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Original Article

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Analysis of actionable gene fusions in a large cohort of Chinese patients with colorectal cancer

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Abstract

Background: The prevalence of gene fusion is extremely low in unselected patients with colorectal cancer (CRC). Published data on gene fusions are limited by relatively small sample sizes, with a primary focus on Western populations. This study aimed to analyse actionable gene fusions in a large consecutive Chinese CRC population.

Methods: This study included 5,534 consecutive CRC patients from the Genecast database. Genomic profiling was performed using a panel of 769 cancer-related genes. Data for 34 CRC patients with actionable gene fusions were also collected from cBioPortal and ChimerSeq.

Results: Among 5,534 CRC patients, 54 (0.98%) had actionable gene fusions, with *NTRK1/2/3* being the most common fusion (0.38%), accounting for 38.9% (21/54) of those with fusions. Actionable gene fusion enrichment was higher in patients with microsatellite instability-high (MSI-H) (6.7% vs. 0.5%, $P < 0.001$), *RAS/BRAF* wildtype (2.0% vs. 0.2%, $P < 0.001$) and *RNF43* mutation (7.7% vs. 0.4%, $P < 0.001$) than in patients with microsatellite stability/MSI-low, *RAS/BRAF* mutation and *RNF43* wildtype, respectively. When these markers were combined, the fusion detection rate increased. Among patients with *RAS/BRAF* wildtype and MSI-H, fusions were detected in 20.3% of patients. The fusion detection rate further increased to 37.5% when *RNF43* mutation was added. The fusion detection rate was also higher in colon cancer than in rectal cancer. No significant differences in clinical or molecular features were found in patients with actionable gene fusions between the Genecast, cBioPortal, and ChimerSeq databases.

Conclusions: Approximately 1% of the unselected Chinese CRC population carries actionable gene fusions, mostly involving *NTRK*. Actionable gene fusions are more prevalent in MSI-H, *RAS/BRAF* wildtype, or *RNF43*-mutated CRC, as well as in colon cancer. Mapping of these molecular markers can markedly increase the fusion detection rate, which can help clinicians select candidates for fusion testing and targeted therapy.

Keywords: actionable gene fusions; colorectal cancer; Chinese population

Introduction

Colorectal cancer (CRC) is one of the most commonly diagnosed cancers worldwide and is the third and fifth leading cause of cancer-related death in the United States and China, respectively [1]. The molecular characteristics of CRC have been emphasized in recent years, as they can significantly affect patient prognosis and determine therapeutic options [2]. In addition, the optimal therapeutic strategy for CRC depends on both patient characteristics and molecular features [3].

Approximately 40% of CRC patients carry *KRAS* gene mutations, whereas only approximately 4% of patients carry *NRAS* mutations. *BRAF* mutations are present in 10%–15% of patients. *RAS* mutations have been shown to be negative biomarkers for targeted anti-epidermal growth factor receptor (EGFR) therapies and are associated with shorter survival. Novel and specific *BRAF* and *KRAS* inhibitors appear to be promising for treating CRC [4]. A microsatellite instability-high (MSI-H) status is found in 3%–5% of metastatic CRCs, and patients with MSI-H CRC tend to have a favourable prognosis and a high rate of response to immunotherapy [5]. The major guidelines recommend testing for *RAS* and *BRAF* mutations as well as the microsatellite status. Emerging biomarkers, such as the *HER2* and *NTRK* fusions, have also been proposed for testing in CRC [6, 7].

Rare gene fusions, which are considered diagnostic and prognostic markers, have been identified in various cancer types [8]. With the emergence of new targeted therapies, these fusions have become potential therapeutic targets [8]. For example, larotrectinib and entrectinib have received Food and Drug Administration approval for tumour-agnostic indications due to their remarkable

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clinical activity in a broad spectrum of advanced solid tumours harbouring *NTRK* gene fusions [9, 10]. In addition, other inhibitors for *ALK*, *RET*, or *ROS1* fusions (initially developed for non-small cell lung cancers) are now available for patients with metastatic CRC through basket clinical trials [11, 12]. Therefore, efficient detection and comprehensive characterization of gene fusions are of critical clinical significance.

Gene fusions involving *ALK*, *RET*, and *ROS1* may represent new targets for CRC therapy [13–15]. However, the prevalence of gene fusions in CRC is extremely low, accounting for less than 1% of unselected CRC cases [16]. Moreover, universal screening of CRC patients for gene fusion is impractical and expensive. Nevertheless, a significant percentage of patients in a selected population might harbour gene fusions. Recent research has revealed that gene fusions are significantly enriched in specific molecular subtypes of MSI-H and *RAS/BRAF* wildtype CRC [17–20]. Combining MSI and *RAS/BRAF* testing might narrow the population for gene fusion testing, making this approach feasible and cost-effective.

However, published data on gene fusions are limited by relatively small sample sizes, and studies have primarily focused on Western populations. Therefore, the objective of this study was to analyse gene fusions in a large consecutive Chinese CRC population using targeted next-generation sequencing (NGS). Additionally, clinicopathological and molecular genetic features were examined to identify the possible enrichment of fusions.

Materials and methods

Patient selection

We retrospectively analysed the genomic alteration data from 5,534 CRC patients who consecutively underwent genetic testing at Genecast Medical Laboratory (Wuxi, Jiangsu, China) between January 2020 and August 2023 (Genecast database). Clinicopathological data, including age at diagnosis, sex, and primary tumour site, were collected. In addition, 34 CRC patients with actionable gene fusions from the cBioPortal (https://www.cbioportal.org/) and ChimerSeq databases (https://www.kobic.re.kr/chimerdb/) were included to examine differences between the Chinese and Western populations [21, 22]. The study was conducted according to the ethics principles of the Declaration of Helsinki, and patients were informed of the study by each investigator and did not express opposition.

Deoxyribonucleic acid (DNA) extraction

Tumour DNA was extracted from formalin-fixed paraffin-embedded (FFPE) CRC specimens by using a Direct FFPE DNA Kit (Qiagen #A31133) according to the manufacturer's protocols.

DNA panel sequencing

The extracted tumour DNA was sheared with a Covaris LE220 instrument, and a KAPA Hyper Preparation Kit was used to prepare libraries with the fragmented DNA. Then, targeted region selection was performed with an IDT xGen Hybridization and Wash Kit. A customized DNA panel including 769 cancer-related genes was designed by Genecast Medical Laboratory and used for hybridization-based NGS to detect single-nucleotide variations, insertions and deletions, copy number alterations and rearrangements. This DNA panel also covers common genetic variations associated with CRC (Supplementary Table S1). The prepared libraries were sequenced by using an Illumina NovaSeq 6000. All the raw Illumina sequence data were demultiplexed and trimmed into clean data using Trimmomatic. Clean reads were aligned to the human reference genome (hg19) by using the bwa mem algorithm. The sequencing panel can detect four types of genomic alterations, including gene fusions using FusionMap, single-nucleotide variants and small insertions and deletions using VarDict and ANNOVAR, and copy number variations using CNVkit [23–26]. Moreover, NGS-based algorithms can be used to determine the microsatellite status, MSI-H status, microsatellite stability status, or microsatellite instability-low (MSS/MSI-L) status [27, 28].

Determination of gene fusions

The detected gene fusions were classified into two categories: definite gene fusions without functional or available drugs and actionable gene fusions with available drugs. Only reported clinically actionable gene fusions were considered, mainly involving the following genes: *NTRK1/2/3*, *RET*, *ALK*, *BRAF*, *ROS1*, and *FGFR1/2/3* [9, 29–33].

Statistical analysis

Continuous variables are presented as the medians and interquartile ranges (IQRs) and were compared using the Wilcoxon rank-sum test. The distributions of categorical variables are presented as frequencies and percentages and were compared by using Fisher's exact test and the chi-square test. All the statistical analyses were performed by using SPSS version 24.0 software (SPSS, Inc., Chicago, IL). $P < 0.05$ (two-sided) was considered to indicate statistical significance.

Results

Prevalence and spectrum of actionable gene fusions in CRC

Our analysis of 5,534 consecutive unselected CRC patients revealed actionable gene fusions in 54 patients (0.98% of the total patients). The most commonly detected actionable gene fusions were *NTRK1/2/3* in 21 patients (0.38%), accounting for 38.9% (21/54) of the patients with fusions. The other actionable fusion genes detected included *RET* in 14 patients (0.25%), *ALK* in 8 patients (0.14%), *BRAF* in 6 patients (0.11%), *FGFR2/3* in 4 patients (0.07%), and *ROS1* in 1 patient (0.02%) (Figure 1A). The distribution of actionable gene fusions in the 54 patients is presented in Figure 1B. All the fusion genes preserve the portion encoding the kinase domain of the cancer driver gene and are classified as tier I (variants with strong clinical significance) or tier II (variants with potential clinical significance) according to the Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer [34]. The fusion breakpoints of the 54 actionable gene fusions are shown in Supplementary Table S2.

Molecular genetic features of CRC patients with or without gene fusions

Among CRC patients with actionable gene fusions, the top 10 most frequently mutated genes were *TP53* (74.1%), *RNF43* (57.4%), *KMT2D* (42.6%), *PTPRS* (35.2%), *ATR* (31.5%), *FBXW7* (31.5%), *RAD50* (29.6%), *MSH3* (29.6%), *KMT2A* (25.9%), and *APC* (25.9%). Among CRC patients without actionable gene fusions, the top 10 co-mutated genes were *TP53* (77.4%), *APC* (72.2%), *KRAS* (49.8%), *PIK3CA* (20.5%), *SMAD4* (17.6%), *FBXW7* (16.9%), *HMCN1* (11.8%), *SOX9* (11.9%), *ARID1A* (10.5%), and *ATM* (10.4%) (Figure 2).

Notably, *TP53* was the most commonly co-mutated gene in both groups. In addition, *RNF43* was the second most common co-mutated gene, while the key receptor tyrosine kinase (RTK)-RAS driver genes *KRAS*, *NRAS*, and *BRAF* were not commonly mutated in CRC patients with actionable gene fusions.

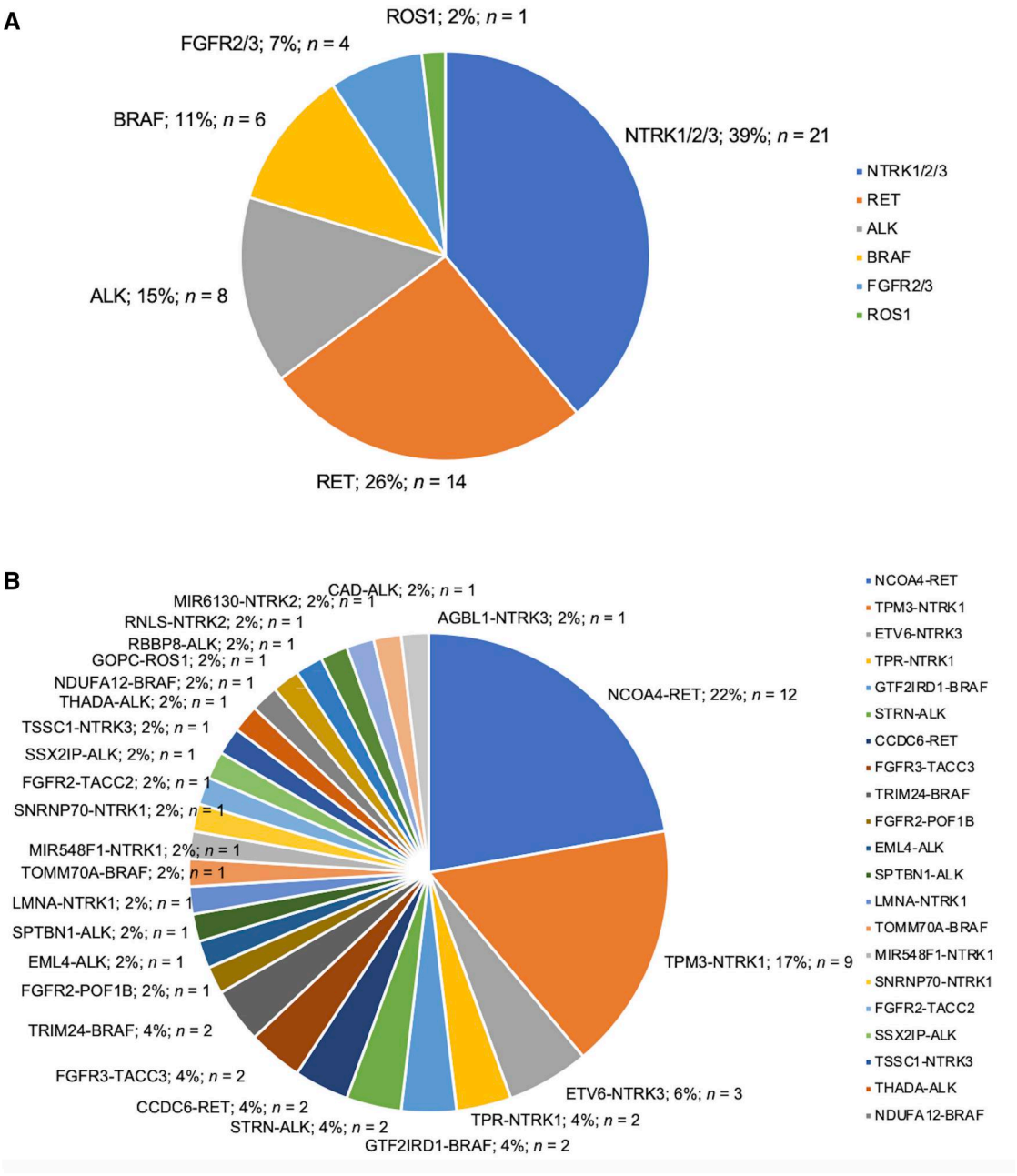

Figure 1. Molecular profiling of actionable gene fusions in the 54 colorectal cancer patients displayed by number and proportions of patients. (A) Molecular profiling of different genes. (B) distribution of actionable gene fusions.

Relationships between MSI, the *RAS/BRAF* status, *RNF43* status, and actionable gene fusions

Of the 5,534 CRC patients, 406 (7.3%) presented with the MSI-H phenotype. Patients with MSI-H tumours were more likely to have actionable gene fusions than those with MSS/MSI-L tumours (6.7% vs. 0.5%, $P < 0.001$). In addition, among the 3,273 patients with *RAS* or *BRAF* mutations (accounting for 59.1% of the cohort), 0.2% ($n = 8$) had actionable gene fusions, and 2.0% ($n = 46$) of patients with wildtype *RAS* and *BRAF* carried actionable gene fusions ($P < 0.001$). Additionally, 404 (7.3%) of the CRC patients had *RNF43* mutations, 7.7% ($n = 31$) of them had actionable gene fusions, whereas 0.4% ($n = 23$) of the CRC patients with wildtype *RNF43* harboured actionable gene fusions ($P < 0.001$) (Figure 3, Table 1).

When these markers were combined, the detection rate of gene fusions increased. For *RAS/BRAF* wildtype and MSI-H patients, fusions were detected in 20.3% (24/118) of patients. Furthermore, when combined with the *RNF43* mutation, the fusion detection rate further increased to 37.5% (24/64) (Figure 3).

Comparison of clinicopathological and molecular features of CRC patients in the Genecast database versus those in the cBioPortal and ChimerSeq databases

Our analysis included 5,534 CRC patients, consisting of 54 patients with actionable gene fusion from the Chinese population in the Genecast database. In addition, 34 CRC patients with actionable gene fusions from the Western population in the

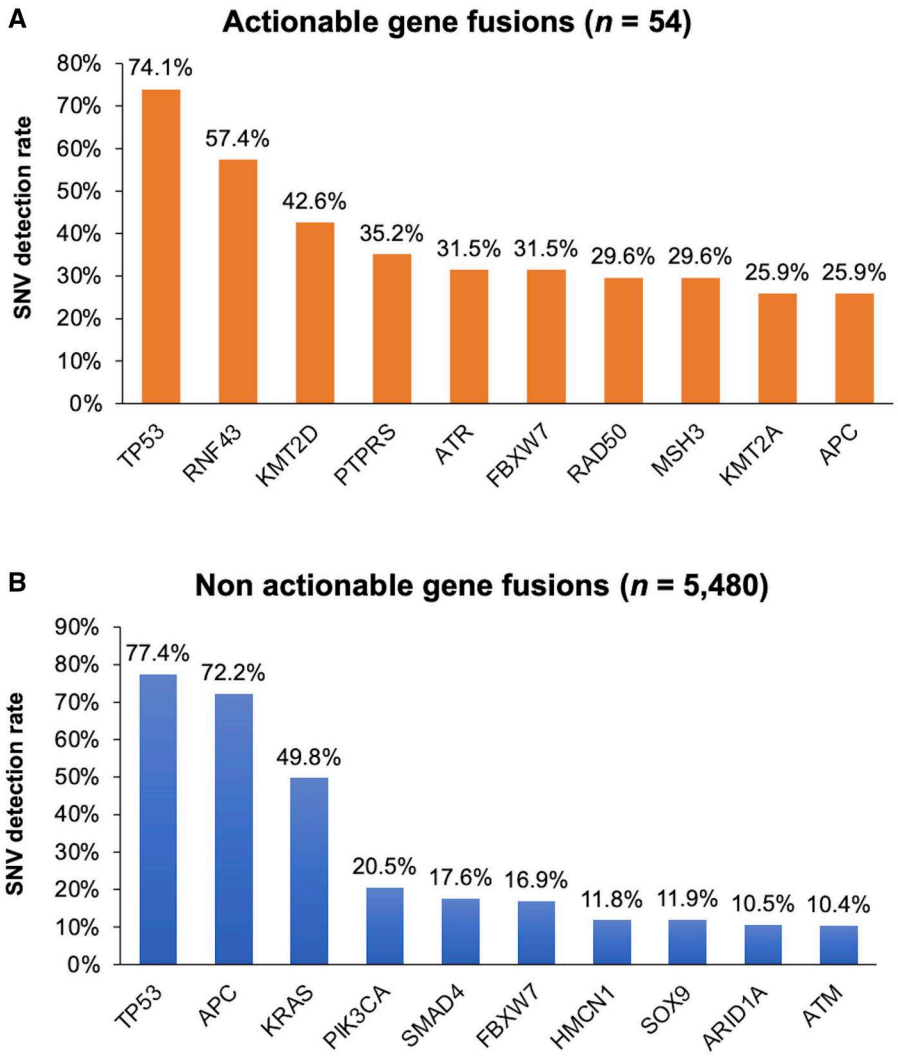

Figure 2. The top 10 most frequently co-mutated genes among 5,534 colorectal cancer patients with (A) or without (B) actionable gene fusions.

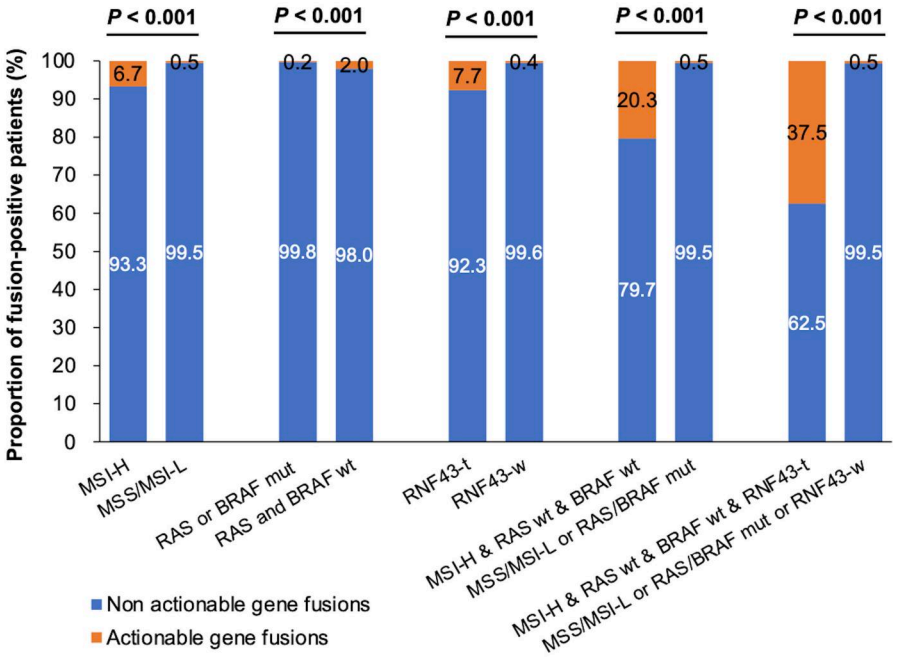

Figure 3. Detection rates of actionable gene fusions between different molecular groups among 5,534 colorectal cancer patients.

Table 1. Comparison of features in colorectal cancer patients between Genecast database and cBioPortal and ChimerSeq database

Characteristic	Genecast database		cBioPortal and ChimerSeq database	P1 value	P2 value
	Non-actionable gene fusion (*n* = 5,480)	Actionable gene fusion (*n* = 54)	Actionable gene fusion (*n* = 34)		
Age, years, median (IQR)	62 (53–70)	67 (59–71)	67 (57–74)	0.005	0.777
Gender, *n* (%)				0.035	0.195
Male	3,309 (60.4)	25 (46.3)	11 (32.4)		
Female	2,171 (39.6)	29 (53.7)	23 (67.6)		
Location, *n* (%)				<0.001	0.167
Colon	3,295 (60.1)	49 (90.7)	21 (61.8)		
Rectum	2,167 (39.5)	5 (9.3)	6 (17.6)		
NA[a]	18 (0.3)	0	7 (20.6)		
MSI status, *n* (%)				<0.001	0.105
MSI-H	379 (6.9)	27 (50.0)	9 (26.5)		
MSS/MSI-L	5,022 (91.6)	26 (48.1)	19 (55.9)		
Unknown	79 (1.4)	1 (1.9)	6 (17.6)		
RAS/BRAF, *n* (%)				<0.001	1.000
RAS and *BRAF* wildtype	2,215 (40.4)	46 (85.2)	29 (85.3)		
RAS or *BRAF* mutant	3,265 (59.6)	8 (14.8)	4 (11.8)		
Unknown	0 (0)	0	1 (2.9)		
RNF43, *n* (%)				<0.001	0.224
Wildtype	5,107 (93.2)	23 (42.6)	19 (55.9)		
Mutant	373 (6.8)	31 (57.4)	15 (44.1)		

IQR = interquartile range, MSI-H = microsatellite instability-high, MSS/MSI-L = microsatellite stability/microsatellite instability-low, NA = not applicable.
*P*1 = Genecast database: Non-actionable gene fusion vs. Gene fusion.
*P*2 = Gene fusion in Gencast database vs. Gene fusion in cBioPortal and ChimerSeq database.
[a] Eighteen patients from the Genecast database have double primary cancer of the colon and rectum.

cBioPortal and ChimerSeq databases were included to examine differences between the Chinese and Western populations.

Clinical characteristics were similar between the two populations with actionable gene fusions. The median age was 67 years, and the patients were predominantly female in both populations. Actionable gene fusions were more commonly located in the colon than in the rectum (Genecast: 90.7% vs. 9.3%; cBioPortal and ChimerSeq: 61.8% vs. 17.6%) (Table 1).

According to the Genecast database, compared with patients without actionable gene fusions, patients with actionable gene fusions were older ($P = 0.005$) and more frequently female ($P = 0.035$). Furthermore, the tumours were more predominantly located in the colon ($P < 0.001$) and were more likely to have MSI-H, *RAS/BRAF* wildtype, or *RNF43* mutation (all $Ps < 0.001$) (Table 1).

Among the molecular features identified in the cBioPortal and ChimerSeq database, the most commonly detected actionable gene fusions involved *NTRK* in 17 patients (accounting for 50.0% of the fusion-positive patients), followed by *BRAF* ($n = 4$, 11.7%), *ERBB2* ($n = 4$, 11.7%), *RET* ($n = 2$, 5.9%), *ALK* ($n = 2$, 5.9%), *ERBB3* ($n = 2$, 5.9%), *FGFR2* ($n = 2$, 5.9%), and *ROS1* ($n = 1$, 2.9%) (Figure 4). The molecular features, including the MSI-H status, *RAS/BRAF* status, and *RNF43* mutation status, were comparable between the two populations with actionable gene fusions (Table 1).

Discussion

We investigated the prevalence of actionable gene fusions in 5,534 Chinese CRC patients from the Genecast database, the largest cohort reported to date. We found an overall actionable gene fusion incidence of 0.98% among unselected patients with CRC. Interestingly, a greater incidence of actionable gene fusions was detected in the MSI-H, *RAS/BRAF* wildtype, or *RNF43*-mutated CRC populations, and the fusion detection rate increased significantly to 37.5% when these molecular markers were combined.

A recent comprehensive analysis revealed that gene fusions were present in 0.9% of 2,314 CRC patients, which is consistent with the prevalence rate of 0.98% found in our study [16]. Our findings combined with those of prior studies confirmed the low frequency of these genomic alterations in an unselected CRC population. Among the actionable gene fusions detected, the most common alteration involved *NTRK*, accounting for 38.9% of the patients with actionable gene fusions. This finding aligns with a study of 295,000 patients with solid tumours in which *NTRK* fusions were detected in 0.22% of 34,590 patients [35].

Previous studies have documented the significant enrichment of gene fusions in MSI-H and *RAS/BRAF* wildtype CRC, with rates ranging from 26% to 67% [17–20]. We found a similar pattern of enrichment in our study, which further supports the role of gene fusions as important oncogenic drivers in CRC. We speculate that gene fusions might serve as a major mechanism of RTK-RAS oncogenic activation and that they are mutually exclusive with *RAS* and *BRAF* mutations [3, 17]. Notably, the fusion rates of *NTRK* (the most commonly detected actionable gene fusion in our study) in patients with MSI-H and MSS/MSI-L were 3.69% (15/406) and 0.12% (6/5,048), respectively ($P < 0.0001$). Hence, identifying *NTRK* fusions in MSI-H patients could offer a new approach, particularly for those who do not respond to immunotherapy.

We observed that among CRC patients with actionable gene fusions, *RNF43* was the second most frequently co-mutated gene. Accordingly, patients with *RNF43* mutations had a greater frequency of gene fusion (7.7%). The possibility of using this single molecular biomarker to select gene fusions even exceeds that of MSI-H or *RAS/BRAF* wildtype, with detection rates of 6.7% and 2.0%, respectively. Additionally, the combination of MSI-H and wildtype *RAS/BRAF* increased the gene fusion detection rate to 20%. When *RNF43* was added, the detection rate further increased to 37%. Therefore, *RNF43* is a potentially valuable biomarker for selecting among CRC patients with actionable gene fusions.

Previous studies have reported that *RNF43* is the most commonly mutated gene in mismatch repair-deficient CRC tumours harbouring gene fusions [17]. Consistent with this result, another study documented a high incidence of *RNF43* mutations in CRC

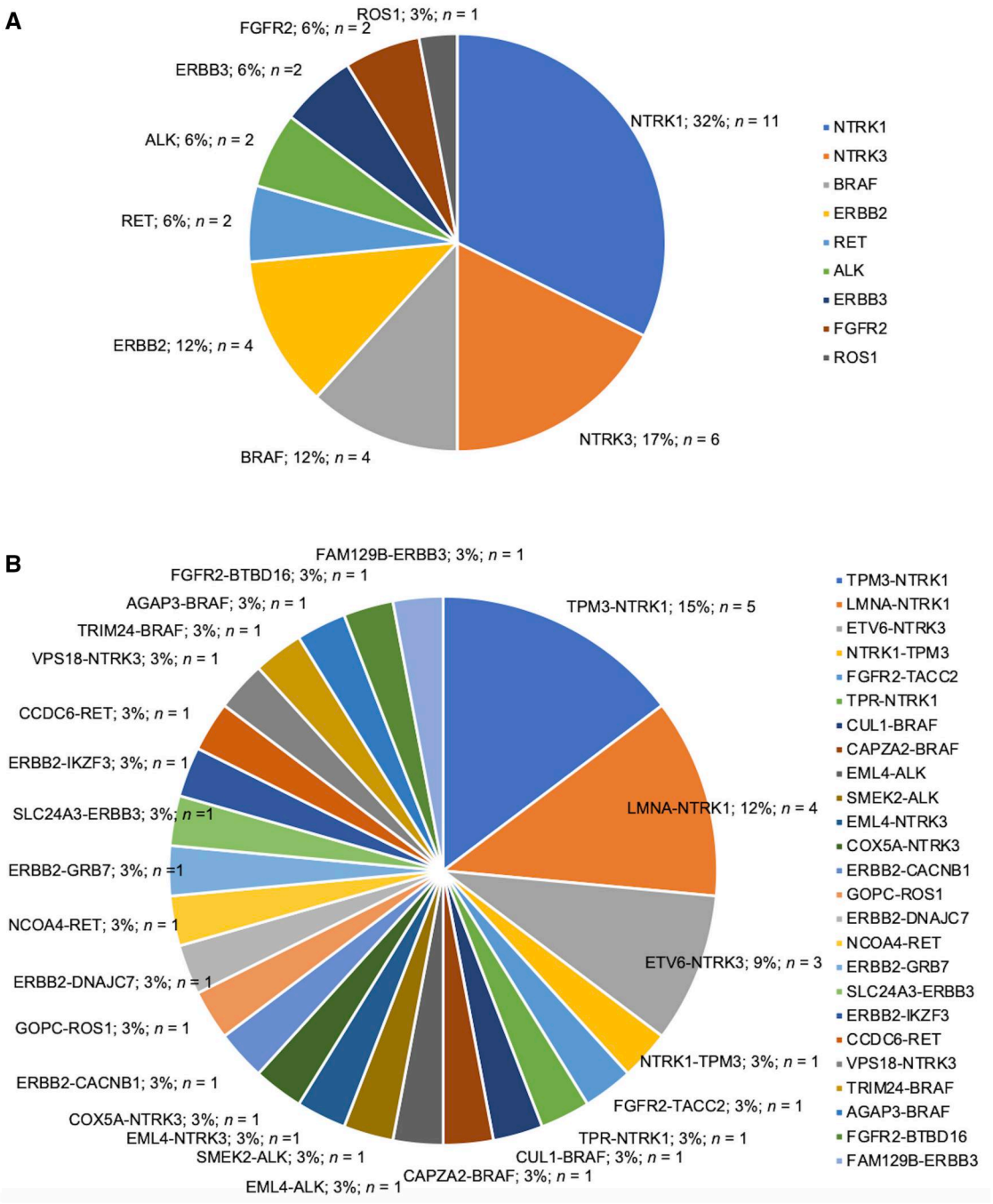

Figure 4. Actionable gene fusion in the 34 colorectal cancer patients from the public cancer database displayed by number and proportions of patients. (A) Molecular profiling of different genes; (B) distribution of actionable gene fusions.

tumours with *ALK*, *ROS1*, and *NTRK* rearrangements in the absence of concomitant *BRAF* V600E mutations. In addition, *RNF43* mutation enrichment in MSI-H CRC has been reported (64.7% vs. 5.9%, $P < 0.001$) [15]. *RNF43* is a key gene involved in the Wnt signalling pathway [36]. We hypothesized that a correlation might exist between the Wnt pathway and positive gene fusions in CRC. Further studies should be conducted to clarify the intrinsic molecular mechanism involved.

Previous studies have indicated that CRC patients with gene fusions are typically older and have poorly differentiated and right-sided colon tumours [15, 16]. Similarly, our study revealed that fusion-positive CRC patients tended to be older ($P = 0.005$) and had tumours predominantly located in the colon ($P < 0.001$). Notably, the fusion detection rate was greater for colon cancer than for rectal cancer (1.5% vs. 0.23%). Nevertheless, due to missing data, our study did not distinguish between right-sided and left-sided colon tumours. Moreover, no significant differences in clinical or molecular features were observed between Chinese and Western populations.

Gene fusions have been suggested to be associated with poor clinical outcomes [15]. Preclinical and preliminary clinical data suggest that patients with CRC tumours harbouring gene fusions might represent a population unlikely to respond to anti-EGFR treatment but might benefit from selective targeted agents [15, 20, 37]. Several clinical trials have reported the favourable efficacy of targeted therapies for patients with gene fusions, such as entrectinib for *NTRK*

fusion and selpercatinib for *RET* fusion, which might reverse the poor prognosis [9, 12]. Further prospective clinical trials are warranted to confirm this evidence, leading to a possible change in the treatment mode for these selected populations.

Currently, testing for *RAS/BRAF* mutations and the MSI status is recommended for patients with CRC by major guidelines. With the recognition of the predictive value of *RNF43* mutations in *BRAF* V600E metastatic CRC receiving anti-*BRAF/EGFR* combinatory therapies, routine testing for *RNF43* mutations would be acceptable [38]. Our findings showed that actionable gene fusions are more prevalent in MSI-H, *RAS/BRAF* wildtype, or *RNF43*-mutated CRC. For patients who have failed to traditionally chemotherapy or targeted therapy and have limited therapeutic options, their mutational status of *RAS*, *BRAF*, and *RNF43* along with MSI status might indicate the possibility of carrying actionable gene fusions and the necessity for additional RNA sequencing. Therefore, conducting *RAS/BRAF* and *RNF43* testing for actionable gene fusions is rational, as our findings suggest potential candidates for positive actionable gene fusions.

Our study has several limitations. The NGS data from the Genecast database included 796 cancer-related genes, and gene fusions may have been missed via whole-exome/genome sequencing. Furthermore, we did not collect data on treatments or outcomes. Therefore, we were unable to analyse the therapeutic and prognostic value of the gene fusions in our cohort. Despite these limitations, our study includes the largest number of patients to date and provides insights into the genomic landscape of gene fusions in the Chinese CRC population.

Conclusions

Approximately 1% of the unselected Chinese CRC population carries actionable gene fusions, the most common of which involve alterations in *NTRK*. A higher frequency of actionable gene fusions is observed in the MSI-H, *RAS/BRAF* wildtype, or *RNF43*-mutated CRC population, as well as in those with colon cancer. Mapping of these molecular markers can markedly increase the fusion detection rate, which can help clinicians select candidates for fusion testing and targeted therapy.

Supplementary Data

Supplementary data is available at *Gastroenterology Report* online.

Authors' Contributions

L.S. and X.-C.W. directed, conceived of, and designed the project. J.-J.Q., E.-L.Z., and L.-J.Z. collected the data. J.-J.Q., F.-R.K., J.L., Z.-H.W., and T.X. performed the analysis. F.-R.K. drafted the manuscript. X.-C.W. supervised the study and revised the manuscript. All authors read and approved the final manuscript.

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Conflicts of Interest

J.-J.Q., E.-L.Z., and L.-J.Z. are employees of Genecast Biotechnology Co., Ltd. The other authors declare that there are no conflicts of interest in this study.

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