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Frequent KIT Mutations in Human  
Gastrointestinal Stromal TumorsSUBJECT AREAS:  
PROGNOSTIC MARKERS  
CANCER GENETICS  
CANCER GENOMICS  
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Identifying gene mutations in individual tumors is critical to improve the efficacy of cancer therapy by matching targeted drugs to specific mutations. Gastrointestinal stromal tumors (GIST) are stromal or mesenchymal subepithelial neoplasms affecting the gastrointestinal tract and frequently contain activating gene mutations in either KIT or platelet-derived growth factor A (PDGFRA). Although GIST is highly responsive to several selective tyrosine kinase inhibitors, combined use of inhibitors targeting other mutations is needed to further prolong survival in patients with GIST. In this study, we aim to screen and identify genetic mutations in GIST for targeted therapy using the new Ion Torrent next-generation sequencing platform. Utilizing the Ion Ampliseq Cancer Panel, we sequenced 737 loci from 45 cancer-related genes using DNA extracted from formalin-fixed and paraffin-embedded (FFPE) samples of 121 human gastrointestinal stromal tumors, set up stringent parameters for reliable variant calling by filtering out potential raw base calling errors, and identified frequent mutations in the KIT gene. This study demonstrates the utility of using Ion Torrent sequencing to efficiently identify human cancer mutations. This may provide a molecular basis for clinically developing new drugs targeting these gene mutations for GIST therapy.

Gastrointestinal stromal tumor (GIST) is a stromal or mesenchymal subepithelial neoplasm affecting the gastrointestinal (GI) tract. Population-based studies showed an annual incidence of 14.5 per million in Swedish, 11 per million in Icelandic<sup>1</sup>, 11.1 per million in French, 19.6 per million in Swiss<sup>2</sup>, and 14.2 per million in Italian populations. Epidemiologic studies show the annual incidence of GIST in United States to be at least 4,000 to 6,000 new cases per year, or roughly 7 to 20 cases per million people<sup>3,4</sup>. While GISTs may occur at any age, they are rare in children. GISTs commonly originate from the stomach (55%), small intestine (35%), and rectum (5%). Esophageal and colonic GISTs are rare, and these tumors also rarely occur outside the alimentary tract such as in the omentum, mesentery, and peritoneum, and are called extragastrointestinal GISTs or “E-GISTs.” GIST may originate from interstitial cells of Cajal (ICC) or from their stem cell-like precursors, although this is not certain<sup>5,6</sup>. Because of their relatively broad morphologic spectrum, GISTs were formerly called leiomyomas, leiomyosarcomas, and leiomyoblastomas of the gastrointestinal tract, until they were found to have clinical, histopathological, and molecular biological features that differentiated them from other soft tissue tumors.

GIST frequently contains oncogenic mutations in one of two receptor tyrosine kinases: KIT or PDGFRA (platelet-derived growth factor receptor alpha)<sup>7,8</sup>. KIT and PDGFRA proteins are growth factor receptors, which are activated by ligands such as PDGF-AA and stem cell factor, respectively triggering cell pathways that up-regulate proliferation, down-regulate apoptosis, and control cell differentiation, adhesion, and motility in normal conditions. Approximately 95% of GISTs express the CD117 antigen, an epitope of the KIT receptor tyrosine kinase<sup>7,9</sup>, hence the most commonly used marker for GIST is CD117. Mutations of KIT and PDGFRA lead to constitutive activation of these cell pathways leading to spontaneous proliferation and uncontrolled growth of a tumor. The downstream events following activation of mutant KIT or mutant PDGFRA are very similar<sup>10,11</sup>. Different mutations can be found in different exons or in different regions of a single exon occurring as point mutations, deletions and insertions in the KIT (exon: 9, 11, 13 and 17) and PDGFRA (exon: 12, 14 and 18) genes.



**Table 1 | Mutations (including Missense point mutations/deletion/insertion) frequencies in 45 genes (737 loci) in female and male GISTs. The p-value of Fisher exact test result of KIT mutations in female and male is 0.429**

Genes	Number of samples with mutations (Mutation frequency in 121 samples)	Number of female samples with mutations (Mutation frequency in 59 female samples)	Number of male samples with mutations (Mutation frequency in 62 male samples)
ABL1	0(0.0%)	0(0.0%)	0(0.0%)
AKT1	0(0.0%)	0(0.0%)	0(0.0%)
ALK	0(0.0%)	0(0.0%)	0(0.0%)
APC	0(0.0%)	0(0.0%)	0(0.0%)
ATM	0(0.0%)	0(0.0%)	0(0.0%)
BRAF	1(0.8%)	0(0.0%)	1(1.6%)
CDH1	1(0.8%)	0(0.0%)	1(1.6%)
CDKN2A	0(0.0%)	0(0.0%)	0(0.0%)
CSF1R	0(0.0%)	0(0.0%)	0(0.0%)
CTNNB1	0(0.0%)	0(0.0%)	0(0.0%)
EGFR	1(0.8%)	1(1.7%)	0(0.0%)
ERBB2	0(0.0%)	0(0.0%)	0(0.0%)
ERBB4	1(0.8%)	1(1.7%)	0(0.0%)
FBXW7	0(0.0%)	0(0.0%)	0(0.0%)
FGFR1	0(0.0%)	0(0.0%)	0(0.0%)
FGFR2	0(0.0%)	0(0.0%)	0(0.0%)
FGFR3	0(0.0%)	0(0.0%)	0(0.0%)
FLT3	3(2.5%)	2(3.4%)	1(1.6%)
GNAS	0(0.0%)	0(0.0%)	0(0.0%)
HNF1A	0(0.0%)	0(0.0%)	0(0.0%)
HRAS	0(0.0%)	0(0.0%)	0(0.0%)
IDH1	0(0.0%)	0(0.0%)	0(0.0%)
JAK3	0(0.0%)	0(0.0%)	0(0.0%)
KDR	0(0.0%)	0(0.0%)	0(0.0%)
KIT	60(49.6%)	25(42.4%)	35(56.5%)
KRAS	1(0.8%)	1(1.7%)	0(0.0%)
MET	0(0.0%)	0(0.0%)	0(0.0%)
MLH1	0(0.0%)	0(0.0%)	0(0.0%)
MPL	0(0.0%)	0(0.0%)	0(0.0%)
NOTCH1	0(0.0%)	0(0.0%)	0(0.0%)
NPM1	0(0.0%)	0(0.0%)	0(0.0%)
NRAS	0(0.0%)	0(0.0%)	0(0.0%)
PDGFRA	1(0.8%)	1(1.7%)	0(0.0%)
PIK3CA	0(0.0%)	0(0.0%)	0(0.0%)
PTEN	1(0.8%)	1(1.7%)	0(0.0%)
PTPN11	0(0.0%)	0(0.0%)	0(0.0%)
RB1	0(0.0%)	0(0.0%)	0(0.0%)
RET	0(0.0%)	0(0.0%)	0(0.0%)
SMAD4	0(0.0%)	0(0.0%)	0(0.0%)
SMARCB1	0(0.0%)	0(0.0%)	0(0.0%)
SMO	0(0.0%)	0(0.0%)	0(0.0%)
SRC	0(0.0%)	0(0.0%)	0(0.0%)
STK11	0(0.0%)	0(0.0%)	0(0.0%)
TP53	1(0.8%)	0(0.0%)	1(1.6%)
VHL	1(0.8%)	0(0.0%)	1(1.6%)

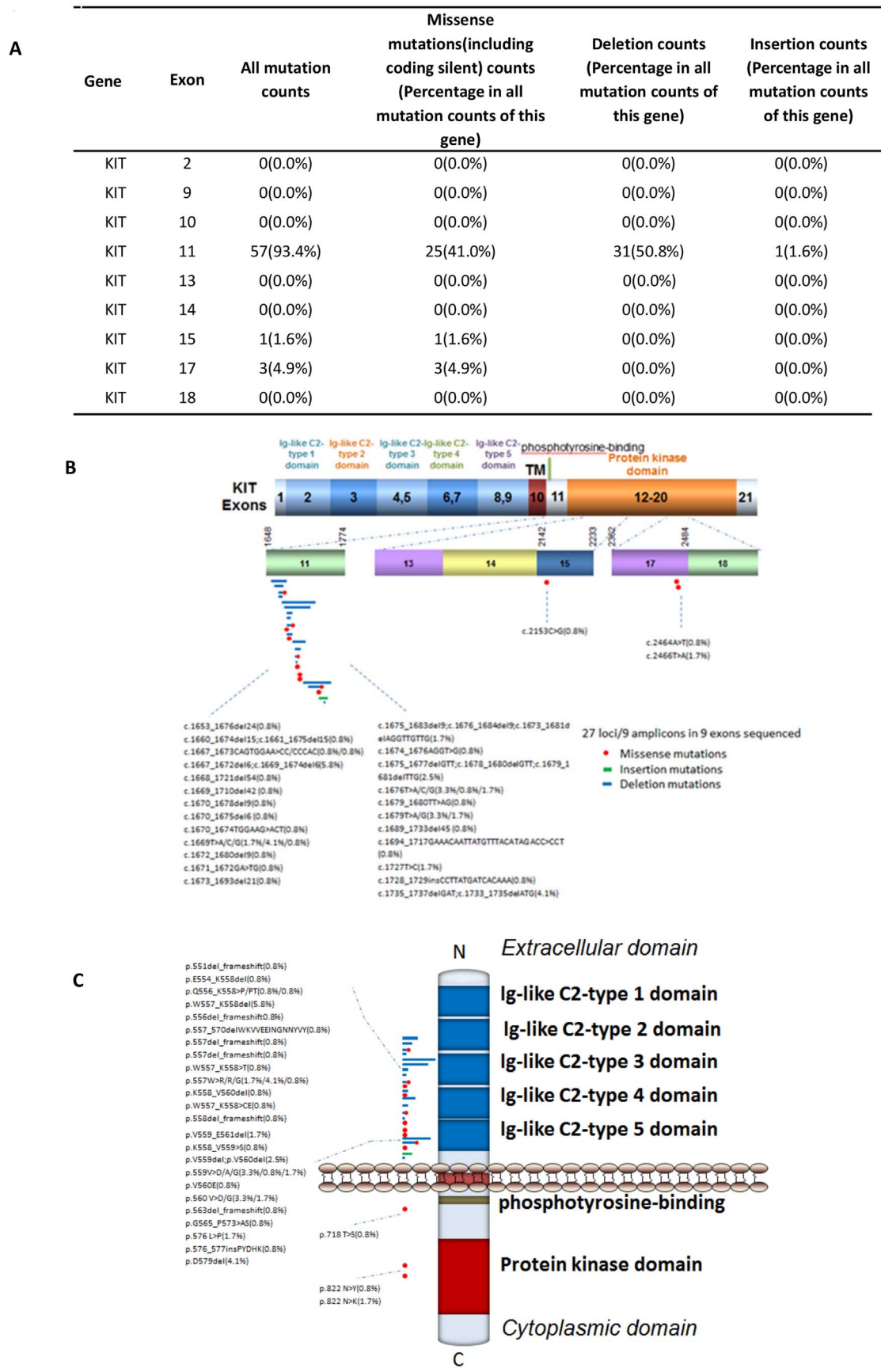
However, some GISTs have no detectable KIT or PDGFRA mutations and fewer than 5% of GIST occur as symptoms of syndromic diseases, such as neurofibromatosis type 1 (NF1), Carney triad syndrome, and other familial diseases<sup>8,12</sup>.

Accurate detection of mutations in GIST is critical for targeted therapy with drugs, such as KIT/PDGFR tyrosine kinase inhibitors (TKI)<sup>8,13,14</sup>. Next-generation sequencing technologies have revolutionized cancer genomics research by providing an unbiased and comprehensive method of detecting somatic cancer genome alterations<sup>15</sup>. These technologies have several advantages over Sanger sequencing by capillary electrophoresis, including the ability to sequence gigabases of nucleotides to detect individually unique mutations<sup>16</sup>. However, routine usage of these technologies leaves us with several limitations such as the cost of entry, long processing time, and sample scalability. A new sequencing technology, Ion Torrent (Life Technologies, Carlsbad, CA, USA), has substantially circumvented many of these issues. The Ion Torrent method relies on standard

DNA polymerase sequencing with unmodified dNTPs and uses semiconductor-based detection of hydrogen ions released during every cycle of DNA polymerization<sup>17</sup>. Each nucleotide incorporation into the growing complementary DNA strand causes the release of a hydrogen ion that is sensed by a hypersensitive ion sensor<sup>17</sup>. Ion Torrent Personal Genome Machine (PGM) can currently generate 10–100 Mb pairs (Mbp) of sequence data on various chips within several hours of instrument's run time. In this study, we sequenced 737 loci of 45 cancer-related genes from 121 human gastrointestinal stromal tumors using the Ion Ampliseq Cancer Panel, set up stringent parameters for reliable variant calling by filtering out potential raw base calling errors, and found frequent missense mutations in KIT gene consistent to that of other reports.

## Results

**Mutation analysis of human gastrointestinal stromal tumors (GISTs) with Ion Ampliseq Cancer Panel.** The sample set for this



**Figure 1 | Mutation distribution in the exons and functional domains of KIT.** (A). Frequencies of detected mutations in different exons. (B). Mutation distribution in exons. (C). Mutation distribution in functional domains.



Table 2 | Single and multiple mutations in 121 GISTs

Mutations combination (including missense point mutations/deletion/insertion)	Number of samples with mutation combination	Percentage in all sequenced samples
Single and more	66	54.50%
Double and more	7	5.80%
Three and more	0	0.00%
No missense, deletion, insert or substitution-nonsense	55	45.50%

study was GISTs from 121 Chinese patients, and 76 samples were of gastric origin and 45 samples had an intestinal origin (Table 1). Ion Ampliseq technology was used to identify mutations in 189 amplicons covering 737 loci of 45 tumor suppressor genes and oncogenes in these tumor samples. The sequenced data were processed and mutations identified using Torrent Suite Software v3.0 with a plug-in “variant caller.” To generate reliable variant calling, three filtering steps were used to eliminate erroneous base calling (Supplementary figure 1), as described in the Materials and Methods. The mean read length was 74 bp, and the average sequence per sample was approximately 27 Mb. With normalization to 300,000 reads per specimen, there was an average of 1,630 reads per amplicon (range: 174 to 4024) (Supplemental figure 2A), and 183/189 (96.8%) amplicons averaged at least 100 reads, and 177/189 (93.7%) amplicons averaged at least 300 reads (Supplemental figure 2B).

**Identification of frequent mutations in a set of cancer-related genes in GISTs.** The incurred mutations were of both gastric and intestinal origin (Supplementary Table 1). Of the 45 oncogenes and tumor suppressor genes sequenced in the 121 GISTs from Chinese patients, mutations were detected in the genes BRAF, CDH1, EGFR, ERBB4, FLT3, KRAS, PDGFRA, PTEN, TP53, and VHL, and the most common mutations occurred in the KIT gene (Table 1). A detailed list of missense, point mutations, insertions, and deletions identified in this study is provided in Supplementary Table 2.

**KIT mutations in GISTs.** Accumulating evidence indicates that activating mutations of tyrosine kinases KIT or PDGFRA are the initiating event in GISTs. Activation of KIT or PDGFR leads to downstream signaling in the PI3K, Ras, and Jak/Stat pathways resulting in increased cell proliferation and inhibition of apoptosis<sup>18</sup>. Accordingly, 60 of the 121 GIST tumors sequenced in this study had KIT gene mutations: exons 2, 9, 10, 11, 13, 14, 15, 17, and 18 were found to be mutated along its transmembrane and cytoplasmic domain but not along its extracellular domain (Fig. 1A–C). Mutations of exon 11 (94.4%), consisting of the regulatory domain of the enzyme included nucleotide deletions in 31 cases (50.80%), missense mutations in 25 cases (41.0%) and insertion in 1 case. Different size deletions in Kit can affect KIT intron 10- exon-11 splice-acceptor sites. Exon 17 encoding the kinase activation loop was mutated in only 3 cases (4.9%) with a substitution of A2466 for T2466 or a substitution of T2464 for A2464. These data support the critical role of KIT mutations in the development and progression of GISTs.

**Multiple mutations and mutation hotspots in GISTs.** Identifying single or combination of mutations for delivering individualized treatment with a single or combination of target agents against the detected mutational combinations has been an effective cancer therapy in the recent years. 54.5% of samples had at least one or more missense mutations, 5.8% had at least two or more missense mutations, and 45.5% of samples incurred no deleterious mutations (Table 2). It is known that functional SNPs may contribute to the development of GISTs, and Table 3 and Table 4 illustrate the incurrence of SNPs in combination with other mutations in our sample set. CD34 and CD117 expression levels can help to predict the prognosis of GIST patients, and mutations found in CD117<sup>+</sup> and CD117<sup>-</sup> GISTs and CD34<sup>+</sup> and CD34<sup>-</sup> GISTs are summarized in Table 5 and Table 6, respectively. There was a significant reduction in the survival rate between patients with one CD34 mutation compared to patients with no CD34 mutations (Table 7 and Fig. 2), and between patients with triple CD117 mutations compared to those with double CD117 mutations (Table 8 and Fig. 3).

## Discussion

Cancer is an accumulation of many genetic changes within cells, and mutation patterns vary widely among different types of cancers and among patients with the same cancer. These mutations can be used to direct targeted drug therapy, an approach has significantly changed the treatment of cancer over the past 10 years. Drugs like small-molecule inhibitors or monoclonal antibodies that target these genetic alterations specifically have been developed, and such drugs act as a potential therapy for many common malignancies and are generally better tolerated with fewer side effects over the traditional cytotoxic chemotherapy.

Since the development of targeted therapy in the last decade, screening the genetic mutations of cancer cells has been of keen interest to direct the use of targeted anticancer drugs. Because of this, identifying mutations in each individual tumor can be highly beneficial to the patient, but has yet to be a feasible option. The first near-complete human genome sequence assembly required more than 12 years of sequencing and more than \$3 billion<sup>19</sup>. The urgent need for alternative sequencing approaches employing a lower cost and faster pace led to the development of Next generation sequencing (NGS) methods such as Illumina HiSeq and Miseq, 454 Pyrosequencing, Helicos Heliscope, SOLiD Sequencing, and Ion Torrent Sequencing<sup>20–22</sup>. These new next-generation sequencing strategies are believed to enable high-throughput screening and detection of cancer cell genomes and epigenomes at much faster rates and

Table 3 | Single and multiple mutations and SNPs in 121 GISTs

Mutations and SNPs combination (including missense point mutation/deletion/insertion/SNP)	Number of samples with mutation/snp combination	Percentage in all sequenced samples
Single and more	88	72.70%
Double and more	30	24.80%
Three and more	4	3.30%
No missense, deletion, insert or substitution-nonsense	33	27.30%



Table 4 | List of multiple mutations and SNPs within more than one GIST patients

Genes with Mutations combination	CDS and amino acid change	Numbers of patients with this mutation combination
MLH1 SNP and KIT mutation	c.1151T > A p.V384D; c.1667_1672del6/c.1669_1674del6 p.W557_K558del	3
MLH1 SNP and KIT SNP	c.1151T > A p.V384D; c.1621A > C p.M541L	2
KIT SNP and KIT mutation	c.1621A > C p.M541L; c.1735_1737delGAT/c.1733_1735delATG p.D579del	2
MLH1 SNP and MET SNP	c.1151T > A p.V384D; c.1124A > G p.N375S	2

Table 5 | Mutations (including Missense point mutations/deletion/insertion) frequencies in 45 genes (737 loci) in CD117+ and CD117- GISTs

Genes	Number of samples with mutations (Mutation frequency in 121 samples)	Number of unknown samples with mutations (Mutation frequency in 57 unknown samples)	Number of CD117(+) samples with mutations (Mutation frequency in 17 CD117(+) samples)	Number of CD117(++) samples with mutations (Mutation frequency in 23 CD117(++) samples)	Number of CD117(+++) samples with mutations (Mutation frequency in 21 CD117(+++) samples)	Number of CD117(-) samples with mutations (Mutation frequency in 3 CD117(-) samples)
ABL1	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
AKT1	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
ALK	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
APC	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
ATM	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
BRAF	1(0.8%)	0(0.0%)	1(5.9%)	0(0.0%)	0(0.0%)	0(0.0%)
CDH1	1(0.8%)	0(0.0%)	0(0.0%)	0(0.0%)	1(4.8%)	0(0.0%)
CDKN2A	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
CSF1R	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
CTNNB1	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
EGFR	1(0.8%)	0(0.0%)	1(5.9%)	0(0.0%)	0(0.0%)	0(0.0%)
ERBB2	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
ERBB4	1(0.8%)	1(1.8%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
FBXW7	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
FGFR1	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
FGFR2	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
FGFR3	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
FLT3	3(2.5%)	0(0.0%)	1(5.9%)	0(0.0%)	2(9.5%)	0(0.0%)
GNAS	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
HNF1A	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
HRAS	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
IDH1	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
JAK3	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
KDR	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
KIT	60(49.6%)	27(47.4%)	8(47.1%)	12(52.2%)	12(57.1%)	1(33.3%)
KRAS	1(0.8%)	1(1.8%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
MET	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
MLH1	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
MPL	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
NOTCH1	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
NPM1	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
NRAS	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
PDGFRA	1(0.8%)	0(0.0%)	0(0.0%)	0(0.0%)	1(4.8%)	0(0.0%)
PIK3CA	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
PTEN	1(0.8%)	0(0.0%)	1(5.9%)	0(0.0%)	0(0.0%)	0(0.0%)
PTPN11	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
RB1	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
RET	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
SMAD4	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
SMARCB1	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
SMO	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
SRC	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
STK11	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
TP53	1(0.8%)	1(1.8%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
VHL	1(0.8%)	1(1.8%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)


**Table 6 | Mutations (including Missense point mutations/deletion/insertion) frequencies in 45 genes (737 loci) in CD34+ and CD34– GISTs**

Genes	Number of samples with mutations in 121 samples (Mutation frequency)	Number of unknown samples with mutations (Mutation frequency in 56 unknown samples)	Number of CD34(+) samples with mutations (Mutation frequency in 20 CD34(+) samples)	Number of CD34(++) samples with mutations (Mutation frequency in 16 CD34(++) samples)	Number of CD34(+++) samples with mutations (Mutation frequency in 17 CD34(+++) samples)	Number of CD34(–) samples with mutations (Mutation frequency in 12 CD34(–) samples)
ABL1	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
AKT1	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
ALK	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
APC	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
ATM	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
BRAF	1(0.8%)	0(0.0%)	0(0.0%)	1(6.2%)	0(0.0%)	0(0.0%)
CDH1	1(0.8%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	1(10.0%)
CDKN2A	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
CSF1R	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
CTNNB1	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
EGFR	1(0.8%)	0(0.0%)	1(5.0%)	0(0.0%)	0(0.0%)	0(0.0%)
ERBB2	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
ERBB4	1(0.8%)	1(1.8%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
FBXW7	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
FGFR1	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
FGFR2	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
FGFR3	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
FLT3	3(2.5%)	0(0.0%)	1(5.0%)	1(6.2%)	1(5.9%)	0(0.0%)
GNAS	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
HNF1A	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
HRAS	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
IDH1	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
JAK3	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
KDR	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
KIT	60(49.6%)	26(46.4%)	11(55.0%)	9(56.2%)	9(52.9%)	5(41.7%)
KRAS	1(0.8%)	1(1.8%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
MET	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
MLH1	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
MPL	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
NOTCH1	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
NPM1	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
NRAS	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
PDGFRA	1(0.8%)	0(0.0%)	0(0.0%)	0(0.0%)	1(5.9%)	0(0.0%)
PIK3CA	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
PTEN	1(0.8%)	0(0.0%)	1(5.0%)	0(0.0%)	0(0.0%)	0(0.0%)
PTPN11	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
RB1	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
RET	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
SMAD4	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
SMARCB1	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
SMO	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
SRC	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
STK11	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
TP53	1(0.8%)	1(1.8%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
VHL	1(0.8%)	1(1.8%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)

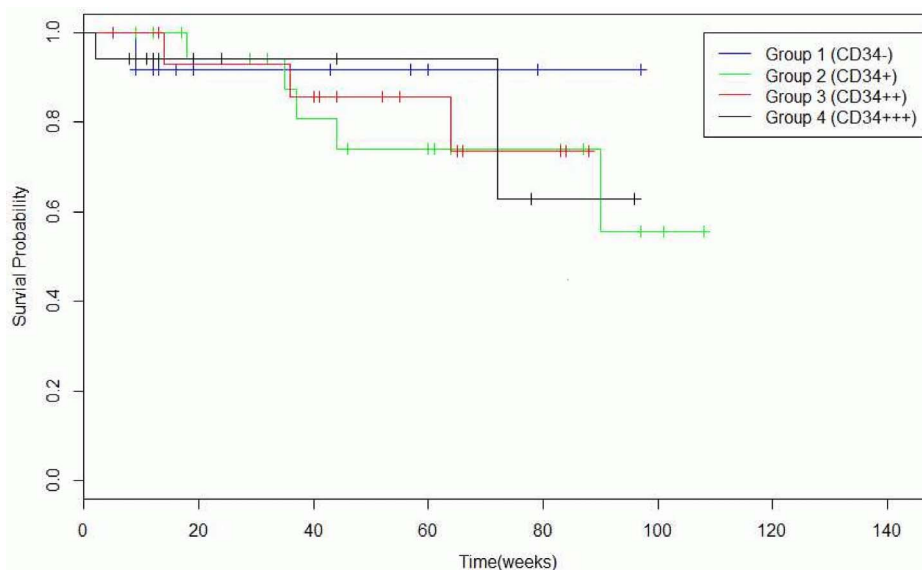
reduced sequencing costs per base<sup>23</sup>, facilitating the advancement of personalized medicine for cancer patients. In addition, the cost and complexity associated with the 4-color optical detection used in all other NGS platforms is circumvented in Ion Torrent sequencing through the employment of Post Light sequencing technology.

This study aimed to identify mutation patterns in human GISTs to demonstrate the possibility of using Ion Torrent sequencing to direct personalized cancer treatment in the near future.

In our sample group of 121 GISTs, 189 amplicons covering 737 loci of 45 oncogenes and tumor suppressor genes from 121 human

**Table 7 | Survival time of patients with CD34+ and CD34– GISTs**

	Number of samples	Survival time (average, range)	P-value (log-rank test)
CD34(–)	12	38 (9–97+ months)	CD34– and CD34+: 0.526
CD34(+)	20	55 (9+–108+ months)	
CD34(++)	16	52 (5+–88+ months)	CD34++ and CD34+++ : 0.935
CD34(+++)	17	29 (2–96+ months)	



**Figure 2** | Survival probability in weeks of patients with CD34+ and CD34- GISTs.

GISTs were sequenced using the Ion Torrent PGM platform. Analysis revealed a unique pattern of distribution and co-occurrence (Tables 2–4 and Tables 7 and 8): KIT was the most frequently mutated gene, and MLH1 and MET SNPs frequently occurred in combination with the KIT mutations. While KIT, PDGFR, and PIK3CA mutations have previously been reported, mutations in the MET and MLH1 genes in GISTs are previously not well known. Interestingly, our tumor group harbored mutations in the exon 11 of KIT but mutations along the PDGFR gene were rare. Defects in genes that control mismatch repair (MMR) such as MLH1, MSH2, and PMS2 generally exhibit microsatellite instability, leading to the formation of tumors such as endometrial and colon cancer<sup>24</sup>. MET signaling is affected by mutations in this gene, and dysregulation of the MET signaling pathway occurs in a wide range of human cancers<sup>25</sup>.

Recent data indicate that GIST patients respond differently to tyrosine kinase inhibitors (drugs like Gleevec and Sutent), depending on the specific mutations displayed by their tumors. Gleevec (Imatinib) is considered as the standard treatment of metastatic GIST, for approximately 65–70% of patients being treated with this drug achieve a partial response, but 15–20% maintain stable disease<sup>26–29</sup>. Reports indicate that GIST patients respond better to Gleevec if their tumors harbor KIT mutations in exon 11<sup>10,30</sup> versus those with mutations in exon 9<sup>31</sup>, or if they have GIST negative for KIT expression. The response to Gleevec for PDGFR mutations also depends on the specific mutations involved, but data indicates that 35% of patients with PDGFR mutations benefit from the drug<sup>32</sup>. Hence, Gleevec is shown to benefit virtually all patients with metastatic GIST regardless of the mutational status of the tumor; however, patients with particular GIST mutations on exon 18 (D842V) are resistant to Gleevec. In addition, newly acquired secondary mutations can also confer drug resistance to Gleevec. Such patients who had grown resistant to Gleevec and with KIT mutations in exon 9 responded well to Sutent, another tyrosine kinase inhibitor<sup>33</sup>.

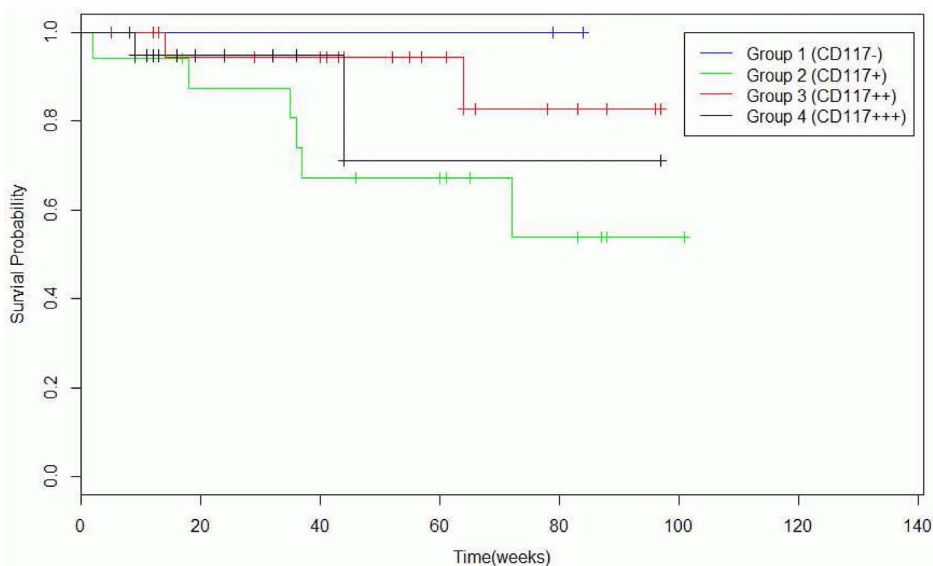
Sunitinib (SU11248), Masitinib, Nilotinib, and Dasatinib are other oral small molecule inhibitors of multiple receptor tyrosine kinases including KIT, PDGFR, VEGFR FLT3, CSF-1R, and RET<sup>5,34,35</sup>. The metastases resistant to drugs are often with new mutations that were not detected in primary tumors<sup>36–38</sup>. For example, drug resistant metastases may lose KIT expression, potentially mimicking other types of tumors<sup>39</sup>. This demands the constant need for the evaluation of GISTs for new mutations for appropriate drug-selection against the specific mutations harboring a tumor.

Frequent SNPs were detected along MLH1 and MET genes in our sample set and are often associated with the development of GISTs when they co-occur with other mutations. While drugs against tyrosine kinases have either entered clinical trials or are being tested massively today, drug targets against MLH1 and MET mutations are still in early stages. Martin et al. suggest that MSH2 and MLH1 deficiencies are associated with increased expression of POLB and POLG, respectively, and therefore DNA sequencing-based detection and IHC detection of these latter proteins in tumor biopsies may serve as a predictive biomarker for the application of POLG or POLB inhibitors<sup>40</sup>. Several MET inhibitors have been tested to date; they are broadly divided into those inhibiting either the ligand or receptor of HGF, and they occur as monoclonal antibodies or small-molecule kinase inhibitors<sup>25</sup>. Tivantinib (ARQ197), a selective, non-adenosine triphosphate-competitive inhibitor targeting MET tyrosine kinase, and abozantinib (XL184), a dual c-Met/VEGFR2 inhibitor, are under early clinical evaluation<sup>41</sup>. However, validated biomarkers for proper evaluation of MET mutations such as copy number and amplification are not yet available<sup>25</sup>, and high throughput DNA sequencing techniques may be highly beneficial to investigate along this line for developing personalized drug-targets against MET mutations.

Our current study identifies several mutations and mutation combinations in GISTs from Chinese patients using the Ion Torrent sequencing platform. As more experience and information is gained from the next generation technologies, it is necessary to expand our

**Table 8** | Survival time of patients with CD117+ and CD117- GISTs

	Number of samples	Survival time (average, range)	P-value(log-rank test)
CD117(-)	3	58 (12+–84+ months)	CD117++ and CD117+++ : 0.367
CD117(+)	17	52 (2–101+ months)	
CD117(++)	23	49 (5+–97+ months)	
CD117(+++)	21	24 (8+–97+ months)	



**Figure 3** | Survival probability in weeks of patients with CD117+ and CD117– GISTs.

understanding in the sensitivity of individualized therapies to specific mutations. So, gathering a complete profile of mutations in GISTs or any specific tumors for the application of personalized and tailored targeted therapy is critical for the development of improved cancer treatments. We believe a faster and more cost effective genotyping tool such as Ion Torrent sequencing will be greatly beneficial for the assignment of such specific therapeutics in the near future.

## Methods

**Ethics statement.** The study has been approved by the Human Research Ethics Committee of Nanjing First Hospital, China. For Formalin fixed and paraffin embedded (FFPE) tumor samples from the tumor tissue bank at the Department of Pathology of the hospital, the institutional ethics committee waived the need for consent. All samples and medical data used in this study have been irreversibly anonymized.

**Patient information.** Tumor samples used in the study were collected from Nanjing First Hospital, China. A total of 121 FFPE tumor samples from GIST patients were analyzed (Supplementary Table 4). DNA was isolated from FFPE samples after deparaffinization and extraction of 3–5  $\mu\text{m}$  thick paraffin sections in xylene and by using the QIAamp DNA Mini Kit (Qiagen) per the manufacturer’s instruction.

**Variant calling.** Data from the PGM runs were processed initially using the Ion Torrent platform-specific pipeline software Torrent Suite to generate sequence reads, trim adapter sequences, filter, and remove poor signal-profile reads. The Torrent Suite Software v3.0 with a plug-in “variant caller v3.0” program was used to generate initial variant calling from the Ion AmpliSeq sequencing data. In order to eliminate erroneous base calling, four filtering steps were used to generate final variant calling (Supplementary Fig. 1). The first filter was set at an average total coverage depth of  $>100$ , each variant coverage of  $>20$ , a variant frequency of each sample  $>10\%$ , and  $P$ -value  $<0.01$ . The second filter was set for base calling  $<4$  bases homopolymer tracts and  $>3$  bases from the terminus of amplicons, due to an observation of false positive mutations at the ends of some reads. The third filtering step was employed by visually examining mutations using Integrative Genomics Viewer (IGV) software (<http://www.broadinstitute.org/igv>) or Samtools software SAMtools software (<http://samtools.sourceforge.net>), as well as by filtering out possible strand-specific errors, like a mutation that was only detected in the “+” or “–” DNA strand, but not in both strands. The last filter step eliminated variants in amplicon AMPL339432 (PIK3CA, exon13, chr3:178938822–178938906), for it is not uniquely matched in human genome. False deletion data were generated from the JAK2 gene locus from our sequencing runs with the Ion AmpliSeq Cancer Panel; thus the sequencing data from this locus were excluded from further analysis.

Variant calling is a subjective process and considerably influenced by the particular software used and the specific parameters employed to filter out raw error base calling. Various next-generation sequencing platforms, including the Ion Torrent sequencing, can generate wrong base calling. The data generated using the Ion Torrent PGM platform were reported to have a raw error rate of  $\sim 1.8\%$ <sup>42</sup>, largely due to the inability of calling the correct number of bases in homopolymers  $>8$  bases long.

**Somatic mutations.** To distinguish between somatic and germline mutations, detected mutations were compared to variants in the 1000 Genomes Project<sup>43</sup> and 6500 exomes of the National Heart, Lung, and Blood Institute Exome Sequencing Project<sup>44</sup>.

**Bioinformatical and experimental validation.** To assess reappearing mutations in GIST, we used the COSMIC (version 64)<sup>45</sup>, MyCancerGenome database (<http://www.mycancergenome.org/>), and other publications (Supplementary Table 1). Additionally, Sanger’s sequencing was used to confirm some detected missense mutations (Supplementary Table 3).

**Statistical analysis.** We selected reappearing somatic missense/in-del mutations in the GIST to perform the statistical analysis. Some categorical data were compared using Fisher’s exact tests. Log-rank statistics were used to determine whether patients’ survival was influenced by CD34 and CD117. Survival analyses were also performed using Kaplan-Meier survival curves. All statistical analyses were performed using R version 3.0.2.

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## Author contributions

S.C. and J.C. designed the study. Z.X., X.H., C.T., F.L., D.Z., S.J., H.S., H.D., G.Z., Z.L., Z.D., B.G., H.Y., C.Y., L.W., Z.S., Y.L., D.G., X.Z., X.W., X.W., L.H., Y.Z., J.Y., Y.G. and C.T. performed experiments and data analysis. C.T., V.N. and L.J. prepared the figure and tables. V.N., L.J., X.H. and S.C. wrote the main manuscript text. All authors reviewed the manuscript.

## Additional information

**Supplementary information** accompanies this paper at <http://www.nature.com/scientificreports>

**Competing financial interests:** Authors Chuaning Tang, Hua Ye, Feng Lou, Dandan Zhang, Shouwen Jiang, Hong Sun, Haichao Dong, Guangchun Zhang, Zhiyuan Liu, Zhishou Dong, Baishuai Guo, He Yan, Chaowei Yan, Lu Wang, Ziyi Su, and Yangyang Li are paid employees of San Valley Biotechnology, Inc. Authors Zhi Xu, Xinying Huo, Vijayalakshmi Nandakumar, Dongying Gu, Xiaojing Zhang, Xiaomin Wu, Xiaowei Wei, Lingzhi Hong, Yangmei Zhang, Jinsong Yang, Yonglin Gong, Cuiju Tang, Lindsey Jones, Xue F. Huang, Si-Yi Chen, and Jinfei Chen declare no potential conflict of interest.

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