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c-kit proto-oncogene exon 8 in-frame deletion plus insertion mutations in acute myeloid leukaemia

Mamdooh Gari,[1] Anne Goodeve,[1] Gill Wilson,[1] Peter Winship,[1] Stephen Langabeer,[2] David Linch,[2] Elisabeth Vandenberghe,[1] Ian Peake[1] and John Reilly[1] [1]*Molecular Haematology Unit, Division of Molecular and Genetic Medicine, Royal Hallamshire Hospital, Sheffield, and* [2]*Department of Haematology, University College London Medical School, London*

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Summary. Genomic DNA from 60 cases of acute myeloid leukaemia (AML) was screened for mutations in the c-kit gene. DNA from all 21 exons was subjected to polymerase chain reaction (PCR) amplification and analysis by conformation sensitive gel electrophoresis (CSGE); exons showing altered CSGE patterns were then sequenced. Mutations were identified only in those patients with inv(16) (3/7 cases) or t(8;21) (1/2 cases) and comprised three in-frame deletion plus insertion mutations (exon 8) and one point mutation (exon 10, GTA → ATA, Val530Ile). Exons 8 and 10 were then analysed in 31 further cases of inv(16) ($n = 14$) and t(8;21) ($n = 17$), revealing four additional exon 8 in-frame deletion plus insertion mutations, all of which were in cases of inv(16). All exon 8 in-frame deletion plus insertion mutations ($n = 7$) involved the loss or repacement of the codon for Asp419 which is highly conserved cross species and is located in the receptor's extracellular domain. The high frequency of the c-kit proto-oncogene exon 8 deletion plus insertion mutations in AML suggests an essential role for this region of the receptor's extracellular domain. The association with inv(16) invites speculation as to the link between these two changes in the pathogenesis of AML.

Keywords: c-kit, oncogene, mutation, AML, CSGE.

Human c-kit, the cellular counterpart of v-kit derived from the Hardy-Zuckerman 4-feline sarcoma virus, is located on chromosome 4 and encodes a 145 kD receptor tyrosine kinase (RTK) that belongs to the same subfamily (type III RTK) as the platelet-derived growth factor receptors (PDGFRs), c-fms and the flt3/flk-2 receptor (Besmer *et al*, 1986; Qui *et al*, 1988; Ulrich & Schlessinger, 1990; Rosnet *et al*, 1993). Type III RTKs share sequence homology and have a similar overall structure, with five immunoglobulin-like repeats in the extracellular domain, a single transmembrane domain and a cytoplasmic kinase domain that is split by a kinase insert sequence into the adenosine triphosphate (ATP)-binding and phosphotransferase regions (Yarden & Ulrich, 1988). The kinase activity of c-kit is tightly regulated by its ligand, stem cell factor (SCF) (Broudy, 1997). SCF binding promotes c-kit dimerization and transphosphorylation of c-kit at specific tyrosine residues that can serve as docking sites for src-homology-2 (SH2) domain-containing signalling and adaptor proteins (Ulrich & Schlessinger, 1990). Such signal transduction plays an important role in the proliferation, differentiation, migration and survival of haemopoietic stem cells, as well as mast cells, neural crest-derived melanocytes and germ cells (Furitsu *et al*, 1993).

Gain-of-function point mutations in c-kit have been identified in a variety of mast cell lines, haemopoietic cells from patients with systemic mastocytosis and idiopathic myelofibrosis, and gastrointestinal stromal tumours. Two mutations, Val560Gly and Asp816Val, located in the tyrosine kinase domain, have been reported in the human mast cell leukaemia cell line HMC-1 (Furitsu *et al*, 1993), and Asp816Val has also been found in cells from patients with aggressive mast cell disease (Nagata *et al*, 1995; Longley *et al*, 1996). Substitution of aspartic acid 817 for tyrosine corresponding to human codon 816, results in ligand-independent activation in both the rat tumour mast cell line RBL-2H3 (Tsujimura *et al*, 1995) and the murine mastocytoma cell line (P-815) (Tsujimura *et al*, 1994). A recent report detailing an Asp816Tyr substitution in the phosphotransferase domain of c-kit provides the first direct evidence for mutations in this protein leading to the development of human acute leukaemia (Beghini *et al*, 1998). These data

Correspondence: Dr J. T. Reilly, Molecular Haematology Unit, M floor, Royal Hallamshire Hospital, Sheffield, S10 2JF. e-mail: j.t.reilly@sheffield.ac.uk.

strengthen a number of earlier observations suggesting that the receptor could be involved in the pathogenesis of acute myeloid leukaemia (AML). For example, c-kit is detectable in blast cells from most cases of AML (Ikeda *et al*, 1991) and the addition of SCF results in the proliferation of some human leukaemia cell lines, such as M07E, and also in AML blasts (Wang *et al*, 1989). Furthermore, c-kit has been reported to be activated, i.e. phosphorylated, in the absence of exogenous SCF in a proportion of AML cases (Kanakura *et al*, 1993).

In addition, Kimura *et al* (1997) reported a mutation in the extracellular domain of c-kit (Asp52Asn) in two patients with idiopathic myelofibrosis and suggested that the substitution conferred enhanced ligand sensitivity. Recently, two groups have reported receptor activation resulting from small juxtamembrane domain deletions. Tsujimura *et al* (1996) reported a seven amino acid deletion (Thr573-His579) in a murine mast cell line (FMA3), and Hirota *et al* (1998) reported c-kit activation in human gastrointestinal stromal tumours resulting from deletions located within an 11-amino acid stretch (Lys550-Val560).

On the basis of these findings, we hypothesized that c-kit mutations might have a role in acute myeloid leukaemia. We have screened the c-kit gene for mutations by conformation sensitive gel electrophoresis (CSGE) and direct sequencing in a total of 91 cases of acute myeloid leukaemia. We report the presence of acquired mutations in exon 8, with consistent loss of the codon for Asp419, in 7·7% of a selected population of patients with AML.

MATERIALS AND METHODS

Patient and normal DNA samples. Samples of genomic DNA were initially obtained from the marrow at presentation of 60 cases of AML entered into the Medical Research Council (MRC) AML X trial. The cases were classified, according to the FAB criteria (Bennett *et al*, 1976), as: M0 ($n=4$), M1 ($n=10$), M2 ($n=10$), M3 ($n=10$), M4 ($n=10$), M5 ($n=10$) and M6 ($n=6$). Standard cytogenetic analysis demonstrated inv(16) ($n=7$), t(8;21) ($n=2$), t(15;17) ($n=10$), other abnormalities ($n=25$) and a normal karyotype ($n=16$). Subsequently, DNA from a further 31 cases was obtained from patients entered into the MRC AML XII trial, with either inv(16) ($n=14$) or t(8;21) ($n=17$). One case (patient 3) was analysed both at presentation and during remission.

Genomic DNA was also prepared from the peripheral blood of 70 normal individuals using the Nucleon Biosciences BACC II kit.

Genomic DNA amplification. Genomic DNA was amplified using the polymerase chain reaction (PCR). The entire coding sequence and intron/exon boundaries corresponding to exons 2–20 of the c-kit gene were amplified independently, using previously reported oligonucleotide primers (Spritz *et al*, 1992). Exons 1 and 21 (including the polyadenylation site) were amplified using the following primers: exon 1 forward 5′-GCTGCACTTGGGCGAGAG-3′ (6207–6224) and exon 1 reverse 5′-CTGGCGGGTACCACCCTCC-3′ (6392–6410) exon 21 forward 5′-GCCTTTTGTTGCTATGTTCG-3′ (86555–86574) and exon 21 reverse 5′-GGTGTTCATACTCTGTCC TTTGTTC-3′ (87197–87221). The numbers in parentheses correspond to the numbering system for the genomic c-kit DNA sequence reported by Andre *et al* (1997). All PCR reactions comprised the following; 16·6 mM $(NH_4)_2SO_4$, 67 mM Tris-HCl (pH 8·8), 300 ng of each primer, 200 μM dNTPs (Pharmacia), $MgCl_2$ at 1·0–4·0 mM, 500 ng genomic DNA and 1 U Taq polymerase (Bioline) in a final volume of 50 μl. Samples were initially denatured at 95°C for 5 min and DNA amplification was achieved by 35 cycles of denaturation (94°C, 1 min), annealing (52°C, 30 s) and extension (72°C, 30 s).

Mutation detection. Conformation sensitive gel electrophoresis (CSGE) was used to screen for mutations as described (Williams *et al*, 1998). Briefly, PCR products were denatured by heating to 95°C for 5 min and then incubated at 65°C for 30 min. These heteroduplexed PCR products were then electrophoresed on 10% polyacrylamide gels; 99:1 acrylamide (BDH):bis-acrolypiperazine (BAP; Fluka), 10% ethylene glycol (Sigma), 15% formamide (Sigma) and 0·5× TTE buffer (1× TTE = 89 mM Tris, 28·5 mM taurine, 0·2 mM EDTA).

Samples displaying abnormal CSGE profiles compared to that obtained from a normal individual were directly sequenced using a Thermosequenase cycle sequencing kit (Amersham) and the appropriate γ-ATP ^{32}P (NEN) radiolabelled primer. Sequencing reactions were size fractionated on 6% denaturing polyacrylamide gels, which were then dried and subjected to autoradiography.

Exon 8 radiolabelled PCR. An 80 bp fragment spanning the 5′ region of exon 8 was amplified with the following forward and reverse primers respectively 5′-GTTTTCCTGTAGCAAA ACCAG-3′ (71818–71838) and 5′-GAATCCTGCTGCCACA CATT-3′ (71878–71897). Prior to amplification, 300 ng of the reverse primer was 5′ end labelled to a specific activity of 24·79 kBq/mmol using T4 polynucleotide kinase (NBL gene science) and γ-ATP ^{32}P (NEN). Otherwise, the PCR conditions were as described above using a $MgCl_2$ concentration of 1 mM. The radiolabelled PCR was undertaken for samples showing an exon 8 deletion plus insertion mutation (patients 1, 2 and 3) and the products were size-fractionated on 8% denaturing polyacrylamide gels. Resulting band intensities were quantitated on a phosphorimager (Bio-Rad, GS-250).

Exon 8 RT-PCR. RT-PCR was undertaken on RNA obtained from three patients showing inv(16) ($n=2$), t(8,21) ($n=1$) with exon 8 deletion plus insertion mutations. cDNA was synthesized from 1 μg RNA using random hexamer priming, essentially as described (Cross *et al*, 1993). cDNA prepared from 125 ng of RNA was used for each PCR reaction using forward primer 5′-ATTCTGACGTCAATGCTGCC-3′ (1208–1227) and reverse primer 5′-GAATCCTGCTGCCACACATT-3′ (1301–1320). In this instance the numbering system for the cDNA sequence was as reported by Yarden *et al* (1987). RT-PCR was performed in a 25 μl reaction volume using the above conditions with the exception of 1·5 mM $MgCl_2$ and 10 pmol of each primer. After 35 cycles of amplification (95°C for 30 s, 55°C for 1 min, 72°C for 1 min), 5 μl of each reaction mixture was size-fractionated on an 8% polyacrylamide gel and visualized under UV light after staining with ethidium bromide.

RESULTS

Identification of a novel polymorphism in codon 546 of the c-kit gene

All 21 exons and the polyadenylation site of the c-kit gene from 60 cases of AML were analysed for sequence differences compared to a normal individual using CSGE. A number of samples were found to have abnormal CSGE profiles, corresponding to previously described polymorphisms in exon 10 (ATG-CTG; codon 541) (Paguette *et al*, 1996; Riva & Larizza, 1996), exon 17 (ACT-ATT; codon 798) (Bowen *et al*, 1993) and exon 18 (CTG-CTC; codon 862) (Spritz *et al*, 1992). In addition, a silent A to G transition in exon 10 (AAA-AAG; codon 546) was identified in two patients. Subsequent analysis of 70 normal individuals identified this as a previously uncharacterized polymorphism in the normal population with a rare 'G' allele frequency of 0·05 (95% confidence interval 0·013–0·087).

The codon for Asp419 is lost in a high proportion of AML cases

c-kit mutations were detected in four of the initial 60 cases of AML. Three cases, inv(16) ($n=2$), t(8;21) ($n=1$) were found to have small, in-frame, deletion plus insertion mutations within exon 8, with consistent loss of the codon for Asp419 (Figs 1 and 2), and one patient with inv(16) showed a

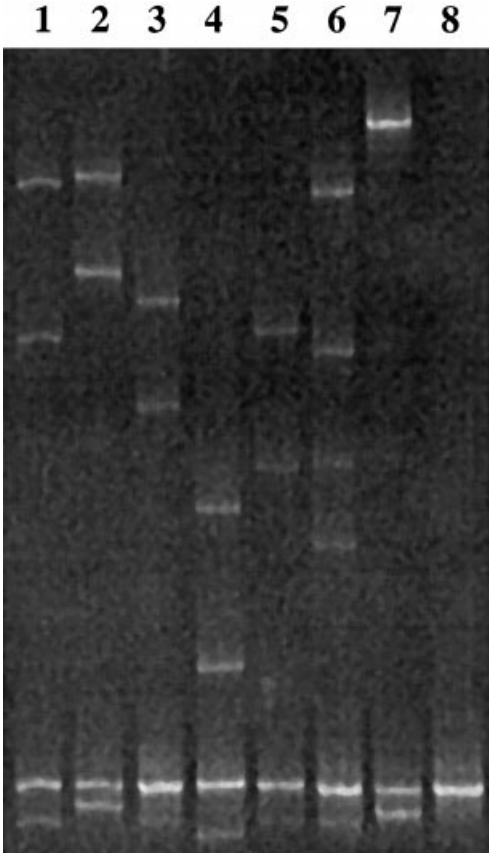

Fig 1. Exon 8 mutations in the c-kit gene of seven AML patients. The CSGE gel shows exon 8 amplified from seven AML patients (lanes 1–7) displaying an abnormal CSGE profile compared to that seen in a normal individual (lane 8).

Normal		416	417	418	**419**	420	421	422
		Leu	Thr	Tyr	Asp	Arg	Leu	Val
	5'	CTG	ACT	TAC	GAC	AGG	CTC	GTG 3'
Patient 1		Leu	**Ile**	Arg	Leu	Val		
	5'	CTG	A**T**C	AGG	CTC	GTG 3'		
			(del CTTACGA ins T)					
Patient 2		Leu	Thr	Tyr	**Phe**	Val		
	5'	CTG	ACT	TAC	**T**TC	GTG 3'		
			(del GACAGGC ins T)					
Patient 3		Leu	**Val**	Arg	Leu	Val		
	5'	CTG	**GTG**	AGG	CTC	GTG 3'		
			(del ACTTACGAC ins GTG)					
Patient 4		Leu	**Arg**	**Gly**	Arg	Leu	Val	
	5'	CTG	A**GA**	**GG**C	AGG	CTC	GTG 3'	
			(del CTTACGA ins GAGG)					
Patient 5		Leu	**Ile**	**Arg**	Leu	Val		
	5'	CTG	A**TA**	**C**GG	CTC	GTG 3'		
			(del CTTACGACA ins TAC)					
Patient 6		Leu	**Asn**	Arg	Leu	Val		
	5'	CTG	A**AT**	AGG	CTC	GTG 3'		
			(del CTTACGAC ins AT)					
Patient 7		Leu	Thr	Tyr	**Phe**	**Phe**	**Asp**	**Gly**	Leu	Val
	5'	CTG	ACT	TAC	**TTC**	**TTC**	**GAC**	**G**GG	CTC	GTG 3'
		(del GACA ins TCCTCCGACG)								

Fig 2. Small in-frame deletion plus insertion mutations in the c-kit gene of seven patients with AML. The nucleotide changes in each patient is indicated, in parentheses, underneath the resulting novel sequence. Bold underlined text indicates inserted nucleotides and amino acids.

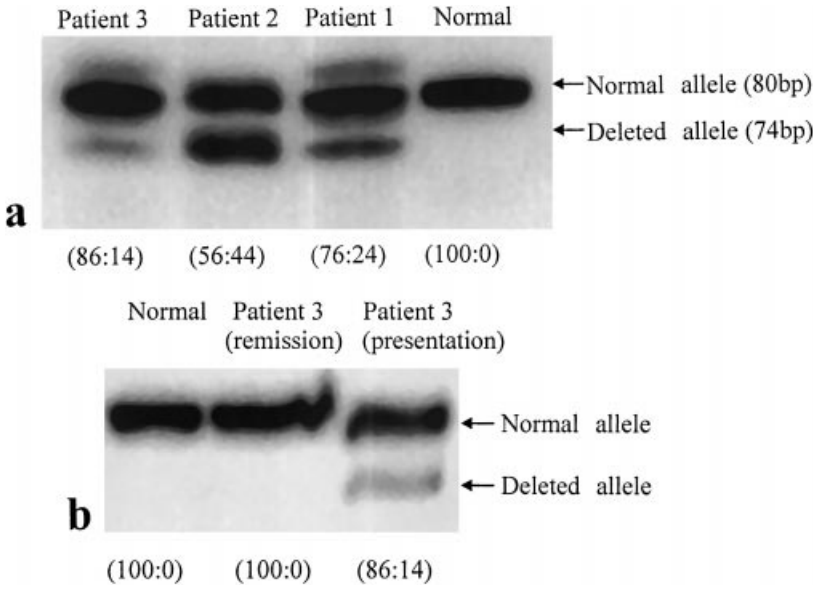

Fig 3. Relative intensities of normal and abnormal exon 8 amplification products of the c-kit gene in AML patients. (a) Autoradiograph of 8% denaturing polyacrylamide gel showing bands (lanes 1–3) corresponding to wild-type and deleted alleles in patients with exon 8 deletion plus insertion mutations and a normal individual. (b) Similar analysis demonstrating loss of the deleted allele in a sample taken from patient 3 while in clinical remission. Figures in parentheses at the foot of each lane represent the ratio of normal to deleted allele quantitated by phosphorimager analysis. Normal and deleted alleles are indicated by the arrows.

Val530Ile (GTA to ATA) substitution within the transmembrane region (exon 10). In view of the apparent cytogenetic association, further samples of inv(16) (14 cases) and t(8;21) (17 cases) were studied. Four of these further cases of inv(16) showed similar small deletion plus insertion mutations of exon 8, again resulting in loss of the codon for Asp419 (Fig 2). These data confirmed that the small in-frame deletion plus insertion mutations were more common in those AML patients with inv(16) than those without ($P < 0{\cdot}001$). Overall, c-kit mutations were detected in 7·7% of patients studied with AML, a figure increasing to 33% in cases with inv(16).

The small in-frame deletion plus insertion mutations are acquired somatically and expressed at the RNA level

The acquired nature of the small in-frame deletion plus insertion mutations was confirmed by radiolabelled PCR of genomic DNA across the mutated region from patients 1, 2 and 3. The data from these quantitative PCRs are shown in Fig 3(a). A significant departure from the expected 50:50 ratio of normal:deleted band intensities, which would result from inheritance of the mutation in two of the three patients (patient 1, 76:24; patient 3, 86:14), suggested that these abnormalities were somatically acquired. The case for patient 2, with a 56:44 ratio, was less clear cut. Conclusive proof that the mutation was of somatic origin in patient 3 was provided by the observation that a genomic DNA sample taken while the patient was in clinical remission failed to show any trace of the abnormal DNA fragment following exon 8 amplification (Fig 3b).

Finally, RT-PCR analysis of RNA from patients 1, 2 and 3 using primers spanning the exon 7 and 8 boundary (to ensure that only amplified product from RNA and not genomic DNA was being analysed) demonstrated that the mutated alleles were indeed expressed at the RNA level (Fig 4).

Cross-species sequence comparison shows the Asp 419 codon to be a highly conserved residue in c-kit

The amino acid sequence of c-kit from several species was compared with human c-kit across the region surrounding codon Asp419 (Fig 5). These data demonstrated the highly

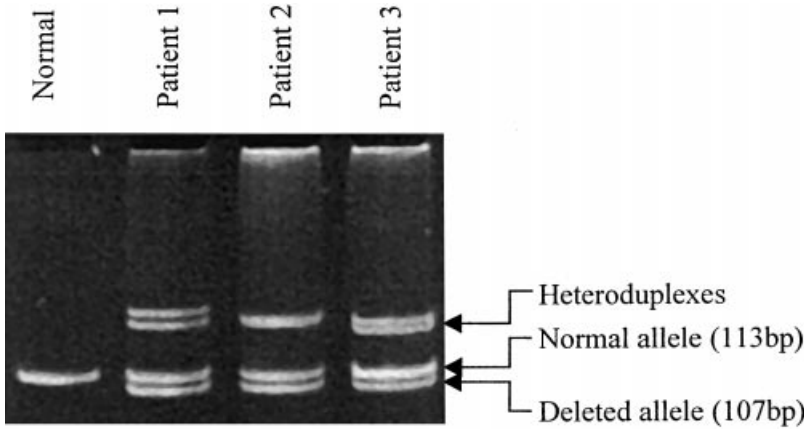

Fig 4. RNA encoding exon 8 of the c-kit gene from a normal individual and patients 1, 2 and 3 was amplified by RT-PCR and size-fractionated on an 8% polyacrylamide gel. Expression of the mutated allele was confirmed by the presence of both normal and deleted alleles in each patient.

	404			419		428
Human	AF	NVYVN	TKPEIL	TY**D**RL	VNGML	Q**C**
Mouse	TF	NVYVN	TKPEIL	TY**D**RL	INGML	Q**C**
Rat	TF	DVYVN	TKPEIL	TY**D**RL	MNGRL	Q**C**
Bovine	TF	DVYVN	TKPEIL	TH**D**RL	MNGRL	Q**C**
Goat	TF	NVNVN	TKPEIL	TH**D**RL	VNGML	Q**C**
Cat	TL	NVYVN	TKPEIL	TH**E**SL	VSGIL	Q**C**
Chicken	TF	NVYVK	TKPEIL	TL**D**ML	GNDIL	Q**C**

Fig 5. Cross-species comparison of amino acid sequences within the c-kit extracellular domain. The sequence is shown for amino acid residues 404–428 of the amino terminal of the fifth immunoglobulin-like domain. The boxed area indicates the region from within which amino acid residues, including the invariantly deleted Asp 419 (highlighted), were deleted in AML patients 1–7. Cysteine 428, one of the two cysteines which forms a disulphide bond across the fifth immunoglobulin-like domain, is also highlighted.

conserved nature of this region of the fifth immunoglobulin-like domain, which includes the Asp419 codon deleted or replaced in our patients. This highlights the probable structural/functional importance of this area of c-kit.

DISCUSSION

This study demonstrates that c-kit deletion plus insertion mutations occur in approximately one third of cases of AML with inv(16) karyotype. This cytogenetic variant occurs in approximately 5% of all AML cases, giving an overall incidence for this specific c-kit mutation subtype in AML of around 2%. Although c-kit mutations have been reported previously in a single case of acute myeloid leukaemia, a number of observations suggest that the receptor may be more frequently involved in the pathogenesis of this disease (Beghini *et al*, 1998). Firstly, the c-kit product is detectable in blast cells from most cases of AML; secondly, the addition of stem cell factor results in the proliferation of some human leukaemic cell lines, such as M07E, and AML blasts and, lastly, c-kit has been reported to be activated, i.e. phosphorylated, in the absence of exogenous SCF in a proportion of AML cases (Wang *et al*, 1989; Ikeda *et al*, 1991; Kanakura *et al*, 1993).

The functional significance of the exon 8 deletion plus insertion mutations at present is unclear. c-kit contains five immunoglobulin-like repeats in the extracellular domain, a single transmembrane domain and a cytoplasmic domain that is split by a kinase insert sequence into the adenosine triphosphate (ATP) binding and phosphotransferase regions (Yarden & Ulrich, 1988). Monoclonal antibody inhibition studies and the analysis of chimaeric mouse–human and deletion mutant kit proteins has enabled the identification of important functional elements in the extracellular region of the receptor (Blechman & Yarden, 1995). The three N-terminal immunoglobulin-like units are involved in ligand binding, the fourth immunoglobulin-like unit is functionally important for receptor dimerization, whereas the function of the fifth domain remains unclear. It is of interest, therefore, that the deletion–insertion mutations described in this study are located in the fifth immunoglobulin-like domain, providing the first evidence in support of an important role for this region of the protein. The structural importance of the deleted region is suggested by the involved amino acids, especially Asp 419, being highly conserved in the c-kit proteins of man (Yarden *et al*, 1987), rat (Tsujimura *et al*, 1991), mouse (Qui *et al*, 1988), cattle (Kubota *et al*, 1994), chicken (Sasaki *et al*, 1993) and goat (Tanaka *et al*, 1997). c-kit receptor activation due to small deletions, albeit in the juxta-membrane domain, has been previously reported. Tsujimura *et al* (1996) described a seven amino acid deletion (Thr573-His579) in a murine mast cell line (FMA3), and Hirota *et al* (1998) reported c-kit activation in human gastrointestinal stromal tumours resulting from deletions located within an 11-amino acid stretch (Lys550-Val560). The role of the fourth immunoglobulin-like domain in activating the transforming potential of the related receptor, c-fms, has been highlighted (van Daalen Wetters *et al*, 1992). Mutations of amino acids 301 and 374 were necessary, but not sufficient, for c-fms dimerization and subsequent cell transformation, in the absence of the ligand (Carlberg & Rohrschneider, 1994). The fifth immunoglobulin-like domain therefore may have some, as yet undefined, function, or with domain 4 may co-operate in receptor dimerization, and Asp 419 changes may therefore lead to possible constitutive activation of c-kit. It is interesting that AML patients with inv(16) karyotype have a complete response rate to treatment of 70–80% and median duration of remission that is significantly prolonged compared to most other subtypes of acute myeloid leukaemia (Grimwade *et al*, 1998). The influence of the described c-kit mutations in terms of prognosis remains at present unclear.

In conclusion, we report the occurrence of c-kit deletion–insertion mutations in acute myeloid leukaemia and demonstrate their strong association with inv(16) karyotype. By inference from other studies of c-kit and related receptors, the changes reported here would suggest a gain-of-function mutation. However, the biological significance and transforming capacity of these deletion–insertion mutations are at present unclear and are currently being addressed in our laboratory by receptor tyrosine phosphorylation studies and by transfection of mutant-type c-kit genes into human cell lines. Clear and decisive information from such experiments should lead to a greater understanding of kit structure–function relationships as well as the

mechanisms underlying regulation of normal and malignant myelopoiesis.

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REFERENCES

Andre, C., Hampe, A., Lachaume, P., Martin, E., Wang, X.P., Manus, V., Hu, W.X. & Galibert, F. (1997) Sequence analysis of two genomic regions containing the KIT and the FMS receptor tyrosine kinase genes. *Genomics*, **39,** 216–226.

Beghini, A., Larizza, L., Cairoli, R. & Morra, E. (1998) c-kit activating mutations and mast cell proliferation in human leukemia. (Letter). *Blood*, **92,** 701–702.

Bennett, J.M., Catovsky, D., Daniel, M.T., Flandrin, G., Galton, D.A.G., Gralnick, H. & Sultan, G. (1976) Proposals for the classification of the acute leukaemias. *British Journal of Haematology*, **33,** 451–458.

Besmer, P., Murphy, J.E., George, P.C., Qui, F., Bergold, P.J., Ledermann, L., Synder, H.W., Brodeur, D., Zuckermann, E.E. & Hardy, W.D. (1986) A new acute transforming feline retrovirus and relationship of its oncogene v-kit with the protein kinase gene family. *Nature*, **320,** 415–421.

Blechman, J.M. & Yarden, Y. (1995) Structural aspects of receptor dimerization. c-Kit as an example. *Annals of the New York Academy of Sciences*, **766,** 344–362.

Bowen, D.T., Padua, R.A., Burnett, A.K., deCastro, C.M. & Kaufman, R.E. (1993) Two new polymorphisms but no mutations of the KIT gene in patients with myelodysplasia at positions corresponding to human FMS and murine W locus mutational hot spots. *Leukemia*, **7,** 1883–1885.

Broudy, V.C. (1997) Stem cell factor and hematopoiesis. *Blood*, **90,** 1345–1364.

Carlberg, K. & Rohrschneider, L. (1994) The effect of activating mutations on dimerization, tyrosine phosphorylation and internalization of the macrophage colony stimulating factor receptor. *Molecular Biology of the Cell*, **5,** 81–95.

Cross, N.C.P., Hughes, T.P., Feng, L., O'Shea, L., Bungey, J., Marks, D.I., Ferrant, A., Martiat, P. & Goldman, J.M. (1993) Minimal residual disease after allogeneic bone marrow transplantation for chronic myeloid leukaemia in first chronic phase: correlations with acute graft-versus-host disease and relapse. *British Journal of Haematology*, **84,** 67–74.

Furitsu, T., Tsujimura, T., Tono, T., Ikeda, H., Kitayama, H., Koshimizu, U., Sugahara, H., Butterfield, J.H., Ashman, L.K., Kanayama, Y., Matsuzawa, Y., Kitamura, Y. & Kanakura, Y. (1993) Identification of mutations in the coding sequence of the proto-oncogene c-kit in a human mast cell leukemia cell line causing ligand-independent activation of c-kit product. *Journal of Clinical Investigation*, **92,** 1736–1744.

Grimwade, D., Walker, H., Oliver, F., Wheatly, K., Harrison, C., Harrison, G., Rees, J., Hann, I., Stevens, R., Burnett, A. & Goldstone, A., on behalf of the Medical Research Council adult and children's leukaemia working parties (1998) The importance of diagnostic cytogenetics on outcome in AML: analysis of 1,612 patients entered into the MRC AML 10 trial. *Blood*, **92,** 2322–2333.

Hirota, S., Isozaki, K., Moriyama, Y., Hashimoto, K., Nishida, T., Ishiguro, S., Kawano, K., Hanada, M., Kurata, A., Takeda, M., Tunio, G.M., Matsuzawa, Y., Kanakura, Y., Shinomura, Y. & Kitamura, Y. (1998) Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. *Science*, **279,** 577–580.

Ikeda, H., Kanakura, Y., Tamaki, T., Kuriu, A., Kitayama, H., Ishikawa, J., Kanayama, Y., Yonzawa, T., Tarui, S. & Griffin, J. (1991) Expression and functional role of the proto-oncogene c-kit in acute myeloblastic leukemia cells. *Blood*, **78,** 2962–2968.

Kanakura, Y., Ikeda, H., Kitayama, H., Sugahara, H. & Furitsu, T. (1993) Expression, function and activation of the proto-oncogene c-kit product in human leukemia cells. *Leukemia and Lymphoma*, **10,** 35–41.

Kimura, A., Nakata, Y., Katoh, O. & Hyodo, H. (1997) c-kit point mutation in patients with myeloproliferative disorders. *Leukemia and Lymphoma*, **25,** 281–287.

Kubota, T., Hikono, H., Sasaki, E. & Sakurai, M. (1994) Sequence of a bovine c-kit proto-oncogene cDNA. *Gene*, **141,** 305–306.

Longley, B.J., Tyrrell, L., Lu, S., Ma, Y., Langley, K., Ding, T., Duffy, T., Jacobs, P., Tang, L.H. & Modlin, I. (1996) Somatic c-kit activating mutation in urticaria pigmentosa and aggressive mastocytosis: establishment of clonality in a human mast cell neoplasm. *Nature Genetics*, **12,** 312–314.

Nagata, H., Worobec, A.S., Chad, K.O., Chowdhury, B.A., Tannenbaum, S., Suzuki, Y. & Metcalf, D.D. (1995) Identification of a point mutation in the catalytic domain of the proto-oncogene c-kit in peripheral blood mononuclear cells of patients who have mastocytosis with an associated hematologic disorder. *Proceedings of the National Academy of Sciences of the United States of America*, **92,** 10560–10564.

Paguette, R.L., Hsu, N.C. & Koeffler, H.P. (1996) Analysis of c-kit integrity in aplastic anemia. *Blood Cells, Molecules and Diseases*, **22,** 159–168.

Qui, F., Ray, P., Brown, K., Barker, P.E., Jhanwar, S., Ruddle, F.H. & Besmer, P. (1988) Primary structure of c-kit: relationship with the CSF-1/PDGF receptor kinase family-oncogenic activation of v-kit involves deletion of extracellular domain and C terminus. *EMBO Journal*, **7,** 1003–1011.

Reith, A.D. & Bernstein, A. (1991) Molecular biology of the W and Steel loci. *Genes and Phenotypes* (ed. by K. E. Davies and S. Tilghman), Vol. 3, p. 105. Cold Spring Harbor Laboratory, New York.

Riva, P. & Larizza, L. (1996) A novel potentially diagnostic polymorphism (4894 A/C) in exon 10 of the human c-kit proto-oncogene. *Molecular and Cellular Probes*, **10,** 387–388.

Rosnet, O., Schiff, C., Pébusque, M.-J., Marchetto, S., Tonnelle, C., Toiron, Y., Birg, F. & Birnbaum, D. (1993) Human FLT3/FLK2 gene: cDNA cloning and expression in hematopoietic cells. *Blood*, **82,** 1110–1119.

Sasaki, E., Okamura, H., Chikamune, T., Kanai, Y., Watanabe, M., Naito, M. & Sakurai, M. (1993) Cloning and expression of the chicken c-kit proto-oncogene. *Gene*, **128,** 257–261.

Spritz, R.A., Giebel, L.B. & Holmes, S.A. (1992) Dominant negative and loss of function mutations of the c-kit (mast/stem cell growth factor receptor) proto-oncogene in human piebaldism. *American Journal of Human Genetics*, **50,** 261–269.

Tanaka, S., Yanagisawa, N., Tojo, H., Kim Y., J., Tsujimura, T., Kitamura, Y., Sawasaki, T. & Tachi, C. (1997) Molecular cloning of cDNA encoding the c-kit receptor of Shiba goats and a novel alanine insertion specific to goats and sheep in the kinase insert region. *Biochimica et Biophysica Acta*, **1352,** 151–155.

Tsujimura, T., Furitsu, T., Morimoto, M., Isozaki, K., Nomura, S., Matsuzawa, Y., Kitamura, Y. & Kanakura, Y. (1994) Ligand-independent activation of c-kit receptor tyrosine kinase in a murine

mastocytoma cell line P-815 generated by a point mutation. *Blood*, **83,** 2619–2626.

Tsujimura, T., Furitsu, T., Morimoto, M., Kanayama, Y., Nomura, S., Matsuzawa, Y., Kitamura, Y. & Kanakura, Y. (1995) Substitution of an aspartic acid results in constitutive activation of c-kit receptor tyrosine kinase in a rat tumour mast cell line RBL-2H3. *International Archives of Allergy and Immunology*, **106,** 377–385.

Tsujimura, T., Hirota, S., Nomura, S., Niwa, Y., Yamazaki, M., Tono, T., Morii, E., Kim, H., Kondo, K., Nishimune, Y. & Kitamura, Y. (1991) Characterization of Ws mutant allele of rats: a 12-base deletion in tyrosine kinase domain of c-kit gene. *Blood*, **78,** 1942–1946.

Tsujimura, T., Morimoto, M., Hashimoto, K., Moriyama, Y., Kitayama, H., Matsuzawa, Y., Kitamura, Y. & Kanakura, Y. (1996) Constitutive activation of c-kit in FMA3 murine mastocytoma cells caused by deletion of seven amino acids at the juxtamembrane domain. *Blood*, **87,** 273–283.

Ullrich, A. & Schlessinger, J. (1990) Signal transduction by receptors with tyrosine kinase activity. *Cell*, **61,** 203–212.

van Daalen Wetters, T., Hawkins, S.A., Roussel, M.F. & Sherr, C.J. (1992) Random mutagenesis of CSF-1 receptor (FMS) reveals multiple sites for activating mutations within the extracellular domain. *EMBO Journal*, **11,** 551–557.

Wang, C., Curtis, J.E., Geissler, E.N., McCulloch, E.A. & Minden, M.D. (1989) The expression of the proto-oncogene c-kit in acute myeloblastic leukemia cells. *Blood*, **78,** 2962–2968.

Williams, I.J., Abuzenadah, A., Winship, P.R., Preston, F.E., Dolan, G., Wright, J., Peake, I.R. & Goodeve, A.C. (1998) Precise carrier diagnosis in families with haemophilia A: use of conformation sensitive gel electrophoresis for mutation screening and polymorphism analysis. *Thrombosis and Haemostasis*, **79,** 723–726.

Yarden, Y., Kuang, W.J., Yang-Feng, T., Coussens, L., Munemitsu, S., Dull, T.J., Chen, E., Schessinger, J., Francke, U. & Ullrich, A. (1987) Human proto-oncogene c-kit: a new cell surface receptor tyrosine kinase for an unidentified ligand. *EMBO Journal*, **6,** 3341–3351.

Yarden, Y. & Ullrich, A. (1988) Growth factor receptor tyrosine kinases. *Annual Review of Biochemistry*, **57,** 443–478.