Laboratory Investigation (2012) 92, 451–457

Characterization of novel germline c-*kit* gene mutation, KIT-Tyr553Cys, observed in a family with multiple gastrointestinal stromal tumors

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We found a novel type germline mutation at exon 11 of the c-*kit* gene, which results in a substitution of Tyr to Cys at codon 553 of the c-*kit* gene product (KIT-Tyr553Cys), in a 68-year-old female patient with multiple gastrointestinal stromal tumors (GISTs). In the present study, we carried out mutational analysis in her family members to determine the carriers and characterized the mutation by introducing the corresponding mutation (murine KIT-Tyr552Cys) into expression vector possessing murine c-*kit* cDNA. Mutational analysis of peripheral blood leukocytes of her family members revealed that a 44-year-old son had the same mutation, but at present he had neither apparent symptoms nor images of multiple GISTs. By transfection with the expression vector possessing the murine mutant c-*kit* cDNA, interleukin-3-dependent Ba/F3 murine lymphoid cells started growing autonomously without any growth factors, indicating that the mutation was considered to be of gain-of-function. Imatinib, a small molecule of tyrosine kinase inhibitor, effectively inhibited autophosphorylation of KIT-Tyr552Cys. Nilotinib, another small molecule of the KIT inhibitor, also effectively inhibited autophosphorylation of KIT-Tyr552Cys. In fact, proliferation of Ba/F3 cells expressing KIT-Tyr552Cys was effectively inhibited by both imatinib and nilotinib. These findings indicate that the novel type human KIT-Tyr553Cys mutation is the cause of the present familial and multiple GISTs, and that both imatinib and nilotinib might effectively inhibit the growth of GISTs developing in the patients of this family.

Laboratory Investigation (2012) **92,** 451–457; doi:10.1038/labinvest.2011.165; published online 14 November 2011

KEYWORDS: exon 11; gastrointestinal stromal tumor; germline mutation; imatinib; juxtamembrane domain; nilotinib; tyrosine kinase inhibitor

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the human gut. Most GISTs are positive for KIT,[1] a receptor tyrosine kinase (TK), which is encoded by the c-*kit* proto-oncogene.[2,3] Interstitial cells of Cajal (ICCs) in the gastrointestinal wall, which are considered to regulate the gastrointestinal motility through their spontaneous impulse generation,[4] are also positive for KIT.[5–7] Some other markers of GISTs such as CD34,[1] embryonic form of smooth muscle myosin heavy chain,[8] nestin,[9] DOG1,[10] and ETV1[11] have been demonstrated to be common markers of ICCs. Therefore, GISTs are now considered to originate from ICCs or their precursor.

KIT consists of an extracellular domain with five immunoglobulin-like repeats, a transmembrane domain, a juxtamembrane domain, and TK I and II domains split by the kinase insert.[2,3] Stem cell factor (SCF) is a ligand for KIT,[12] and the SCF–KIT system has a crucial role in development of melanocytes, erythrocytes, germ cells, mast cells and ICCs.[5–7,13,14] Somatic gain-of-function mutations of the c-*kit* gene are observed in some types of human tumors such as GISTs,[1,15] mast cell tumors,[16,17] germ cell tumors,[18] and malignant melanomas.[19,20] Most of the mutations are detected in four different exons, ie, exon 9, exon 11, exon 13, and exon 17.[1,15–20] These mutations essentially result in the constitutive activation of KIT. In sporadic GISTs, the most frequent mutations are located at exon 11.[1,15] In rarer cases, the mutations are found at exon 9 encoding the extracellular domain,[15,21,22] at exon 13 encoding the TK I domain,[15,21,23]

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Received 8 September 2011; revised 7 October 2011; accepted 10 October 2011

or at exon 17 encoding the TK II domain.[15,21,23] In melanomas, most of the mutations are also present at exon 11.[19,20] However, most of them are located at exon 17 in both human mast cell tumors and germ cell tumors.[16–18]

Imatinib, one of the TK inhibitors, potently inhibits KIT activity and is now used for the treatment of advanced GISTs.[24] Imatinib is well known to show remarkable inhibitory effect to most exon 11 c-*kit* gene mutations but not to most exon 17 c-*kit* gene mutations.[25] In fact, it has remarkable effect to most GISTs with exon 11 c-*kit* gene mutations.[26] But secondary resistance to imatinib often develops during long-term use.[27] Most common mechanism of the secondary resistance is considered to be addition of second mutation at exon 13 or exon 17 of the c-*kit* gene.[28] Sunitinib, another TK inhibitor, is now used for imatinib-resistant GISTs, but the effect is limited.[29] Therefore, many other reagents including another TK inhibitor, nilotinib, are under development for GIST treatment.

We published the first case of familial and multiple GISTs with germline mutation at exon 11 of the c-*kit* gene in 1998.[30] To our knowledge, ~20 families with germline c-*kit* gene mutations and multiple GISTs have been reported so far.[31–49] Thirteen families including the first case have the c-*kit* gene mutation at exon 11,[30,31,33,34,36–40,43,44,46,49] three families at exon 13,[32,45,48] three families at exon 17[35,42,47] and one family at exon 8.[41] In addition to multiple GISTs, patients of these families have hyperplasia of ICCs in the gut wall. Recently, we found a 68-year-old Japanese woman who had an operation for multiple mesenchymal tumors of the stomach, ileum and the cecum. These tumors were KIT-positive GISTs, and she also had hyperplasia of ICCs in the stomach and small intestine. Mutational analysis of her GIST tissues and peripheral blood leukocytes revealed that she had a novel type of germline c-*kit* gene mutation, KIT-Tyr553Cys, at exon 11. In the present study, we carried out mutational analysis in the family members to determine the carriers, and characterized the novel type germline mutation including inhibitory effect of TK inhibitors such as imatinib and nilotinib.

MATERIALS AND METHODS

Analysis of Germline c-*kit* Gene Mutation Using Genomic DNA

Genomic DNA was extracted from peripheral blood using GenTLE Kit (Takara, Otsu, Japan), according to the manufacturer's instructions. DNA fragment of exon 11 of the c-*kit* gene was amplified by PCR using forward primer (5′-GAGTG CTCTAATGACTGAGA-3′) and reverse primer (5′-AAAGGT GACATGGAAAGCCC-3′), and was sequenced directly by using the same primers. Exons 9, 13 and 17 of genomic c-*kit* DNA were also amplified by PCR as described previously.[50] Present analysis was performed after taking informed consent from the family members, under the approval of the authors' institutional ethical committees, and according to fundamental principles of research on the human genome recommended by the Japanese Ministry of Education, Culture, Sports, Science and Technology.

Generation of Ba/F3 Cells Expressing Murine KIT-Tyr552Cys

Murine c-*kit* gene mutation (murine KIT-Tyr552Cys) corresponding to human c-*kit* gene mutation (human KIT-Tyr553Cys) was introduced into expression vector possessing murine c-*kit* cDNA using site-directed mutagenesis as described previously.[1] The expression vector was transfected into interleukin-3 (IL-3)-dependent Ba/F3 murine lymphoid cell line, and selection of the transfectant was done as described previously.[1] The stable transfectant was cloned with the method of limiting dilution.

Proliferation Profile of Ba/F3 Cells Expressing Murine KIT-Tyr552Cys

To evaluate the proliferation profile of Ba/F3 cells expressing murine KIT-Tyr552Cys in the presence or absence of factors, MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium inner salt) colorimetric assay was performed using CellTiter 96 AQueous One Solution Cell Proliferation Assay (G3580, Promega Corporation, Madison, WI, USA), according to the manufacturer's instruction. Briefly, cells were plated in a 96-well plate at a concentration of 1.5×10^4 cells/well and cultured with various concentrations of recombinant mouse (rm) IL-3 (0, 0.01, 0.1, 1 and 10 ng/ml) or rmSCF (0, 1, 10, 100, and 1000 ng/ml) for 48 h. Then cells were further cultured for 2 h in the presence of MTS. The optical density was measured with a test wavelength of 490 nm and a reference wavelength of 650 nm. Ba/F3 cells expressing another murine exon 11 c-*kit* gene mutation, KIT-del-(Val558&Val559), corresponding to human exon 11 c-*kit* gene mutation, KIT-del-(Val559&Val560), which are known to show factor-independent growth,[1] and those expressing murine wild-type c-*kit* gene, which are known to show factor-dependent growth,[1] were used as comparisons.

Effect of KIT Inhibitors on Autophosphorylation of Murine KIT-Tyr552Cys

To evaluate the effect of imatinib (a generous gift from Novartis, Basel, Switzerland) and nilotinib (a generous gift from Novartis) on autophosphorylation of murine mutant KIT-Tyr552Cys, 3×10^6 Ba/F3 cells expressing the mutation were cultured at various concentrations of imatinib (0, 0.001, 0.01, 0.1, 1 and 10 μM) or nilotinib (0, 0.001, 0.01, 0.1, 1 and 10 μM) for 90 min. Then, cells were collected and lysed as described previously.[1] Phosphorylation of KIT was detected by rabbit polyclonal anti-phospho-c-KIT antibody (pTyr823, Affinity BioReagents, Golden, CO, USA). Reprobing was done with rabbit polyclonal anti-KIT antibody (A4502, Dako, Glostrup, Denmark). Ba/F3 cells expressing another murine exon 11 c-*kit* gene mutation, KIT-del-(Val558&-Val559), corresponding to human c-*kit* gene mutation,

KIT-del-(Val559&Val560), which is known to be sensitive to imatinib,[25] were used as a comparison. Ba/F3 cells expressing murine c-*kit* gene mutation, KIT-Val653Ala, corresponding to human c-*kit* gene mutation, KIT-Val654Ala, which is known to be resistant to imatinib,[51] were also used as another comparison.

Effect of KIT Inhibitors on Proliferation of Ba/F3 Cells Expressing Murine KIT-Tyr552Cys

To evaluate the effect of imatinib and nilotinib on proliferation of Ba/F3 cells expressing murine mutant KIT-Tyr552Cys, MTS colorimetric assay was performed using CellTiter 96 AQueous One Solution Cell Proliferation Assay (Promega). Briefly, cells were plated in a 96-well plate at a concentration of 1.5×10^4 cells/well and cultured with various concentrations of imatinib (0, 0.001, 0.01, 0.1, 1 and 10 μM) or nilotinib (0, 0.001, 0.01, 0.1, 1 and 10 μM) for 48 h. Then the cells were further cultured for 2 h in the presence of MTS. The optical density was measured with a test wavelength of 490 nm and a reference wavelength of 650 nm. Ba/F3 cells expressing another murine c-*kit* gene mutation, KIT-6 codons-(549–554) to Ile, corresponding to human c-*kit* gene mutation, KIT-6 codons-(550–555) to Ile, were used as a control. Ba/F3 cells expressing murine c-*kit* gene mutation, KIT-Val653Ala, corresponding to human c-*kit* gene mutation, KIT-Val654Ala, were also used as another control.

RESULTS

Analysis of Germline c-*kit* Gene Mutation in Family Members

We examined whether family members of the proband (case 6 in Figure 1) had the germline mutation, KIT-Tyr553Cys, by direct sequencing of genomic DNA from peripheral blood leukocytes. A son (44-year-old, case 7 in Figure 1) of the proband had the same germline mutation of the c-*kit* gene at exon 11 (data not shown). On the other hand, a brother (80-year-old, case 3 in Figure 1), a sister (76-year-old, case 4 in Figure 1) and a daughter (40-year-old, case 8 in Figure 1) of the proband did not show any mutations at exons 9, 11, 13 and 17 (data not shown). A sister (72-year-old, case 5 in Figure 1) of the proband did not allow us to examine the mutation. As the father (case 1 in Figure 1) and mother (case 2 in Figure 1) of the proband died from senility at age of 88 and 90 years, respectively, we could not analyze genomic DNA of them. There was a possibility that the son's two children (a 13-year-old son and a 10-year-old daughter, case 9 and case 10 in Figure 1, respectively) had the germline mutation, but we could not carry out the mutational analysis in them because of their young age. All family members examined including the proband did not report dysphagia, hyperpigmentation and symptoms suggesting mast cell tumor, germ cell tumor or neurofibromatosis type 1.

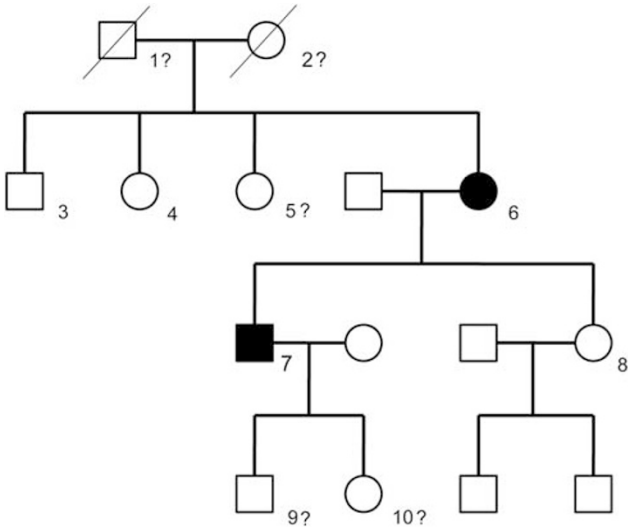

Figure 1 Pedigree of the family. Case 6 is the proband. Filled symbols show the family members with germline c-*kit* gene mutation. Squares and circles indicate males and females, respectively. Symbols with '/' mean dead cases at the time of investigation. Persons who have not taken mutational test because of refusal (cases 5, 9 and 10) or death (cases 1 and 2) are indicated by '?'.

Proliferation Profile of Ba/F3 Cells Expressing Murine KIT-Tyr552Cys

Murine c-*kit* gene mutation (murine KIT-Tyr552Cys) corresponding to human c-*kit* gene mutation (human KIT-Tyr553Cys) was introduced into expression vector possessing murine c-*kit* cDNA, and it was stably transfected into the IL-3-dependent Ba/F3 cells. MTS colorimetric assay was carried out to clarify the proliferation profile of the transfectant in the presence or absence of rmIL-3 and rmSCF. Transfectant expressing murine wild-type KIT and that expressing another exon 11 murine mutant KIT, which is proved to be factor-independent cells,[1] were used as controls. Ba/F3 cells with wild-type KIT responded to both rmIL-3 and rmSCF (Figures 2a and b). On the other hand, Ba/F3 cells with murine mutant KIT-Tyr552Cys grew autonomously without rmIL-3 and rmSCF as observed in those with another exon 11 mutant KIT (Figures 2a and b).

Effect of KIT Inhibitors on Autophosphorylation of Murine KIT-Tyr552Cys

Ba/F3 cells expressing murine mutant KIT-Tyr552Cys were treated with imatinib or nilotinib, and autophosphorylation of the mutant KIT was examined. Transfectant with another exon 11 mutant KIT, which is known to be sensitive to imatinib,[25] and that with exon 13 mutant KIT, which is known to be resistant to imatinib,[51] were also treated with the reagents as controls. Autophosphorylation of mutant KIT-Tyr552Cys was inhibited by imatinib at the concentration of 0.1 μM as well as that of another exon 11 mutant KIT (Figure 3). In contrast, autophosphorylation of murine exon 13 mutant KIT was not inhibited at the concentration of

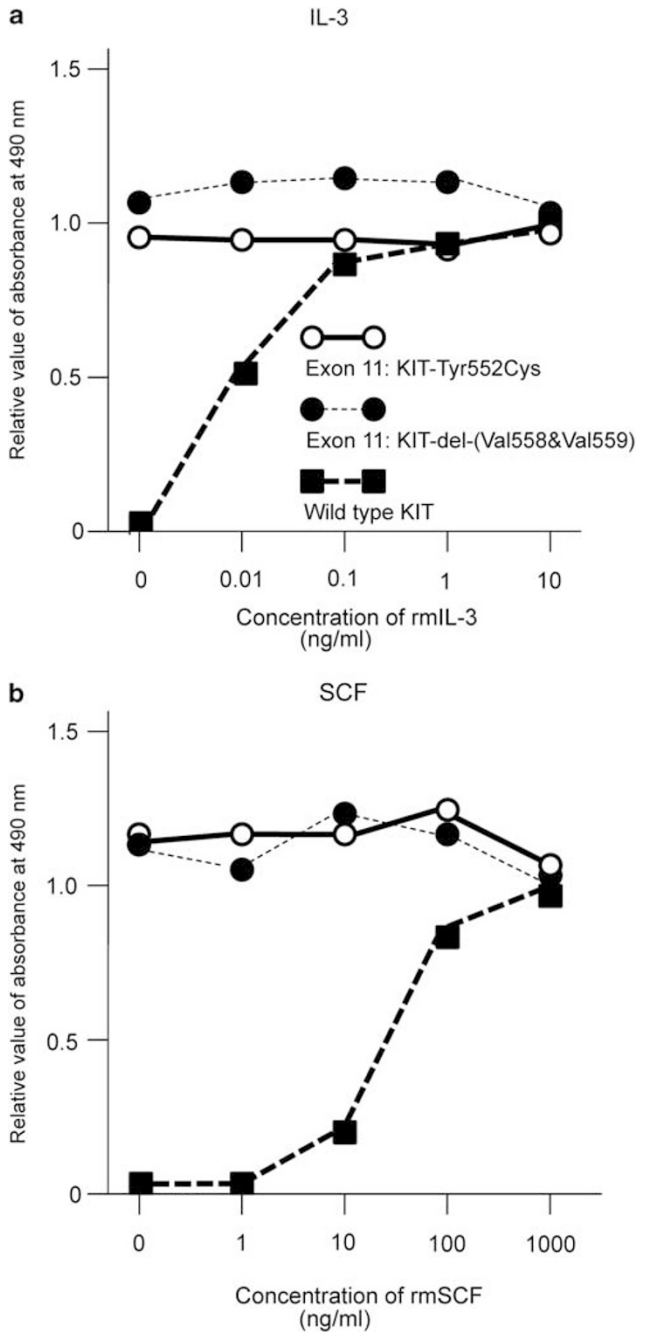

Figure 2 Autonomous proliferation of Ba/F3 cells with murine KIT-Tyr552Cys. Ba/F3 cells with mouse wild-type c-*kit* cDNA and those with murine KIT-del-(Val558&Val559) were used as controls. (**a**) MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium inner salt) colorimetric assay in the presence or absence of recombinant mouse (rm) interleukin-3 (IL-3). (**b**) MTS colorimetric assay in the presence or absence of rm stem cell factor (SCF). Ba/F3 cells with wild-type KIT responded to both rmIL-3 and rmSCF, but those with KIT-Tyr552Cys and KIT-del-(Val558&Val559) grew autonomously without rmIL-3 and rmSCF. Ba/F3 cells with mutant KIT-Tyr552Cys (○), Ba/F3 cells with mutant KIT-del-(Val558&Val559) (●), and Ba/F3 cells with wild KIT (■). Data are expressed as the mean of six wells. The values of vertical axis are expressed as the relative ones when the mean value of the optical density on 10 ng/ml is regarded as 1.0 in case of rmIL3 and that on 1000 ng/ml is regarded as 1.0 in case of rmSCF.

0.1 μM and was completely inhibited just at the concentration of 10 μM (Figure 3). When nilotinib was used instead of imatinib, autophosphorylation of mutant KIT-Tyr552Cys was similarly inhibited at the concentration of 0.1 μM (Figure 4). Autophosphorylation of another exon 11 mutant KIT was also inhibited at the concentration of 0.1 μM, and that of the murine exon 13 mutant KIT was completely inhibited just at the concentration of 10 μM (Figure 4).

Effect of KIT Inhibitors on Proliferation of Ba/F3 Cells Expressing Murine KIT-Tyr552Cys

MTS assay was carried out to assess the inhibitory effect of imatinib and nilotinib on the proliferation of transfectant with mutant KIT-Tyr552Cys. Ba/F3 cells expressing another exon 11 mutant KIT and those expressing imatinib-resistant exon 13 mutant KIT were also examined as controls. Imatinib completely inhibited autonomous proliferation of both Ba/F3 cells expressing mutant KIT-Tyr552Cys and those expressing another exon 11 mutant KIT at the concentration of 0.1 μM.

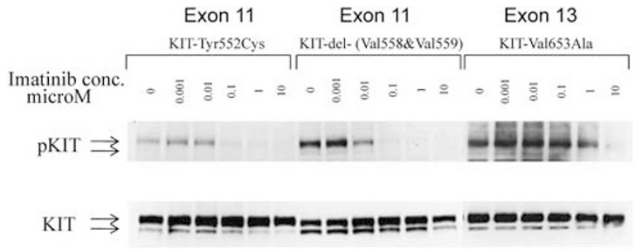

Figure 3 Effect of imatinib on autophosphorylation of murine KIT-Tyr552Cys. Autophosphorylation of mutant KIT was examined after 90 min incubation of Ba/F3 cells expressing the mutant KIT at concentrations of 0, 0.001, 0.01, 0.1, 1 and 10 μM of imatinib. Imatinib-sensitive murine KIT-del-(Val558&Val559) and imatinib-resistant murine KIT-Val653Ala were also examined as controls. Autophosphorylation of KIT-Tyr552Cys was almost completely inhibited at a concentration of 0.1 μM as well as that of KIT-del-(Val558&Val559). In contrast, autophosphorylation of KIT-Val653Ala was not even at 1 μM.

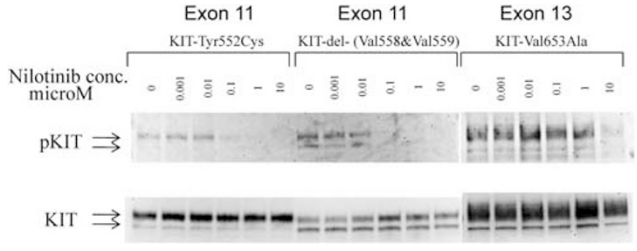

Figure 4 Effect of nilotinib on autophosphorylation of murine KIT-Tyr552Cys. Autophosphorylation of mutant KIT was examined after 90 min incubation of Ba/F3 cells expressing the mutant KIT at concentrations of 0, 0.001, 0.01, 0.1, 1 and 10 μM of nilotinib. Imatinib-sensitive murine KIT-del-(Val558&Val559) and imatinib-resistant murine KIT-Val653Ala were also examined as controls. Similarly in imatinib, autophosphorylation of KIT-Tyr552Cys and KIT-del-(Val558&Val559) was almost completely inhibited at a concentration of 0.1 μM; on the other hand, that of KIT-Val653Ala was just at 10 μM.

However, autonomous proliferation of Ba/F3 cells expressing exon 13 mutant KIT was inhibited just at the concentration of 10 μM (Figure 5). Nilotinib also completely inhibited autonomous proliferation of BaF/3 cells expressing mutant KIT-Tyr552Cys at the concentration of 0.1 μM. Autonomous proliferation of Ba/F3 cells with another exon 11 mutant KIT was also inhibited at 0.1 μM, but that with exon 13 mutant KIT was just at the concentration of 10 μM (Figure 6).

DISCUSSION

So far, ~20 families with germline c-*kit* gene mutations and multiple GISTs have been reported,[30–49] and the mutations were detected at exon 11 in 12 families.[30,31,33,34,36–40,43,44,46,49] KIT-Val559Ala mutation is the most frequent type, which has been detected in five families.[33,34,38,39,49] Recently, we found a germline mutation at exon 11, KIT-Tyr553Cys, in a 68-year-old woman with multiple GISTs. To our knowledge, this type of mutation has not been reported yet. In the present study, we carried out analysis of the novel germline mutation in the family members to determine the carriers, and characterized the mutation including inhibitory effect of TK inhibitors such as imatinib and nilotinib.

We found that a 44-year-old son of the proband had the germline mutation, but at present he did not show apparent symptoms and images of multiple GISTs. The penetration rate of multiple GIST development in persons with various types of germline c-*kit* gene mutations appears to be nearly 100%. As multiple GISTs and symptoms associated with the tumors often develop in over 50-year-old persons, the son must be closely followed hereafter for development of multiple GISTs.

Most of the somatic gain-of-function mutations of the c-*kit* gene are detected at exon 9, exon 11, exon 13 or exon 17 in GISTs, mast cell tumors, seminomas or melanomas.[1,15–23] Similarly, germline loss-of-function mutations of the c-*kit* gene in various W mutant mice are often observed at exon 11, exon 13 or exon 17.[52] Mutation of murine c-*kit* gene at codon 582, KIT-Gln582Lys, corresponding to human KIT-Gln583Lys is a cause of W^{37} loss-of-function mutant mice.[52]

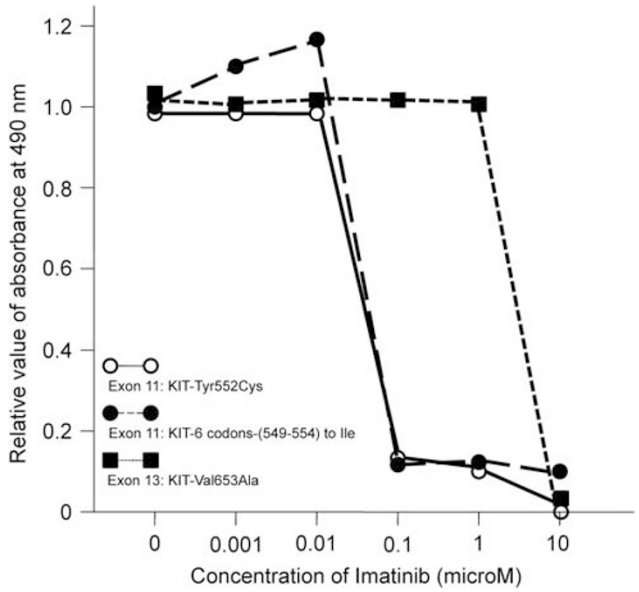

Figure 5 Effect of imatinib on *in vitro* cell proliferation of Ba/F3 cells with murine KIT-Tyr552Cys. Cells were incubated for 48 h at concentrations of 0, 0.001, 0.01, 0.1, 1 and 10 μM of imatinib, and MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium inner salt) colorimetric assay was carried out. Ba/F3 cells expressing KIT-Tyr552Cys, those expressing imatinib-sensitive murine KIT-6 codons-(549–554) to Ile, and those expressing imatinib-resistant murine KIT-Val653Ala were used. Proliferation of both BaF3 cells expressing KIT-Tyr552Cys and those expressing KIT-6 codons-(549–554) to Ile was completely inhibited at the concentration of 0.1 μM of imatinib. On the other hand, proliferation of Ba/F3 cells expressing KIT-Val653Ala was inhibited just at the concentration of 10 μM. Ba/F3 cells with murine KIT-Tyr552Cys (○), Ba/F3 cells with murine KIT-6 codons-(549–554) to Ile (●) and Ba/F3 cells with murine KIT-Val653Ala (■). Data are expressed as the mean of six wells. The values are expressed as the relative ones when each mean value of the optical density on 0 μM of imatinib is regarded as 1.0.

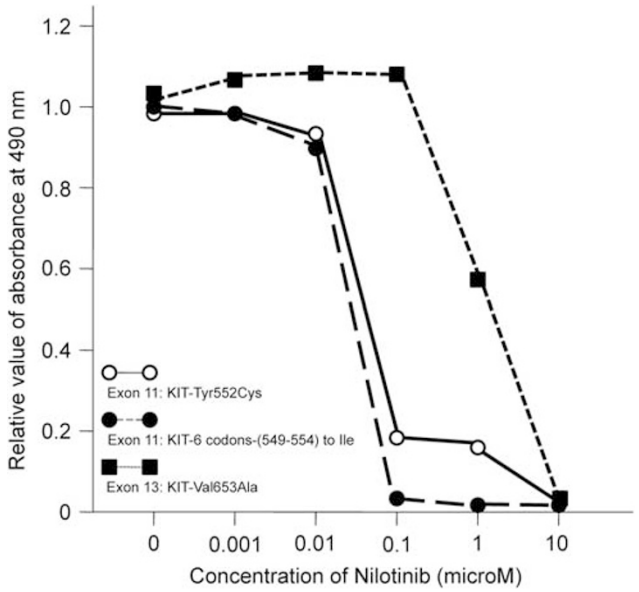

Figure 6 Effect of nilotinib on *in vitro* cell proliferation of Ba/F3 cells with murine KIT-Tyr552Cys. Cells were incubated for 48 h at concentrations of 0, 0.001, 0.01, 0.1, 1 and 10 μM of nilotinib, and MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium inner salt) colorimetric assay was carried out. Similarly in imatinib, Ba/F3 cells expressing KIT-Tyr552Cys, those expressing imatinib-sensitive murine KIT-6 codons-(549–554) to Ile and those expressing imatinib-resistant murine KIT-Val653Ala were used. Nilotinib completely inhibited proliferation of both BaF3 cells expressing KIT-Tyr552Cys and those expressing KIT-6 codons-(549–554) to Ile at the concentration of 0.1 μM. In contrast, proliferation of Ba/F3 cells expressing KIT-Val653Ala was inhibited just at the concentration of 10 μM. Ba/F3 cells with murine KIT-Tyr552Cys (○), Ba/F3 cells with murine KIT-6 codons-(549–554) to Ile (●) and Ba/F3 cells with murine KIT-Val653Ala (■). Data are expressed as the mean of six wells. The values are expressed as relative ones when each mean value of the optical density on 0 μM of nilotinib is regarded as 1.0.

Therefore, it does not always mean that the c-*kit* gene mutations at exon 11 are of gain-of-function. In the present study, we clarified that transfection of the murine c-*kit* gene mutation, KIT-Tyr552Cys, corresponding to human c-*kit* gene mutation, KIT-Tyr553Cys, converted IL-3-dependent Ba/F3 cells to factor-independent cells. This suggests that the c-*kit* gene mutation, KIT-Tyr553Cys, is of gain-of-function and the cause of familial and multiple GISTs in the present case.

The proband showed ICC hyperplasia, which is nearly always observed in patients with familial and multiple GISTs. Although hyperpigmentation is often seen and mast cell tumor is sometimes seen in previously reported patients with exon 11 germline c-*kit* gene mutation,[30,33,34,36–38,44,48] the present patient and her son, a possible carrier, did not show those phenotypes. Dysphagia is also a symptom that is often seen in exon 17 germline c-*kit* gene mutation,[35,42] but is rarely seen in exon 11 germline ones.[36] In fact, the present patient and her son also did not have the symptom. Those phenotypes and symptoms might not be associated with this type of germline c-*kit* gene mutation.

As described above, various types of exon 11 germline c-*kit* gene mutations have been reported,[30,31,33,34,36–40,43,44,46,49] but KIT-Tyr553Cys mutation has not been reported yet. Even in somatic c-*kit* gene mutation in sporadic GISTs, substitution of amino acid at codon 553 appears to be very rare. We experienced only one case of sporadic GIST with substitution at codon 553 in over 300 sporadic GISTs examined. Moreover, the mutation is not Tyr553Cys but Tyr553Asn (unpublished data). In malignant melanoma, a case with Tyr553Asn mutation has been reported.[20]

Most of the exon 11 mutations of the c-*kit* gene are imatinib sensitive.[53] However, we reported that certain type of the exon 11 mutation observed in mast cell tumor, Val559Ile, is resistant to imatinib.[54] In the present study, we clarified whether murine KIT-Tyr552Cys corresponding to human KIT-Tyr553Cys is sensitive to imatinib and nilotinib. Phosphorylation of the KIT-Tyr552Cys was nearly completely inhibited at the concentration of 0.1 μM of both imatinib and nilotinib, as well as that of another exon 11 mutant KIT that is proved to be sensitive to imatinib. In fact, proliferation of the cells expressing KIT-Tyr552Cys was nearly completely inhibited at the concentration of 0.1 μM of both imatinib and nilotinib, as well as that expressing another exon 11 mutant KIT. The novel type of the human KIT-Tyr553Cys mutation is considered to be sensitive to both imatinib and nilotinib.

In summary, we found a carrier with the novel type of the c-*kit* gene mutation in the family, and showed that the mutation is imatinib and nilotinib sensitive. When the patients of the family need the treatment by imatinib or nilotinib, these drugs might be effective on control of GISTs.

DISCLOSURE/CONFLICT OF INTEREST

Dr Hirota has received donations for research support from Novartis. All authors except Dr Hirota do not have any conflict of interest declared.

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