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Epidemiology and molecular biology of gastrointestinal stromal tumors (GISTs): a population-based study in the South of Switzerland, 1999-2005

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Summary. Introduction. Gastrointestinal stromal tumors (GISTs) are characterized at the molecular level by *c-kit* or *PDGFRA* oncogene mutations. Although GISTs raised major interest in past decades, population-based studies are still rare. Materials and Methods. All GISTs diagnosed in Southern Switzerland (1999-2005) were identified using Ticino Cancer Registry and analysed for *c-kit* and PDGFRA mutations. Clinical and molecular features were studied. Results. Annual incidence of GISTs was 1.47 cases/100,000 inhabitants (median age: 64 years; median size: 6.0 cm). Most GISTs arose in the stomach (60.5%). The malignancy risk was very-low/low in 47% of patients. DNA sequences showed a gene alteration in either *c-kit* or *PDGFRA* genes in 72.5% of patients. Mutations occurred mostly in *c-kit* exon 11 (60%). No mutations in *c-kit* exons 13 or 17 were found. An equal number of alterations in exons 12 and 18, and no mutations in exon 14 were observed in the PDGFRA gene. Discussion. This is the first comprehensive population-based study of GISTs incidence and molecular biology characterization in Central Europe. Our incidence data showed higher age-standardized rates compared to other European countries. The gene mutation spectrum differed when compared to the literature. This is relevant to improve the molecular profile knowledge based on Cancer Registry data.

Key words: Gastrointestinal stromal tumors, c-kit, PDGFRA, Epidemiology, Cancer registry

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Introduction

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal neoplasms of the gastrointestinal tract. In the past they were classified heterogeneously under various terms, including leiomyoma, leiomyosarcoma, leiomyoblastoma, angiosarcoma, gastrointestinal autonomic nerve tumors (GANT), etc (Graadt van Roggen et al., 2001; Miettinen et al., 2002; Yamanda, 2003; Rubin, 2006). The recognition that these tumors express CD117 (tyrosine kinase receptor kit) in about 95% of patients increased diagnostic accuracy considerably (Kindblom et al., 1998; Graadt van Roggen et al., 2001; Miettinen and Lasota, 2001).

At a molecular level, GISTs are characterized by hyperactivating somatic mutations either in *c-kit* or PDGFRA genes (Hirota et al., 1998; Rubin et al., 2001; Corless et al., 2005). These mutations occur mostly in the tyrosine kinase or juxtamembrane domains, or in the extracellular region immediately adjacent to the plasma membrane. GISTs are one of the most attractive neoplasms for targeted biological therapy. In fact, Imatinib (also named Gleevec, Glivec or STI571) binds c-kit or PDGFRA proteins and inhibits their ability to promote tumor growth (Demetri et al., 2002). Each type of mutation leads to a different drug sensitivity or resistance (Corless et al., 2004; Penzel et al., 2005; Andersson et al., 2006) and is therefore an important predictive factor that must be considered, together with other well characterized prognostic factors, such as size, location, mitotic index of tumors, and presence of synchronous metastatic disease, before making treatment decisions.

Although GISTs raised enormous interest over the past decade, population-based studies providing data about tumor incidence are rare (Goettsch et al., 2005;

Nilsson et al., 2005; Tryggvason et al., 2005; Steigen and Eide, 2006; Mucciarini et al., 2007; Rubió et al., 2007) and there is a lack of information on the characteristic molecular alterations (Steigen et al., 2007). This is somewhat surprising since knowledge on molecular events involved in GISTs development obtained from population-based studies may be essential for the evaluation of clinical studies with targeted therapies. Thus, the present retrospective study, based on cases surgically resected in Ticino (Southern Switzerland) between 1999 and 2005, is the first report of both clinical and molecular data concerning a Central European population.

Materials and methods

Retrieval of patients

The Ticino Cancer Registry is a population-based cancer registry located in the South of Switzerland and covers a Caucasian population of about 320.000 inhabitants.

All potential patients with GIST tumors diagnosed in Ticino in the 7-year period between 1999 to 2005 were retrieved. GISTs have been recorded as such in Ticino since 2002, when the Cancer Registry started adopting the third version of the International Classification of Diseases for Oncology, which identifies gastrointestinal stromal tumors as a distinct entity (Fritz et al., 2000). However, in order to include all cases possible in the study, a manual search was performed both for cases occurring between 1999 and 2001 and for data between 2002 and 2005. Histopathologic findings were collected, through a comprehensive computer search of topography and morphology codes (ICDO, III edition, WHO) identifying the disease (Fritz et al., 2000). The information sources were the files of the Institute of Pathology of Locarno, the unique diagnostic laboratory available in Ticino, which has received all surgical pathology specimens of this nature since 1978; the Cancer Registry of Ticino and the a-priori defined sources usually consulted by a population-based cancer registry (Jensen et al., 1991). An additional search by location and morphology code was performed using the Canton Information System and the Autopsy registry.

The search by topography codes consisted of selecting all sites of the gastrointestinal tract, as well as the intra-abdominal, retroperitoneal, mesenteric, omental and pelvic regions. Morphology codes included all types of benign and malignant mesenchymal neoplasms. As recently reported by Miettinen and colleagues, a further search was performed including specific terms, such as leiomyoma, leiomyosarcoma, leiomyoblastoma, haemangioma, haemangioendothelioma, angiosarcoma, schwannoma, peripheral nerve sheath tumor, gastrointestinal autonomic nerve tumor (GANT), epitheloid leiomyoma or leiomyiosarcoma, mesenchymal tumor, glomus tumor, neurofibroma, fibromatosis, desmoid, inflammatory pseudotumor, inflammatory myofibroblastic tumor, solitary fibrous tumor, stromal sarcoma, undifferentiated sarcoma, sarcoma not otherwise specified (NOS) of the gastrointestinal tract and dedifferentiated liposarcoma (Miettinen et al., 2002). An additional search consisted of extracting from the Pathology data-base all reports of surgical resection involving the gastro-intestinal tract.

To estimate the malignancy risk of the GISTs included in the studies, we used the consensus criteria recommended by the National Institutes of Health (NIH) in 2001, i.e. tumor size and mitotic rate per 50 high power field (hpf) (Fletcher et al., 2002).

Accordingly, each tumor was assigned to one of 4 groups: very low risk (<2 cm diameter and <5 mitoses/50 hpf), low risk (2-5 cm and <5 mitoses/50 hpf), intermediate risk (<5 cm and 6-10 mitoses/50 hpf, or 5-10 cm and <5 mitoses/50 hpf) and high risk (>5 cm and >5 mitoses/50 hpf, or >10 cm regardless of mitotic rate, or any size and >10 mitoses/50 hpf) (Fletcher et al., 2002).

Immunohistochemistry analysis

Immunohistochemical reactions were performed with a Benchmark automatic immunostaining device (Ventana Medical System, Tucson, AZ, USA) according to the manufacturer's instructions. Three-µm thick tissue sections were incubated after heat induced antigen retrieval with antibodies against CD117 [clone A4502, DakoCytomation (Glostrup, Denmark), 1:50 dilution]. Positive and negative controls were included in each slide run.

Molecular analysis

Primary tumors were surgically resected, fixed in 10% buffered formalin, embedded in paraffin and, after histopathological diagnosis, archived at the Institute of Pathology, Locarno, Switzerland. All formalin-fixed paraffin-embedded tumor blocks were reviewed for quality and tumor content, and a single representative tumor block from each case, containing at least 70% neoplastic cells, was selected for immunohistochemical and molecular analyses.

Genomic DNA was extracted using the QIAamp Mini kit (Qiagen, Chatsworth, CA, USA) according to the manufacturer's instructions.

We searched for *c-kit* mutations in exons 9, 11, 13, 14 and 17, and for *PDGFRA* mutations in exons 12, 14 and 18, according to previous studies (Miselli et al., 2007). Briefly, polymerase chain reaction (PCR) conditions and profile were common to all exons. One hundred ng of genomic DNA were amplified in a final reaction volume of 25 µl containing 1X PCR Buffer II (Applied Biosystems, Foster City, CA, USA), 2.5 µM $MgCl_2$ (Applied Biosystems), 0.2 mM dNTPs (Amersham), 0.04 mM dUTP (Amersham), 0.4 µm of each primer, 1U of uracil-N-glycosilase (UNG) (Roche) and 1.25 U of AmpliTaq Gold (Applied Biosystems).

PCRs were carried out with an initial pre-PCR step at 50°C for 2 min for UNG incubation, another pre-PCR step at 96°C for 10 min for UNG inactivation and AmpliTaq Gold activation, followed by 40 cycles at 96°C for 30 sec, at the specific annealing temperature for 1 min, and at 72°C for 1 min, followed by a final elongation step at 72°C for 7 min. PCR products were electrophoresed on 1.8% agarose gel and visualized with ethidium bromide staining under UV-light. After purification with the QIAquick PCR purification kit (QIAGEN), PCR products were subjected to automated sequencing by ABI PRISM 3100 (Applied Biosystems) and analyzed with Chromas software. Each sequence reaction was performed at least twice, starting from independent PCR reactions. In each case, the detected mutation was confirmed in the sequence as sense and antisense strands.

Statistical methods

Incidence rates were calculated as crude rate and age-standardized rate on the European population (Esteve et al., 1994). This method removes the strong correlation existing between patient age and tumor occurrence, deleting all effects of demographic change on the Ticino population, and therefore allows comparisons with other population-based studies. The age-standardized rate is provided for the whole period (1999-2005). Statistically significant associations were detected through the Fisher's exact test; p-value less than 0.05 was considered significant. The statistical analysis was performed by means of the SAS System V.9.1 Software.

Ethical considerations

This was a retrospective study; no study-driven clinical intervention was performed and no material was sent to external institutions. Thus, a simplified Institutional Review Board approval for retrospective studies was obtained and patient consent was not considered to be necessary.

Results

One hundred potential GISTs were retrieved in the 1999-2005 period and all histological material was reviewed by a pathologist. Fifty-seven patients were excluded, 5 because they did not reside in Ticino at the time of diagnosis, and 52 because they did not fulfill the microscopical criteria of GIST. In the remaining 43 GISTs included in the study, CD117 expression was strong in 40 cases (93%), weak in 2 (4.6%) and not evaluated in one case (2.3%) because of unavailability of paraffin blocks.

Patient and tumor characteristics are summarized in Table 1. The series included 19 men (44.2%) and 24 women (55.8%), with a median age of 64 years (range 39-96). Patients were younger than 49 at time of diagnosis in 14% of the cases, between 50 and 69 in 49%, and older than 70 in 37%. The majority of GISTs arose in the stomach (60.5%) or in the small intestine (25.6%), whereas the remaining cases were equally distributed between colon, rectum and retroperitoneoum. Only 4 patients (9.3%) had metastases at the time of diagnosis: 3 in the liver (histologically confirmed) and one in the bones (diagnosed clinically). Median tumor size was 6.0 cm (range 0.4-22.0 cm). In particular, 39% of the tumors measured less than 5 cm, 34% ranged between 5 and 10 cm, and 27% measured more than 10 cm. Gastric tumors were generally smaller than non-gastric GISTs (4.3 cm vs. 8.0 cm). The estimated malignancy risk was very low in 9 patients (21%), low in 11 (26%), intermediate in 6 (14%), and high in 17 (39%).

Additional malignant tumors were detected in 13 patients (30.2%). In 5 patients (11.6%) the tumor developed before GIST diagnosis; 7 patients (16.3%) had a synchronous cancer, and in one patient (2.3%) a second tumor was diagnosed after the GIST. In this context it is noteworthy that eight GISTs (19%) were incidentally detected after surgical procedures for other causes. None of these cases were classified as high risk, whereas 23 out of 35 (63%) primarly diagnosed GISTs were assigned to intermediate or high risk categories ($p<0.0038$).

The mean annual number of cases in Ticino for the period considered was 6.1. This result, expressed in terms of annual crude incidence rate and European age-standardized rate, is for both sexes 1.96 and 1.47 cases per 100,000 inhabitants/year, respectively. Comparing age-specific rate curves by sex we noticed that most cases were concentrated between 55 and 69 years of age in men, whereas the curve reaches a peak between 70-79 years and over 85 years in women (data not shown). Incidence rates, evaluated in two different periods, were

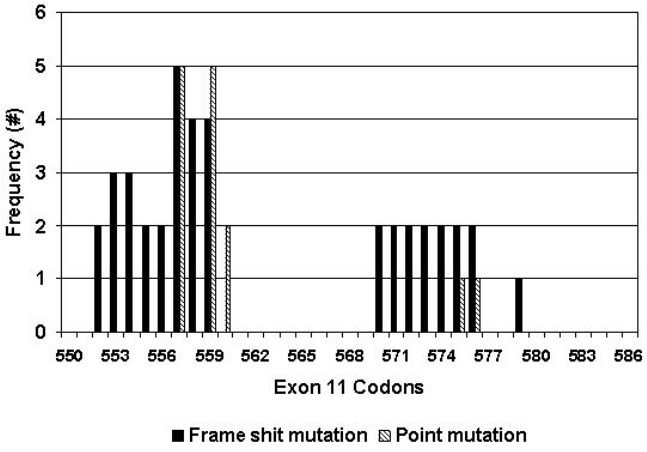

Fig. 1. Involvement of *c-kit* exon 11 codons by mutations in 40 GISTs from Ticino (Southern Switzerland) between 1999 and 2005.

1.44 and 1.51 in 1999-2002 and in 2003-2005, respectively. At the end of follow-up (December 31, 2006) there were a total of 27 living patients (62.8%). Of 16 patients who had died (37.2%), eight died of GIST (18.6%), 3 of a second malignant disease (7.0%), three of suicide (7.0%) and two (4.6%) of unknown causes.

The molecular data concerning the *c-kit* and *PDGFRA* genes are reported in Table 2. One patient (# 16) was excluded because of the unavailability of paraffin blocks. The amplification procedures on two patients failed because of poor DNA quality. Thus, DNA sequences were available for 40 patients. Overall, 29 patients showed at least a gene alteration in *c-kit* or *PDGFRA* genes (72.5%). The vast majority occurred in *c-kit* exon 11 (24 cases), with 14 point mutations and 12 frame-shift mutations. Point mutations occurred predominantly at codons 557 (all changes W557R) and 559 (3 changes V559D and 2 changes V559A). Two point mutations occurred at codon 560 (one change V560D and one V560G), one at codon 575 and one at codon 576. As regards *c-kit* exon 11 frame-shift mutations, all alterations detected in the present cohort of patients were codon deletions (Δ), predominantly involving codons 557-559. In particular, 2 cases showed Δ557-558, 2 cases Δ557-559 and 2 cases Δ570-576. In the other exons of the c-kit gene, point mutations were exclusively found in exon 9, consisting of the well characterized duplication of codons 502-503, and the missense mutation at codon 476 (S476I), which were observed once each. Case # 18 showed the silent T461T mutation. No alterations were detected in exons 13, 14 and 17. The analysis of exon 17 revealed three polymorphisms, two located in the 5'-untranslated region (-97 and -67 positions) and one in the coding sequence (at codon 798). At position -97, the vast majority of patients (n=35) were homozygous for T, three were homozygous for A and 2 were heterozygous. At position -67, G homozygosity was found in 30 patients (75%), A homozygosity in one case and heterozygosity in the 9 remaining patients. The C→T

Table 1. Patient and tumor characteristics of GISTs in Ticino (Southern Switzerland), 1999-2005, and in other population-based studies.

Clinical Variable	Ticino (Switzerland)1999-2005	Western Sweden 1983-2000[1]	Girona (Spain) 1994-2001[2]	Iceland 1990-2003[3]
Number of patients	43	288	46	57
Age				
median (yrs)	64	69	63	66
range	(39 - 96)	(10 - 92)	(26 - 90)	(24 - 90)
Sex				
female	24 (55.8%)	144 (50%)	24 (52.2%)	24 (42.1%)
male	19 (44.2%)	144 (50%)	22 (47.8%)	33 (57.9%)
Size of tumors				
median (cm)	6.0 cm	7.0 cm	-	4.6 cm
range	(0.4 - 22.0)	(0.5 - 35.0)		(0.4 - 20.0)
NIH risk categories				
I	9 (21%)	48 (16.7%)	15 (32.6%)‡	13 (24.1%)
II	11 (26%)	96 (33.3%)		18 (33.3%)
III	6 (14%)	55 (19%)	14 (30.4%)	10 (18.5%)
IV	17 (39%)	89 (31%)	17 (37.0%)†	13 (24.1%)
Tumor primary location				
Oesophagus	0 (0%)	0 (0%)	0 (0%)	2 (3.5%)
Stomach	26 (60.5%)	170 (59%)	23 (50.0%)	35 (61.4%)
Small intestine	11 (25.6%)	97 (33.7%)	20 (43.5%)	17(29.8%)
Colon-Rectum	4 (9.3%)	18 (6.3%)	1 (2.2%)	2 (3.5%)
Other *	2 (4.6%)	3 (1.0%)	2 (4.3%)	1 (1.8%)
Clinical behaviour at diagnosis				
Nonmetastatic	39 (90.7%)	259 (90%)	-	49 (86%)
Metastastatic	4 (9.3%)	29 (10%)	-	8 (14%)
Time of follow-up				
median (yr)	2.6 years	-	4.5 years	2.6 years
range	0- 7.8	-	0.25-10.3	0.08-12.1
Number of Deaths				
all causes	13 (30.2%)	-	16 (34.8%)	18 (31.6%)
GIST related	7 (16.3%)	-	-	5 (11.6%)
Incidence Rates				
(European ASR)	1.47	1.45	0.9	1.1

*: appendix, retroperitoneum, omentum; ‡: for Girona (Spain), we considered together very low and low risk cases; † for Girona (Spain), we considered together high risk and overtly malignant cases. [1]: Nilsson et al., 2005; [2]: Rubió et al., 2007; [3]: Tryggvason et al., 2005.

polymorphism in codon 798 (I798I) was found to be heterozygous in 5 cases.

Three patients showed two concomitant mutations for the c-kit gene: two cases had both a missense and a frame-shift mutation in exon 11, and one patient carried a missense mutation at codon 559 of exon 11 (V559D) and the silent T461T mutation in exon 9.

Analysis of the PDGFRA gene showed the well known V561D alteration in one patient, the missense mutation at codon 581 (C→T, P581S) in one patient and polymorphism in four patients (3 cases had the silent G→A (P567P) and one case the silent mutation C→T (I565I)) in exon 12. One patient showed the prognostically adverse D842V mutation, one the previously described deletion of codons 843-846, and two cases the silent C→T (V842V) nucleotide mutation in exon 18. No alterations were found in exon 14. If we limit the analysis to missense and frame-shift mutations, c-kit and PDGFRA gene alterations were mutually exclusive, with the exception of patient # 6, who carried a c-kit exon 11 deletion of codons 553-556 and the not yet described P581S missense mutation in *PDGFRA*

Table 2. Molecular characteristics of GISTs, Ticino (Southern Switzerland) 1999-2005.

Obs	Year of Diagnosis	*c-kit* exon 9	*c-kit* exon 11	*c-kit* exon 13	*c-kit* exon 14	*c-kit* exon 17	PDGFRA exon 12	PDGFRA exon 14	PDGFRA exon 18	*c-kit*	*PDGFRA*
1	1999	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
2	1999	WT	Δ559	WT	WT	WT	WT	WT	WT	MUT	WT
3	1999	duplA502-Y503	WT	WT	WT	WT	P567P ‡	WT	WT	MUT	WT
4	1999	WT	Δ559	WT	WT	WT	I565I ‡	WT	WT	MUT	WT
5	1999	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
6	1999	WT	Δ553…556+W557R	WT	WT	WT	P581S	WT	WT	MUT	MUT
7	1999	WT	Δ570…576	WT	WT	WT	WT	WT	WT	MUT	WT
8	2000	WT	Δ557-558	WT	WT	WT	WT	WT	WT	MUT	WT
9	2000	WT	Δ557-558-559	WT	WT	WT	WT	WT	WT	MUT	WT
10	2000	WT	WT	WT	WT	WT	WT	WT	Δ843…846	WT	MUT
11	2000	WT	W557R	WT	WT	WT	WT	WT	WT	MUT	WT
12	2000	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
13	2000	WT	L576P	WT	WT	WT	WT	WT	WT	MUT	WT
14	2001	ne *	ne *	ne *	ne *	ne *	ne *	ne *	ne *	ne *	ne *
15	2001	WT	WT	WT	WT	WT	WT	WT	D842V	WT	MUT
16	2001	na †	na †	na †	na†	na †	na †	na †	na †	na †	na †
17	2001	S476I	WT	WT	WT	WT	WT	WT	WT	MUT	WT
18	2001	T461T ‡	V559D	WT	WT	WT	WT	WT	WT	MUT	WT
19	2001	WT	V559A	WT	WT	WT	WT	WT	WT	MUT	WT
20	2002	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
21	2002	WT	V559D	WT	WT	WT	WT	WT	WT	MUT	WT
22	2002	WT	Δ570…576+Q575R	WT	WT	WT	WT	WT	WT	MUT	WT
23	2002	WT	V559A	WT	WT	WT	WT	WT	WT	MUT	WT
24	2003	ne *	ne *	ne *	ne *	ne *	ne *	ne *	ne *	ne *	ne *
25	2003	WT	V559D	WT	WT	WT	WT	WT	V824V ‡	MUT	WT
26	2003	WT	WT	WT	WT	WT	P567P ‡	WT	WT	WT	WT
27	2003	WT	Δ552…554	WT	WT	WT	WT	WT	WT	MUT	WT
28	2003	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
29	2003	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
30	2004	WT	Δ579	WT	WT	WT	WT	WT	WT	MUT	WT
31	2004	WT	Δ552…557	WT	WT	WT	WT	WT	WT	MUT	WT
32	2004	WT	W557R	WT	WT	WT	WT	WT	V824V ‡	MUT	WT
33	2004	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
34	2004	WT	V560G	WT	WT	WT	WT	WT	WT	MUT	WT
35	2004	WT	Δ557-558-559	WT	WT	WT	WT	WT	WT	MUT	WT
36	2005	WT	W557R	WT	WT	WT	P567P ‡	WT	WT	MUT	WT
37	2005	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
38	2005	WT	W557R	WT	WT	WT	WT	WT	WT	MUT	WT
39	2005	WT	Δ557-558	WT	WT	WT	WT	WT	WT	MUT	WT
40	2005	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
41	2005	WT	WT	WT	WT	WT	V561D	WT	WT	WT	MUT
42	2005	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
43	2005	WT	V560D	WT	WT	WT	WT	WT	WT	MUT	WT
Number (%) of mutated cases		2 (5%)	24 (60%)	0 (0%)	0 (0%)	0 (0%)	2 (5.0%)	0 (0%)	2 (5.0%)	26 (65%)	4 (10%)

*ne: not evaluable; † na: not available; ‡ polymorphism

exon 12.

Discussion

This report is the first population-based study for Central Europe and the first molecular characterization of a population-based cohort of GISTs.

Poor knowledge of these neoplasms, as well as controversy regarding their classification and immunophenotype have considerably hampered previous retrospective population-based studies. This is highlighted by the work of Goettsch et al. who, considering cases between 1995 and 2003, showed a progressive increase in GISTs incidence rates until 2002, when evaluation of CD117 expression was introduced into routine histopathological practice (Goettsch et al., 2005). In this context, the stability of the incidence rates observed in the present series indirectly confirms the completeness of the data.

The number of GISTs included in the present cohort is comparable to those of published Northern and Southern European population-based studies, even though in the Swedish report a larger cohort was analyzed over a longer period of time (Table 1) (Nilsson et al., 2005; Tryggvason et al., 2005; Rubió et al., 2007). The age-standardized rate of 1.47 in Ticino is similar to that observed in Western Sweden (1.45 cases per 100.000 inhabitants/year) and higher than those of Iceland and Spain (1.1 and 0.9 cases per 100.000 inhabitants/year, respectively) (Nilsson et al., 2005; Tryggvason et al., 2005; Rubió et al., 2007). With respect to patient and tumor characteristics, we observed a substantial overlap of our data with those of the other population-based studies, with slight differences. For instance, GISTs are more common in females in Ticino (55.8% of cases), North Italy (53.2%), Western Sweden (50%) and Spain (52.2%) whereas in Iceland male patients are predominant (57.9% of cases) (Nilsson et al., 2005; Tryggvason et al., 2005; Mucciarini et al., 2007; Rubió et al., 2007). If we consider the aggressiveness of tumors, as expressed by NIH criteria (Fletcher et al., 2002), 20 Ticino cases (47%) were cumulatively classified as very low or low risk. Our figure is similar to that obtained in the Northern European studies (50% of patients in Western Sweden and 57.4% of patients in Iceland) and higher than Spanish data (33% of patients) (Nilsson et al., 2005; Tryggvason et al., 2005; Rubió et al., 2007). In this setting, the analysis of molecular data may be very helpful and justify our effort to characterize a population-based cohort of patients by molecular analysis of the *c-kit* and *PDGFRA* genes. Overall, we observed a mutation of either *c-kit* or *PDGFRA* genes in 72.5% of GISTs, a percentage slightly lower than those reported in the literature, but comparable to results obtained from the population-based Northern Norway study, as well as to those reported in a large meta-analysis (Table 3) (Corless et al., 2004; Joensuu, 2006; Steigen et al., 2007). According to the published data, we found that the vast majority of GISTs carried a mutation in c-kit exon 11. We did not detect mutations in either *c-kit* exons 13 or 17, which are reported to be very rare (about 1% of all GISTs analyzed) and may therefore be underrepresented in our cohort of 43 patients. A more detailed analysis of the data, however, revealed differences with published studies concerning *c-kit* exon 11. In fact, nucleotide insertions or deletions of this exon typically occur in codons 557-559, while point mutations are quite rare and occur mainly at codons 557, 559, 560 and 576. In the present cohort of patients, however, we observed a higher number of point mutations than frame-shift alterations (14 vs 12, respectively). The spectrum of point mutations detected in our study is in agreement with recent large meta-analysis data, whereas frame-shift mutations were exclusively represented by deletions, in contrast to the literature, where a consistent number of nucleotide insertions has been reported (Corless et al., 2004; Joensuu, 2006; Steigen et al., 2007). Therefore, we conclude that, in a context of similar percentages of c-kit mutated cases, patients from Ticino display a peculiar spectrum of mutations in exon 11.

As regards the *PDGFRA* gene, we observed an equal number of alterations in exons 12 and 18, whereas both in the Northern Norway population-based study and in a large meta- analysis, mutations of *PDGFRA* exon 18 were reported to be more common than at exon 12 (Table 3) (Corless et al., 2004; Joensuu, 2006; Steigen et al., 2007). In addition to the previously reported types of mutations (Table 2), we found one case (# 6) carrying a missense mutation that leads to the *P581S* codon change. This mutation has never been described and the functional consequences, as with many other mutations, remain unknown. Interestingly, we also detected three cases carrying the CCG to CCA nucleotide change, *P567P* in *PDGFR* exon 12. This type of alteration has been described in 2 out of 28 medulloblastomas (Gilbertson et al., 2006), and the authors suggested that this silent mutation, detected in none of 150 healthy individuals, might be associated with medulloblastoma development. In agreement with this work, we think that

Table 3. Comparison between molecular characteristics in Ticino (Southern Switzerland, Central Europe), in Northern Norway (Northern Europe) and in a non population-based meta-analysis.

	Ticino (Switzerland) 1999-2005	Northern Norway 1974-2003[1]	Joensuu H.[2]
c-kit exon 9	5%	6%	5-15%
c-kit exon 11	60%	65%	60-70%
c-kit exon 13	0%	3%	~ 1%
c-kit exon 17	0%	1%	~ 1%
PDGFRA exon 12	5%	2%	~ 1%
PDGFRA exon 14	0%	-	rare, <1%
PDGFRA exon 18	5%	8%	~ 5%
Wild Type	27.5%	15%	10-15%

[1]: Steigen et al., 2007; [2]: Joensuu, 2006.

P567P might play a role in GIST development as well.

Finally, no statistically significant differences were observed in the distribution of c-kit or PDGFRA mutation vs wild-type cases according to gender (male vs female), malignancy risk (very low / low risk vs intermediate / high risk) and tumor primary location (gastric vs non-gastric).

In conclusion, our data show that GISTs from Ticino (Central Europe) display clinical features similar to those observed in other population-based studies from Europe, particularly from Northern Europe. On the contrary, at molecular level some peculiarities are apparent, mainly in the percentage of *PDGFRA* exon 12 mutations, and in the type of alterations of *c-kit* exon 11. These data therefore suggest that environmental factors might lead to different types of alterations in the genes involved in GIST development.

Since the specific type of *c-kit* or *PDGFRA* gene mutation is directly linked to response to targeted therapy with Imatinib, at present physicians are encouraged to enrol eligible patients into clinical studies. However, we strongly believe that population-based Cancer Registry molecular classification of GISTs, as reported in Ticino, is still lacking, although it is essential for the confirmation of clinical trial findings, the optimization of cancer treatment or clinical implications and the promotion of linkage between tissue bank specimen and Cancer registry data.

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