RESEARCH ARTICLE

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# Spectrum of the *KIT* Gene Mutations in Gastrointestinal Stromal Tumors in Arab Patients

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## Abstract

**Background:** Gastrointestinal stromal tumors are the most common mesenchymal tumors of the gastrointestinal tract, which originate from the interstitial cells of Cajal. These tumors are characterized by expression of CD117 and CD34 antigens and activating mutations in the *KIT* and *PDGFRA* genes. While *KIT* and *PDGFRA* mutations have been extensively studied in other populations, the spectrum of mutations in Arab patients remains unknown. The study aimed at determining the distribution of *KIT* and *PDGFRA* mutations and phenotypic characterization of the gastrointestinal stromal tumors in Arab patients. **Methods:** Sanger sequencing was used to analyze 52 archived gastrointestinal stromal tumors for mutations in the *KIT* and the *PDGFRA* genes. Tumor descriptions were obtained from the clinical reports of patients. **Results:** In these patients, most tumors occur in the stomach, followed by the rest of the digestive tract. A vast majority of tumors express the CD117 and CD34 antigens. Sequencing of the *KIT* and *PDGFRA* genes identified five non-synonymous mutations and 26 deletions (25 novel) in exon 11 of the *KIT* gene. All non-synonymous mutations and deletions affect the juxta-membrane domain, which is known to inhibit ligand-independent activation of the *KIT* receptor. No mutations were found in the *PDGFRA* gene. **Conclusions:** Molecular profiling of the gastrointestinal stromal tumors in Arab patients identified a unique spectrum of mutations in exon 11 of the *KIT* gene. These data are important for the diagnosis and management of patients of Arab ethnic origin.

**Keywords:** Gastrointestinal stromal tumors- *KIT* mutations- Arab patients- CD117

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## Introduction

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal tract. These tumors are found in the esophagus, stomach, small intestine, colon, rectum, and mesentery. GISTs originate from the interstitial cells of Cajal, which regulate peristalsis in the gastrointestinal tract (Sepe and Brugge, 2009; Chi et al., 2010). After patients display symptoms, GISTs are usually diagnosed by computed tomography, positron emission tomography, endoscopy or endoscopic ultrasonography techniques (Sepe and Brugge, 2009). Histologic examination of GISTs shows spindle-shaped, epithelioid, or mixed cell morphology (Biasco et al., 2009; Haller et al., 2010). Most GISTs express CD117 (*KIT*) and/or CD34 antigens (Lasota and Miettinen, 2008; Sepe and Brugge, 2009; Hirota 2018). In GISTs, several somatic or germ-line mutations have been described in the *KIT* and *PDGFRA* genes, which encode for the highly homologous type-3 receptor tyrosine kinases, v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog (*KIT*) and the platelet-derived growth factor receptor alpha (*PDGFRA*), respectively. Mutations in these genes cause ligand-independent tyrosine kinase activity resulting in receptor autophosphorylation and signaling, which lead to cell proliferation and GIST formation (Hirota et al., 1998; Lasota and Miettinen 2008; Tryggvason et al., 2010). In GISTs, both *KIT* and *PDGFRA* mutations activate the same downstream signaling pathways, which result in tumor formation (Lasota and Miettinen, 2008).

Mutation screening of GISTs is of great biological and clinical significance. While histopathology and CD117 or CD34 immunohistochemistry are sufficient to diagnose GISTs in majority of the cases, the response to imatinib and sunitinib treatment, and survival of patients depend upon specific mutations in GISTs (Debiec-Rychter et al, 2006; Lasota and Miettinen 2008; Heinrich et al.,

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2008; Heinrich et al., 2008). Although most GISTs carry mutations in exon 11 of the *KIT* gene, a minority of GISTs (less than 5%) which lack *KIT* mutations carry mutations in the *PDGFRA* gene (Thacoor 2018). Mutations in exon 18 account for most of the *PDGFRA* mutated GISTs (Mavroeidis et al., 2018). GIST with exon 9 *KIT* mutations and the exon 18 *PDGFRA* mutations show lesser sensitivity to imatinib treatment (Heinrich et al., 2003, Mavroeidis et al., 2018). Deletions of the codon 557-558 of the *KIT* may be associated with poor prognosis (Wardelmann et al., 2003). In the *KIT* and *PDGFRA*, while most studies have described point mutations, deletions spanning critical exons and splice regions have been rarely mentioned.

In this study, we have analyzed 52 archived GIST tissues from patients of Arab origin to determine the spectrum of mutations in the *KIT* and *PDGFRA* genes. We describe point mutations and deletions in exon 11 of the *KIT* gene. In addition, we provide clinical and histopathological features of GISTs in these patients.

## Materials and Methods

*Tissues*

Archived paraffin-embedded GISTs form 30 male and 22 female patients collected during 1991 and 2006 were used. The institutional review committee approved the study. The mitotic count (number of mitotic cells per 50 high power fields) information was available from the clinical histories of 47 patients.

*Immunohistochemistry*

Immunostaining for CD117 and CD34 was performed using mouse monoclonal antibodies (Novocastra Laboratories, Newcastle upon Tyne, UK). A horseradish peroxidase conjugated anti-mouse secondary antibody and DAB chromogen were used to detect immune complexes.

*Mutation analysis*

DNA was extracted from paraffin-embedded GIST tissues. Regions encompassing exons 9, 11, 13, and 17 of the *KIT* and exons 12 and 18 of the *PDGFRA* genes were PCR amplified using the primers described in Supplementary Table 1. DNA sequencing was performed on automated Applied BioSystems Sequencers 3100/3730. All sequences were compared with the reference sequences of the *KIT* (GI:148005048) and the *PDGFRA* (GI:172072625) genes from the GenBank. To detect deletions in the *KIT* gene, multiple sets of primers were used to PCR amplify the regions spanning intron 10, exon 11, and intron 11.

*Statistical analyses*

The Fisher exact test was used to analyze the mutation distribution among tissues and mitotic counts.

## Results

*Clinical details of GISTs*

The age range of the patients in this cohort is 26-90 years (mean age = 60±1.8 SEM) with most patients

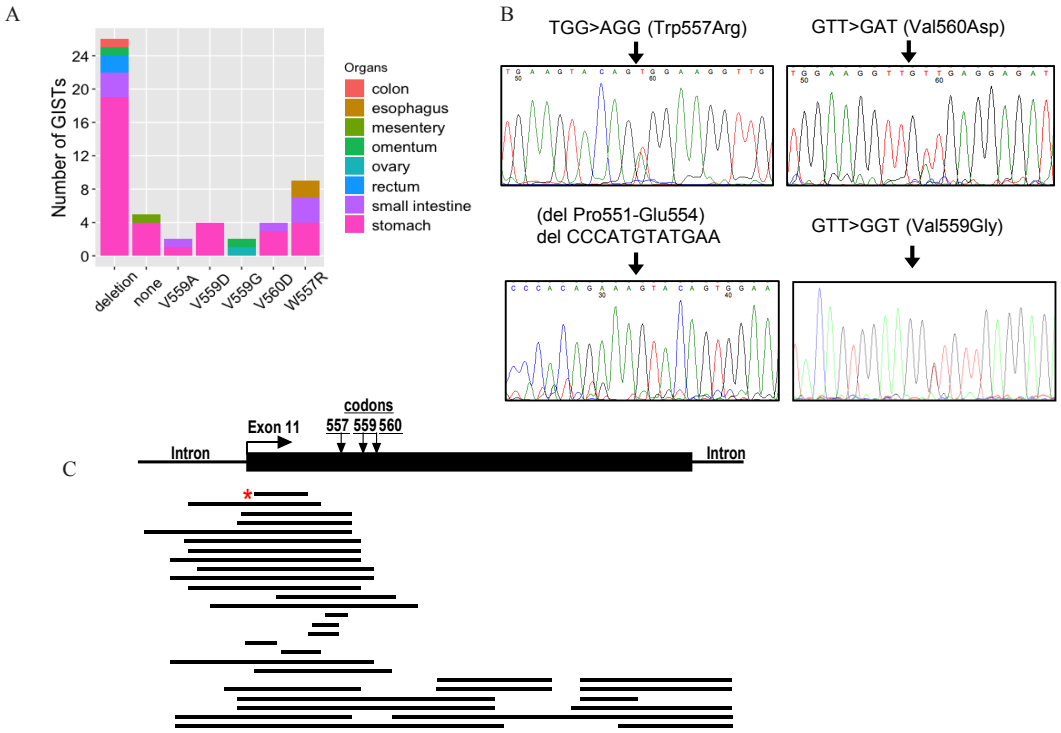


Figure 1. legend? Figure 1. Organ distribution and molecular features of GISTs. (A) Location of GISTs and the distribution of point mutations and deletions in exon 11 of the KIT gene. (B) Chromatograms show representative point mutations and a deletion producing the p.Trp557Arg, p.Val560Asp, p.Val559Gly , and deletion of p.Pro551 to p.Glu554 amino acids, respectively. (C) Locations of deletions within exon 11 of the KIT gene are shown. Locations of codons with non-synonymous changes p.Trp557, p.Val559, and p.Val560, are identified. The asterisk denotes a twelve-nucleotide in-frame deletion.

Table 1. Types of Mutations in the *KIT* Gene and Tissue Distribution of GISTs

| | Deletion | Substitution | No mutation | Total |
|---|---|---|---|---|
| Stomach | 19 | 12 | 4 | 35 |
| Small intestine | 3 | 5 | 0 | 8 |
| Rectum/Colon | 3 | 0 | 0 | 3 |
| Mesentery/Omentum | 1 | 1 | 1 | 3 |
| Esophagus | 0 | 2 | 0 | 2 |
| Ovary | 0 | 1 | 0 | 1 |
| Total | 26 | 21 | 5 | 52 |

diagnosed at 60-70 years of age. Most tumors are of gastric origin (67.3%), followed by small intestine (15.4%), mesentery (5.8%), rectum and colon (5.8%), esophagus (3.8%), and the ovary (1.9%). GISTs were identified by histopathology of tumor biopsies. Cell morphology in most of the GISTs is spindle-shaped (77%) followed by epithelioid (21%), and mixed appearances (1.9%). Among the 52 GISTs, CD117 is expressed in 47 tumors (90.4%). CD34 expression was examined only in 36 GISTs. In this subset of tumors, 31 (86%) GISTs show expression of the CD34 antigen. Among the five CD34 negative GISTs, two are positive and three are negative for CD117 expression.

*Mutation analysis*

In 47 tumors (~90%), five non-synonymous mutations and 26 deletions were identified (Figure 1, and Tables 1 and 2). These mutations affect the exon 11 of *KIT* gene, which encodes for the juxtamembrane domain of *KIT* receptor. All mutations and deletions are in heterozygous states except for a near homozygous deletion of twelve nucleotides in a single GIST that was found in the

Table 2. Novel and Recurrent Deletions and Missense Mutations Found in the *KIT* Gene

| Nucleotide change | Amino acid change | Details |
|---|---|---|
| Missense mutations | | |
| c.1669T>A | p.Trp557Arg | Recurrent |
| c.1676T>A | p.Val559Asp | Recurrent |
| c.1676T>C | p.Val559Ala | Recurrent |
| c.1676T>G | p.Val559Gly | Recurrent |
| c.1679T>A | p.Val560Asp | Recurrent |
| Single deletions | | |
| c.1651_1662del12 | p.Pro551_Glu554del | homozygous in frame deletion/Recurrent |
| c.1648-12_1665del30 | p.Lys550_Phe591del | splice site deletion/Novel |
| c.1648_1672del25 | p.Lys550_Lys558del | new splice acceptor site/Novel |
| c.1648-1_1672del26 | p.Lys550_Lys558del | new splice acceptor site/Novel |
| c.1648-22_1672del47 | p.Lys550_Phe591del | splice site deletion/Novel |
| c.1648-13_1674del40 | p.Lys550_Phe591del | splice site deletion/Novel |
| c.1648-12_1674del39 | p.Lys550_Phe591del | splice site deletion/Novel |
| c.1648-16_1674del43 | p.Lys550_Phe591del | splice site deletion/Novel |
| c.1648-10_1677del40 | p.Lys550_Phe591del | splice site deletion/Novel |
| c.1648-16_1677del46 | p.Lys550_Phe591del | splice site deletion/Novel |
| c.1648-12_1674del39 | p.Lys550_Phe591del | splice site deletion/Novel |
| c.1656_1682del27 | p.Met552_Glu561del | in frame deletion/Novel |
| c.1648-7_1687del47 | p.Lys550_Phe591del | splice site deletion/Novel |
| c.1666_1671del6 | p.Gln556_Trp557del | in frame deletion/Novel |
| c.1664_1669del6 | p.Tyr553_Val555delinsGly | in frame del/insertion/Novel |
| c.1663_1671del9 | p.Val555_Trp557del | in frame deletion/Novel |
| c.1649_1655del7 | p.Lys550_Met552del | new splice acceptor site/Novel |
| c.1657_1665del9 | p.Tyr553_Val555del | in frame deletion/Novel |
| c.1648-16_1677del46 | p.Lys550_Phe591del | splice site deletion/Novel |
| c.1651_1681del31 | p.Lys550_Phe591del | splice site broken/Novel |
| Multiple deletions | | |
| c.1693_1728del36, c.1738_1774+?del | | in frame deletion/Novel |
| c.1648-4_1674del31, c.1693_1728del36,c.1738_1774+?del | p.Lys550_Phe591del | splice site deletion/Novel |
| c.1648-1_1710del64, c.1738_1755del18 | p.Lys550_Phe591del | splice site deletion/Novel |
| c.1648-1_1710del64, c.1735_1774+?del | p.Lys550_Phe591del | splice site deletion/Novel |
| c.1648-15_1672del40, c.1682_1774+?del | p.Lys550_Phe591del | splice site deletion/Novel |
| c.1648-15_1713del81, 1750_1774+?del | p.Lys550_Phe591del | splice site deletion/Novel |

Human Splicing Finder program (version 2.4) was used to determine the creations of new splice acceptor sites.

Table 3. Mitotic Count Analysis of the 47 GISTs and Mutations

| Mutation | Mitotic counts per 50 High Power Fields | | | Total |
|---|---|---|---|---|
| | 0-4 | 5-9 | ≥10 | |
| Deletions | 10 | 3 | 12 | 25 |
| Trp557Arg | 4 | 3 | 2 | 9 |
| Val559Ala | 1 | 0 | 0 | 1 |
| Val559Asp | 1 | 1 | 1 | 3 |
| Val559Gly | 0 | 2 | 0 | 2 |
| Val560Asp | 1 | 1 | 1 | 3 |
| None | 2 | 0 | 2 | 4 |

mesentery of a 68-year-old female (Table 2). These mutations include unique deletions in 26 GISTs and five single nucleotide non-synonymous changes in 21 GISTs. Deletions range from 5 to 104 nucleotides and include in-frame deletions, splice site deletions, and creation of new splice acceptor sites (Figure 1c, and Table 2). Among deletions, twenty GISTs contained single region deletions, five contained two deletions, and one GIST was identified with three regions deleted. The deletions mostly clustered around the 5' sequences of exon 11, which encodes for the juxtamembrane domain of the *KIT* receptor (Figure 1c). In eleven GISTs, intron 10/exon 11 splice sites were deleted. In five GISTs, both intron 10/exon 11 and exon 11/intron 11 splice sites were deleted. In three GISTs, deletion of the intron 10 acceptor splice site results in new splice acceptor sites, with strong consensus values as predicted by the Human Splicing Finder program (Desmet et al., 2009). The three deletions with new splice acceptor sites encode for in frame amino acid deletions in the juxtamembrane domain of the *KIT* receptor. In other GISTs, splice site deletions likely result in exclusion of the exon 11 encoding amino acids from the juxtamembrane domain of *KIT* receptor.

The non-synonymous single nucleotide mutations that were found in the present study include c.1669T>A, c.1676T>A, c.1676T>C, c.1676T>G, and c.1679T>A which encode for the p.Trp557Arg, p.Val559Asp, p.Val559Ala, p.Val559Gly, and p.Val560Asp substitutions, respectively (Figure 1). Among these non-synonymous mutations, the p.Trp557Arg encoding mutation was more common than the mutations encoding for p.Val559Asp, p.Val559Ala, p.Val559Gly, and p.Val560Asp changes. Duplications, inversions, deletions/insertions, and other complex mutations were not found in the present study. No mutation was found in other exons of the *KIT* and the *PDGFRA* genes that were sequenced. Five tumors (~9%) were negative for either *KIT* or *PDGFRA* genes mutation. No significant differences were present in tissue distribution of GISTs with respect to point mutations and deletions in the *KIT* gene (P>0.05).

Not enough information was available about the treatment of these patients whose tumors were collected and archived over 16 years. Therefore, no relationship could be established about the drug responsiveness of specific mutations.

*Mitotic index*

Mitotic counts were determined for 47 GISTs (Table 3). Of the GISTs with deletions in the *KIT* gene, 48% had a mitotic index of >10 and 52% had <10. In GISTs with single point mutations of codons 557, 559, and 560, only 22% GISTs had a mitotic index of >10 and 78% had <10. Although not statistically significantly different (P>0.05), the data shows a trend of a higher mitotic index of tumors with deletions in the *KIT* gene.

## Discussion

In this study, we have described the age of presentation, organ distribution, and molecular characterization of GISTs in Arab patients. DNA sequencing of the *KIT* and *PDGFRA* genes revealed non-synonymous point mutations and deletions in exon 11 of the *KIT* gene. These mutations produce single amino acid substitutions and deletions in the juxtamembrane domain of the *KIT* receptor (Mol et al., 2004).

In this cohort of patients, most GISTs occurred in the stomach and then in the intestinal tract. Most tumors exhibited spindle-shaped cell morphology, some tumors exhibited epithelioid appearance, and a single GIST showed mixed cell populations. Most tumors showed expression of the CD117 and CD34 antigens, which are diagnostic markers for GIST (Biasco et al., 2009). In five tumors CD117 expression was not detected. It is notable that expression of CD117 (*KIT*) is independent of the mutations in GISTs, which cause functional activation of the *KIT* or *PDGFRA* receptors. As reviewed previously, CD117 and CD34 are only expressed in ~ 95% and 60-70% of GISTs, respectively (Lasota and Mietttinen 2008; Sepe and Brugge 2009). The observations in the present study agree with the histopathological description of GISTs in previous studies.

In tumors with deletions, ~48% had a mitotic index of >10 compared to only 22% in tumors with single amino acid substitutions. These data indicate that deletions in exon 11 of the *KIT* gene are more deleterious in our cohort of patients.

The five non-synonymous mutations and most of the 26 deletions were found around the 5' sequences of exon 11of the *KIT* gene. This mutation 'hotspot' region encodes for the juxtamembrane domain, which normally inhibits ligand-independent activation of the kinase function of the *KIT* receptor. Mutations and deletions in this domain result in constitutive tyrosine kinase activity of *KIT* in the absence of its ligand, Stem Cell Factor (Hirotaetal.,1998;LasotaandMiettinen,2008).Theconstitutive kinase activity of *KIT* confers autophosphorylation of tyrosine and activation of downstream signaling pathways, cell proliferation, and progression towards GIST. In addition to unique single deletions in twenty GISTs, multiple deletions in exon 11 of the *KIT* gene were found in six GISTs. These could either be independent mutations in different cells of the tumor or may have arisen as secondary mutations following treatment of patients with drugs. Secondary deletions in GISTs have been described previously (Lasota and Miettinen, 2008).

In the GIST with a twelve-nucleotide homozygous deletion, loss of heterozygosity and subsequent homozygosity of the mutation may have ensued. This phenomenon has been described in GISTs (Lasota et al., 2007). The twelve nucleotide deletion encodes for the p.Pro551_Glu554del which has been described in other patients (Andersson et al., 2006). The creation of new splice acceptor sites in three deletions produces in-frame deletions of amino acids in exon 11 of the *KIT* gene. This is consistent with the *KIT* mutations as being causative in these GISTs.

While most of the deletions are unique, the five amino acid substitutions have been described in other studies (Hirota et al., 2000; Beghini et al., 2001; Maeyama et al, 2001; Morey et al., 2002; Martin et al, 2005; Haller et al., 2007; Keun Park et al., 2008; Kontogianni-Katsarou et al; 2008; Forbes et al., 2010). The two CD117 positive GISTs in the esophagus and a single GIST in the ovary harbored the p.Trp557Arg and the p.Val559Gly encoding mutations, respectively. In a previous study conducted in USA, p.Val559 was the most frequently affected amino acid in *KIT* followed by p.Val560 and p.Trp557 amino acids (Lasota and Miettinen, 2008). In the present study, GISTs with single nucleotide mutations exhibited most substitutions involving the p.Trp557, followed by p.Val559 and p.Val560 codons. No mutation was found in five GISTs. The absence of mutations in *KIT* and *PDGFRA* has been described in <10% of sporadic GISTs (Lasota and Miettinen, 2008). The p.Ala502_Tyr503dup which was described as being rare (6.6%) in stomach GISTs in the European and the United States studies and frequent (32%) in the combined Asian studies, was not found in our patients (Lasota and Miettinen, 2008). Furthermore, insertions, inversions, insertion/deletions, and other complex mutations were not found in the present study (Martin et al., 2005; Lasota et al., 2007; Keun Park et al., 2008; Kontogianni-Katsarou et al., 2008; Lasota and Miettinen, 2008). These findings suggest a different frequency and spectrum of mutations in Arab patients. The single nucleotide polymorphisms and point mutations may affect some molecular aspects of genes and response to treatment (Ebrahimi et al., 2017; Mavroeidis et al., 2018; Soleimani et al., 2017).

The present study is the only description of molecular characterization of GISTs in the Arab population. This study revealed tissue distribution, the age of onset, and the expression of CD117 and CD35 antigens similar to other reports, but the frequency and nature of various mutations differed from previous studies. Altogether, this unique study of Arab patients provides useful information for molecular and cellular characterization of GISTs, which can aid in the management of patients from this distinct ethnicity.

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