


## BRIEF REPORT

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# The novel *KIT* exon 11 germline mutation K558N is associated with gastrointestinal stromal tumor, mastocytosis, and seminoma development

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## Abstract

Familial gastrointestinal stromal tumors (GIST) are dominant genetic disorders that are caused by germline mutations of the type III receptor tyrosine kinase *KIT*. While sporadic mutations are frequently found in mastocytosis and GISTs, germline mutations of *KIT* have only been described in 39 families until now. We detected a novel germline mutation of *KIT* in exon 11 (p.Lys-558-Asn; K558N) in a patient from a kindred with several GISTs harboring different secondary somatic *KIT* mutations. Structural analysis suggests that the primary germline mutation alone is not sufficient to release the autoinhibitory region of *KIT* located in the transmembrane domain. Instead, the *KIT* kinase module becomes constitutively activated when K558N combines with different secondary somatic mutations. The identical germline mutation in combination with an additional somatic *KIT* mutation was detected in a second patient of the kindred with seminoma while a third patient within the family had a cutaneous mastocytosis. These findings suggest that the K558N mutation interferes with the juxtamembranous part of *KIT*, since seminoma and mastocytosis are usually not associated with exon 11 mutations.

## KEYWORDS

germline mutation, GIST, *KIT*, mastocytosis, seminoma

## 1 | INTRODUCTION

Receptor tyrosine kinases (RTKs) are ubiquitously expressed enzymes that control several pivotal cellular processes including cell migration, cell proliferation, and apoptosis.<sup>1</sup> The modular structure of RTKs consists of an immunoglobulin-like extracellular domain, a small helical transmembrane domain, a juxtamembrane domain, and an intracellular domain containing the kinase module. *KIT* is a proto-oncogene RTK that is expressed in hematopoietic cells, interstitial cells of Cajal, gametogenic cells, and melanocytes.<sup>2–4</sup> Binding of *KIT* to its ligand, stem cell factor (SCF), leads to receptor dimerization, activation of *KIT*'s intrinsic kinase function, and autophosphorylation of different

tyrosine residues within the intracellular domain.<sup>5</sup> This then initiates multiple intracellular signaling cascades including PI3K, MAPK, and SRC signaling. Thus, activating somatic mutations of *KIT* can lead to various malignancies including gastrointestinal stromal tumors (GIST), seminoma, and mastocytosis.<sup>6</sup>

Approximately 90% of nonsyndromic GISTs<sup>7</sup> and 80% of mastocytoses<sup>8</sup> are associated with mutations of *KIT*, whereas syndromic GISTs like Carney–Stratakis syndrome are linked to mutations in the succinate dehydrogenase gene.<sup>9</sup> While most of these mutations are sporadic, 39 families with germline mutations of *KIT* have been reported (a detailed description of these families can be found in Table S1). Familial GISTs are autosomal dominant genetic disorders generally characterized

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by hyperpigmentation or dysphagia in their clinical appearance, although each germline mutation can present differently.<sup>10</sup> Thus, penetrance of the germline alteration varies between family members and organ systems. Most *KIT* mutations lead to constitutive activation and autophosphorylation of the receptor in the absence of ligand binding.<sup>11,12</sup> The majority of mutations responsible for *KIT*-associated tumors are located in exon 11 encoding the juxtamembraneous domain of *KIT*. A better understanding of *KIT* function in different tumor entities is necessary to optimize the treatment of *KIT*-associated diseases. Kindreds with germline mutations often hold the key to understanding gene function of in different tissue types. In the present study, we describe a novel germline mutation of *KIT* in exon 11 encoding the juxtamembraneous domain that leads to different kinds of *KIT*-associated neoplasia.

## 2 | MATERIALS AND METHODS

### 2.1 | Patients and DNA extraction

The index patient (Patient 1) is a 60-year old with eight gastric GISTs. The four largest GISTs (2 cm–4.3 cm in size) were laparoscopically resected, while the other smaller GISTs (<1.5 cm) were placed under continuous surveillance over a period of more than 3 years and showed no significant progression. The normal gastric tissue showed no Cajal cell hyperplasia, though manometric measurements revealed unspecific contractions of the esophagus. The son of Patient 1 (Patient 2) was diagnosed with a seminoma at the age of 29 roughly 2 years prior to the diagnosis of Patient 1. A grandchild of Patient 1 was diagnosed with cutaneous mastocytosis 4 years ago at the age of 2. Blood samples were investigated in the other unaffected family members. All family members were offered genetic counseling and endoscopic and magnetic resonance imaging surveillance.

Subsequent to microdissection, GIST and seminoma samples were formalin-fixed and paraffin-embedded. Genomic DNA was extracted using the Maxwell RSC Blood DNA Kit after pre-treatment with a THG1-Thioglycerol/incubation buffer mix for 10 min at 80°C and subsequent incubation with proteinase K at 65°C overnight (Promega GmbH, Walldorf, Germany). DNA was quantified by quantitative PCR (TaqMan RNase P Detection Reagents Kit, Life Technologies, Darmstadt, Germany).

### 2.2 | Multiplex PCR-based panel sequencing

Libraries were prepared with the Ion AmpliSeq Cancer Hotspot Panel v2 and the Ion AmpliSeq Library Kit 2.0, according to the manufacturer's recommendations. Subsequently, libraries were templated and enriched with the Ion OneTouch 2 and the Ion One Touch ES automated systems. Sequencing was performed using semiconductor-sequencing technology (Ion Torrent PGM). Data were analyzed using the Torrent Server Variant Caller (v 5.6) and the Ion Reporter Software (v5.10) (Thermo Fisher Scientific, Darmstadt, Germany). We

filtered for somatic, nonsynonymous, exonic variants, and splice site variants in the flanking regions, showing an allele frequency of more than 5%. Variants in the general reported population with a minor allele frequency of more than 0.5% in 1000 Genomes and dbSNP were excluded.

### 2.3 | Mapping of mutations in the *KIT* crystal structure

Mutations of the autoinhibited form of *KIT* (pdb code 1t46, PMID 15123710) were mapped and visually inspected using the pymol software.

## 3 | RESULTS

### 3.1 | Panel sequencing reveals a novel germline mutation of *KIT* in kindred with additional mutations in different *KIT*-associated neoplasias

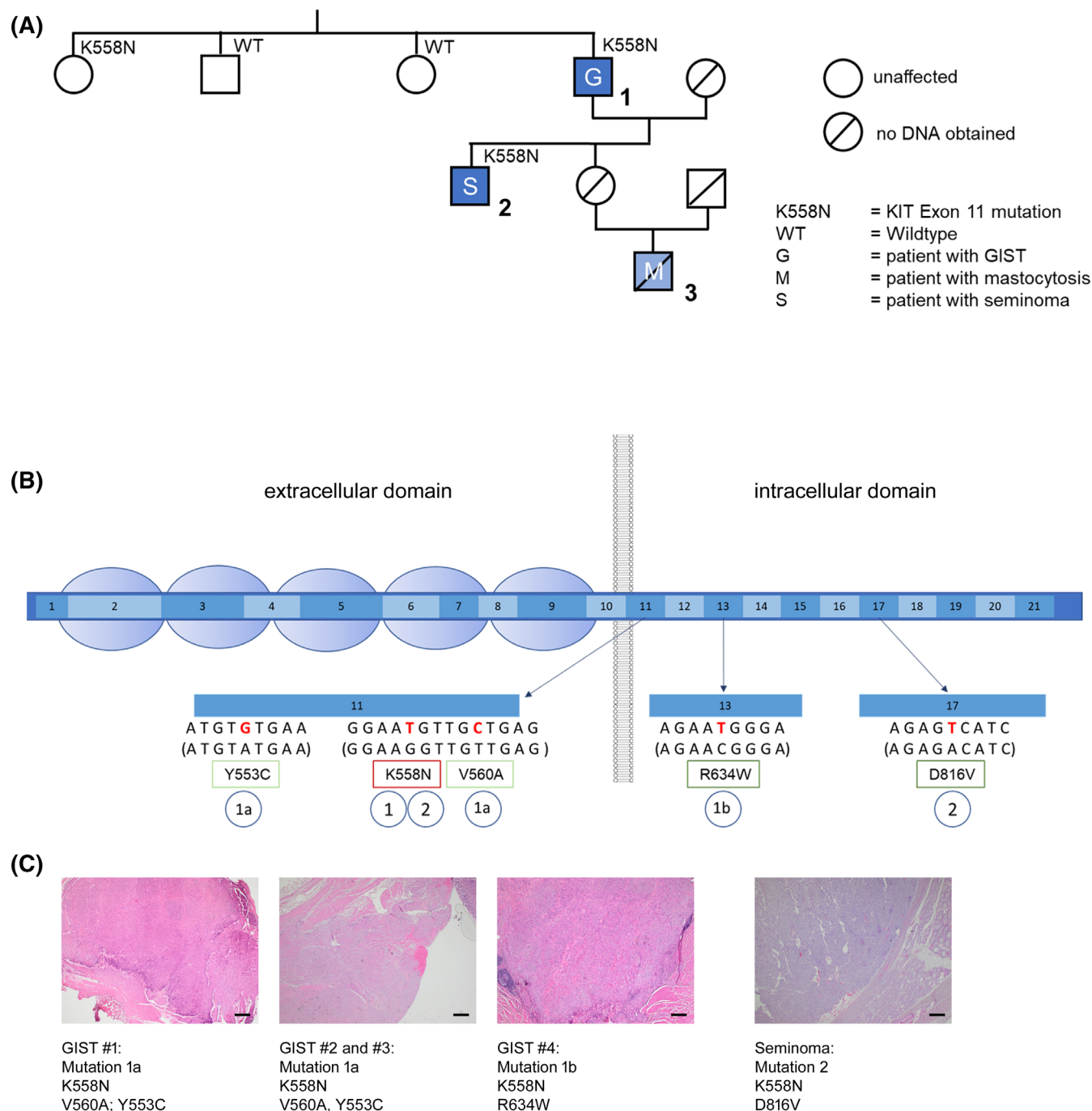
Within the kindred, we found an increase in *KIT*-associated diseases (Figure 1). The pedigree demonstrated *KIT*-associated tumors such as GIST, seminoma, and cutaneous mastocytosis in three generations. Panel sequencing demonstrated that all tumors and healthy tissue of the resection margin showed a mutation in exon 11 with an allele frequency of 50% indicating a heterozygous germline mutation in the family (Table S2).

Consequently, all resected tumors within the family were further analyzed. All gastric tumors of Patient 1 carried the K558N mutation in exon 11. Three of four GISTs showed an additional mutation (p.V560A in exon 11) while in the fourth tumor a different additional mutation was detected in *KIT* exon 13 (p.R634W) (Figure 2). All GISTs were smaller than 5 cm and showed a mitotic count of less than 1/5 mm<sup>2</sup> suggesting a very low risk for disease progression according to Miettinen.

In the second generation, Patient 2 was diagnosed with a seminoma. Analysis of their tumor specimen demonstrated an activating co-mutation in the *KIT* gene in exon 17 (p.D816V) (Figure 2A). In the third generation, Patient 3 was diagnosed with yet another *KIT*-associated disease—a cutaneous mastocytosis). However, the family declined testing for the germline mutation or further investigations of tissue samples.

### 3.2 | Structural mapping indicates a clustering of all mutations around the auto-inhibitory region of *KIT*

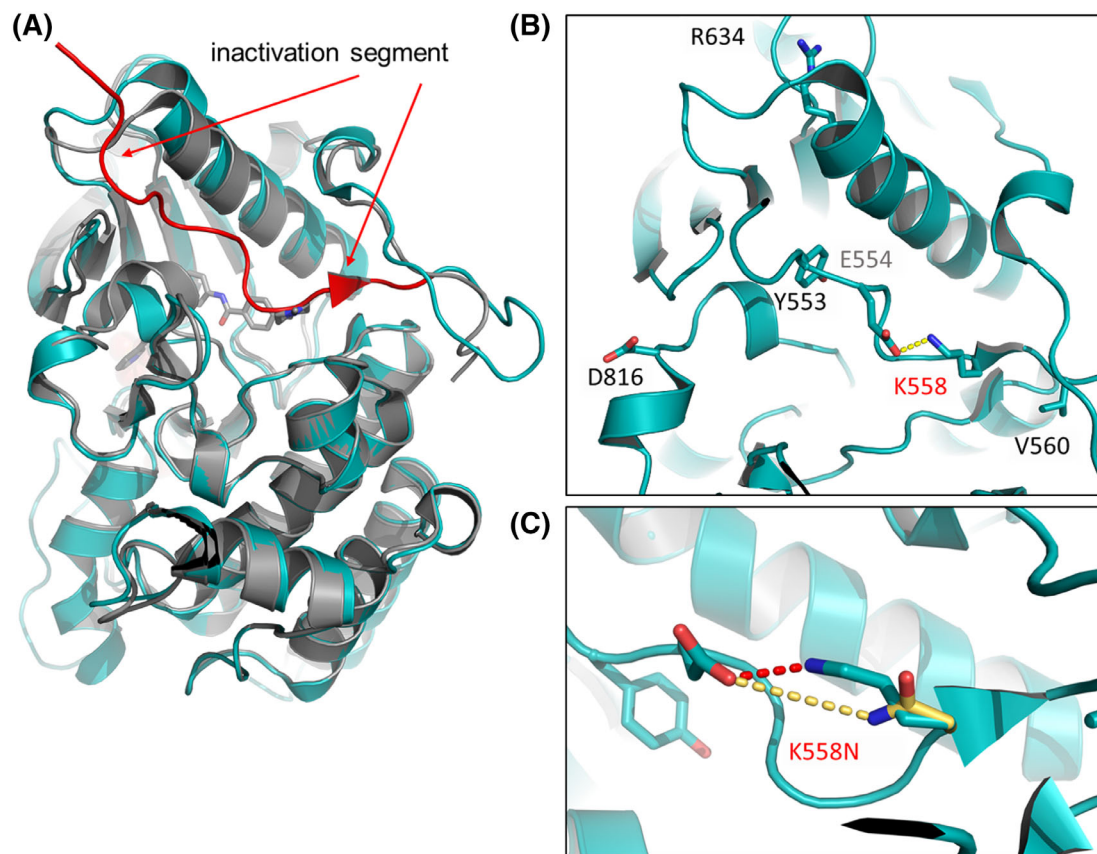
Structural mapping of the *KIT* kinase mutations was performed to locate and analyze the variants in a structural model of the *KIT* kinase domain (1t46) and thus to discern their influence on protein structure. This analysis revealed that the p.K558N mutation in exon 11 is located in the autoinhibitory, juxtamembraneous domain of *KIT* (Figure 2B).



**FIGURE 1** (A), Pedigree of kindred with familial *KIT*-associated tumors is shown. Blue symbols indicate family members with a *KIT*-associated disease. Family members that inherited the germline mutation were marked with K558N, while those without were marked with WT. A dash indicates that the family members declined a genetic investigation. Numbers point to the description in the case report. Squares, males; circles, females. (B) A schematic overview of *KIT* is shown with the mutations identified in the different exons and different tumors of the patients. All tumors showed a heterozygous mutation of the germline mutation p.K558N in exon 11. In Patient 1, three GISTs showed secondary mutations of p.Y553C and p.V560A in exon 11, while another GIST showed a secondary mutation of p.R634W in exon 13. The seminoma specimen of Patient 2 showed an additional mutation of *KIT* p.D816V in exon 17 together with the germline mutation of p.K558N in exon 11. (C) Hematoxylin and eosin staining of the tumors demonstrate the typical features of GISTs and seminoma. In Patient 1, two of the four GIST (#2 and #3) were in close proximity and therefore resected in one specimen. The correlating mutations in the tumors are listed directly under the histological images (scale 200  $\mu$ m)

The p.K558N mutation potentially leads to a disruption or weakening of the salt bridge between K558 and E554, which supports the conformation of the autoinhibitory segment (Figure 2C). However, K558N

alone might not be sufficient to destabilize this region and thus to lead to an autoactivation of the *KIT* kinase (Figure 2D). Looking at the clustering of the additional *KIT* mutations either in or around this



**FIGURE 2** (A). Model of the KIT kinase domain. The left panel shows a superposition of the active (gray) and inactive (cyan) form of c-KIT (1t45, 1t46) with the inactivation segment highlighted in red. The protein is shown in cartoon mode. (B) The panel shows the location of the amino acids that are affected by the single mutations. The exchanges have not been modeled in the figure. (C) Modeling of p.K558N revealed that the mutation alone is not sufficient to destabilize the autoinhibitory, juxtamembraneous domain of KIT

inhibitory segment, it is likely that only the combination of the primary and the respective secondary mutation is strong enough to release autoinhibition.

## 4 | DISCUSSION

In the present study, we describe a family with a novel germline mutation located in exon 11 of the *KIT* gene. Usually, these families are discovered by accumulation of *KIT*-associated tumors or diseases in different family members. In our described kindred, only half of the family members with the germline mutation showed a *KIT*-associated disease. For the first time, a germline mutation of *KIT* in exon 11 was found to be associated with a seminoma and probably with cutaneous mastocytosis. In total, 25 families with a germline mutation of *KIT* in exon 11 have been identified.<sup>13</sup> These germline mutations within the *KIT* gene were usually gain-of-function mutations. In contrast, our kindred harbored a *KIT* mutation in exon 11 which alone is evidently not sufficient to cause tumorigenesis. The fact that only 50% of family members with the detected K558N mutation showed a phenotype may point to its role as a predisposing cofactor, which needs additional *KIT* mutations to induce *KIT*-associated tumors.

The mutation was not biochemically characterized and therefore its effect on *KIT* protein function remains unknown. Furthermore, most of the additional mutations detected in the gastric GISTs and the seminoma have already been described in sporadic *KIT*-associated tumors. While the p.Y553C and p.V560A mutations in exon 11 have been described previously in sporadic GISTs,<sup>14,15</sup> and the p.D816V mutation in exon 17 is commonly found in mastocytosis and seminoma,<sup>8</sup> the p.R634W mutation in exon 13 is novel and its significance in GIST pathogenesis requires further study.

Our observations may provide insight into the biological relevance of the p.K558N *KIT* mutation. For example, it could potentially predispose the development of additional mutations that lead to *KIT*-associated tumors. This hypothesis is somehow limited by the fact that in the kindred, only Patient 1 developed multiple GISTs with two different *KIT* mutations while no other kindred in the same generation has so far developed *KIT*-associated tumors. In addition, the two patients in the second and third generations did not develop further tumors which may be due to their relatively young age. This is in line with the median age of 40.6 years in patients from other families with germline GISTs (Table S1).

Our results suggest that *KIT*-associated tumors develop only when the p.K558N mutation occurs in combination with additional

sporadic mutations. This is in line with eight other reports of the p.K558N mutation in sporadic KIT-associated tumors<sup>16–24</sup> in which the mutation in GISTs was always found together with a secondary mutation (Table S3). In particular, the patient reported by Kikuchi et al.<sup>23</sup> presented with multiple sporadic GISTs. The association of the p.K558N mutation with the V560 deletion and the V654A mutation in the same patient further supports our hypothesis of a predisposing role for the K558N mutation.

The pathogenic potential of the p.K558N mutation is further underlined by its association with secondary imatinib resistance.<sup>17</sup> Usually, GISTs with multiple mutations in *KIT* in exon 11 are sensitive to RTK-inhibition with Imatinib, whereas the individual mutation predicts response rate. However, in other tumors with similar mutations, sensitization to Imatinib treatment does not occur. For example, mutations in *KIT* have been identified in various melanomas, but they are resistant to Imatinib treatment.<sup>25</sup>

Taken together, structural analysis revealed in our kindred that secondary mutations cluster with the primary mutation either in or around the auto-inhibitory region of *KIT*. We conclude that only the combination of both mutations leads to the necessary destabilization of the region to release the autoinhibitory function of *KIT*.

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## AUTHOR CONTRIBUTIONS

Michael Meir, Katja Maurus, and Jochen Kuper contributed to the study concept and experiments, drafted figures and wrote the manuscript. Christoph-Thomas Germer and Andreas Rosenwald contributed to the study concept and drafted the manuscript. Eva Wardelmann contributed to the case study as reference pathologist and revised the manuscript. Armin Wiegering contributed to the study concept and experiments, drafted figures, and drafted the manuscript.

## DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article.

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#### SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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