ELECTRONIC LETTER

Mitotic recombination as evidence of alternative pathogenesis of gastrointestinal stromal tumours in neurofibromatosis type 1

Douglas R Stewart, Christopher L Corless, Brian P Rubin, Michael C Heinrich, Ludwine M Messiaen, Lisa J Kessler, Paul J Zhang, David G Brooks

J Med Genet 2007;**44**:e61 (http://www.jmedgenet.com/cgi/content/full/44/1/e61). doi: 10.1136/jmg.2006.043075

Background: Neurofibromatosis type 1 (NF1) is a neurocutaneous disorder resulting in the growth of a variety of tumours, and is inherited in an autosomal dominant pattern. Gastrointestinal stromal tumours (GISTs) are mesenchymal tumours that commonly harbour oncogenic mutations in *KIT* or *PDGFRA* and are thought to arise from the interstitial cells of Cajal (ICC; the pacemaker cells of the gut).
Aim: To characterise two patients with NF1 and GISTs.
Methods: Two patients were genotyped for germline mutations in *NF1*. GISTs from both patients were genotyped for somatic mutations in *KIT* and *PDGFRA*. Loss of heterozygosity (LOH) of *NF1* in one GIST was assessed by genotyping seven microsatellite markers spanning 2.39 Mb of the *NF1* locus in the tumour and in genomic DNA. The known germline mutation in *NF1* was confirmed in GIST DNA by sequencing. The copy number of the mutated *NF1* allele was determined by multiplex ligand-dependent probe amplification.
Results: GISTs from both patients were of wild type for mutations in *KIT* and *PDGFRA*. In the GIST with adequate DNA, all seven markers were informative and showed LOH at the *NF1* locus; sequencing of *NF1* from that GIST showed no wild-type sequence, suggesting that it was lost in the tumour. Multiplex ligand-dependent probe amplification analysis showed that two copies of all *NF1* exons were present.
Conclusions: This is the first evidence of mitotic recombination resulting in a reduction to homozygosity of a germline *NF1* mutation in an NF1-associated GIST. We hypothesise that the LOH of *NF1* and lack of *KIT* and *PDGFRA* mutations are evidence of an alternative pathogenesis in NF1-associated GISTs.

Neurofibromatosis type 1 (NF1) is a common neurocutaneous disorder secondary to mutations in the tumour suppressor gene *NF1*, and is inherited in an autosomal dominant pattern.[1] A variety of benign and malignant gastrointestinal tumours have been reported in *NF1*, including gastrointestinal stromal tumours (GISTs).[2 3] The GIST is the most common mesenchymal tumour of the gastrointestinal tract in the general population but is still rare,[4] with an estimated incidence of 10–20 cases/million.[5] The incidence of GIST in NF1 is unknown. Until 1998, a diagnosis of GIST subsumed a heterogeneous collection of tumours (in both the general and NF1 population), including true GISTs, peripheral nerve sheath tumours, true smooth muscle tumours and others.[3 6]

In 1998, Hirota *et al*[7] reported that *KIT* expression in sporadic GISTs was accompanied by mutations in the proto-oncogene receptor tyrosine kinase *KIT*. This hallmark expression of *KIT* (CD117, a transmembrane receptor for the growth factor stem cell factor), combined with molecular testing of mutations in *KIT*[8] and *PDGFRA*,[9] has greatly clarified the diagnosis and nosology of GISTs.[10] It is hypothesised that GISTs arise from either the interstitial cells of Cajal or a precursor (henceforth, both possibilities abbreviated ''ICC''), which are autonomic nerve-related gastrointestinal pacemaker cells that regulate gastrointestinal motility.[6] Although most sporadic GISTs are benign (low risk), malignant (high risk) GISTs are typically unresponsive to conventional cancer chemotherapy. The tyrosine kinase inhibitor imatinib mesylate (Gleevec, Novartis, East Hanover, New Jersey, USA) has produced dramatic clinical results in patients with widely metastatic chemotherapy-resistant sporadic GISTs.[11 12]

Since 1998, true GISTs (tumours with positive staining for *KIT*) have been reported in NF1[13]; most lack *KIT* or *PDGFRA* mutations. In this paper, we report two cases of *KIT* and *PDGFRA* mutation-negative GISTs in patients with NF1. In one patient, we document the first evidence of loss of heterozygosity (LOH) of *NF1* by mitotic recombination. The LOH of *NF1* in GISTs in NF1 has recently been reported.[14] The LOH by mitotic recombination is common in neurofibromas[15]; it has not been reported in other tissues in NF1. We consider the implications of *NF1* haploinsufficiency and the subsequent LOH in the ICC and propose an alternative pathogenesis for GISTs in NF1.

CASE REPORTS

Patient 1

Patient 1 presented at 34 years of age with a history of progressive left leg pain and weakness unresponsive to conservative therapy. NF1 had been diagnosed when he was a child. No other family members had a history of NF1 or GISTs. He denied any gastrointestinal symptoms.

In 1995, at age 27 years, he presented with acute peritonitis. At surgery he was found to have a ruptured cyst in the mid-jejunum. The pathology report diagnosed a spindle cell schwannoma of the mid-gut.

Magnetic resonance imaging of his leg showed a large, heterogeneous solid mass in the presacral space, originating from the left S1 nerve root. At surgery, a 20×15×11 cm mass was resected from the pelvis and identified as a high-grade malignant peripheral nerve sheath tumour (MPNST). Frequent mitotic figures (>15/hpf) and extensive tumour necrosis were observed. Immunohistochemical staining with adequate controls showed focally positive S-100 and CD34 staining, but negative KIT and smooth muscle actin (SMA) staining. Multiple, small adjacent masses were also noted studding the peritoneum (all <3 cm). They were not direct extensions of the major pelvic tumour. They exhibited a typical

Abbreviations: GIST, gastrointestinal stromal tumour; ICC, interstitial cells of Cajal; LOH, loss of heterozygosity; MLPA, multiplex ligand-dependent probe amplification; MPNST, malignant peripheral nerve sheath tumour; NF1, neurofibromatosis type 1; SMA, smooth muscle actin

immunohistostaining pattern for GISTs, with strong, diffuse positive staining for KIT, focally positive for CD34 (seen in 70% of GISTs)[10] and negative for S-100. Few mitotic figures were seen.

Re-examination of the ruptured jejunal spindle cell cystic tumour that was resected in 1995 and diagnosed as a schwannoma showed abundant KIT immunostaining, suggesting that this tumour was, in fact, a GIST. Rupture of the tumour probably seeded the peritoneum with slow-growing tumour cells, which resulted in the multiple peritoneal GISTs that were observed 7 years later. Non-NF1-associated GISTs can present as a cystic mass[16] and can disseminate throughout the peritoneum in ascitic fluid.[17]

The patient's pain improved after resection of the MPNST. He received a course of direct beam radiation to the MPNST. In consultation with his oncologist, he started treatment with imatinib mesylate. Side effects included fatigue and weight gain, and the drug was held once because of leucopenia secondary to concurrent radiation therapy. He was treated with imatinib mesylate for approximately 26 months.

The patient developed recurrent pain and weakness in the left lower extremity, and repeat magnetic resonance imaging and subsequent laparatomy showed extensive MPNST recurrence in the pelvis. A small bladder wall mass was a benign fibrous nodule, with no evidence of positive CD34 or KIT staining. Despite debulking of the MPNST and additional chemotherapy, the patient died about 6 months after the recurrence of the symptoms.

Patient 2

Patient 2 is a 62-year-old woman with NF1 and a history of breast cancer. A mass at the ileocecal junction was found on routine screening colonoscopy at age 56 years and the patient was referred for a laparotomy. The pathology report diagnosed the mass as polypoid fat with reactive changes. Two subserosal nodules in the jejunum were also incidentally noted, and biopsy performed on one of these (<0.5 cm nodule) showed a GIST based on positive staining for KIT and CD34 and negative staining for SMA and S-100. No mitotic activity or necrosis was seen.

At the time of the GIST diagnosis, the patient was receiving chemotherapy for breast cancer. No GIST-specific chemotherapy was given. A positron emission tomography scan at age 60 years did not find evidence of metabolically active disease. The patient remains asymptomatic of gastrointestinal complaints attributable to GISTs.

MATERIALS AND METHODS

All investigations were performed under protocols approved by the University of Pennsylvania School of Medicine and the National Human Genome Research Institute Institutional Review Board.

Tumour immunohistostaining

The tissue was fixed in 10% neutralised formalin and embedded in paraffin wax. Immunostaining for S-100, CD34, SMA and KIT was performed on the paraffin-wax-embedded tumour tissue using standard immunohistochemical protocol as follows. Sections of 5 μm thickness were cut and deparaffinised in xylene and rehydrated in graded alcohol. Endogenous peroxidase activity was blocked by 3% hydrogen peroxidase in methanol for 20 min. Antibodies to S-100 (polyclonal, 1:4000, Dako, Carpinteria, California, USA), KIT (polyclonal, 1:75, Dako), CD34 (Myo10/8G12, 1:80, Becton-Dickinson, Franklin Lakes, New Jersey, USA) and SMA (1A4, 1:500, Dako) were used. Primary antibodies were incubated for 1 h at room temperature. Immunohistochemical detection was performed on a DAKOCytomation Autostainer using the EnVision+HRP DAB System (DAKOCytomation). A positive control was used for each antibody. The primary antibody substituted by normal serum of the same species was used as a negative control.

Analysis of *NF1* gene mutation

The total coding region of the *NF1* gene was analysed by reverse transcription-polymerase chain reaction and in vitro transcription/translation as reported previously.[18]

Analysis of *KIT* and *PDGFRA* gene mutation in formalin-fixed, paraffin-wax-embedded tissue

Tumour DNA was extracted from two formalin-fixed, paraffin-wax-embedded GISTs (one from each patient) according to the manufacturer's directions (PureGene, Gentra Systems, Minneapolis, Minnesota, USA). Using methods previously described,[19 20] exons 9, 11, 13 and 17 of the *KIT* gene were amplified by polymerase chain reaction and screened for mutations using denaturing high-performance liquid chromatography. Likewise, *PDGFRA* exons 12, 14 and 18 were analysed by the same approach.

Analysis of *NF1* LOH, resequencing to determine loss of wild-type allele and multiplex ligand-dependent probe amplification to demonstrate mitotic recombination in GIST DNA

Seven microsatellite markers spanning 2.39 Mb (table 1) in, and flanking, *NF1* were genotyped in genomic (blood) and GIST DNA from patient 1.[21–24] (Analysis of the GIST from patient 2 was precluded by inadequate DNA.) Sequencing of exon 27a from the GIST DNA from patient 1 confirmed the presence of the mutated allele. To determine the number of copies of the mutated allele, multiplex ligand-dependent probe amplification (MLPA) was performed, with appropriate controls, using the MLPA P081/082 kit, V.04 (MRC Holland, Amsterdam, The Netherlands).[25]

RESULTS

Mutations in *NF1*

Patient 1: cDNA sequencing of fragment 3 (exons 19–29) showed (4537C→T) mutation in exon 27a, which predicts R1513X. Patient 2: a mutation in exon 30 of the *NF1* gene was identified, 5747delC, which predicts L1920X. Both mutations were confirmed by sequencing in genomic DNA.

Mutations in *KIT* and *PDGFRA*

Analyses of exons 9, 11, 13 and 17 of the *KIT* gene and exons 12, 14 and 18 of the *PDGFRA* gene in tumour DNA from GISTs from both patients 1 and 2 did not show mutations in either gene.

Table 1 Microsatellite markers in and near *NF1* used to test for loss of heterozygosity

Marker	Alleles from genomic DNA	Allele from GIST DNA
D17S841	265/269	265
Alu	400/396	400
IVS27GT	271/280	271
IVS27CAGT	189/199	189
IVS38	179/177	179
3′NF1-1	246/241	246
3′NF1-2	159/167	159

GIST, gastrointestinal stromal tumour; NF1, neurofibromatosis type 1.

Table 2 Summary of features in sporadic and NF1-associated gastrointestinal stromal tumours

Feature	Sporadic GISTs	NF1-associated GISTs
Incidence	10–20/million/year general population	Unknown
Mean age at presentation	~60 years	~50 years
Seen at younger ages?	Rare <40 years (except in familial GIST)	Common <40 years
Sex	M=F	M=F
Multiple or synchronous tumours?	Uncommon	Very common
Anatomical site	50–60% gastric; 25–30% small intestine; 10% colon/rectum; 5% oesophagus	40–60% jejunal; 25–75% other small intestine; 2–25% gastric
Anatomical site prognosis	Gastric may be more favourable; small intestine, rectum may be less favourable	Jejunum more favourable; duodenum less favourable
Presence of ICC hyperplasia?	Rare in sporadic GIST; common in familial GISTs	Common
Size of tumour at presentation (all sites)	Smaller	Larger
Risk profile at diagnosis	30% overtly malignant	Usually low risk; often indolent course
Morphology	70% spindle; 20% epitheloid; 10% mixed	Typically spindle; minority with epitheloid features
KIT/PDGFRA mutations	~85–90% with *KIT* or *PDGFRA* mutations	Uncommon; reported *KIT* and *PDGFRA* mutations are atypical

GIST, gastrointestinal stromal tumour; ICC, interstitial cell of Cajal.

Analysis of *NF1* LOH, resequencing to determine loss of wild-type allele and MLPA to show mitotic recombination in GIST DNA

All seven markers covering 2.39 Mb were informative in patient 1. A LOH was observed in the tumour for all markers analysed (table 1). Sequencing of exon 27a on the DNA sample from the GIST from patient 1 showed the known mutation 4537C→T and no wild-type sequence, suggesting that the wild-type allele was lost in the tumour. MLPA analysis showed that two copies of all *NF1* exons were present. With the exception of the control at chromosome 1p36, all positive controls showed appropriate signal strength. At chromosome 1p36, decreased signal strength suggested the loss of one copy.

DISCUSSION

Since 1998, the discovery of KIT expression in GISTs has improved tumour phenotyping and has strengthened the evidence of the association of GIST and NF1.[13 26 27] There are no estimates on the incidence of GIST in NF1 using post-1998 diagnostic criteria, although some studies have found cases of NF1 enriched in GIST populations.[28 29] The two patients described here typify the GIST phenotype in NF1: both had multiple, low-risk GISTs originating from the jejunum. Both tested tumours lacked *KIT* or *PDGFRA* mutations. Patient 1 originally presented with bowel perforation at age 27 years; patient 2 was diagnosed incidentally at 56 years. Table 2 summarises the differences between published reports of sporadic and NF1-associated GISTs.[10 13 26 27] These argue that NF1-associated GISTs have a distinct pathogenesis.

This argument is predicated on: (1) a general lack of typical *KIT/PDGFRA* mutations in *NF1*-associated GISTs[13 26 27]; (2) the evidence of *NF1* LOH observed in this report and others[14]; and (3) the presence of ICC hyperplasia in the small bowel of individuals with NF1.[13 26–28 30] Each of these is discussed.

Mutations in *KIT* are present in approximately 78% of sporadic GISTs, primarily in exon 11 (65%) and exon 9 (10%). An additional 8% of GISTs have mutations in *PDGFRA*, most commonly in exon 18.[10] Only seven NF1-associated GISTs have been reported with a mutation in *KIT* or *PDGFRA*. Takazawa *et al*[26] reported four missense mutations not previously documented among thousands of sporadic GISTs examined to date, but the importance of these mutations is unknown. Cheng *et al*[29] reported two patients with NF1-associated GIST who had *KIT* exon 11 mutations that overlap with those observed in familial GIST kindreds. Given that individuals with a germline *KIT* exon 11 mutation may have skin hyperpigmentation, clinical confusion with NF1 is not inconceivable. Similarly, Yantiss *et al*[30] reported an identical *KIT* exon 11 mutation observed in three separate GISTs from one patient. However, sequencing non-tumour DNA for a germline mutation in *KIT* was not performed. In summary, NF1-associated GISTs lack typical *KIT* and *PDGFRA* mutations.

This report is the first documentation (outside a neurofibroma) of mitotic recombination of *NF1* leading to a LOH and subsequent tumour formation. *NF1* LOH secondary to somatic *NF1* mutations has been recently reported in NF1-associated GISTs.[14] Multiple mechanisms can lead to LOH, including deletion, non-disjunctional chromosome loss with or without reduplication, gene conversion, point mutation, epigenetic inactivation and mitotic recombination.[31] Gene conversion and chromosomal loss with reduplication are theoretically possible in the GIST from patient 1 in this report. However, mitotic recombination as a mechanism of LOH has been previously observed in NF1-associated neurofibromas.[15]

Lastly, hyperplasia of the ICC in the myenteric plexus of individuals with NF1 and GIST is observed both in the vicinity of a GIST and in the macroscopically normal bowel.[13 26–28 30] In familial GIST (kindreds harbouring germline *KIT* or *PDGFRA* mutations), this diffuse proliferation of the ICC throughout the bowel is common.[5 32–34] The proliferation is polyclonal, suggesting that it is a hyperplasia rather than a neoplasia,[35] and is regarded as a precursor lesion to GISTs. Presumably, additional *KIT* activations or cytogenetic changes prompt the neoplastic emergence of a GIST from the background hyperplasia.[19] Precursor lesions in sporadic GISTs have not been well characterised,[33] if they exist at all.

In NF1, ICC hyperplasia is the probable precursor lesion for GISTs.[27 36] The ICC are of mesodermal and neural crest origin.[37] Murine *Nf1*-haploinsufficient melanocytes and mast cells (both of neural crest origin) have increased proliferation in response to Steel factor, the murine ligand for KIT.[38] Thus, ICC hyperplasia in NF1 is plausibly secondary to *NF1* haploinsufficiency.

We hypothesise that if ICC hyperplasia is secondary to *NF1* haploinsufficiency, then subsequent LOH of *NF1* leads to emergence of a GIST. The LOH of *NF1* is probably a necessary, but not sufficient, prerequisite for tumour development. In our patient 1, the reduced signal strength of the MLPA chromosome 1p36 positive control may be a cytogenetic change that aided the emergence of the GIST. Loss of chromosome 1p (and other cytogenetic changes) have been observed in other NF1-associated GISTs.[14]

Our hypothesis of alternate pathogenesis is compatible with the predominant small bowel distribution (especially in the jejunum) of GISTs in patients with NF1. Sporadic GISTs typically arise in the stomach, secondary to a somatic mutation

Key points

- Neurofibromatosis type 1 (NF1) is a neurocutaneous disorder resulting in the growth of a variety of tumours, and is inherited in an autosomal dominant pattern. Gastrointestinal stromal tumours (GISTs) are mesenchymal tumours that commonly harbour oncogenic mutations in *KIT* or *PDGFRA* and are thought to arise from the interstitial cells of Cajal (ICC; the pacemaker cells of the gut).
- We genotyped two patients for germline mutations in *NF1*. GISTs from both patients were genotyped for somatic mutations in *KIT* and *PDGFRA*. We also assessed loss of heterozygosity (LOH) of *NF1* in one GIST.
- GISTs from both patients were of wild type for mutations in *KIT* and *PDGFRA*. In the GIST with adequate DNA, seven informative microsatellite markers spanning 2.39 Mb showed LOH at the *NF1* locus. Sequencing of *NF1* from that GIST showed no wild-type sequence, suggesting that it was lost in the tumour. The multiplex ligand-dependent probe amplification analysis showed that two copies of all *NF1* exons were present.
- This is the first evidence of mitotic recombination resulting in a reduction to homozygosity of a germline *NF1* mutation in an NF1-associated GIST. We hypothesise that the LOH of *NF1* and lack of *KIT* and *PDGFRA* mutations are evidence of an alternative pathogenesis in NF1-associated GISTs. This hypothesis is compatible with the multiple, marked differences between sporadic and NF1-associated GISTs.

in *KIT* or *PDGFRA*. The higher rate of sporadic gastric carcinoma (*v* small intestine cancers) in the general population suggests that the stomach is a relatively larger source of mutagens (eg, *Helicobacter pylori*, nitrite exposure, smoked or salted foods).[39] Although gastric GISTs are observed in patients with NF1, precursor lesions (in the form of ICC hyperplasia) in the small intestine correspondingly increase the risk of tumour development. The notion of ICC hyperplasia as a precursor lesion is also compatible with the multiple and synchronous GISTs observed in patients with NF1, and may explain the younger age of onset of these tumours.

As ICC hyperplasia is implicated in dysmotility in cases of familial GISTs,[40] it may plausibly underlie the gastrointestinal dysmotility commonly seen in NF1.[3 41 42] Phenotyping with KIT and CD34 of the myenteric plexus hyperplasia[3 41 42] and intestinal neuronal dysplasia[43 44] observed in individuals with severe constipation and NF1 should clarify the role of the ICC in this important clinical problem.

Authors' affiliations

Douglas R Stewart, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland, USA
Christopher L Corless, Michael C Heinrich, Department of Pathology, Oregon Health and Science University Cancer Institute, Portland, Oregon, USA
Brian P Rubin, Department of Anatomic Pathology, University of Washington Medical Center, Seattle, Washington, USA
Ludwine M Messiaen, Department of Genetics, University of Alabama at Birmingham, Birmingham, Alabama, USA
Lisa J Kessler, Department of Medical Genetics, Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania, USA
Paul J Zhang, Department of Pathology, Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania, USA
David G Brooks, Merck and Co, Whitehouse Station, Pennsylvania, USA

Funding: This research was supported in part by the Intramural Research Program of the National Human Genome Research Institute, National Institutes of Health.

Competing interests: None.

The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products or organisations imply endorsement by the US Government.

Correspondence to: Dr D R Stewart, National Human Genome Research Institute, National Institutes of Health, 49 Convent Drive, Bldg 49, Room 4A62, Bethesda, MD 20892, USA; drstewart@mail.nih.gov

Received 4 April 2006
Revised 13 June 2006
Accepted 13 July 2006

REFERENCES

1 Friedman JM, Gutmann DH, MacCollin M, Riccardi VM, eds. *Neurofibromatosis: phenotype, natural history and pathogenesis*, 3rd edn. Baltimore: Johns Hopkins University Press, 1999.
2 Case records of the Massachusetts General Hospital. Weekly clinicopathological exercises. Case 15-1989. A 52-year-old man with neurofibromatosis and jaundice. *N Engl J Med* 1989;**320**(15):996–1004.
3 **Fuller CE**, Williams GT. Gastrointestinal manifestations of type 1 neurofibromatosis (von Recklinghausen's disease). *Histopathology* 1991;**19**(1):1–11.
4 **Duffaud F**, Blay JY. Gastrointestinal stromal tumors: biology and treatment. *Oncology* 2003;**65**(3):187–97.
5 **Maeyama H**, Hidaka E, Ota H, Minami S, Kajiyama M, Kuraishi A, Mori H, Matsuda Y, Wada S, Sodeyama H, Nakata S, Kawamura N, Hata S, Watanabe M, Iijima Y, Katsuyama T. Familial gastrointestinal stromal tumor with hyperpigmentation: association with a germline mutation of the c-kit gene. *Gastroenterology* 2001;**120**(1):210–5.
6 **Miettinen M**, Furlong M, Sarlomo-Rikala M, Burke A, Sobin LH, Lasota J. Gastrointestinal stromal tumors, intramural leiomyomas, and leiomyosarcomas in the rectum and anus: a clinicopathologic, immunohistochemical, and molecular genetic study of 144 cases. *Am J Surg Pathol* 2001;**25**(9):1121–33.
7 **Hirota S**, Isozaki K, Moriyama Y, Hashimoto K, Nishida T, Ishiguro S, Kawano K, Hanada M, Kurata A, Takeda M, Muhammad Tunio G, Matsuzawa Y, Kanakura Y, Shinomura Y, Kitamura Y. Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. *Science* 1998;**279**(5350):577–80.
8 **Rubin BP**, Singer S, Tsao C, Duensing A, Lux ML, Ruiz R, Hibbard MK, Chen CJ, Xiao S, Tuveson DA, Demetri GD, Fletcher CD, Fletcher JA. KIT activation is a ubiquitous feature of gastrointestinal stromal tumors. *Cancer Res* 2001;**61**(22):8118–21.
9 **Heinrich MC**, Corless CL, Demetri GD, Blanke CD, von Mehren M, Joensuu H, McGreevey LS, Chen CJ, Van den Abbeele AD, Druker BJ, Kiese B, Eisenberg B, Roberts PJ, Singer S, Fletcher CD, Silberman S, Dimitrijevic S, Fletcher JA. Kinase mutations and imatinib response in patients with metastatic gastrointestinal stromal tumor. *J Clin Oncol* 2003;**21**(23):4342–9.
10 **Corless CL**, Fletcher JA, Heinrich MC. Biology of gastrointestinal stromal tumors. *J Clin Oncol* 2004;**22**(18):3813–25.
11 **Demetri GD**, von Mehren M, Blanke CD, Van den Abbeele AD, Eisenberg B, Roberts PJ, Heinrich MC, Tuveson DA, Singer S, Janicek M, Fletcher JA, Silverman SG, Silberman SL, Capdeville R, Kiese B, Peng B, Dimitrijevic S, Druker BJ, Corless C, Fletcher CD, Joensuu H. Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. *N Engl J Med* 2002;**347**(7):472–80.
12 **Joensuu H**, Roberts PJ, Sarlomo-Rikala M, Andersson LC, Tervahartiala P, Tuveson D, Silberman S, Capdeville R, Dimitrijevic S, Druker B, Demetri GD. Effect of the tyrosine kinase inhibitor STI571 in a patient with a metastatic gastrointestinal stromal tumor. *N Engl J Med* 2001;**344**(14):1052–6.
13 **Miettinen M**, Fetsch JF, Sobin LH, Lasota J. Gastrointestinal Stromal Tumors in Patients With Neurofibromatosis 1: A Clinicopathologic and Molecular Genetic Study of 45 Cases. *Am J Surg Pathol* 2006;**30**(1):90–96.
14 **Maertens O**, Prenen H, Debiec-Rychter M, Wozniak A, Sciot R, Pauwels P, De Wever I, Vermeesch JR, de Raedt T, De Paepe A, Speleman F, van Oosterom A, Messiaen L, Legius E. Molecular pathogenesis of multiple gastrointestinal stromal tumors in NF1 patients. *Hum Mol Genet* 2006;**15**(6):1015–23.
15 **Serra E**, Rosenbaum T, Nadal M, Winner U, Ars E, Estivill X, Lazaro C. Mitotic recombination effects homozygosity for NF1 germline mutations in neurofibromas. *Nat Genet* 2001;**28**(3):294–6.
16 **Chang CY**, Wang HP, Mao TL, Hu RH, Yang PM, Lai MY, Lin JT. Unusual sonographic appearance of a gastrointestinal stromal tumor presenting as a large multilocular cystic mass. *J Clin Ultrasound* 2004;**32**(4):200–3.
17 **Wong NA**, Broadbent MR, Paterson-Brown S, al-Nafussi A. Gastrointestinal stromal tumor in ascitic fluid. A case report. *Acta Cytol* 2002;**46**(4):723–7.
18 **Messiaen LM**, Callens T, Mortier G, Beysen D, Vandenbroucke I, Van Roy N, Speleman F, Paepe AD. Exhaustive mutation analysis of the NF1 gene allows identification of 95% of mutations and reveals a high frequency of unusual splicing defects. *Hum Mutat* 2000;**15**(6):541–55.

19 **Heinrich MC**, Blanke CD, Druker BJ, Corless CL. Inhibition of KIT tyrosine kinase activity: a novel molecular approach to the treatment of KIT-positive malignancies. *J Clin Oncol* 2002;**20**(6):1692–703.
20 **Corless CL**, McGreevey L, Haley A, Town A, Heinrich MC. KIT mutations are common in incidental gastrointestinal stromal tumors one centimeter or less in size. *Am J Pathol* 2002;**160**(5):1567–72.
21 **Lopez Correa C**, Brems H, Lazaro C, Estivill X, Clementi M, Mason S, Rutkowski JL, Marynen P, Legius E. Molecular studies in 20 submicroscopic neurofibromatosis type 1 gene deletions. *Hum Mutat* 1999;**14**(5):387–93.
22 **Xu GF**, Nelson L, O'Connell P, White R. An Alu polymorphism intragenic to the neurofibromatosis type 1 gene (NF1). *Nucleic Acids Res* 1991;**19**(13):3764.
23 **Lazaro C**, Gaona A, Xu G, Weiss R, Estivill X. A highly informative CA/GT repeat polymorphism in intron 38 of the human neurofibromatosis type 1 (NF1) gene. *Hum Genet* 1993;**92**(4):429–30.
24 **Lazaro C**, Gaona A, Estivill X. Two CA/GT repeat polymorphisms in intron 27 of the human neurofibromatosis (NF1) gene. *Hum Genet* 1994;**93**(3):351–2.
25 **Wimmer K**, Yao S, Claes K, Kehrer-Sawatzki H, Tinschert S, De Raedt T, Legius E, Callens T, Beiglbock H, Maertens O, Messiaen L. Spectrum of single- and multiexon NF1 copy number changes in a cohort of 1,100 unselected NF1 patients. *Genes Chromosomes Cancer* 2006;**45**(3):265–76.
26 **Takazawa Y**, Sakurai S, Sakuma Y, Ikeda T, Yamaguchi J, Hashizume Y, Yokoyama S, Motegi A, Fukayama M. Gastrointestinal stromal tumors of neurofibromatosis type I (von Recklinghausen's disease). *Am J Surg Pathol* 2005;**29**(6):755–63.
27 **Andersson J**, Sihto H, Meis-Kindblom JM, Joensuu H, Nupponen N, Kindblom LG. NF1-associated gastrointestinal stromal tumors have unique clinical, phenotypic, and genotypic characteristics. *Am J Surg Pathol* 2005;**29**(9):1170–6.
28 **Kindblom LG**, Remotti HE, Angervall L, Mele-Kindblom JM. Gastrointestinal pacemaker cell tumor - a manifestation of neurofibromatosis 1 (abstract). *Mod Pathol* 1999;**12**:77A.
29 **Cheng SP**, Huang MJ, Yang TL, Tzen CY, Liu CL, Liu TP, Hsiao SC. Neurofibromatosis with gastrointestinal stromal tumors: insights into the association. *Dig Dis Sci* 2004;**49**(7-8):1165–9.
30 **Yantiss RK**, Rosenberg AE, Sarran L, Besmer P, Antonescu CR. Multiple gastrointestinal stromal tumors in type I neurofibromatosis: a pathologic and molecular study. *Mod Pathol* 2005;**18**(4):475–84.
31 **Tischfield JA**. Loss of heterozygosity or: how I learned to stop worrying and love mitotic recombination. *Am J Hum Genet* 1997;**61**(5):995–9.
32 **O'Riain C**, Corless CL, Heinrich MC, Keegan D, Vioreanu M, Maguire D, Sheahan K. Gastrointestinal stromal tumors: insights from a new familial GIST kindred with unusual genetic and pathologic features. *Am J Surg Pathol* 2005;**29**(12):1680–3.
33 **Agaimy A**, Wünsch PH. Sporadic Cajal cell hyperplasia is common in resection specimens for distal oesophageal carcinoma A retrospective review of 77 consecutive surgical resection specimens. *Virchows Arch* 2006;**448**:288–94.
34 **Hirota S**, Isozaki K. Pathology of gastrointestinal stromal tumors. *Pathol Int* 2006;**56**(1):1–9.
35 **Chen H**, Hirota S, Isozaki K, Sun H, Ohashi A, Kinoshita K, O'Brien P, Kapusta L, Dardick I, Obayashi T, Okazaki T, Shinomura Y, Matsuzawa Y, Kitamura Y. Polyclonal nature of diffuse proliferation of interstitial cells of Cajal in patients with familial and multiple gastrointestinal stromal tumours. *Gut* 2002;**51**(6):793–6.
36 **Rubin BP**. Gastrointestinal stromal tumours: an update. *Histopathology* 2006;**48**(1):83–96.
37 **Camborova P**, Hubka P, Sulkova I, Hulin I. The pacemaker activity of interstitial cells of Cajal and gastric electrical activity. *Physiol Res* 2003;**52**(3):275–84.
38 **Ingram DA**, Yang FC, Travers JB, Wenning MJ, Hiatt K, New S, Hood A, Shannon K, Williams DA, Clapp DW. Genetic and biochemical evidence that haploinsufficiency of the Nf1 tumor suppressor gene modulates melanocyte and mast cell fates in vivo. *J Exp Med* 2000;**191**(1):181–8.
39 **Kumar V**, Cotran RS, Robbins SL. *Robbins basic pathology*, 7th ed. Philadelphia, PA: Saunders, 2003.
40 **Jeng YM**, Mao TL, Hsu WM, Huang SF, Hsu HC. Congenital interstitial cell of cajal hyperplasia with neuronal intestinal dysplasia. *Am J Surg Pathol* 2000;**24**(11):1568–72.
41 **Hochberg FH**, Dasilva AB, Galdabini J, Richardson EP, Jr. Gastrointestinal involvement in von Recklinghausen's neurofibromatosis. *Neurology* 1974;**24**(12):1144–51.
42 **Riccardi VM**. Von Recklinghausen neurofibromatosis. *N Engl J Med* 1981;**305**(27):1617–27.
43 **Wu JF**, Chen HL, Peng SS, Yu TW, Lee WT, Chang MH. Neurofibromatosis type I and intestinal neuronal dysplasia type B in a child: report of one case. *Acta Paediatr Taiwan* 2003;**44**(4):232–4.
44 **Feinstat T**, Tesluk H, Schuffler MD, Krishnamurthy S, Verlenden L, Gilles W, Frey C, Trudeau W. Megacolon and neurofibromatosis: a neuronal intestinal dysplasia. Case report and review of the literature. *Gastroenterology* 1984;**86**(6):1573–9.