A multicenter study assessing the prevalence of germline genetic alterations in Chinese gastric cancer patients

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Abstract

Background: Approximately 10% of patients with gastric cancer (GC) have a genetic predisposition for the disease. To date, knowledge regarding germline mutations in predisposing genes in the Chinese GC population is scarce. The aim of this study was to determine the spectrum and distribution of predisposing gene mutations among Chinese GC patients known to have hereditary high-risk factors for cancer.

Methods: Forty patients from among ten families were recruited from seven medical institutions in China. Next-generation sequencing was performed on 171 genes associated with cancer predisposition. For probands with pathogenic/likely pathogenic germline variants, Sanger sequencing was used to validate the variants in the probands and their relatives.

Results: Sequencing indicated that 25% (10/40) of the patients carried a combined total of ten pathogenic or likely pathogenic germline variants involving nine different genes: CDH1 (n = 1), MLH1 (n = 1), MSH2 (n = 1), CHEK2 (n = 1), BLM (n = 1), EXT2 (n = 1), PALB2 (n = 1), ERCC2 (n = 1), and SPINK1 (n = 2). Five of these variants have not previously been reported. In addition, a total of 129 variants of uncertain significance were identified in 27 patients.

Conclusions: This study found that 25% of Chinese GC patients with hereditary high-risk factors have deleterious germline alterations. This result may indicate a unique genetic background of GC among Chinese patients.

Background

Gastric cancer (GC) is the third most common cause of cancer-related mortality worldwide[1]. Approximately 10% of GC cases are associated with strong familial clustering and can be attributed to genetic risk. It is now established that 1–3% of GC cases arise as a result of inherited cancer predisposition syndromes including hereditary diffuse gastric cancer (HDGC), Lynch syndrome (LS), Li-Fraumeni syndrome (LFS), Peutz-Jeghers syndrome (PJS), hereditary breast and ovarian cancer (HBOC), MUTYH-associated adenomatous polyposis (MAP), familial adenomatous polyposis (FAP), juvenile polyposis syndrome (JPS), and PTEN hamartoma tumor syndrome[2]. Since the discovery of the GC susceptibility gene CDH1 in 1998[3], more than 20 GC-associated susceptibility genes have
been identified, including CDH1, MLH1, MSH2, MSH6, PMS2, EPCAM, TP53, STK11, BRCA1, BRCA2, MUTYH, APC, SMAD4, BMPR1A, and PTEN[4]. Nevertheless, because of the low incidence of individual predisposing gene mutations, conventional approaches such as Sanger sequencing may yield false-negative results as a result of limited coverage. However, multiple gene testing is now commercially and clinically available for cancer risk assessment because of the widespread application of next-generation sequencing (NGS). Especially for CDH1-negative families that do, however, meet the HDGC criteria, multiple gene sequencing of germline DNA can be used to identify novel variants and risk alleles of varying penetrance.

The marked differences in ethnicity, diet, and living habits between Chinese and Caucasian populations suggest that Western genetic screening guidelines may not be suitable for Eastern populations. To date, research on hereditary gastric cancer in the Chinese population has been scarce. It is important to identify patients with genetic aberrations because these may influence their clinical management; however, the underlying genetic susceptibility to gastric cancer remains largely unknown. Thus, this study aimed to explore the frequency and spectrum of predisposing gene germline variants among Chinese GC patients with hereditary high-risk factors for cancer. For patients with pathogenic or likely pathogenic germline variants, Sanger sequencing was applied to validate the variants in the probands and their family members.

Methods
Study population
Between January 2017 and August 2018, gastric adenocarcinoma patients with hereditary high-risk factors were recruited from seven hospitals throughout six provinces in China (Supplementary Table S1). Patients who met one of the following criteria for high risk were included: 1) onset age \( \leq 30 \) years, regardless of the family history; 2) onset age \( \leq 35 \) years, and GC histologically classified as signet ring cell carcinoma (SRCC) or mucinous adenocarcinoma, regardless of the family history; 3) onset age \( \leq 50 \) years, and at least one first-degree relative diagnosed with malignant tumors; 4) at least two first- or second-degree relatives diagnosed with malignant tumors, with at least one of these being a first-degree relative; 5) diagnosed with more than two primary malignant tumors, with one of
these having an onset age ≤ 50 years; 6) tissue specimens showing microsatellite instability or deficient mismatch repair. A total of 40 patients from among ten families met these criteria and were included in the study.

**Sequencing Panel Design**

Our solution-phase panel was designed to cover all of the exon regions (including parts of the intron regions) of 171 cancer-predisposing genes that were selected following a thorough literature review and a review of unpublished data (Supplementary Table S3).

**Next-generation sequencing (NGS), bioinformatics, and variant filtering**

Genomic DNA extraction, NGS, bioinformatic analysis, and variant filtering and annotation are described in detail in the Supplementary Information.

**Statistical Analyses**

Statistical analyses were performed with SPSS 21.0 software. Medians with interquartile ranges of abnormally distributed data were used for continuous variables (diagnosed ages of patients) and rank tests for analyses. Categorical variables were presented as proportions. Mutation rates and proportions were compared and analyzed with the Chi-square (χ²) test.

**Results**

**Patient cohort and characteristics**

Forty GC patients and their families were recruited from seven medical centers (Supplementary Table S1) across six provinces in China. The clinical characteristics of the patients are presented in Table 1. The patients’ median age at initial GC diagnosis was 37.5 years (range, 24–76 years), and 26 patients (65%) exhibited an advanced disease stage (III–IV) at diagnosis. The 40 GC cases were histologically segregated into 22 (55%) cases of adenocarcinoma, 16 (40%) of signet ring cell carcinoma, and 2 (5%) of mucinous adenocarcinoma. Overall, 29 (72.5%) patients had a family history of malignancies.
Table 1
Clinical characteristics of the patients included in this study

| Characteristic                       | No.of patients (%) |
|--------------------------------------|--------------------|
| All cases                           | 40 (100)           |
| Sex                                  |                    |
| Male                                 | 23(57.5)           |
| Female                               | 17(42.5)           |
| Median age at diagnosis(range)       | 37.5y (24–76)      |
| Age at diagnosis                     |                    |
| ≤ 30 y                               | 16(40)             |
| 31 ~ 40 y                            | 6(15)              |
| 41 ~ 50 y                            | 2(5)               |
| 51 ~ 60 y                            | 12(30)             |
| > 60 y                               | 4(10)              |
| Tumor stage                          |                    |
| I                                    | 6(15)              |
| II                                   | 8(20)              |
| III                                  | 15(37.5)           |
| IV                                   | 11(27.5)           |
| Histologic types                     |                    |
| Adenocarcinoma                       | 22(55.0)           |
| Mucinous adenocarcinoma              | 2(5)               |
| Signet ring cell carcinoma           | 16(40.0)           |
| Family history                       |                    |
| Yes                                  | 29(72.5)           |
| no                                   | 11(27.5)           |

Pathogenic Or Likely Pathogenic Germline Variants

Among the 40 patients, a total of 10 patients (25%) were found to carry pathogenic or likely
pathogenic variants (Fig. 1a). Two probands carried mismatch repair (MMR) pathogenic variants
(MLH1 = 1, MSH2 = 1), which are associated with Lynch syndrome. Three probands carried pathogenic
or likely pathogenic variants of homologous recombination repair (HRR) genes (BLM = 1, PALB2 = 1,
CHEK2 = 1). One proband had a CDH1 variant associated with HDGC, which was, therefore, likely
pathogenic. Another two probands had likely pathogenic variants of other genes known to be
associated with a genetic predisposition to cancer (EXT2 = 1, ERCC2 = 1). Two patients were identified
to bear SPINK1 mutations that have not been reported previously (Fig. 1b). Patients with pathogenic
or likely pathogenic variants are listed in Table 2.
Table 2
Details of pathogenic/likely pathogenic variants detected via next-generation sequencing

| Family Code | Cancer                  | Sex/Age at Diagnosis GC | Family History of Malignancies | Gene   | Transcript ID | DNA       | Protein        | Variant Type |
|-------------|-------------------------|-------------------------|-------------------------------|--------|---------------|-----------|----------------|--------------|
| NG5         | GC                      | M/25                    | Yes                           | BLM    | NM_000057     | c.1105C > T| p.Q369*        | Nonsense     |
| XJ1         | GC                      | M/30                    | no                            | PALB2  | NM_024675     | c.1684 +1G > A| NA             | Splice       |
| LZ2         | MPCC, EC, pleural fibrosarcoma, esophageal leiomyosarcoma | F/45                    | Yes                           | MLH1   | NM_000249     | c.790 +1G > A| NA             | Splice       |
| BZ1         | GC, BC                  | F/54                    | Yes                           | ERCC2  | NM_000400     | c.1532G > A| p.R511Q        | Missense     |
| BZ2         | GC, Renal cancer        | M/63                    | Yes                           | SPINK1 | NM_000267     | c.194 +2T > C| NA             | Splice       |
| BZ3         | GC, EC                  | F/68                    | Yes                           | MSH2   | NM_000251     | c.610G > T | p.G204*        | Nonsense     |
| BZ5         | GC                      | M/34                    | Yes                           | CDH1   | NM_004360     | c.1475_1479del GAGTG | p.V493Sfs*42 | Frameshift |
| BZ10        | GC, Thyroid cancer      | F/37                    | No                            | SPINK1 | NM_000267     | c.194 +2T > C| NA             | Splice       |
| BZ13        | GC                      | F/24                    | Yes                           | EXT2   | NM_000401     | c.630delC    | p.S211Lfs*92  | Frameshift   |
| BZ16        | GC                      | M/26                    | no                            | CHEK2  | NM_007194     | c.1553_1554insG| p.S518Rfs*7 | Frameshift   |

GC: Gastric cancer; MPCC: multiple primary colorectal carcinoma, CRC: colorectal cancer; EC: endometrial cancer; BC: breast cancer;

Clinicopathological Associations Among Mutation Carriers

The enrollment criteria for these 40 patients are shown in details in Supplementary Table S2. No differences were found in the age of onset, family history or clinical stage between the patients carrying the pathogenic/likely pathogenic variants and patients with variants of uncertain significance (VUS). However, for patients with pathogenic/likely pathogenic mutations, multiple onset primary malignancies were significantly enriched (50% vs 7.69%, Fisher Exact P = 0.0105; see Table 3).
### Table 3
Inclusion criteria with pathogenic/likely pathogenic variants

| Criteria                                                                 | Probands with (Likely)Pathogenic variants | Ratio in pathogenic group | Ration in non-pathogenic group | P-Value (Fisher Exact) |
|--------------------------------------------------------------------------|-------------------------------------------|---------------------------|---------------------------------|------------------------|
| 1) onset age ≤ 30 years, regardless of the family history               | NG5,XJ1,BZ13,BZ16                         | 40.00%                    | 36.67%                          | > 0.99                 |
| 2) onset age ≤ 35 years, and GC histologically classified as signet ring cell carcinoma (SRCC) or mucinous adenocarcinoma, Regardless of the family history | NA                                        | NA                        | 6.67%                          | > 0.99                 |
| 3) onset age ≤ 50 years, and at least one first-degree relative was diagnosed with malignant tumors | NG5,LZ2,BZ5                               | 30.00%                    | 16.67%                          | 0.3878                 |
| 4) at least two first- or second-degree relatives were diagnosed with malignant tumors, and at least one of them is first-degree relative | NG5,LZ2,BZ1,BZ2,BZ3                       | 50.00%                    | 56.67%                          | 0.4727                 |
| 5) the patient was diagnosed with more than two primary malignant tumors, and one of the onset age ≤ 50 years | LZ2,BZ1,BZ2,BZ3,BZ10                      | 50.00%                    | 6.67%                           | 0.0105                 |
| 6) tissue specimen was microsatellite instability or deficient mismatch repair | LZ2                                      | 10.00%                    | 3.33%                           | 0.4423                 |

**Variants Of Uncertain Significance**

Among the other 30 patients, a total of 129 VUS were identified in 27 patients (Supplementary Table S4), consisting of 111 missense variants, 3 frameshift variants, 3 splice variants, 7 non-frameshift deletions, 2 non-frameshift insertions, and 3 nonsense variants. Based on the ACMG 2015 guidelines[5] and eight in silico function predictors, 28 germline mutations were identified in 16 patients as putative high-risk VUS (Fig. 2). These included VUS of three DNA damage repair (DDR) genes (EPCAM, MLH1, and CHEK2).

**Familial Pedigrees And Sanger Sequencing**

For the ten probands carrying pathogenic or likely pathogenic germline variants, Sanger sequencing was performed to validate these variants in the probands and their first- and second-degree relatives. The familial pedigrees of probands with germline pathogenic or likely pathogenic mutations are
shown in Fig. 3. The proband of the LZ2 family, harboring an MLH1 pathogenic mutation, was diagnosed with seven metachronous tumors (descending colon cancer at age 41; gastric adenocarcinoma, endometrial cancer, and rectal cancer at age 45; ascending colon cancer at age 52; pleural fibrosarcoma at age 55; and esophageal fibrosarcoma at age 62). A large proportion of her family members suffered from colorectal cancer, which is a typical presentation of Lynch syndrome. Her son and two nephews did not carry the same variant based on Sanger sequencing verification (Fig. 3a). The proband of the BZ3 family, bearing the MSH2 pathogenic mutation, was diagnosed with GC and endometrial cancer at age 68. All of her siblings were diagnosed with either colorectal cancer, gastric cancer, prostate cancer or endometrial cancer. Three children were verified as positive for the MSH2 mutation by Sanger sequencing, and the eldest daughter was diagnosed with breast cancer at age 34 (Fig. 3b). The proband of the BZ5 family bearing the CDH1 mutation was diagnosed with GC at age 34. His elder brother was diagnosed with GC at the same age. His mother and nephew harbored the same mutation (Fig. 3c). For patient BZ1 with subsequent onset of breast cancer and gastric cancer, the ERCC2 mutation was discovered together with the MUTYH mutation. In addition, her mother was confirmed to have colorectal cancer and another first-degree relative was found to harbor breast cancer. However, her mother and son did not carry the same mutation (Fig. 3d). The proband of the NG5 family harboring the BLM likely pathogenic mutation was diagnosed with GC at age 25. The patient had a family history of cancer through his mother’s lineage but his father harbored the same mutation as the patient instead of his mother, who was diagnosed with breast cancer at age 45 (Fig. 3e). The patient of the BZ13 family with early-onset GC harbored an EXT2 likely pathogenic variant inherited from his mother. The patient had a family history of one second-degree relative with GC (Fig. 3f). The patient of the BZ16 family harboring the CHEK2 likely pathogenic mutation was diagnosed with GC at age 25 and did not have a family history of cancer. His mother and twin brother had the same variant as verified by Sanger sequencing, while his father and aunt did not carry this variant (Fig. 3g). The proband of the XJ1 family harboring the PALB2 mutation was diagnosed with GC at age 30 without a family history. The results of Sanger sequencing verification for his parents showed that this variant was inherited from his father (Fig. 3h). The probands of the BZ2 family and
BZ10 family each harboring the SPINK1 pathogenic mutation were both diagnosed with two primary cancers. The patient of the BZ2 family was diagnosed with renal clear cell carcinoma at age 62 and was subsequently diagnosed with breast cancer at age 63. He had a family history of cancer but his younger brother and younger sister did not carry the same variant based on Sanger sequencing verification (Fig. 3i). The patient of the BZ10 family was diagnosed with thyroid cancer at age 31 and GC at age 37 (Fig. 3j).

Discussion
Although research on hereditary GC in China is limited, investigations on other neoplasms such as breast cancer, ovarian cancer, and prostate cancer have suggested that the genetic spectrum of Chinese patients with hereditary tumors may be different from that in Caucasians. The heterogeneous clinical features of hereditary tumor syndromes and the atypical presentation of cancer family history hampers attempts to summarize and cluster genotypes and phenotypes with a traditional single-gene resolution approach. In this study, patients with hereditary high-risk factors for cancer were enrolled and clinical information including age of diagnosis, special histologic types, family history of malignant tumors, and microsatellite status was used to explore the frequency and spectrum of germline variants of cancer-predisposing genes. This is the first multicenter research study in China aiming to reveal GC-related germline variants in CDH1 and other putative cancer susceptibility genes through targeted next-generation sequencing of high-risk GC patients. This prospective study indicates that one in every four GC patients with hereditary high-risk factors may bear pathogenic/likely pathogenic cancer susceptibility gene variants. We identified deleterious germline variants involving nine different genes: MLH1 (n = 1), MSH2 (n = 1), CDH1 (n = 1), BLM (n = 1), PALB2 (n = 1), EXT2 (n = 1), CHEK2 (n = 1), ERCC2 (n = 1), and SPINK1 (n = 2).

Compared with previous studies, the present spectrum of germline variants derived from Chinese gastric patients demonstrated a distinct pattern. Although genes such as CDH1, MSH2, and PALB2 were also identified in previous studies[6–9], there is a marked difference in the types of variants involved and other mutated loci were identified here for the first time.

We identified two pathogenic variants in the MMR genes, which are associated with Lynch syndrome
First, the proband of the LZ2 family carried the MLH1 splicing variant (c.790 + 1G > A), which results in the loss of amino acids 227–295 in the MLH1 protein and has been shown functionally to render MLH1 defective in mismatch repair activity[10]. This variant has been reported in individuals with LS and colorectal cancer[11, 12]. In addition, multiple clinical diagnostic laboratories/reputable databases classify this variant as pathogenic. Second, the proband of the BZ3 family carried the MSH2 nonsense mutation (c.610G > T, p.G204*). Sheng et al.[13] detected this variant in one HNPCC family and classified it as a pathogenic mutation.

Both of the above families met the clinical criteria for LS. The lifetime risks for LS-associated cancers are highest for colorectal cancer at 52–82%, followed by an endometrial cancer risk of 25–60% in women, a 6–13% risk for gastric cancer, and 4–12% for ovarian cancer[14]. Both probands had multiple primary malignant tumors.

Clinically defined HDGC is characterized by early-onset, multigenerational diffuse GC and lobular breast cancer. Clinical criteria for HDGC were established by the International Gastric Cancer Linkage Consortium (IGCLC)[2]. CDH1 is a cancer predisposition gene mutated in families meeting the criteria for clinically defined HDGC, with approximately 40% of HDGC families having germline mutations in CDH1. For example, Hansford et al.[8] identified 47 distinct pathogenic mutations in 183 index cases meeting the clinical criteria for HDGC (25.7%), and among these, 31 cases carried pathogenic CDH1 mutations. The CDH1 germline mutation rate is negatively correlated with the morbidity of GC worldwide. In countries with low morbidity, such as Canada, the United States, the United Kingdom, and the Netherlands, the CDH1 mutation rate can be as high as 51.6% in patients meeting the HDGC clinical criteria[15]. However, in Japan, which has the highest gastric cancer morbidity, the CDH1 mutation rate is 15.4%[16]. There were 15 families meeting the HDGC clinical criteria of IGCLC2015 in our study but only one proband carried the CDH1 gene germline mutation (c.1475_1479delGAGTG, p.V493Sfs*42). Thus, the CDH1 mutation rate in our study was 6.7%.

The ERCC2 missense mutation (c.1532G > A, p.R511Q) is not described in any of the queried databases, but it was predicted in silico to be pathogenic when using the DANN, GERP, dbNSFP, FATHMM, LRT, MetaLR, MetaSVM, MutationAssessor, MutationTaster, PROVEAN, and SIFT bioinformatic
tools. Therefore, this variant was classified as likely pathogenic.

The BLM gene is the causative gene of Bloom syndrome (BS). Bloom syndrome is an autosomal recessive disorder characterized by proportionate pre- and postnatal growth deficiency; sun sensitivity; telangiectatic, hypo- and hyperpigmented skin; predisposition to malignancy; and chromosomal instability[17]. This variant was classified as likely pathogenic.

The EXT2 gene is the causative gene of hereditary multiple exostoses (HME). HME is an autosomal dominant disorder characterized by multiple exostoses most commonly arising from the juxtaepiphysyal region of the long bones[18]. The EXT2 frameshift variant was classified as likely pathogenic.

The CHEK2 variant (c.1553_1554insG, p.S518Rfs*7) is a well-described, lower penetrance mutation that is mainly associated with breast cancer but also colorectal cancer and prostate cancer. This frameshift variant results in the loss of almost 10% of the protein sequence and a functional study reported that the missing region includes amino acid residues Pro515-Pro522, which is a nuclear localization signal (NLS)[19] Thus, this variant was classified as likely pathogenic.

PALB2 colocalizes with BRCA2 in nuclear foci, promoting its localization and stability in nuclear structures, and enabling its recombinational repair and checkpoint functions. A previous study showed that PALB2 is a breast cancer susceptibility gene[20]. The PALB2 splicing mutation (c.1684 + 1G > A) results in abnormal splicing of the mRNA, which affects protein function. This variant was detected in one patient with high-risk neuroblastoma and classified as likely pathogenic in accordance with a previous report[21].

The SPINK1 splicing mutation (c.194 + 2T > C) affects a donor splice site in intron 4 of the SPINK1 gene. It is predicted to affect mRNA splicing, resulting in a significantly altered protein due to either exon skipping, shortening, or the inclusion of intronic material. Experimental studies have shown that this splice site variant completely abolishes the expression of SPINK1 mRNA and protein in cell cultureThis variant is recurrent in individuals of Asian descent with chronic pancreatitis[22, 23]. Loss-of-function variants of SPINK1 are known to be pathogenic. Multiple clinical diagnostic laboratories have, therefore, classified this variant as pathogenic.
In summary, of the nine pathogenic/likely pathogenic mutations found in this study, four mutations have been reported in previous studies, while the other five mutations are considered novel mutations.

We found a total of 129 variants of uncertain significance (VUS) in 27 of the patients. Most of these VUS were missense mutations, while 27 VUS were predicted in silico to be high-risk variants. The pathogenic classification of two of these VUS is controversial as described below. We used Sanger sequencing to validate these VUS in the two patients. The familial pedigrees of these two patients are shown in Fig. 3.

MUTYH-associated polyposis (MAP) is an autosomal recessive disease that usually appears in patients with an attenuated polyposis phenotype. This syndrome is associated with biallelic mutations in the MUTYH gene. MAP typically presents with multiple colorectal adenomas and an increased risk for colorectal cancers. Gastric cancer among these patients is uncommon; it is reported in only 2% of cases[24]. The proband of the BZ1 family carried the MUTYH splicing mutation (c.934-2A > G), which alters a conserved intronic nucleotide and causes aberrant splicing based on in vitro studies[25]. However, whether this alteration causes a biological loss of function of the MUTYH protein in humans is uncertain. This variant has been widely studied in East Asian populations and is frequently reported in individuals with colorectal cancer. Only one homozygous patient with gastric cancer has been described with this mutation, while the rest of the reported patients harbor this variant in the heterozygous state[26–28]. Multiple clinical diagnostic laboratories/reputable databases have classified this variant as a VUS or likely pathogenic. This conflicting evidence has prevented the pathogenicity or neutrality of this variant from being established with certainty, and it was, therefore, classified here as a VUS. Additional studies are needed to clarify the significance of this variant.

The proband of the BZ14 family carried the CHEK2 missense mutation (c.1111C > T, p.H371Y). Liu et al[29]. have reported that the c.1111C > T variant confers a significantly increased risk of breast cancer in the Chinese population but the clinical significance of this association has not been established. Additional, smaller studies in Asian populations have identified this variant in breast cancer cases as well as the controls[30, 31]. In vitro functional studies have shown that this missense
change causes a decrease in phosphorylation and enzymatic activity compared with the wild-type CHEK2 protein. However, the decreased activity caused by this variant is not as pronounced as the effect caused by a known kinase-disruptive variant[29]. In silico analyses support that this variant does not alter protein structure or function. Although there is some indication that this variant could cause disease, the evidence is insufficient at this time to prove this conclusively. Therefore, based on these data and the proband’s pedigree, we classified it as a VUS.

There are many differences in ethnicity, diet, and living habits between China and Western countries. Therefore, we cannot simply refer to the relevant foreign screening criteria for hereditary gastric cancer. One of the purposes of this study was to establish screening criteria for hereditary gastric cancer in China. This study referenced the clinical criteria for HDGC and LS as screening criteria. We enrolled patients with hereditary high-risk factors including the age of diagnosis, special histologic types, family history of malignant tumors, and microsatellite status. We observed that patients who had multiple onset primary malignancies seemed to be more likely to have pathogenic germline mutations(50% vs 7.69%, Fisher Exact P = 0.0105; see Table 3).

Our study has several limitations that should be noted. The size of the cohort recruited in this study was limited; thus, we were unable to unequivocally define the disease-causing variants and, therefore, the GC-predisposing genes. A large number of VUS were disclosed with the application of next-generation sequencing. Several candidate VUS were considered to be potentially pathogenic based on certain ACMG criteria and bioinformatic prediction. Thus, further functional studies in vitro and in vivo should be performed to correctly classify these variants.

Conclusions
This prospective multicenter study enrolled 40 patients with hereditary high-risk factors for cancer to explore the prevalence of germline genetic alterations in cancer susceptibility genes by next-generation sequencing. In total, we found that 25% of patients carried deleterious germline mutations in nine of the 171 genes tested. The CDH1 gene mutation rate was 6.7% in the 15 families meeting the HDGC clinical criteria, which is significantly lower than that in Western countries. This may be indicative of the unique genetic background of GC in Chinese patients. Because patients with
pathogenic or likely pathogenic germline variants have a dismal clinical outcome and a higher rate of multi-cancer occurrence, genetic counseling, genetic screening, and family surveillance and management should be strongly recommended for those patients with hereditary high-risk factors. By screening populations with hereditary high-risk factors, multiple-gene sequencing can be used effectively to discover novel disease-causing genes of hereditary disease.

**Abbreviations**

GC: gastric cancer; HDGC: hereditary diffuse gastric cancer; LS: Lynch syndrome; LFS: Li-Fraumeni syndrome; PJS: Peutz-Jeghers syndrome; HBOC: hereditary breast and ovarian cancer; MAP: MUTYH-associated adenomatous polyposis; FAP: familial adenomatous polyposis; JPS: juvenile polyposis syndrome; NGS: next-generation sequencing; SRCC: signet ring cell carcinoma; MMR: mismatch repair; VUS: variants of uncertain significance; IGCLC: International Gastric Cancer Linkage Consortium; BS: Bloom syndrome; HME: hereditary multiple exostoses; NLS: nuclear localization signal; MAP: MUTYH-associated polyposis.

**Declarations**

**Ethics approval and consent to participate**

This study was approved by the Ethics Committee of Peking University Cancer Hospital and the relevant ethics committees of each of the other participating centers. All procedures were performed in accordance with the ethical standards of the respective committees on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions. The study was also approved by the Institutional Review Board of Beijing Cancer Hospital. All patients and their family members provided written informed consent to participate in this study.

**Consent for publication**

Not applicable.

**Availability of data and materials**

Some or all data used during the study are available from the corresponding author by request.

**Competing interests**

Changbin Zhu, Di Shao, and Yang Ke are employees of BGI Genomics, which produces the panel test
used in this study. The other authors declare that they have no conflicts of interest.

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**Authors' contributions**

LS and XCW directed and designed research. YJZ, YY, TX and QW performed research. CBZ and KY performed DNA sequencing and sequencing data analysis. YJZ wrote the manuscript. CBZ, DS, XTZ and JG revised the manuscript. BRL, JDZ, XBC, ZJW, MQ, XW and LS are the principal investigators from 7 institutions. LS and XCW provided supervision for the study, and revised the manuscript; and all authors have read and approved the manuscript as submitted.

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Germline mutations in cancer susceptibility genes. a. Distribution of germline mutations in Chinese GC patients. b. Germline profiles for GC patients with pathogenic/likely pathogenic variants.

Germline profiles for 16 patients with possible high-risk VUS.
The familial pedigrees of probands with germline pathogenic/likely pathogenic mutations as determined with next-generation sequencing. Sanger sequencing verification outcomes are also indicated.

Supplementary Files
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Supplementary Table S4. Details of VUS detected.xlsx
Supplementary information.docx