,120] exhibit syndactyly, an essential diagnostic hallmark of the human clinical disorder.

5. Paternal origin of FGFR mutations

To understand why specific nucleotide substitutions occur so frequently in the *FGFR2* and *FGFR3* genes, previous work has focused on the parental origin of *FGFR2* mutations causing Apert, Crouzon and Pfeiffer syndromes, and *FGFR3* mutations causing achondroplasia and Muenke syndromes, and the relationship with parental age. Aided by the localized nature of these mutations, the most commonly used analytical approach has been to study trios of affected children and their unaffected, mutation-negative parents using allele-specific amplification of single nucleotide polymorphisms (SNPs) or other sequence variation lying close to the site of the mutation [121]. The results of these analyses have been unequivocal: in 79 cases of *FGFR2* mutations and 50 cases of *FGFR3* mutations that were informative for parental origin, all 129 mutations originated from the father [80,121–123]. The average paternal age at the birth of the affected child was found to be elevated by two to five years, when compared to the paternal age in the matching general population [80].

6. FGFR mutations in sperm

6.1. Estimates of mutation levels in sperm

The exclusive paternal origin of germline FGFR mutations has led three groups to develop methods to identify these mutations directly in the sperm. Tiemann-Boege et al. studied the *FGFR3*1138G > A achondroplasia mutation using a combination of primer-mismatch PCR amplification and restriction digest (to select mutant *FGFR3* sequences) with allele-specific quantitative amplification of the specific mutant sequence [13]. Goriely et al. studied the *FGFR2*755C > G Apert syndrome mutation using two rounds of restriction digest selection and PCR amplification, followed by Pyrosequencing [14]. Finally, Glaser et al. employed a one-step allele-specific amplification of both the 755C > G and 758C > G *FGFR2* Apert mutations, using a peptide nucleic acid matching the sequence of the wild type allele to mask non-specific amplification [15]. In the sperm of men who did not have a family history of FGFR mutation, these three studies [13–15] obtained strikingly different results both for absolute mutation levels and for variations with donor age. Goriely et al. found an increase in mutation prevalence that closely matched the observed paternal age effect [124] for the 755C > G Apert mutation (Fig. 2A), whereas the observed age relationship was much weaker for the other two studies (Fig. 2A and B) [13,15].

These discrepancies are more likely to be explained by methodological variation between the three studies [13–15] than by fundamental differences in the biology of the mutations under investigation. For example, the number of replicate measurements and amount of DNA used to estimate the mutation level in individual specimens varied widely (5–14 × 1 μg [13], 3 × 10 μg [14] and 7–16 × 0.15 μg [15], equivalent to a maximum of ∼4.0, 8.6 and 0.7 million haploid genomes respectively). The small unit of measurement used by Glaser et al. (0.15 μg, containing ∼43,000 haploid genomes) placed an absolute limit on their ability to detect very rare mutations in individual assays [15]; the larger amount of DNA employed in the other two studies [13,14] suggests that these latter results are likely to be more reliable. However, a limitation in the technique used by Tiemann-Boege et al. [13] was that the lowest mutation level that they could determine was about 1 in 26,000 (38.5 per million): any individual with a lower mutation level would be scored at this background level, obscuring the potential to identify a paternal age effect (from Table 3, the average mutation level for achondroplasia is expected to be ∼1 in 48,000). By contrast, Goriely et al. [14] stated that they could measure a mutation level of 10^{-5} with two-fold accuracy.

Considering the results shown in Fig. 2, I suggest the following interpretation: (1) As Goriely et al. [14] were able

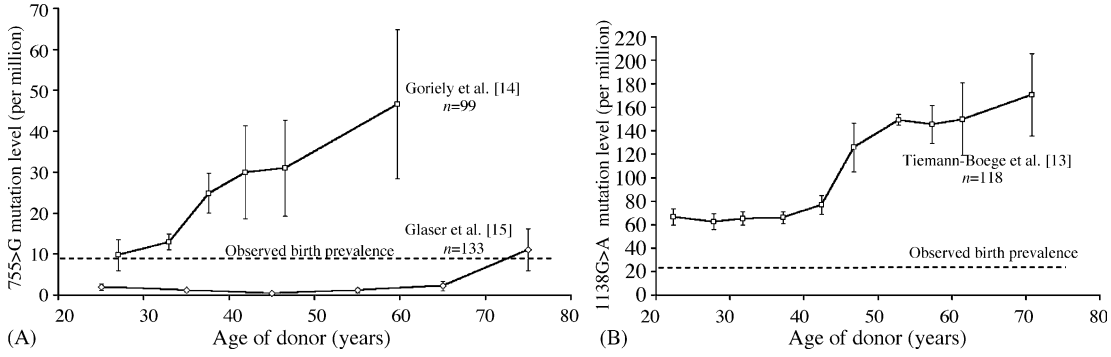

Fig. 2. Comparison of experimentally determined mutation levels in human sperm in relation to donor age. (A) 755C > G mutation in *FGFR2* [14,15]. (B) 1138G > A mutation in *FGFR3* [13]. The values shown are mean mutation level ± standard error of mean, calculated in 5-year age bins (note difference in the scale of mutation levels between A and B). The expected mutation prevalence, calculated by combining birth data (Table 3) and proportion of cases accounted for by the mutation (Table 2), is indicated by the dashed line. At the average paternal age in the population (∼30 years), only the measurements by Goriely et al. [14] correspond to the expected prevalence. Tiemann-Boege et al. [13] also considered a model of their data in which levels estimated at <52.5 per million were set to zero; this would adjust the mean mutation level for the youngest age group plotted in part B down to 49 per million (N. Arnheim, personal communication).

to measure mutation levels above 10^{-5} with two-fold accuracy, they would have estimated average mutation levels quite reliably even in the youngest age group (Fig. 2A). (2) The results of Tiemann-Boege et al. [13] are reliable for mutation levels above $\sim 8 \times 10^{-5}$ but levels below this have been consistently overestimated, accounting for the apparent plateau in mutation levels in the under 45 s (Fig. 2B). (3) The results of Glaser et al. [15] seem unreliable at all ages (Fig. 2A), owing to the small unit sample and perhaps to other methodological problems.

What accounts for the very high absolute levels of some FGFR mutations in sperm? In individuals heterozygous for a common G/A SNP located 118 bp away from the 755C > G *FGFR2* mutation, Goriely et al. [14] estimated the proportions of mutation present on the two alleles and related this to the absolute level of mutation. Surprisingly, most individuals showed a marked bias of mutations on one allele, although there was no preference in the group as a whole for whether this was the G or the A allele of the SNP. This pattern is difficult to reconcile with the prevailing model of multiple accumulating replication errors, which would be expected to homogenise the mutation ratio across the two alleles. Instead Goriely et al. [14] used various arguments to propose that clonal expansion of spermatogonial stem cells within the testis, driven by positive selection of rare FGFR2 mutations conferring gain-of-function to the mutant cell, explains both the high absolute levels attained by specific mutations and the paternal age effect, which they attribute to the cumulative effects of selection acting over the course of time. Of note, a different mutation at *FGFR2* position 755, the C > T transition encoding S252L (already mentioned in Section 2 and Table 3), was also unexpectedly found in sperm at high levels (which were, on average, only 1.66-fold lower than the 755C > G mutation) and showed a paternal age effect. Crucially however, the mutations exhibited much less skewing between the *FGFR2* alleles in heterozygotes for the G/A SNP. This result robustly demonstrated differential selection of the two mutations [14,125].

The model of selection proposed by Goriely et al. [14] accounts for various unusual aspects of human FGFR genetics, including the very high absolute levels of specific mutations, their exclusive paternal origin and the unexpectedly frequent occurrence of double mutations. Selection acting in the context of tumorigenesis is a widely accepted concept and interestingly, the spectrum of *FGFR2* and *FGFR3* mutations identified in human tumors [65,66] matches the distribution of germline mutations quite closely. The possibility of meiotic drive, which would not be mutually exclusive to the above mechanism, seems to be ruled out (at least in the case of the 758C > G *FGFR2* mutation), by the finding that a man with Apert syndrome and heterozygous for this germline mutation produced wild-type and mutant sperm at a ratio indistinguishable from 50:50 [126].

An unexpected observation is that in men over 45 years old, relatively modest rises in mutation prevalence with age are observed for both the 755C > G *FGFR2* mutation [14] and the 1138G > A *FGFR3* mutation [13] (Fig. 2). It was previously suggested, based on birth data, that mutation prevalence increased exponentially with age [124], but there were too few fathers aged over 50 years to validate the hypothesis by reference to this older age group. The sperm mutation data do not support this proposal, suggesting a much shallower rate of rise with age than expected for an exponential distribution. Clearly further data are required to model the age-related processes influencing sperm mutation prevalence.

6.2. Recurrence risk for de novo FGFR mutations

The three studies described above all reported FGFR mutation levels in the sperm of clinically unaffected men who had fathered children harboring the corresponding de novo mutation ($n = 4$, 6 and 15 in refs [13,14,15], respectively). All concur that the mutation levels in such men are relatively low (well under 1 in 10^3). Although the numbers investigated are still fairly small, this suggests that most fathers of children with achondroplasia and Apert syndrome have sperm mutation levels within the normal range and are not true biological outliers. Obviously, the average mutation level in these fathers is still expected to be skewed upwards compared to same-aged men as a whole, because a man producing mutations at a level of 10^{-4} is ten times as likely to father an affected child as a man producing mutations at a level of 10^{-5}.

In conclusion, it seems that FGFR disorders are conditions par excellence where an "apparently sporadic mutation" really is just that, and the healthy parents can be counselled with a low recurrence risk (in practice, <1%). This is supported by empiric data in the case of achondroplasia [127]. A cautionary note is, however, provided by a few cases of recurrence in siblings with Apert syndrome or achondroplasia born to unaffected parents [128,129], as well as by molecularly proven instances of somatic mosaicism for an *FGFR2* mutation [130] and, in two cases (both female), combined somatic and germline (gonosomal) mosaicism for an *FGFR3* mutation [131,132].

7. FGFR diagnostics and genetic counselling

The description of FGFR mutations in bone dysplasias and craniosynostosis has obvious applications in genetic diagnostics; GeneTests (www.genetests.org) lists 13 and 23 laboratories respectively that offer mutation testing for craniosynostosis syndromes (*FGFR2*) and achondroplasia (*FGFR3*). Molecular genetic testing is frequently necessary to establish the correct diagnosis. It is essential for the recognition of Muenke syndrome, for which heterozygosity for the 749C > G (P250R) mutation in *FGFR3* is pathognomonic (Table 2). Owing to its variable, sometimes mild and non-specific features, Muenke syndrome cannot be

confidently diagnosed clinically, yet it is the single most common cause of coronal craniosynostosis [8,9,78,133–135]. Other examples of diagnostic refinement (Table 2) include the recognition that mild and severe forms of Pfeiffer syndrome are caused by FGFR1 and FGFR2 mutations respectively [136], and that a subtype of Crouzon syndrome characterized by the development of acanthosis nigricans, a hyperkeratotic skin disorder, and a high prevalence of choanal atresia and hydrocephalus, is caused by a specific A391E mutation in FGFR3 [137]. Mutation identification may be of value prognostically. For example, apparently non-syndromic coronal craniosynostosis is associated with a worse cosmetic outcome and higher re-operation rate in patients heterozygous for the P250R mutation in FGFR3, than in mutation-negative patients [138–140].

The implementation of FGFR testing in the prenatal diagnostic setting has most commonly been applied to the confirmation of diagnoses suspected on ultrasound scanning in low-risk pregnancies at mid-trimester (from around 18–20 weeks gestation), usually in the context of TD and Apert syndromes [87,89,141–146]. Slightly milder disorders, such as achondroplasia and Pfeiffer syndromes, usually do not become apparent on ultrasound until somewhat later in pregnancy (20–30 weeks): implementation of prenatal genetic diagnosis will depend on national factors relating to the organisation of prenatal services, ethical attitudes, and legal regulations on termination of pregnancy. Clearly such molecular diagnoses are undertaken [87,99,147] and may be very valuable for resolving diagnostic uncertainty [148]; there is considerable under-reporting of such cases. Nevertheless, a majority of affected babies remain undetected until birth or later infancy.

Earlier (from 11–12 weeks gestation) genetic prenatal diagnosis may also be undertaken for an affected parent who is at 50% risk of having an affected child: this has been undertaken for Crouzon syndrome [149], Apert syndrome [99,150], achondroplasia [87,151] and Pfeiffer syndrome caused by the P252R mutation in FGFR1 (author's unpublished data). When the affected parent's phenotype is relatively mild, uptake of prenatal diagnosis is likely to be low, both because of the parent's own perception of the impact of the disease and because phenotypic variability is a significant and unpredictable factor for some of the more common familial mutations such as A344A in FGFR2 [152] and P250R in FGFR3 [9,78,134,135].

For couples at 50% risk of having an affected child, an alternative to prenatal diagnosis is preimplantation diagnosis. An unaffected child was born after successful preimplantation diagnosis of a Crouzon *FGFR2* mutation present in the mother [153]. Attempted preimplantation diagnosis has been reported for three couples at 50% risk of achondroplasia; no liveborn children resulted following four embryo transfers from six attempted cycles [151]. Interestingly, these couples (originating from France) had previously had six terminations of fetuses affected with heterozygous achondroplasia, an experience in marked contrast to the results of a USA-based survey of attitudes to the termination of such pregnancies, which were approved by only 5% of affected individuals or their relatives [154]. In one anecdotal case a couple both affected with achondroplasia, requested preimplantation diagnosis and wished to ensure that their child was also *affected*. This provides cogent illustration of the unexpected ethical difficulties that can arise from the availability of new medical technologies [155].

To date it is clear that knowledge of the molecular bases of FGFR-related disorders has had little impact on their prevalence in the childhood population. It is interesting to speculate whether more widespread prenatal genetic screening of low risk pregnancies for FGFR mutations might be attempted in the near future. The detection of an achondroplasia mutation in maternal plasma has been described at 30 weeks gestation following suspicious ultrasound findings [156]. Given that trisomy 21, which increases steeply in prevalence with maternal age, is currently the primary focus of genetic screening programmes, we can anticipate that supplementary testing of some (paternal age-related) FGFR mutations may become available as assay methods get cheaper, despite the low absolute risks. Balancing this, it should be remembered that the complications of FGFR-related disorders can often be ameliorated with surgery, and that intelligence is normal in many cases.

8. Unsolved questions in FGFR disorders

We have come a long way over the past decade in the study of human phenotypes associated with FGFR disorders. Quite apart from the clinical dividends, this approach has uncovered many fascinating aspects of FGFR biology that would have been very difficult to access by any other means. This review has attempted to illustrate why the human genetics of the FGF receptor mutations is justifiably a paradigm for the application of a phenotype-driven approach to biological investigation.

However, many unsolved questions remain: here are just a few. Are there entirely novel phenotypes waiting to be discovered, that will take us to further pastures of developmental biology? Why have no mutations been found in FGFR4? What is the pathophysiology of the brain, skin and limb malformations associated with FGFR mutations, and what roles do FGFRs play in spermatogenesis? There are many aspects of genotype-phenotype correlation that remain incompletely understood, for example the predominance of IgIII mutations in FGFR2 contrasted with the transmembrane mutations in FGFR3, how equivalent mutations in FGFR1 and FGFR2 have apparently different effects on protein function, and why IgII-III linker mutations (except those to cysteine) are all associated specifically with craniosynostosis. Given the critical roles of FGF receptors in many aspects of early development, perhaps the greatest surprises are that these

gain-of-function mutations in general seem neither lethal in embryogenesis nor associated with a greatly increased risk of cancer in later life. This highlights our ignorance about the precise roles that processes such as limited availability of specific FGF ligands, receptor heterodimerization and downregulation play in different tissues at different times to shape the eventual phenotype.

Finally, the work on FGFR mutations in sperm highlights a novel biological form of selfishness, distinct from previous definitions at the level of the gene [157] or DNA [158]: rather, it opens up the new realm of the selfish testis. The reproductive fitness of FGFR disorders is low, so that fresh mutations will generally be eliminated within a few generations. But this need not inevitably be the case: in other situations, the balance of positive selection in the testis and negative selection in the organism might tip the other way. We are just at the beginning of understanding the biological implications of these findings, which should channel further efforts into the direct study of human sperm and exploration of mutational processes in the testis.

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