

ARTICLE

Novel fibroblast growth factor receptor 3 (*FGFR3*) mutations in bladder cancer previously identified in non-lethal skeletal disorders

Bas WG van Rhijn^{1,2}, Angela AG van Tilborg¹, Irene Lurkin¹, Jacky Bonaventure³, Annie de Vries¹, Jean-Paul Thiery⁴, Theodorus H van der Kwast¹, Ellen C Zwarthoff^{*1} and Francois Radvanyi⁴

¹Department of Pathology, Josephine Nefkens Institute, Erasmus University, 3000 DR Rotterdam, The Netherlands; ²Department of Urology, Erasmus University and University Hospital, 3000 CA Rotterdam, The Netherlands; ³Unité de Recherches sur les Handicaps Génétiques de l'Enfant, INSERM U 393, Hôpital Necker, 75743 Paris Cedex 15, France; ⁴Laboratoire de Morphogenèse Cellulaire et Progression Tumorale, UMR 144, CNRS, Institut Curie, 75248 Paris Cedex 05, France

Activating mutations in the fibroblast growth factor receptor 3 (*FGFR3*) gene are responsible for several autosomal dominant craniosynostosis syndromes and chondrodysplasias i.e. hypochondroplasia, achondroplasia, SADDAN and thanatophoric dysplasia – a neonatal lethal dwarfism syndrome. Recently, activating *FGFR3* mutations have also been found to be present in cancer, i.e. at high frequency in carcinoma of the bladder and rarely in multiple myeloma and carcinoma of the cervix. Almost all reported mutations in carcinomas corresponded to the mutations identified in thanatophoric dysplasia. We here screened a series of 297 bladder tumours and found three *FGFR3* somatic mutations (G380/382R; K650/652M and K650/652T) that were not previously identified in carcinomas or thanatophoric dysplasia. Another novel finding was the occurrence of two simultaneous *FGFR3* mutations in four tumours. Two of the three new mutations in bladder cancer, the G380/382R and the K650/652M mutations, were previously reported in achondroplasia and SADDAN, respectively. These syndromes entail a longer life span than thanatophoric dysplasia. The K650/652T mutation has not previously been detected in patients with skeletal disorders, but affects a codon that has been shown to be affected in some cases of thanatophoric dysplasia, SADDAN and hypochondroplasia. From a clinical perspective, the patients with *FGFR3*-related, non-lethal skeletal disorders might be at a higher risk for development of bladder tumours than the general population.

European Journal of Human Genetics (2002) 10, 819–824. doi:10.1038/sj.ejhg.5200883

Keywords: fibroblast growth factor receptor 3 (*FGFR3*); bladder; cancer; achondroplasia; hypochondroplasia; SADDAN; mutation

Introduction

The fibroblast growth factor receptor 3 (*FGFR3*) belongs to a family of structurally related tyrosine kinase receptors

encoded by four different genes (*FGFR1–4*). These receptors consist of three glycosylated extracellular immunoglobulin-like domains (Ig-like), a transmembrane domain and a split intracellular tyrosine-kinase domain. Ligand binding induces FGFR dimerisation, resulting in autophosphorylation of the kinase domain and interaction with and phosphorylation of effector signalling proteins.^{1,2} Alternative mRNA splicing mechanisms generate many different receptor isoforms, which differ in ligand specificity. The

*Correspondence: EC Zwarthoff, Department of Pathology, Josephine Nefkens Institute, Erasmus University, PO Box 1738, 3000 DR Rotterdam, The Netherlands; Tel: +31-104087929; Fax: +31-104089487; E-mail: zwarthoff@path.fgg.eur.nl

Received 22 January 2002; revised 9 August 2002; accepted 13 August 2002

isoforms *FGFR3b* and *FGFR3c* result from a mutually exclusive splicing event, in which the second half of the third Ig-like domain is encoded by either the 151 nucleotides of exon 8 or the 145 nucleotides of exon 9.³ These two isoforms have different tissue distributions: for example, *FGFR3b* is the main form in epithelial cells whereas *FGFR3c* is the predominant form in chondrocytes.^{3–5}

Point mutations in specific domains of *FGFR3* are associated with autosomal dominant dwarfism and craniosynostosis syndromes such as hypochondroplasia, achondroplasia (the most common form of skeletal dysplasia), severe achondroplasia with developmental delay and acanthosis nigricans (SADDAN), thanatophoric dysplasia (a lethal form of dwarfism), Crouzon syndrome with acanthosis nigricans and Muenke coronal craniosynostosis.^{3,6,7} Several reports have demonstrated that these mutations lead to constitutive activation of the receptor.^{8–11}

In contrast with the inhibitory role on bone growth, an oncogenic role for *FGFR3* in human cancer has emerged. Indeed, somatic activating mutations in *FGFR3* have been reported in multiple myeloma and, more recently, in two epithelial malignancies, i.e. bladder- and cervix carcinomas.^{5,12,13} *FGFR3* mutations are rare in multiple myeloma and cervix carcinomas,^{14,15} whereas their high incidence in bladder carcinomas (74% of non-invasive papillary tumours) suggests that the constitutive activation of *FGFR3* is an important event for bladder tumorigenesis.^{16,17} Nearly all mutations identified in bladder tumours are identical to the activating mutations responsible for thanatophoric dysplasia, a lethal form of dwarfism.^{5,16–18} Only two of the 117 *FGFR3* mutations identified by these groups, the A393E and K652Q mutations, do not correspond to thanatophoric dysplasia mutations.^{17,18} The A393E mutation is identical to a mutation associated with a craniosynostosis syndrome (Crouzon syndrome with acanthosis nigricans) and the K652Q mutation is identical to a mutation associated with hypochondroplasia.^{11,19}

In this report, we describe three new somatic *FGFR3* mutations in bladder tumours (G380/382R, K650/652M and K650/652T) (*FGFR3c* isoform numbering/*FGFR3b* isoform numbering). The G380/382R and the K650/652M mutations have previously been reported in achondroplasia and SADDAN, respectively. The K650/652T mutation has not been reported before in a skeletal disorder. In addition, among the 177 mutated tumours, we observed the occurrence of two concurrent *FGFR3* mutations in four cases.

Materials and methods

FGFR3 mutation analysis was performed in a series of 297 bladder tumours as described.¹⁶ T7 Sequenase v2.0 (Amersham life Science, Inc., Cleveland, OH, USA) was used for sequencing to analyse the four regions of *FGFR3*, located in exons 7, 10, 15 and 19, known to harbour the point mutations previously described in multiple myeloma, bladder- and cervix carcinomas, thanatophoric dysplasia and

SADDAN. None of the 297 patients had a hereditary skeletal disorder documented. The mean patient's age at diagnosis was 65.9 years (range, 23–90). Seventy (24%) of the 297 patients were female. The three new mutations of *FGFR3* and all the 'double' mutations were confirmed on a second PCR product.

Results

In this series of 297 bladder carcinomas, we detected 181 *FGFR3* mutations. Consistent with previous studies,^{5,16–18} most of the mutations (173 out of 181, 96%), were identical to the germinal activating mutations responsible for thanatophoric dysplasia, with the S249C mutation, found in 125 (69%) of the 181 mutations as the most frequent point mutation. The A391/393E mutation, identical to the germinal mutation that causes Crouzon syndrome with acanthosis nigricans and already identified in bladder cancer,¹⁷ was found in four tumours.

We detected three mutations in this series of 297 bladder carcinomas, which were not previously identified in carcinomas or in thanatophoric dysplasia patients (Figure 1). These mutations affected the codons 380/382 and 650/652 (*FGFR3c* isoform numbering/*FGFR3b* isoform numbering). The G380/382R mutation affected the transmembrane domain and resulted in the replacement of a glycine by an arginine residue. This mutation is identical to a germinal activating mutation associated with the most common form of genetic dwarfism, i.e. achondroplasia.^{20–22} The other two mutations (K650/652M and K650/652T), affecting the tyrosine kinase domain, resulted in the replacement of a lysine by a methionine or a threonine. The K650/652M mutation is identical to the germinal activating mutation that causes SADDAN.²³ This novel mutation was found twice in this series of tumours. The matched constitutional DNA contained the wild-type sequence in every case, demonstrating the somatic nature of these mutations in (bladder) cancer. The tumour with the G380/382R mutation also displayed a S249C mutation in *FGFR3*. This patient suffered a recurrence 5 years later and the recurrent tumour was found to contain the same two *FGFR3* mutations.

In addition to the above-mentioned case, three other bladder tumours also contained two distinct *FGFR3* mutations. In each case, the S249C mutation was present. S249C was accompanied by the R248C, G370/372C and A391/393E mutations. The clinico-pathological data of the bladder-carcinoma patients carrying the new and the 'double' mutations are depicted together with the type of *FGFR3* mutations in Table 1. We found no significant differences in the pathological status or the clinical data for the various *FGFR3* mutations in bladder cancer (not shown).

Figure 2 indicates the locations and the nature of the missense mutations associated with skeletal disorders and bladder carcinomas. The frequencies of the various *FGFR3* mutations in bladder cancer are also indicated. These

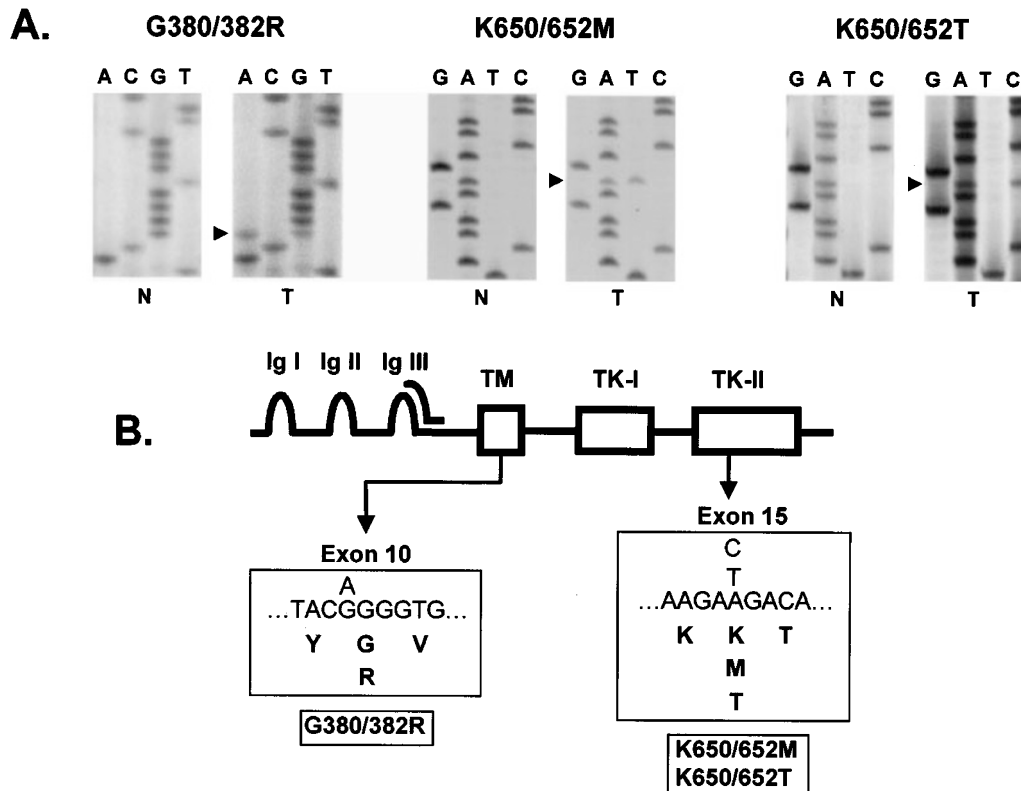


Figure 1 Identification of novel *FGFR3* mutations in bladder cancer. (A) Novel somatic *FGFR3* mutations were identified by PCR–SSCP followed by direct DNA sequencing. The tumour shown on the left side has a G to A transition in exon 10, which changes the sequence of codon 380/382 from GGG (Gly) to AGG (Arg). The tumour in the middle has an A to T transversion in exon 15, which changes the sequence of codon 650/652 from AAG (Lys) to ATG (Met). The tumour on the right side has an A to C transversion in exon 15, which alters the sequence of codon 650/652 from AAG (Lys) to ACG (Thr). Arrowheads indicate the positions of the mutations. The *FGFR3b* isoform, which is expressed in epithelial cells, contains two amino acids more than the *FGFR3c* isoform expressed in bone. Therefore, the G380R, K650M and K650T mutations in *FGFR3c* are equivalent to the G382R, K652M and K652T mutations in *FGFR3b*. Note in addition: while the present study was under evaluation, the K650/652M mutation in bladder cancer was reported by Kimura *et al.*²⁶ N, sequence of normal DNA; T, sequence of tumour DNA. (B) Schematic diagram of the *FGFR3* protein. The areas within the two exons, in which the new missense mutations occurred, are shown in greater detail. The nucleotide substitutions are shown above, the amino acid substitutions are shown at the bottom of the figure.

Table 1 The patient characteristics and the histopathological data corresponding to the novel and the concurrent *FGFR3* mutations in bladder carcinomas. The cases with the numbers 143, 172, 287 and 265 contained two concurrent *FGFR3* mutations in one tumor. The cases 287, 213, 17 and 55 presented with the novel mutations reported here for the first time

Case #	Age/sex	Stage/grade*	Exon	nt Position**	mutation	Predicted effect**
143	77/M	pTa/G2	7	nt742	C→T	R248C
			7	nt746	C→G	S249C
172	65/M	pT3/G3	7	nt746	C→G	S249C
287	68/M	pTa/G1	10	nt1114	G→T	G372C
			7	nt746	C→G	S249C
			10	nt1144	G→A	G382R
265	74/M	pTa/G2	7	nt746	C→G	S249C
			10	nt1178	C→A	A393E
213	74/M	pTa/G2	15	nt1955	A→T	K652M
17	66/M	pTa/G1	15	nt1955	A→T	K652M
55	63/M	pTa/G1	15	nt1955	A→C	K652T

*Pathology according to the TNM97 and the WHO classification guidelines. **The nucleotide (nt) and codon numbering refer to the *FGFR3b* isoform.

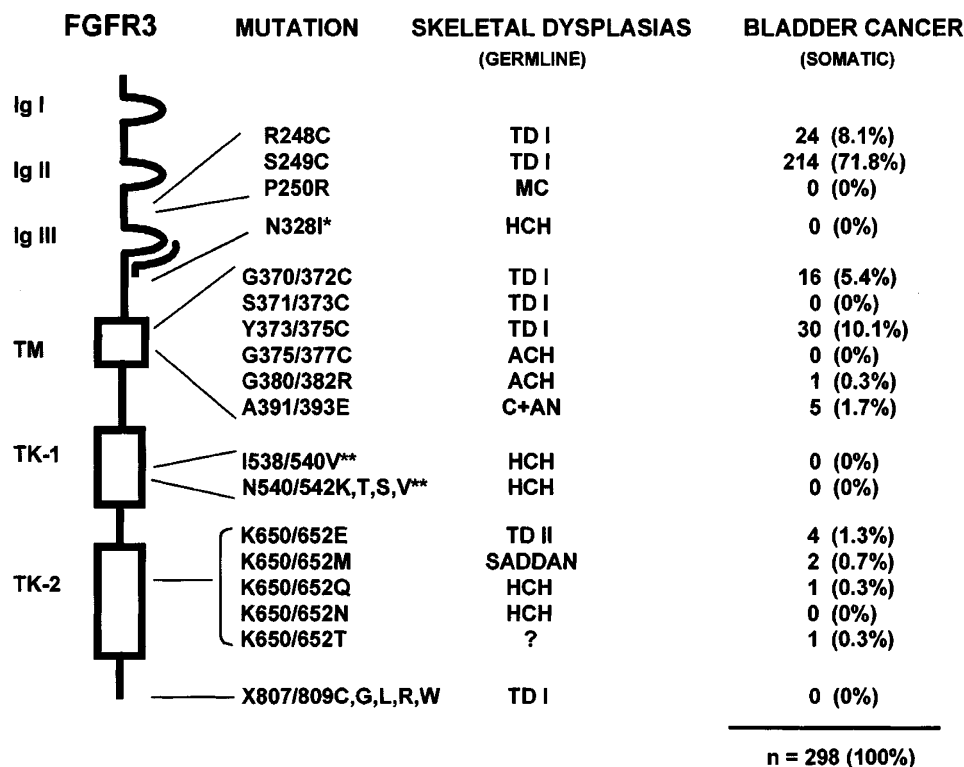


Figure 2 *FGFR3* mutations associated with skeletal disorders and bladder carcinomas. A schematic diagram of the structure of *FGFR3* is shown (Ig I–III, immunoglobulin-like domains; TM, transmembrane domain; TK-1 and -2, tyrosine kinase domains). The locations of the missense mutations associated with skeletal dysplasias (TDI, thanatophoric dysplasia type I; TDII, thanatophoric dysplasia type II; MC, Muenke craniosynostosis; HCH, hypochondroplasia; ACH, achondroplasia; C+AN, Crouzon syndrome with acanthosis nigricans; SADDAN, severe achondroplasia with developmental defect and acanthosis nigricans) and bladder carcinomas are indicated. The positions are numbered according to the *FGFR3c/FGFR3b* numbering. The number of occurrences and the percentage of each *FGFR3* mutation in mutated bladder tumours are given. These data were obtained from 570 bladder tumours of which 273 were reported before^{5,16–18} and 297 were reported here. The different mutations found in skeletal dysplasias have been obtained from the review of Passos-Bueno *et al*⁶ and the work of Bellus *et al*¹¹ and Winterpacht *et al*.²⁷ *This region of *FGFR3b* corresponding to a region of *FGFR3c* where a mutation, associated with hypochondroplasia (N328I) was previously found was only examined in 26 of the 570 bladder tumours studied so far.⁵ **This region containing mutations associated with hypochondroplasia was only examined in 89 of the 570 bladder tumours studied so far.^{5,18}

numbers are based on the previously published series by Cappellen *et al*⁵, Billerey *et al*¹⁶, van Rhijn *et al*¹⁷ and Sibley *et al*¹⁸ (*n*=273 patients) and the series described in this paper (*n*=297 patients).

Discussion

FGFR3 mutations occur frequently in bladder carcinoma.^{5,16–18} The vast majority of somatic *FGFR3* mutations identified in bladder cancer are identical to those found in thanatophoric dysplasia.^{7,9} In the previous published series, only two *FGFR3* mutations (A391/393E and K650/652Q) did not correspond to thanatophoric dysplasia mutations.^{17,18} However, both mutations have been found to be associated with milder types of skeletal dysplasia: the A391/393E mutation with the Crouzon syndrome with acanthosis nigricans and the K650/652Q mutation with hypochondroplasia. In this new series of 297 bladder carcinomas

reported here, we identified 181 *FGFR3* mutations. Ninety-six per cent (173 out of 181) of these somatic mutations have been previously reported to be associated with thanatophoric dysplasia. However, other activating *FGFR3* mutations including the three described here for the first time in bladder cancer (G380/382R, K650/652M and K650/652T), have never been reported to be associated with thanatophoric dysplasia.

The activating G380/382R mutation, which affects the transmembrane domain, is responsible for almost all (~97%) cases of achondroplasia.^{20,22} Achondroplasia is the most common form of non-lethal skeletal dysplasia, affecting approximately one in 15 000 to one in 40 000 live births.⁷ This mutation is here reported in cancer for the first time.

The activating K650/652M mutation, located in the kinase domain, has already been found in SADDAN (severe

achondroplasia with developmental delay and acanthosis nigricans)²³ and in a primary multiple myeloma.¹² Transient transfection studies have demonstrated that the K650/652M mutation results in stronger constitutive activation of *FGFR3* than does the K650/652E mutation responsible for thanatophoric dysplasia.²³ Nevertheless, despite multiple physical and neurological impairments, most SADDAN patients survive past infancy without the need for prolonged ventilator support.^{7,23}

The K650/652T mutation has never been reported before in either skeletal dysplasia or cancer. However, other mutations of the same codon are associated with either thanatophoric dysplasia (K650/652E), SADDAN (K650/652M) or hypochondroplasia (K650/652N and K650/652Q).^{10,11,23} Bellus *et al*¹¹ investigated all the possible amino acid substitutions resulting from single nucleotide changes in the 650/652 codon and showed that the K650/652T mutation leads to constitutive activation of the *FGFR3* tyrosine kinase. This activation was equivalent to that observed with the K650/652N and K650/652Q (hypochondroplasia) mutations but was considerably weaker than observed with the K650/652E and K650/652M mutations associated with thanatophoric dysplasia and SADDAN, respectively.

The identification of mutations in bladder cancers identical (K650/652Q)¹⁸ or similar (K650/652T) (this study) to mutations found in hypochondroplasia suggests that other hypochondroplasia mutations, such as the I538/540V and N540/542K,T,S,V mutations in exon 13 may also occur in this carcinoma.^{6,24} In other words, possible hypochondroplasia mutations in other exons than 15 may have escaped their detection in bladder cancer. Among the 570 bladder tumours studied so far, only 89 have been studied for the hypochondroplasia mutations located in exon 13.^{5,18} No activating mutations were detected in this exon for the 89 cases. Nevertheless, the percentage of mutations not corresponding to thanatophoric dysplasia in bladder cancer might be slightly higher than suggested.

Another novel finding presented here was the occurrence of two simultaneous *FGFR3* mutations in four tumours (case-numbers: 143, 172, 265 and 287, Table 1). The PCR-SSCP analysis of exon 7 in the case with number 404 provided a strong indication that the two concurrent mutations (R248C and S249C), are carried by the two different alleles (not shown). For the remaining three cases, this remains to be determined because these tumours had no loss of heterozygosity at the *FGFR3* locus (not shown) and RNA was not available.

From a clinical perspective, the presence of *FGFR3* mutations in bladder carcinomas, identical to the mutations found in patients with non-lethal skeletal disorders (hypochondroplasia, achondroplasia, SADDAN and Crouzon syndrome) suggests that the patients with these *FGFR3*-related syndromes might have a higher risk to develop bladder cancer than the general population. However, to our

knowledge, there are no reports on a higher incidence of cancer in these patients, so it is possible that the non-lethal *FGFR3* mutations do not sufficiently activate the protein to an extent that is needed for tumour formation or other genes must be affected for bladder tumour formation. On the other hand, the predisposition may have gone unnoticed to clinicians, especially if we consider the relative low prevalence of non-lethal skeletal syndromes (~10000 cases in the USA). For example, the retinoblastoma gene, cloned 18 years ago, has been demonstrated to play a role in many carcinomas, including bladder carcinomas. Yet, it was only recently proven that hereditary retinoblastoma patients have a higher risk to develop lung cancer than unaffected individuals.²⁵

Acknowledgements

This work was supported by the University Hospital Rotterdam as part of a top-down revolving fund project (FED 0930) and by grants from the Maurits and Anna de Kock foundations, the Comité de Paris Ligue Nationale Contre le Cancer (UMR 144, associated laboratory), the CNRS and the Institut Curie. The medical-ethical committee of the Erasmus University and the University Hospital Rotterdam approved the study (MEC 168.922/1998/55).

References

- 1 Johnson DE, Williams LT: Structural and functional diversity in the FGF receptor multigene family. *Adv Cancer Res* 1993; **60**: 1–41.
- 2 Robertson SC, Tynan JA, Donoghue DJ: RTK mutations and human syndromes: when good receptors turn bad. *Trends Genet* 2000; **16**: 265–271.
- 3 Murgue M, Tsunekawa S, Rosenberg I, deBeaumont M, Podolsky DK: Identification of a novel variant of fibroblast growth factor receptor 3 (*FGFR3* IIIb) in human colonic epithelium. *Cancer Res* 1994; **54**: 5206–5211.
- 4 Delezoide AL, Benoist-Lassel C, Legeai-Mallet L *et al*: Spatio-temporal expression of *FGFR* 1, 2 and 3 genes during human embryo-fetal ossification. *Mech Dev* 1998; **77**: 19–30.
- 5 Cappellen D, De Oliveira C, Ricol D *et al*: Frequent activating mutations of *FGFR3* in human bladder and cervix carcinomas. *Nat Genet* 1999; **23**: 18–20.
- 6 Passos-Bueno MR, Wilcox WR, Jabs EW, Sertié AL, Alonso LG, Kitoh H: Clinical spectrum of fibroblast growth factor receptor mutations. *Hum Mutat* 1999; **14**: 115–125.
- 7 Vajo Z, Francomano CA, Wilkin DJ: The molecular and genetic basis of fibroblast growth factor receptor 3 disorders: The achondroplasia family of skeletal dysplasias, Muenke craniosynostosis, and Crouzon syndrome with acanthosis nigricans. *Endocr Rev* 2000; **21**: 23–39.
- 8 Naski MC, Wang Q, Xu J, Ornitz DM: Graded activation of fibroblast growth receptor 3 by mutations causing achondroplasia and thanatophoric dysplasia. *Nat Genet* 1996; **13**: 233–237.
- 9 Webster MK, Donoghue DJ: FGFR activation in skeletal disorders: too much of a good thing. *Trends Genet* 1997; **13**: 178–182.
- 10 Tavormina PL, Shiang R, Thompson LM *et al*: Thanatophoric dysplasia (types I and II) caused by distinct mutations in fibroblast growth factor receptor 3. *Nat Genet* 1995; **9**: 321–328.
- 11 Bellus GA, Spector EB, Speiser PW *et al*: Distinct missense mutations of the *FGFR3* Lys650 codon modulate receptor kinase activation and the severity of the skeletal dysplasia phenotype. *Am J Hum Genet* 2000; **67**: 1411–1421.

- 12 Chesi M, Nardini E, Brents LA *et al*: Frequent translocation t(4;14)(p16.3;q32.3) in multiple myeloma is associated with increased expression and activating mutations of fibroblast growth factor receptor 3. *Nat Genet* 1997; **16**: 260–264.
- 13 Richelda R, Ronchetti D, Baldini L *et al*: A novel translocation t(4;14)(p16.3;q32.3) in multiple myeloma involves the fibroblast growth-factor receptor 3 gene. *Blood* 1997; **90**: 4062–4070.
- 14 Fracchiolla NS, Luminari S, Baldini L, Lombardi L, Maiolo AT, Neri A: *FGFR3* gene mutations associated with human skeletal disorders occur rarely in multiple myeloma. *Blood* 1998; **92**: 2987–2989.
- 15 Wu R, Connolly D, Ngelangel C, Bosch FX, Muñoz N, Cho KR: Somatic mutations of fibroblast growth factor receptor 3 (*FGFR3*) are uncommon in carcinomas of the uterine cervix. *Oncogene* 2000; **19**: 5543–5546.
- 16 Billerey C, Chopin D, Aubriot-Lorton M-H *et al*: Frequent *FGFR3* mutations in papillary non-invasive bladder (pTa) tumors. *Am J Pathol* 2001; **158**: 1955–1959.
- 17 Van Rhijn BWG, Lurkin I, Radvanyi F, Kirkels WJ, Van der Kwast ThH, Zwarthoff EC: The fibroblast growth factor receptor 3 (*FGFR3*) mutation is a strong indicator of superficial bladder cancer with low recurrence rate. *Cancer Res* 2001; **61**: 1265–1268.
- 18 Sibley K, Cuthbert-Heavens D, Knowles MA: Loss of heterozygosity at 4p16.3 and mutation of *FGFR3* in transitional cell carcinoma. *Oncogene* 2001; **20**: 686–691.
- 19 Meyers GA, Orlow SJ, Munro IR, Przylepa KA, Jabs EW: Fibroblast growth factor receptor 3 (*FGFR3*) transmembrane mutation in Crouzon syndrome with acanthosis nigricans. *Nat Genet* 1995; **11**: 462–464.
- 20 Rousseau F, Bonaventure J, Legeai-Mallet L *et al*: Mutations in the gene encoding fibroblast growth factor receptor-3 in achondroplasia. *Nature* 1994; **371**: 252–254.
- 21 Shiang R, Thompson LM, Zhu YZ *et al*: Mutations in the transmembrane domain of *FGFR3* cause the most common genetic form of dwarfism, achondroplasia. *Cell* 1994; **787**: 335–342.
- 22 Bellus GA, Hefferon TW, Ortiz de Luna RI *et al*: Achondroplasia is defined by recurrent G380R mutations of *FGFR3*. *Am J Hum Genet* 1995; **56**: 368–373.
- 23 Tavormina PL, Bellus GA, Webster MK *et al*: A novel skeletal dysplasia with developmental delay and acanthosis nigricans is caused by a Lys650Met mutation in the fibroblast growth factor receptor 3 gene. *Am J Hum Genet* 1999; **64**: 722–731.
- 24 Bellus GA, McIntosh I, Smith EA *et al*: A recurrent mutation in the tyrosine kinase domain of fibroblast growth factor receptor 3 causes hypochondroplasia. *Nat Genet* 1995; **10**: 357–359.
- 25 Kleinerman RA, Tarone RE, Abramson DH, Seddon JM, Li FP, Tucker MA: Hereditary retinoblastoma and risk of lung cancer. *J Natl Cancer Inst* 2000; **92**: 2037–2039.
- 26 Kimura T, Suzuki H, Ohashi T, Asano K, Kiyota H, Eto Y: The incidence of thanatophoric dysplasia mutations in *FGFR3* gene is higher in low-grade or superficial bladder carcinomas. *Cancer* 2001; **92**: 2555–2561.
- 27 Winterpacht A, Hilbert K, Stelzer C *et al*: A novel mutation in *FGFR-3* disrupts a putative N-glycosylation site and results in hypochondroplasia. *Physiol Genomics* 2000; **2**: 9–12.