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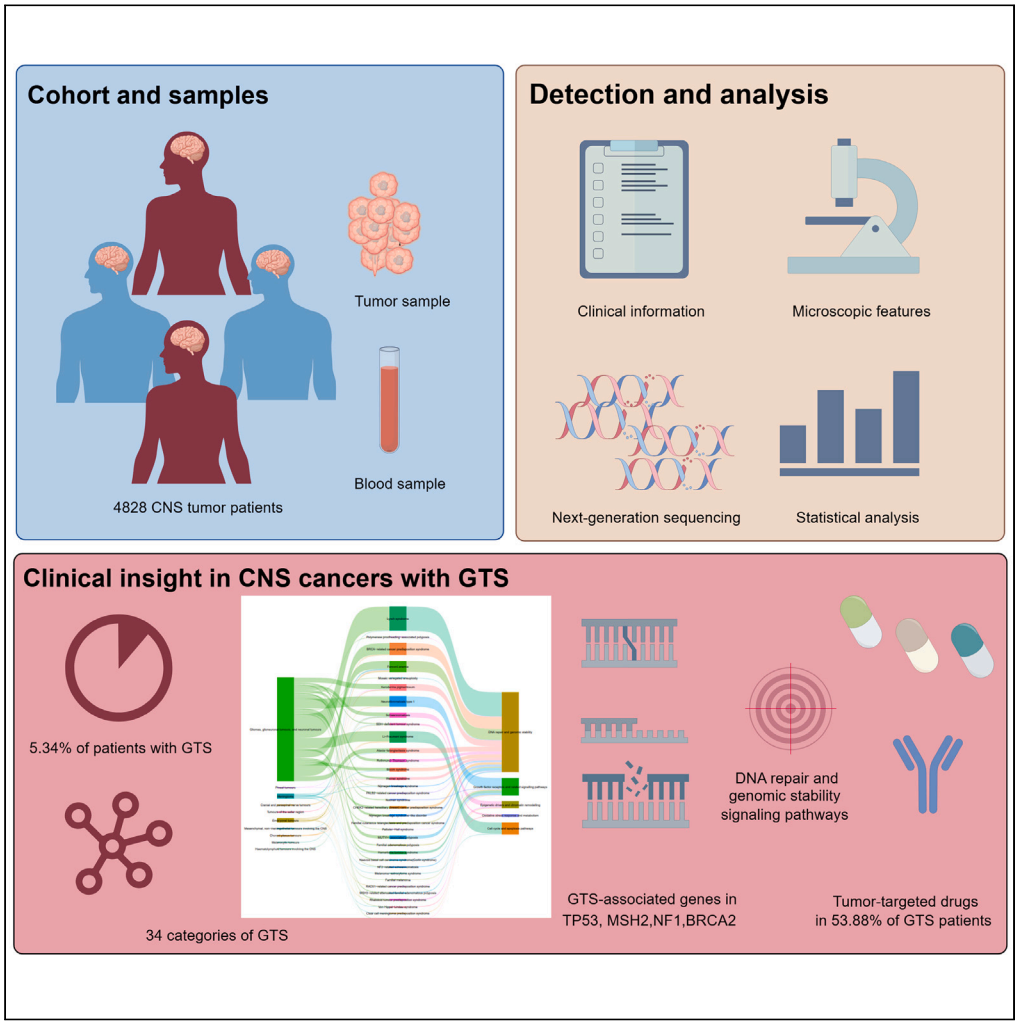

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Highlights

Identified 34 categories of GTS with CNS tumors in a Chinese large-scale cohort

Revealed the genetic and clinical landscape of the CNS tumors with GTS

Revealed high genetic heterogeneity in primary CNS tumors

Found 53.88% of patients with CNS GTS harboring potential therapeutic target mutations

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Genetic and clinical characteristics of genetic tumor syndromes in the central nervous system cancers: Implications for clinical practice

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SUMMARY

Recognizing individuals with Genetic tumor syndromes (GTS) in the primary central nervous system (CNS) tumors is crucial for optimizing proper genetic counseling and improving therapeutics and clinical care. We retrospectively analyzed the GTS in a Chinese CNS tumor cohort and examined the molecular characteristics and their clinical significance for diagnostic and therapeutic purposes. Our study identified 34 categories of GTS in 258 patients with CNS tumors. The gene with the highest germline pathogenic or likely pathogenic mutation frequency was *TP53*, followed by *MSH2*, *NF1*, and *BRCA2*. The top five GTS in CNS tumors showed high genetic heterogeneity GTS analysis reclassifies CNS tumors as "NEC." 53.88% of patients diagnosed with GTS harbor potential precision oncology therapy target mutations. The results of our study deepen our understanding of CNS tumors, provide a reference direction for the future design of clinical trials, and further expect to improve disease entire process management in CNS tumors.

INTRODUCTION

Inherited cancer susceptibility syndrome (GTS) is a type of disease in which patients are predisposed to multiple tumors due to pathogenic or likely pathogenic (P/LP) mutations in germline genes.[1,2] GTS caused approximately 5%–10% of malignancies, with even higher proportions in children and adolescents.[2–5] Emerging studies have revealed that germline variants contribute not only to cancer risk but also to tumor progression by increasing the risk for specific somatic events.[6,7] Additionally, knowledge of germline variation can help to predict drug sensitivity, affect drug toxicity, and then help select therapy methods to minimize side effects.[8–11] In brief, germline variants can predict the risk of developing malignancy, which is useful for individualizing cancer screening and therapy.[1,3,12–15] Next-generation sequencing (NGS) has increased our molecular understanding of somatic and germline mutations in cancers, which is promising for the use of sequencing data to improve clinical decisions.[1,3,16–19] Genetic testing can modify cancer screening and preventive therapies to improve outcomes for relatives of patients with cancer.[20,21] GTS has different pathogenesis, clinical phenotype, diagnosis, treatment strategy, and family management from corresponding sporadic tumors.[22–24] The occurrence of GTS-associated tumors is consistent with the two-hit events theory and GTS-related tumors develop early and tend to be multiple or involve paired bilateral organs.[3,25,26]

GTS also predisposes patients to specific brain tumors, and their pathophysiology influences both surveillance and treatment.[27,28] The fifth edition of the World Health Organization (WHO) Classification of Tumors emphasized that central nervous system (CNS) tumors are frequently associated with various GTS. Additionally, eight new genetic tumor syndromes have been added to the guidelines.[29] The annual incidence of primary brain malignancies in adults is approximately 0.007%, with a 5-year survival of approximately 36%. GTS-related malignancies are relatively rare.[27,30] CNS tumors are the second most prominent type of pediatric malignancy, with approximately 10% harboring a germline variant in the predisposition gene.[31–33] The prominent application of NGS increases the ability to diagnose GTS in patients with primary CNS tumors. Recognition of individuals with GTS in primary CNS tumors is crucial to optimize proper genetic counseling and improve therapeutics and clinical care, and may further directly improve patient outcomes.[27,28,34] The most prevalent GTS associated with primary CNS include neurofibromatosis 1 (NF1), Lynch syndrome (LS), and Li–Fraumeni syndrome (LFS).[35–38] However, the systematic distribution

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Table 1. Characteristics of patients with CNS tumors with genetic tumor syndromes

Characteristics	N (% of total)
Total number of patients	258
Mean age at diagnosis, years (*n*=252)	36.25 (0–81)
Gender	
Female	110 (42.64%)
Male	148 (57.36%)
Cancer type	
Gliomas, glioneuronal tumors, and neuronal tumors	225 (87.21%)
Meningioma	11 (4.26%)
Embryonal tumors	9 (3.49%)
Choroid plexus tumors	3 (1.16%)
Cranial and paraspinal nerve tumors	2 (0.78%)
Mesenchymal, non-meningothelial tumors involving the CNS	2 (0.78%)
Pineal tumors	2 (0.78%)
Tumors of the sellar region	2 (0.78%)
Haematolymphoid tumors involving the CNS	1 (0.39%)
Melanocytic tumors	1 (0.39%)
Lesion location	
Cerebral hemispheres/Supratentorial	116 (44.96%)
Cerebellar/Infratentorial	50 (19.38%)
Ventricle	21 (8.14%)
Sellar	5 (1.94%)
Others	66 (25.58%)
WHO grade	
1	28 (10.85%)
2	42 (16.28%)
3	32 (12.40%)
1–2	6 (2.33%)
3–4	8 (3.10%)
4	137 (53.10%)
Unknown	5 (1.94%)

and molecular landscape of GTS in CNS tumors through adults and children remained limited, although some studies have presented common primary CNS tumors associated with GTS.

Our study retrospectively analyzed 258 cases with CNS tumors diagnosed as GTS by NGS to investigate the molecular characteristics and distribution, which is a large-scale study in a CNS GTS cohort. We analyzed the type of GTS corresponding to the distribution of cancer subtypes and signaling pathways. Additionally, we focused on the molecular landscape of GTS in CNS tumors and analyzed the germline variants and somatic mutations in each GTS. Finally, we individually analyzed the top five GTS. Conclusions drawn from our study provide evidence for the individualization of cancer screening, integrated diagnosis, and therapy directions of GTS in CNS tumors.

RESULTS

Patient enrollment

We retrospectively screened patients from the Simceredx CNS tumor cohort whose tissue and control blood were detected by DNA-based targeted NGS detection. Germline P/LP variants were determined following the ACMG guidelines, and 258 patients were finally diagnosed with GTS. The category of GTS was identified based on the germline P/LP genes. Among the 258 enrolled patients, 148 were males and 110 were females. The median age was 36.25 (range: 0–81) years, of which 78 were $\leq$20 years. The integrated diagnosis of the patient was determined by combining the results of histological pathology and molecular pathology. Among all the cancer types of the enrolled patients, gliomas, glioneuronal tumors, and neuronal tumors accounted for the largest number of 225 (87.21%), followed by meningioma (11, 4.26%), embryonal tumors (9, 3.49%), and choroid plexus tumors (3, 1.16%%). Table 1 shows the baseline characteristics of all the enrolled patients.

Table 2. Category of genetic tumor syndromes in patients with CNS tumors

Category of genetic tumor syndromes	N	Percentage
Lynch syndrome	53	1.10%
[a]BRCA-related cancer predisposition syndrome	27	0.559%
Li-Fraumeni syndrome	25	0.518%
Fanconi anemia	22	0.456%
Neurofibromatosis type 1	22	0.456%
[a]Xeroderma pigmentosum	13	0.269%
[a]Ataxia-telangiectasia syndrome	11	0.228%
Schwannomatosis	8	0.166%
[a]Bloom syndrome	7	0.145%
[a]MUTYH-associated polyposis	6	0.124%
[a]Rothmund-Thomson syndrome	6	0.124%
[a]Werner syndrome	6	0.124%
[a]CHEK2-related hereditary (breast) cancer predisposition syndrome	5	0.104%
[a]Hamartoma tumor syndrome	5	0.104%
[b]Nijmegen breakage syndrome-like disorder	5	0.104%
[a]SDH-deficient tumor syndrome	4	0.0829%
Rhabdoid tumor predisposition syndrome	3	0.0621%
Familial adenomatous polyposis	2	0.0414%
[b]Familial cutaneous telangiectasia and predisposition cancer syndrome	2	0.0414%
NF2-related schwannomatosis	2	0.0414%
[a]Nijmegen breakage syndrome	2	0.0414%
[a]Noonan syndrome	2	0.0414%
[a]PALB2-related cancer predisposition syndrome	2	0.0414%
Von Hippel-Lindau syndrome	2	0.0414%
Fanconi anemia and Schwannomatosis	2	0.0414%
[a]Clear cell meningioma predisposition syndrome	1	0.0207%
[a]Familial melanoma	1	0.0207%
Melanoma-astrocytoma syndrome	1	0.0207%
[a]Mosaic variegated aneuploidy	1	0.0207%
[b]MSH3-related attenuated familial adenomatous polyposis	1	0.0207%
Naevoid basal cell carcinoma syndrome (Gorlin syndrome)	1	0.0207%
[b]Pallister-Hall syndrome	1	0.0207%
[a]Polymerase proofreading-associated polyposis	1	0.0207%
[a]RAD51-related cancer predisposition syndrome	1	0.0207%
Tuberous sclerosis	1	0.0207%
Schwannomatosis and [b]MSH3-related attenuated familial adenomatous polyposis	1	0.0207%

(*Continued on next page*)

Table 2. *Continued*

Category of genetic tumor syndromes	N	Percentage
[a]Bloom syndrome and [a]Xeroderma pigmentosum	1	0.0207%
Lynch syndrome and Neurofibromatosis type 1	1	0.0207%
Naevoid basal cell carcinoma syndrome (Gorlin syndrome) and [a]MUTYH-associated polyposis	1	0.0207%
Total	258	5.34%

[a]Not included in WHO Central Nervous System Tumors (5th ed.).
[b]Not included in WHO Genetic Tumor Syndromes (5th ed.).

The enrolled 258 patients with CNS tumors with GTS had a total of 34 categories of GTS, among which LS has the largest number of patients (*N* = 54), followed by BRCA-related cancer predisposition syndrome (*N* = 27), LFS (*N* = 25), Fanconi anemia (FA, *N* = 22), and NF1 (*N* = 22). Among 258 patients enrolled, seven patients harbored two germline P/LP variants in different genes and were classified as GTS with double mutation. Table 2 shows the specific classification of the GTS.

Distribution landscape of patients with genetic tumor syndromes in central nervous system tumors

Figure 1 shows the correspondence between GTS and cancer types and between GTS and the signal pathway of germline mutations using the Sankey diagram. Table S2 lists the age, gender, germline P/LP variant, GTS, cancer type, integrated diagnosis, and signal pathway of germline mutations for 258 individuals. The histological classification of 258 patients with primary CNS tumors was mainly concentrated in gliomas, glioneuronal tumors, and neuronal tumors (87.2%, 225/258). Molecular characteristics of 34 categories of GTS were identified, including 14 types of GTS listed in the fifth edition of the WHO Classification of Tumors of CNS and 20 other types of GTS, occurring in 72.09% (186/258) and 27.91% (72/258) of patients, respectively. These GTS-associated genes were enriched in DNA repair and genomic stability signaling pathways, growth factor receptors and related signaling pathways, cell cycle and apoptosis pathways, epigenetic drivers and chromatin remodeling, and oxidative stress response and metabolism pathways.

Mutational landscape of patients with central nervous system tumor with genetic tumor syndromes

Germline mutational landscape analysis

We analyzed a total of 265 germline P/LP variants in 258 enrolled patients. Figure 2A shows the oncoprint of all germline P/LP variants in 258 patients. The gene with the highest mutation frequency was *TP53* (9.69%), followed by *MSH2* (9.30%), *NF1* (8.91), and *BRCA2* (7.75%). We then elaborately portrayed a lollipop plot of the *TP53* mutation sites (Figure 2B) and *MSH2* mutation sites (Figure 2C) on the peptide sequence. The germline P/LP variants in *TP53* mainly cluster in the TP53 binding domain and 96% (24/25) of them are SNVs, including missense (17/25), splice-site (3/25), and stop-gained (4/25) variants, which is consistent with a previous report.[39] The germline P/LP variants of *MSH2* are dispersively distributed in the domain, 75% (18/24) of which are frameshift and stop-gained variants.

Somatic mutational landscape

We then analyzed somatic mutations in these 258 patients with GTS with CNS tumors and drew an oncoprint of somatic mutations with >10% mutation frequency genes (Figure 3A). The genes with the top five mutation frequencies were *TP53* (42.25%), *TERT* (30.23%), *CDKN2A* (28.68%), *CDKN2B* (27.52%), and *PTEN* (25,58%). Missense is the most prominent mutation type. Deletion of *CDKN2A/B* and amplification of *CDK4/6, EGFR,* and *MET* are prominent deletion and amplification mutations in CNS tumors with GTS. Lynch syndrome demonstrated significantly more mutations than other syndromes (p value <0.001) by Wilcoxon test.

Analysis of two-hit events

We further analyzed the genes that occurred in both somatic and germline mutations in the same patient. A total of 11 genes were found to have both germline and somatic mutations in the same patient, among which *NF1, MSH6,* and *MSH2* ranked top three in both the total number of somatic mutations and the number of loss of function (LOF) mutations (Figure 3B). Figures 3C–3E respectively show the LOF somatic and germline mutation sites on the peptide sequence of the *NF1, MSH6,* and *MSH2* genes in the lollipop plots.

Clinical and genomic features of the top five genetic tumor syndrome-related central nervous system tumors

Genetic and clinical landscape of Lynch syndrome-related central nervous system tumors: MSH2 is the most prominent germline variant gene in four mismatch repair genes

In our cohort, 54 patients were identified as having LS-related tumors, which were more prominent in CNS WHO high grades 3 and 4 (84.91%, 45/53) than in WHO low grades 1 and 2 (15.09%, 8/53). Except for one case of meningeal melanocytic neoplasms, the remaining 53 patients with LS-related tumors were gliomas. The mean age of patients with LS-related glioma was 40 (range: 3–77) years, with no obvious age peak

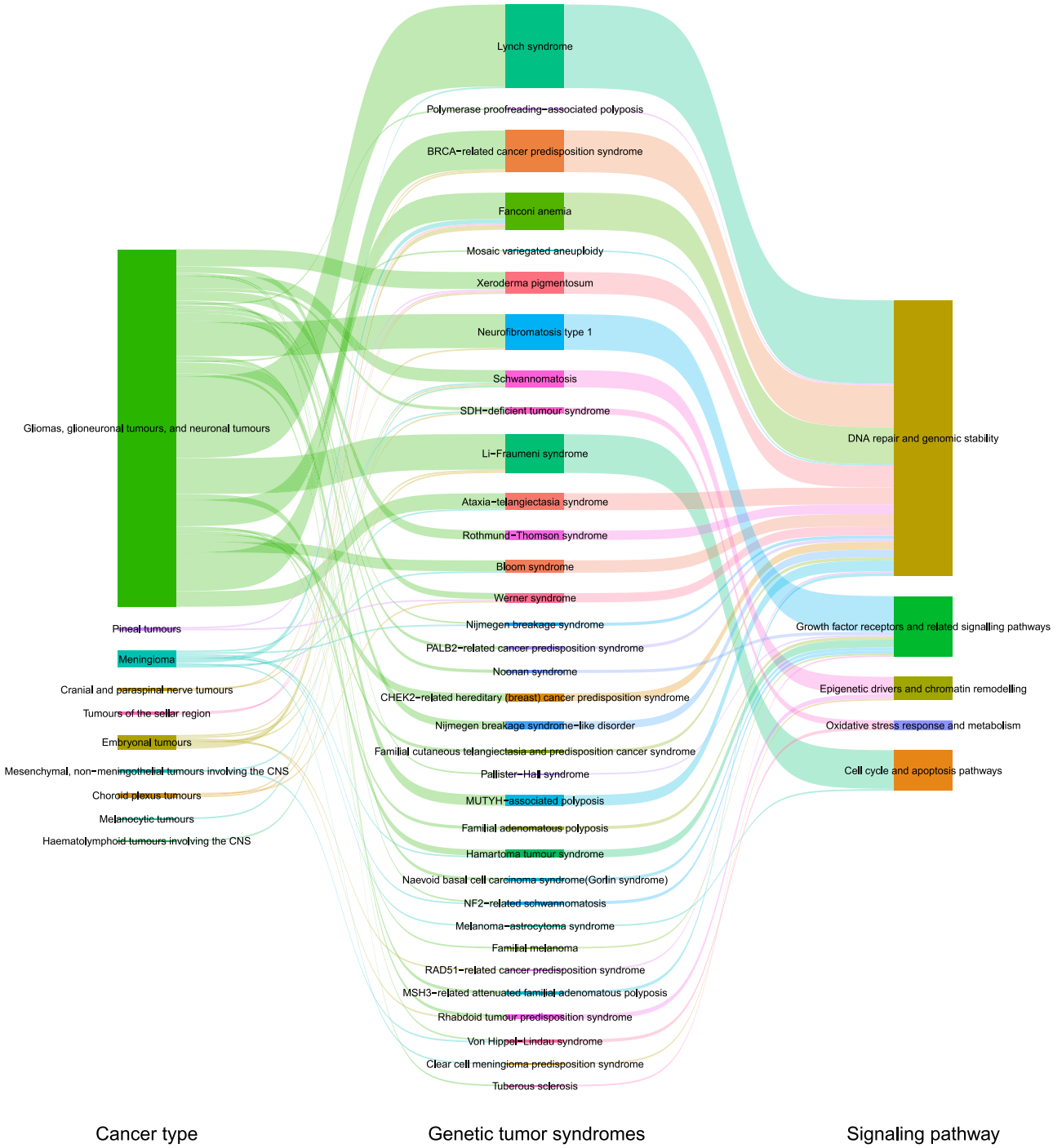

Figure 1. Sankey diagram of the correspondence between inherited cancer susceptibility syndrome (GTS) and cancer types and between GTS and the signal pathway of germline mutations

(Figure 4). Patients with LS-related glioma included glioblastoma, IDH-wildtype (*N* = 18), glioma, IDH-wildtype (*N* = 15), astrocytoma, IDH-mutant (*N* = 10), diffuse pediatric-type high-grade glioma, H3-wildtype and IDH-wildtype (*N* = 6), diffuse midline glioma, H3 K27-altered (*N* = 1), diffuse hemispheric glioma, H3 G34-mutant (*N* = 1), pilocytic astrocytoma (*N* = 1), and high grade astrocytoma with piloid features (*N* = 1). Figure 5A shows the characteristics of LS-related tumors, particularly those that are most prominent in gliomas and associated with typing. *MSH2* and *MSH6* germline variants were most common in LS-related gliomas, which were found in 80.00% (8/10) gliomas with

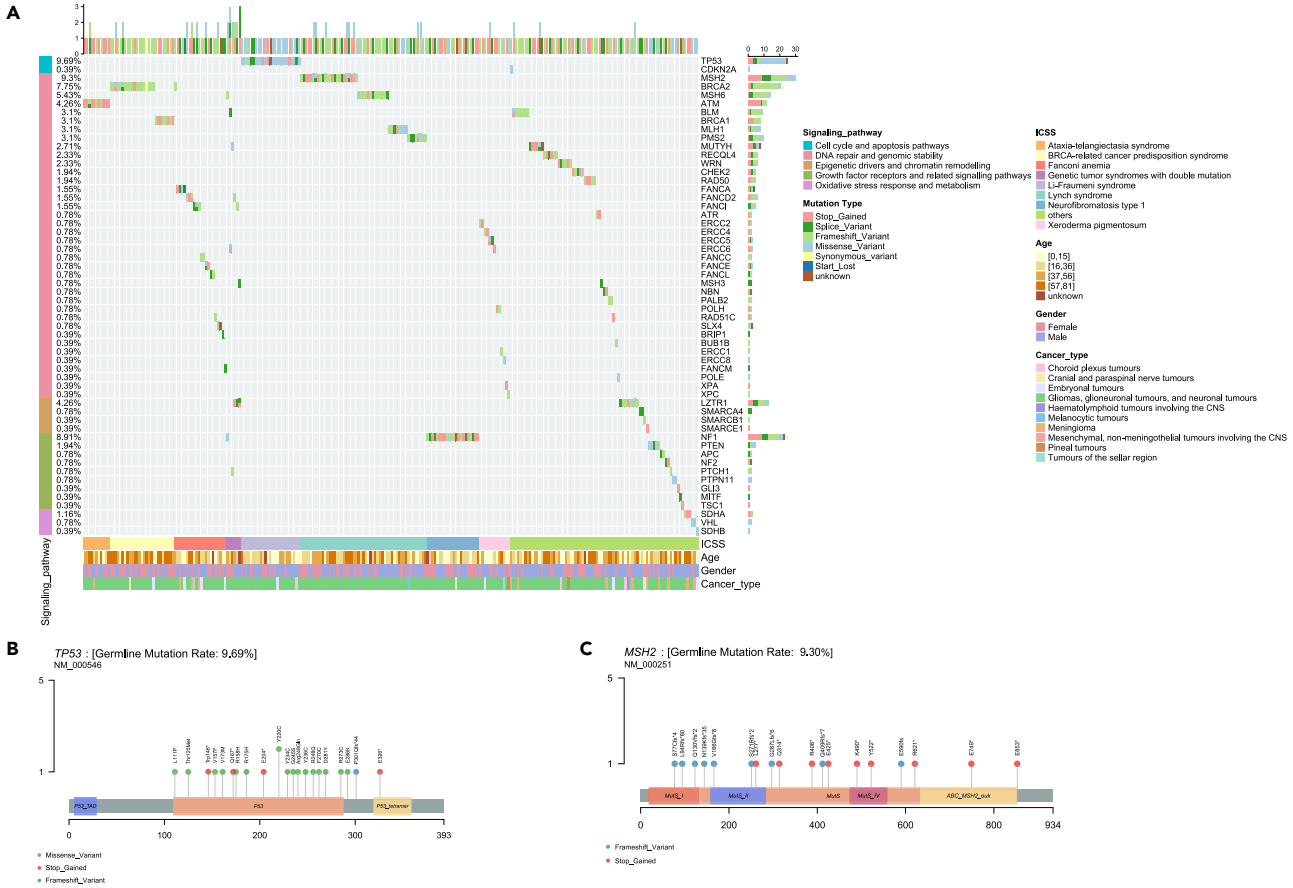

Figure 2. Oncoprint of germline variants in 258 patients with GTS with central nervous system (CNS) tumors and lollipop plot of the top two mutation frequency germline variant genes

(A) Oncoprint of all germline pathogenic and likely pathogenic variants in 258 enrolled patients.

(B) Lollipop plot of *TP53* gene mutation sites on the peptide sequence.

(C) Lollipop plot of *MSH2* gene mutation sites on the peptide sequence.

IDH-mutant and 72.50% (29/40) gliomas with IDH/H3 wild type. *TERT* promoter mutants were absent in LS-related gliomas, and *NF1* (54.72%) was a prominent somatic mutant gene.

Furthermore, unlike typical glioblastoma, LS-related glioma with IDH/H3-wildtype did not have *EGFR/PDGFRA/MYCN* amplification, +7/−10 chromosome copy-number changes, rather with high occurrence in *TP53* mutant, *CDKN2A/B* deletion (Figure 5A). MSI-H, MSI-L, and MSS were found in 26.42% (14/53), 16.98% (9/53), and 56.60% (30/53) patients with LS-related glioma, respectively. *MLH1* and *MSH2* germline variants were most prominent in LS-related glioma with MSI-H (92.86%,13/14). Most patients (12/14) had TMB values of >20 mutations/Mb, which is much higher than the average value of other GTS CNS tumors (Figure 4).

A patient with pilocytic astrocytoma (GP-092) and a patient with meningeal melanocytic neoplasms (GP-053) harbored *PMS2* germline heterozygous deleterious mutations with MSS and low WHO grade, which could be an incidental finding in LS (Figure 5A; Table S2). LS-related H3K27M glioma (GP-044) with *MSH2* LP variant demonstrated TMB-H/MSS, and H3G34 glioma (GP-222) with *MLH1* LP variant exhibited MSS (Table S2). We found a patient with high-grade astrocytoma with piloid features harboring rare double germline variants in the *NF1* and *MSH6* genes (GP-027, Table S2).

Characteristics of BRCA-related central nervous system tumors

In our cohort, 27 patients with BRCA-related CNS tumors were found, with a bimodal age-related peak, one small peak at 0–20 years, and the significant other at an older age of 50–70 years (Figure 4). Except for one patient with schwannoma and one with WNT-activated medulloblastoma, all other patients with BRCA-related CNS tumors had gliomas, of which 13/25 (52.00%) were glioblastomas, IDH-wildtype, 4/25 (16%) were oligodendrogliomas, IDH-mutant, and 1p/19q-codeleted, and 7/25 (28%) were low-grade gliomas (pilocytic astrocytoma, diffuse low-grade glioma, MAPK pathway-altered, pleomorphic xanthoastrocytoma, and spinal ependymoma). The average age of the patients was 59 years, and the prominent somatic mutations were *TERT, PTEN, TP53, CDK6, and CDKN2A/B* in BRCA-related glioblastomas, which was

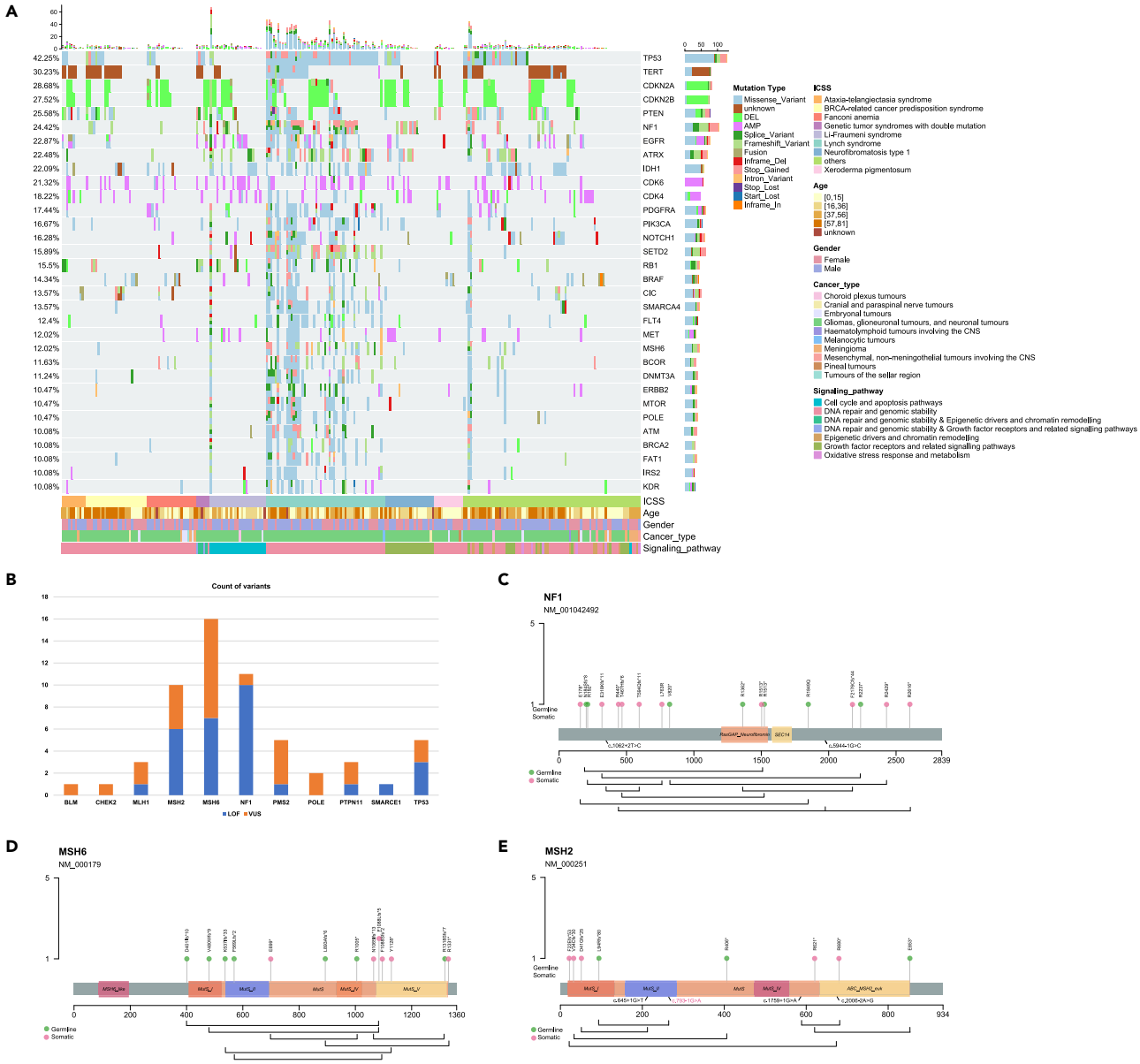

Figure 3. Oncoprint of somatic mutations with >10% mutation frequency genes in 258 patients with CNS tumors with GTS and two-hit events analysis

(A) Oncoprint of somatic mutations with >10% mutation frequency genes in 258 enrolled patients. The unknown in the mutation type of the TERT gene is promoter mutations.

(B) Count of somatic mutations and loss of function (LOF) mutations in a total of 11 genes that had both germline and somatic mutations in the same patient.

(C–E) Lollipop plots of (C) *NF1*, (D) *MSH6*, and (E) *MSH2* gene germline and LOF somatic mutation sites on the peptide sequence. The line below represents the same patient.

similar to the characteristics of elderly sporadic glioblastoma (Figure 5B; Table S2). In other low-grade gliomas, *BRAF* was the common somatic mutation, and fewer tumor-associated somatic mutations were detected. Our results reveal that common somatic mutations are similar between gliomas with and without P/LP germline *BRCA1/2* variants, indicating no significant relationship between *BRCA1/2* gene germline variants and susceptibility to CNS tumors.

High-frequency copy number variants are common events in Li–Fraumeni syndrome-related central nervous system tumors

LFS-related CNS tumors in our study were all gliomas (25), including astrocytoma, IDH-mutant (7) and glioblastoma, IDH-wildtype (10), choroid plexus carcinoma (1), and medulloblastoma (1), which were almost all high-grade tumors. The age of LFS-related CNS tumor

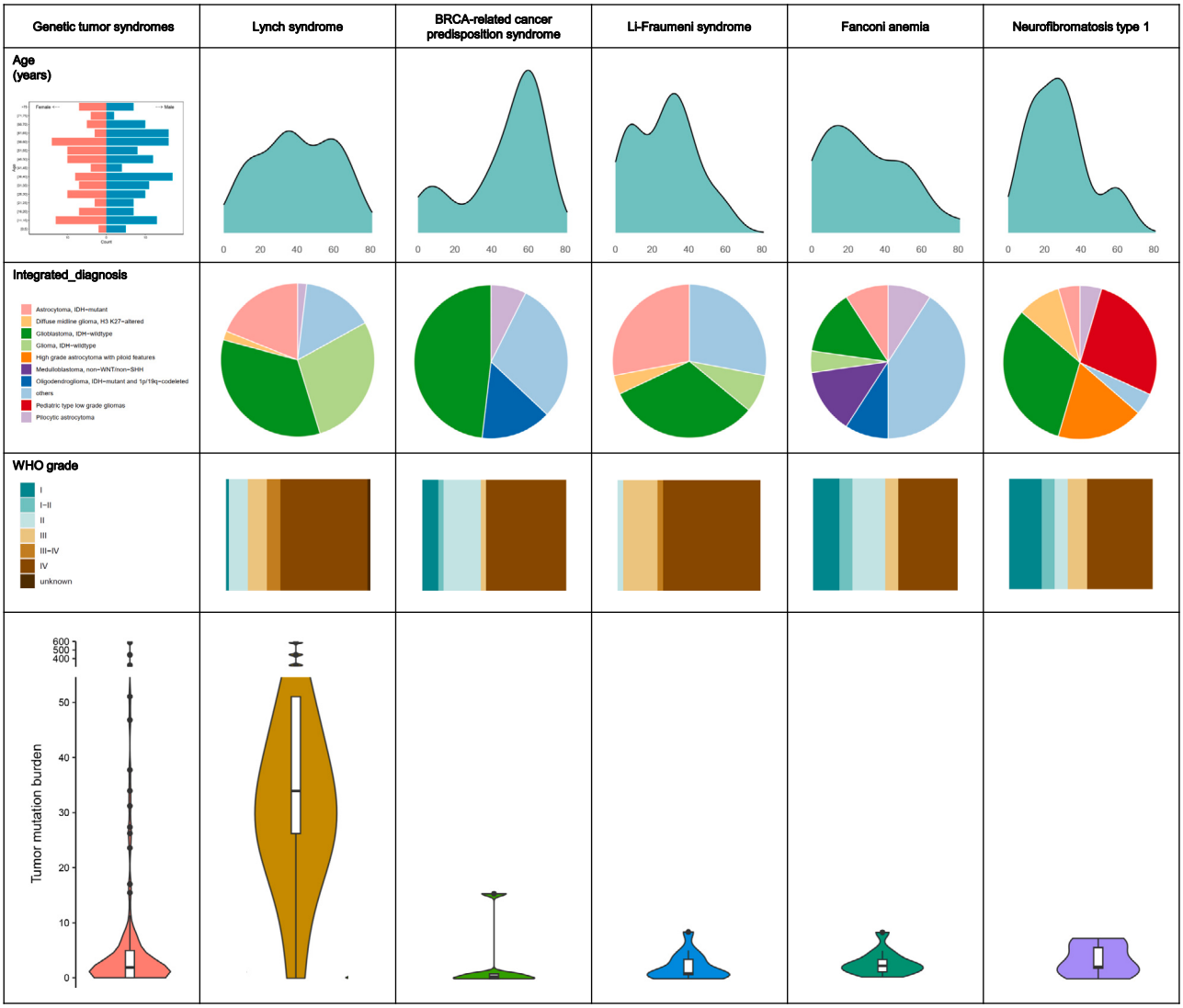

Figure 4. Clinical characteristics of the top five GTS-related CNS tumors, including age, integrated diagnosis, WHO grade, and tumor mutation burden (TMB)

Data are represented as median and percentiles. Center line denotes the median value (50th percentile), the bottom of the box is the 25th percentile, and the top of the box is the 75th percentile.

occurrence demonstrated a continuous bimodal distribution, with the first peak in 0–20 years and the second highest peak in 20–40 years (Figure 4). Copy number variations were very common in LFS-related tumors, including *CDK6, EGFR, CDKN2A/B, CDK4,* and *MYC*. *ATRX* mutations significantly cooccurred with *IDH1* mutations, whereas *TERT* mutations were mutually exclusive (Figure 5C).

FA-related central nervous system tumors require further classification

Patients with FA-related CNS tumors were of complex histological type with mainly gliomas (17/24), which have diverse manifestations. We found 3 patients with medulloblastoma, non-WNT/non-SHH, 3 patients with meningioma, and 1 patient with craniopharyngioma. WHO grade in FA-related CNS tumors ranged from 1 to 4 (Table S2). The peak incidence of FA-related CNS tumors occurred between the ages of 10 and 20 years, and their incidence decreased with age (Figure 4). *FANCA* was the most prominent germline variant gene, accounting for 18.18% (4/22), followed by *FANCD2* (13.63%, 3/22) and *FANCI* (13.63%, 3/22). Except for the deletion of *CDKN2A/B* (36.36%, 31.82%) that was enriched in the FA-related CNS tumors, no other obvious CNS tumor-associated mutation characteristics were observed (Figure 5D).

FA is an autosomal recessive disorder that most frequently results from homozygous or compound heterozygous germline mutations. In our cohort, the FA gene mutation was heterozygous in all 24 patients, and no patient had a definite FA diagnosis. One patient with glioma, NEC (GP-189), was found to have double germline heterozygous variants in *BRCA2* and *SLX4* (Table S2).

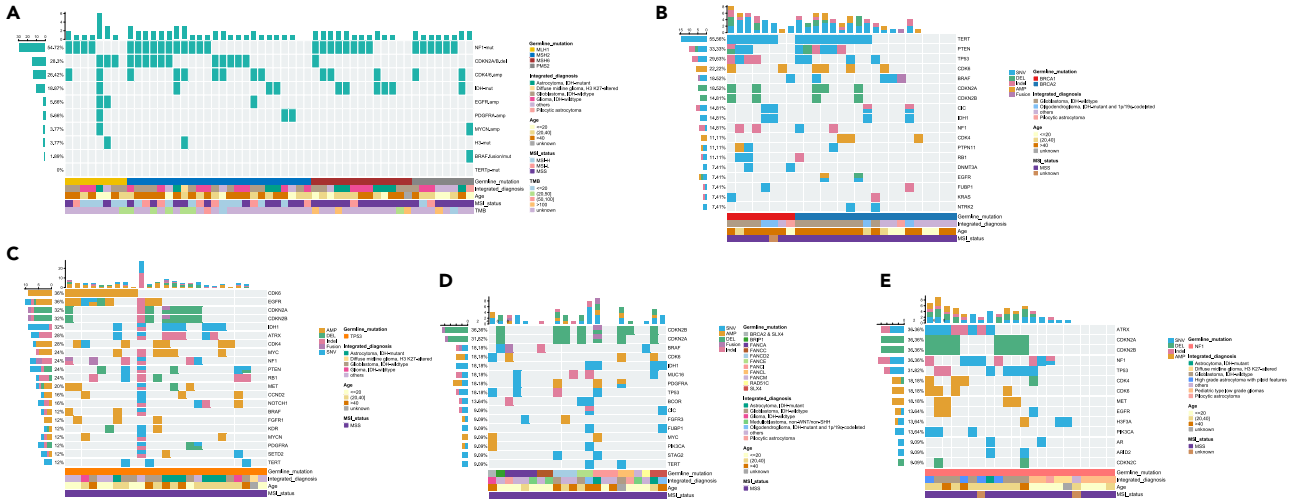

Figure 5. Genomic features of the top five GTS-related CNS tumors

(A) The oncoprint of Lynch syndrome (LS), the most common mutational characteristic in gliomas, especially those associated with typing.

(B) A heatmap of somatic mutations with >7% mutation frequency genes in BRCA-related CNS tumors.

(C) The oncoprint of somatic mutations with >10% mutation frequency genes in Li–Fraumeni syndrome (LFS)-related CNS tumors.

(D) The heatmap of somatic mutations with >9% mutation frequency genes in Fanconi anemia (FA)-related CNS tumors.

(E) A heatmap of somatic mutations with >9% mutation frequency genes in neurofibromatosis 1 (NF1)-related CNS tumors.

Neurofibromatosis 1-related gliomas are driven by different mechanisms

Our study identified 22 patients with NF1-related CNS tumors, including 21 gliomas and 1 neurofibroma. The age distribution of patients with glioma had two main peaks at 20 and 60 years, respectively (Figure 4). Of 7 patients with NF1-related pediatric gliomas (≤20 years), 5 had low-grade gliomas, and high-grade gliomas occurred primarily in adults aged >20 years (Figure 5E). The somatic mutational load of NF1-glioma was associated with age and grade. *ATRX* mutation, *CDKN2A/B* deletion, and *NF1* and *TP53* mutations were enriched in high-grade gliomas in older patients (Figure 5E). However, younger patients demonstrated fewer mutations. *IDH1/2* and *TERT* mutations frequently found in sporadic gliomas were almost absent in NF1-related glioma tumors. H3K27M mutation was found in two patients with high-grade NF1-related CNS tumors, including a 14-year-old child and a patient lacking age information (Table S2). Furthermore, multiple mutations or two hits in the *NF1* gene frequently occurred in NF1-related tumors (Figures 3C and 5E).

Genetic tumor syndromes analysis supports the diagnosis and treatment of central nervous system tumors

The analysis of Genetic tumor syndromes contributes to further "gliomas NEC" classification

Our study found 27 patients with glioma with GTS with unclear subtypes. We reveal an interesting point worth noting. Among clinically diagnosed "glioma, IDH-wildtype, NEC," 68% (15/22) were LS-associated gliomas, whereas the remaining cases were distributed among six other GTS (Figure S1). The assessment of DNA mismatch repair (MMR) deficiency is not typically included in the traditional pathological diagnosis of gliomas, making the clinical identification of these tumors challenging and potentially causing an incomplete disease assessment. Based on this, we temporarily classified LS-related "glioma, IDH-wildtype, NEC" into a specific subtype called "primary MMR-deficient IDH-wildtype glioma."

Five cases had "gliomas NEC" corresponding to different GTSs (Figure S1). A germline P mutation of the *VHL* gene was found in a 29-year-old patient (GP-209) with pathological suspicion of cerebellar glioma, suspecting a strong possibility of hemangioblastoma (Table S2). The pathologist finally confirmed the hemangioblastoma by integrated diagnosis. Our study reveals that combining NGS testing with traditional CNS tumor pathology projects is an effective approach to identifying this type of disease.

Further treatment opportunities can be identified through genetic tumor syndrome analysis

CNS tumors have a complicated classification according to histopathology and molecular pathology. Molecular-targeted therapies have emerged as promising avenues for treating CNS tumors with the in-depth exploration of genomics. Our study provides data to the field of molecular therapy populations by analyzing GTS in CNS tumors. The biomarkers with potential targetable therapies were concentrated in the MMR signal pathway genes of DNA repair and homologous recombination repair (HRR) signal pathway genes of genomic stability and *TSC1, NF1, VHL,* and *SMARCB1*. Our study revealed that 53.88% (139/258) of patients diagnosed with GTS harbored potential precision oncology therapy target mutations (Table 3). Regrettably, clinical trials that investigate molecularly targeted therapy for GTS-related CNS tumors are lacking. Our research data provides support for the development of future clinical trials in this field.

Table 3. Potential precision oncology therapy in our cohort

Precision oncology therapy	Biomarker(s)	Patients
Pembrolizumab	dMMR	53
Pembrolizumab	MSI-H	18
Ipilimumab + Nivolumab	dMMR	53
Ipilimumab + Nivolumab	MSI-H	18
Dostarlimab	dMMR	53
Olaparib	*BRCA1/2* Oncogenic Mutations	28
Olaparib	*ATM, BARD1, BRCA1/2, BRIP1, CDK12, CHEK1/2, FANCL, PALB2, RAD51B, RAD51C, RAD51D, RAD54* Oncogenic Mutations	51
Rucaparib	*BRCA1/2* Oncogenic Mutations	28
Niraparib	*BRCA1/2* Oncogenic Mutations	28
Talazoparib	*BRCA1/2* Oncogenic Mutations	28
Talazoparib	*ATM, ATR, BRCA1/2, CDK12, CHEK2, FANCA, MLH1, MRE11, NBN, PALB2, RAD51C* Oncogenic Mutations	62
Everolimus	*TSC1/2* Oncogenic Mutations	1
Selumetinib	*NF1* Oncogenic Mutations	23
Belzutifan	*VHL* Oncogenic Mutations	2
Tazemetostat	*SMARCB1* Deletion	1

DISCUSSION

Comprehensive analysis of the molecular profile of CNS tumors using DNA-based NGS, especially supplementary analysis for GTS, is highly significant for disease management throughout the entire process in this rapidly changing field. It provides valuable insights into various aspects, including auxiliary differential diagnosis, prognosis prediction, treatment plan optimization, etiology tracing, and tumor genetic risk assessment. Our study is a large-scale study to analyze the clinical and genomic features of CNS tumor-related GTS. Our study identified 258 (5.34%) patients with GTS among 4,828 patients with CNS tumors, in which the rates of 6.48% (47/725) in pediatric tumors (age<=20 years old) and 5.09% (205/4028) in adult tumors. The prevalence of GTS in pan-cancer childhood tumors has been reported to be between 7% and 12%,[5,32] and might higher rates have been reported under multiple detection methods.[40] In CNS tumors, the identification of GTS was reported at 10%.[41] The proportion of GTS in different studies might vary due to differences in enrollment population, cutoff selection of age, detection methods, and platforms.

Germline P/LP variations can not only increase the risk of cancer but also contribute to tumor progression and affect the therapeutic response. The knowledge of germline P/LP variants could further understand molecular processes in the field and identify patients benefiting from differential clinical management. Clinical trials have demonstrated that genetic testing for germline P/LP variations can improve survival through cancer screening, preventive measures, and targeted therapies in non-CNS solid tumors; therefore, low rates of germline genetic screening may contribute to higher cancer mortality rates.[20,21,42,43] However, in CNS solid tumors, the emphasis on genetic screening, genetic counseling, and treatment for patients with GTS is limited. In our study, the age peak, tumor subtype, and mutant genes were different in each GTS, which explains the mechanism of tumorigenesis of each GTS with unique characteristics. Our data reveal high genetic heterogeneity in primary CNS tumors, which also reminds us that focusing on the effects of genetic factors on tumor biological behavior in primary CNS tumors may require a broader focus. The results of our study will help to understand the occurrence of GTS in different CNS tumors and contribute to better genetic screening, genetic counseling, and disease entire process management.

The expansion of the scope of detection, with the application and popularization of NGS technology, has resulted in the discovery of more germline variants of tumor-related genes as anticipated. These results have presented significant challenges while enhancing our understanding of CNS tumors. Reportedly, germline MMR gene variants found in children, adolescents, and young adults redefine a subtype of "primary MMR-deficient IDH-mutant astrocytoma" with a significantly worse clinical outcome than classic IDH-mutant astrocytoma, with a median survival of only 15 months[44] However, the median survival of nine "*de novo* replication repair deficient glioblastoma, IDH-wildtype" adult patients was 36.8 months, which was significantly longer than that of classic IDH-wildtype glioblastoma, among which five patients with immune checkpoint blockade (pembrolizumab or nivolumab).[45] Our study found LS-related CNS tumors in both adults and children and both IDH-mutant and IDH-wildtype. We found LS-related IDH/H3 wild-type glioma, unlike typical glioblastoma, based on our comprehensive analysis. We defined "glioma, IDH-wildtype, NEC" into a specific subtype and temporarily designated this group as "primary MMR-deficient IDH-wildtype glioma." Our retrospective study found a high proportion of "glioma, IDH-wildtype, NEC" in patients with GTSs, which suggested that patients with NEC should pay attention to GTS. CNS tumor patients with GTS may have different carcinogenic causes and subsequent biological behaviors for CNS tumors, and the mechanisms can be explored in the future.

Moreover, seven patients carried double germline variants, including *FANCI/LZTR1, MSH6/NF1, MUTYH/PTCH1, LZTR1/MSH3, BLM/ERCC6, BRCA2/SLX4,* and *FANCD2/LZTR1*, which contained at least one DNA repair and genomic stability pathway gene. We do not know whether it is a *de novo* mutation, incomplete penetrance, or clinical heterogeneity because it cannot be verified through pedigree.

The approval of PARP inhibitors in HRR gene-mutated metastatic castration-resistant prostate cancer has brought attention to FA family genes.[46] Studies have revealed that the impaired FA pathway increases the release of fragmented DNA following X-irradiation and that the altered pathway promotes the development of human cancer.[47] However, there is no consensus on the tumor risk of FA heterozygotes in various population studies.[48,49] The results of our study contribute to the CNS tumor atlas and reveal the possibility of PARP inhibitors in CNS tumors, but larger population analyses are warranted to accurately assess tumor risk.

In conclusion, our study includes a large-scale cohort of patients with CNS tumors with GTS and analyzes the clinical and genomic features as well as their clinical significance. Results in our study of CNS tumors with GTS are expected to improve disease's entire process management, which is an important application of DNA-based NGS in GTS evaluation and management and provides a reference direction for the future design of clinical trials.

Limitations of the study

This study has some limitations, including the retrospective study design with the cancer type of enrolled patients being not random, without a family history and follow-up information, and the management of genes with moderate penetrance that cannot give good and reasonable advice.[50,51] In addition, the targeted capture assay may be bias introduced, specifically germline copy number changes and large genomic rearrangements (LGRs) may not be detected well, and certain genes that are not covered in the assay will be missed. Our study was retrospective and did not include family verification, so the diagnosis of constitutional mismatch repair deficiency (CMMRD) could not be conclusively confirmed. These limitations hinder our ability to conduct a comprehensive analysis of tumor occurrence and development and interfere with the formulation of personalized health management for patients with GTS and their family members. Nevertheless, our research also provides valuable insights for future prospective studies, particularly in the areas of disease diagnosis and treatment plan development based on diverse molecular backgrounds.

RESOURCE AVAILABILITY

Lead contact

Further information and requests should be directed to and will be fulfilled by the lead contact, Jie Gong (gongjie@sdu.edu.cn).

Materials availability

This study did not generate new, unique reagents.

Data and code availability

- The raw sequencing data are available under restricted access due to data privacy laws. Data are available on request sharing by sending requests to the corresponding author Jie Gong (gongjie@sdu.edu.cn), which will need the approval of the institutional ethical committees. Clinical data were not publicly available due to involving patient privacy, but can be accessed from the corresponding author, upon request for 3 years; individual de-identified patient data will be shared for clinical study analyses. The remaining data are available in the article, supplemental information, or Source Data file. The study protocol is provided in the supplemental information file.
- This article does not report the original code.
- Any additional information required to reanalyze the data reported in this work article is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

Conceptualization, J.G., C.W., and J.C.; methodology, J.G., Y.W., N.L., and T.H.; investigation, N.L., T.H., X.Y., and Y.S.; writing – original draft, C.W., J.C., N.L., and T.H.; writing – review and editing, D.C., N.L., and J.G.; funding acquisition, C.J.; resources, J.G., and D.C.; supervision, J.G., and D.C.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Tumor tissues and paired leukocytes samples for analysis were collected from 258 patients recruited in the trial	This manuscript	N/A
Critical commercial assays		
gDNA Tissue Extraction Kit	Concert®	N/A
magnetic universal gDNA kit	TIANGEN®	N/A
Qubit dsDNA HS Assay Kit	Thermo Fisher Scientific Qubit Fluorometer	N/A
Agilent 4,200 TapeStation	Agilent	N/A
KAPA Hyper DNA Library Preparation Kit	Roche Diagnostics	N/A
VAHTSTM Universal DNA Library Prep Kit	Illumina® (Vazyme)	N/A
Software and algorithms		
fastp (V.2.20.0)	Chen et al.[52]	https://github.com/OpenGene/fastp
Burrows-Wheller Aligner algorithm (V.0.7.17)	Li et al.[53]	http://bio-bwa.sourceforge.net/index.shtml
VarDict (V.1.5.7)	Lai et al.[54]	https://github.com/AstraZeneca-NGS/VarDict
InterVar	Li et al.[55]	https://github.com/WGLab/InterVar
Factera (V1.4.4)	Newman et al.[56]	https://fredhutch.github.io/easybuild-life-sciences/updates/2020-12-03-factera/
CNVkit (dx1.1)	Talevich et al.[57]	https://cnvkit-pbgl.readthedocs.io/en/stable/index.html
ANNOVAR	Wang et al.[58]	https://annovar.openbioinformatics.org/en/latest/
R (4.3.1)	The R project for statistical computing	https://cran.r-project.org/
ggsankey (0.0.99999)	GitHub	https://github.com/davidsjoberg/ggsankey
ComplexHeatmap (2.16.0)	Gu et al.[59]	https://bioconductor.org/packages/release/bioc/html/ComplexHeatmap.html
maftools (2.16.0)	Mayakonda et al.[60]	https://bioconductor.org/packages/release/bioc/html/maftools.html

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Human subject

A total of 258 patients diagnosed with genetic tumor syndromes harboring germline P/LP variants from the Simceredx cohort of CNS tumors were enrolled in this study. Baseline characteristics, including age, gender, cancer type, WHO grade, lesion location, and pathological diagnosis of all patients were collected. Among the 258 enrolled patients, 148 were males and 110 were females. The median age was 36.25 (range: 0–81) years, of which 78 were $\leq$20 years. Informed consent from all adult participants or guardians' consent was obtained for all patients in this retrospective study. As part of consenting, adult participants or guardians decided if they wanted to be informed about germline P/LP variants indicative of a GTS or not. All study steps were performed in accordance with the Declaration of Helsinki. This study was approved by the Ethical committee of Qilu Hospital of Shandong University (KYLL-202409 (YJ)-017) and the Ethical committee of the Affiliated Yantai Yuhuangding Hospital of Qingdao University (2024-614).

METHOD DETAILS

Patient cohort and sample collection

In this study, the Simceredx cohort encompassed 4,828 patients with CNS tumors from June 2019 to May 2023, who were subjected to target capture NGS large panel assays in tumor tissues and paired leukocytes. Informed consent from all adult participants or guardians' consent was obtained for all patients in this retrospective study. As part of consenting, adult participants or guardians decided if they wanted to be informed about germline P/LP variants indicative of a GTS or not.

DNA extraction, library construction, and next-generation sequencing

A gDNA Tissue Extraction Kit (Concert®) was used to prepare genomic DNA (gDNA) from formalin-fixed paraffin-embedded tumor blocks/slides or frozen fresh tumor tissues. A magnetic universal gDNA kit (TIANGEN®) was used to prepare paired leukocyte gDNA. A Qubit dsDNA HS Assay Kit was used to quantify the extracted gDNA using a Thermo Fisher Scientific Qubit Fluorometer, and an Agilent 4,200 TapeStation (Agilent) was used to evaluate gDNA quality.

Enzyme treatment was then used to shear 200 ng of extracted eligible gDNA into 200–300 bp fragments. The KAPA Hyper DNA Library Preparation Kit from Roche Diagnostics was used to perform end-repair sheared DNA, and the VAHTSTM Universal DNA Library Prep Kit for Illumina® (Vazyme) was utilized to perform A-tailing and to synthesize indexed paired-end adaptors for the SimcereDx Illumina platform. The size selection function was then used to remove unligated adaptors using Agencourt AMPure XP beads from Beckman Coulter. The ligation products were amplified by polymerase chain reaction (PCR) to form a hybridization prelibrary.

The prepared final qualified DNA libraries were sequenced by 150-bp paired-end according to the manufacturer's instructions on the Illumina NovaSeq6000 platform by the College of American Pathologist (CAP)-accredited central laboratory at Jiangsu Simcere Diagnostics Co., LTD (Nanjing, China).

Bioinformatics analysis of genomic data

The sequencing raw data were converted to FASTQ files, and fastp software (V.2.20.0) was used for quality control to trim the adapter and remove low-quality bases.[52] The Burrows-Wheller Aligner (BWA-MEM v.0.7.17) algorithm (http://bio-bwa.sourceforge.net/index.shtml) was used to align the obtained clean paired-end reads to the hg19 human genome reference (UCSC hg19/GRCh37).[61] Dedup with Error Correct was used to remove PCR duplicate reads.

Determination of the somatic variations

VarDict (v.1.5.7) and InterVar were used to call and annotate single nucleotide variation (SNV) and insertion/deletion (Indel) mutations, respectively. We then filtered the variants in public databases for common single nucleotide polymorphisms, including 1,000 Genome Project (August 2015) and Exome Aggregation Consortium Browser 28. Fusions and copy number variations, including amplification and deletion, were analyzed using Factera (v1.4.4) and CNVkit (dx1.1), respectively. The minimum allelic mutation frequency threshold of SNVs, Indels, and fusions was set at 2%. Subsequently, the Variant Calling Format is annotated by ANNOVAR.

The analysis was performed using a NGS panel that covers the exon and intron regions of more than 362 brain tumor-related genes.[62] Tumor mutation burden (TMB) was calculated by summing all nonsynonymous somatic mutations in the coding region per megabase (muts/Mb), excluding alterations listed as known somatic alterations in COSMIC. Homopolymer repeat loci on the panel with adequate coverage were selected to determine the microsatellite instability (MSI) status, and reads that were successfully mapped to each loci were extracted from the deduplicate BAM file. Msisensor was used to determine the stability of each locus, and it defined the percentage of unstable loci as the MSI score.[62] Any sample with an MSI score greater than the cut-off value ($\geq$ 0.15) was classified as MSI-high.

Determination and classification of germline variations

We analyzed 90 tumor-related predisposition genes for germline data. Table S1 shows the list of the 90 tumor-related predisposition genes. Germline mutations were detected by VarDict (v.1.5.7) and annotated to several public databases, including gnomad (v3.1.2), CLINVAR (202308), dbNSFP (v42a), COSMIC (v98), and the Simceredx database. The germline mutations were filtered based on the annotation, and systematic false-positive mutations were filtered following the background baseline. After filtration, the pathogenicity judgment for each mutation was given based on the results of InterVar and databases such as CLINVAR.

The germline SNV/Indel variants of 90 tumor predisposition genes were analyzed in our study. Germline variants were classified based on the American College of Medical Genetics and Genomics (ACMG) guidelines into benign, likely benign, variants of unknown significance, pathogenic, and likely pathogenic.[63]

QUANTIFICATION AND STATISTICAL ANALYSIS

A total of 258 patients with germline P/LP variants from the Simceredx cohort of CNS tumors were diagnosed with GTS. Baseline characteristics, including age, gender, cancer type, WHO grade, lesion location, and pathological diagnosis of all patients were collected. Genetic sequencing results and pathological features were combined to determine the final integrated diagnosis.

R version 4.3.1 and Excel were used for all analyses and tests. The Sankey diagrams were generated using the R package ggsankey (https://github.com/davidsjoberg/ggsankey). The ComplexHeatmap (R package) was used for the visualization of germline (P/LP) and somatic oncoprints.[59] The maftools R package was used to analyze lollipop plots of the top mutated genes.[60] Other figures were generated using the R package ggplot2 or Excel.