Molecular characterization of choroid plexus tumors reveals novel clinically relevant subgroups

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The authors disclose no potential conflicts of interest.

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ABSTRACT:

Purpose: To investigate molecular alterations in choroid plexus tumors (CPTs) using a genome-wide high-throughput approach, in order to identify diagnostic and prognostic signatures that will refine tumor stratification and guide therapeutic options.

Experimental Design: One hundred CPTs were obtained from a multi-institutional tissue and clinical database. Copy number (CN), DNA methylation and gene expression signatures were assessed for 74, 36 and 40 samples, respectively. Molecular subgroups were correlated with clinical parameters and outcomes.

Results: Unique molecular signatures distinguished choroid plexus carcinomas (CPCs) from choroid plexus papillomas (CPPs) and atypical choroid plexus papillomas (aCPPs); however, no significantly distinct molecular alterations between CPPs and aCPPs were observed. Allele-specific CN analysis of CPCs revealed two novel subgroups according to DNA content: hypodiploid and hyperdiploid CPCs. Hyperdiploid CPCs exhibited recurrent acquired uniparental disomy (aUPD) events. Somatic mutations in TP53 were observed in 60% of CPCs. Investigating the number of mutated copies of p53 per sample revealed a high-risk group of patients with CPC carrying two copies of mutant p53, who exhibited poor 5-year event-free (EFS) and overall survival (OS) compared to patients with CPC carrying one copy of mutant p53. (OS: 14.3%, 95% CI 0.71%-46.5% versus 66.7%, 28.2%-87.8%, respectively, p=0.04; EFS: 0% versus 44.4%, 13.6%-71.9%, respectively, p=0.03). CPPs and aCPPs exhibited favorable survival.

Discussion: Our data demonstrates that differences in CN, gene expression and DNA methylation signatures distinguish CPCs from CPPs and aCPPs; however molecular similarities among the papillomas suggest that these two histological subgroups are indeed a single molecular entity. A greater number of copies of mutated TP53 was significantly associated to increased tumor aggressiveness and a worse survival outcome in CPCs. Collectively, these findings will facilitate stratified approaches to the clinical management of CPTs.
TRANSLATIONAL RELEVANCE:

This report is the first to dissect the aberrant complexity in copy number, methylation, and gene expression of one of the largest cohort of pediatric choroid plexus tumors (CPTs). Our findings revealed molecular homogeneity among choroid plexus papillomas (CPPs) and atypical choroid plexus papillomas (aCPPs), reflecting the favourable survival of these patients and suggesting these histologically distinct subgroups are a single tumor entity. Choroid plexus carcinomas (CPCs) were significantly different from CPPs and aCPPs. Moreover, CPCs exhibited molecular heterogeneity, and patient outcomes varied widely. We identified novel CPC subgroups with significantly distinct copy number signatures suggesting different mechanisms drive CPC development. We identified that patient overall and event-free survival significantly decreased with an increasing number of mutated copies of p53. By defining the molecular landscape of CPTs, this study has provided a comprehensive molecular background on which to explore mechanisms of tumorigenesis and develop stratified approaches to the clinical management of CPTs.
INTRODUCTION

Choroid plexus tumors (CPTs) are rare intraventricular neoplasms accounting for up to 20% of brain tumors in children under two years of age. Three histological subgroups have been described: choroid plexus papilloma (CPP, WHO grade I), atypical choroid plexus papilloma (aCPP, WHO grade II), choroid plexus carcinoma (CPC, WHO grade III). Long-term survival of CPPs is favorable with surgical resection alone (>90%). Conversely, CPCs exhibit a dismal prognosis, with an overall survival of about 30%. Despite aggressive treatment protocols, including surgical resection and combination chemo- and radiation therapy, the clinical behavior of CPCs is variable and most of the few survivors exhibit long-term cognitive and developmental deficits. aCPP, a recently described pathological subgroup, exhibits an intermediate degree of mitotic activity and outcome; however, some cases may be difficult to distinguish from CPC by histology alone.

Over 50% of CPC tumors carry somatic TP53 mutations, and TP53 mutant CPCs have been associated with increased genetic tumor instability and worse prognosis. Germline TP53 mutations have also been observed in CPC patients as CPC is one of the hallmark cancers of the Li-Fraumeni syndrome (LFS), a familial cancer syndrome in which affected family members harbor a mutant copy of the TP53 tumor suppressor gene. Cytogenetic studies of central nervous system (CNS) tumors have revealed high chromosomal instability in more than 90% of CPTs analyzed (Supplementary Table S1). Defining the molecular landscape of CPTs and identifying actionable molecular aberrations has been challenging due in part to the limited number of patients and high-quality samples available for genome-wide studies. Here, we use an integrative molecular
approach to characterize the genomic, transcriptomic and epigenomic landscape of the largest cohort of CPTs to date. The information derived from these analyses creates a molecular foundation on which to develop approaches to improve the clinical management of this devastating disease.

MATERIALS and METHODS

Patients and Sample Preparation

CPT samples and/or clinical data were collected from institutions in Canada, the United States of America, Brazil, Israel, and Germany (see Appendix) in accordance with each institution’s Research Ethics Board. Informed consent was obtained from the parents/legal guardians of all patients. We studied 100 unique tumor samples (58 CPC, 30 CPP and 12 aCPP) from 91 pediatric patients (ages 0.03-16.50 years old) for which TP53 sequence data was available (Table S2). Pathological review of CPTs was conducted by C.H., D.W.E., and B.T.H. when samples were available. In all other institutions, expert neuropathologists critically examined each case. In fifteen CPC cases, immunohistochemical analysis of hHF5/INI1 was conducted and revealed immunopositivity excluding the diagnosis of atypical teratoid/rhabdoid tumors (ATRT). Nucleic acids were derived from fresh frozen (n=75), optimal cutting temperature compound (OCT) (n=9), and formalin-fixed paraffin-embedded (FFPE) (n=12) samples. We received isolated tumor DNA from 4 samples. Twenty-two nucleic acid samples exhibited suboptimal quality and/or quantity, leaving 78 high-quality samples from 73 patients for analysis (Supplementary Fig. S1). Detailed clinical data were obtained for 68 patients. Tumor DNA was extracted using standard phenol-chloroform extraction from
fresh-frozen samples, and the RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE (Ambion, Carlsbad, USA) from FFPE samples. Total RNA was isolated from fresh-frozen samples using the TRIZol method (Invitrogen, Carlsbad, USA) according to the manufacturer’s instructions.

**TP53 Sequencing**

Sequencing of the coding region of *TP53* (exons 2-11) was performed in the molecular diagnostic laboratory at The Hospital for Sick Children (Toronto) by direct Sanger sequencing of whole genome DNA as previously described.(5)

**Microarray Processing & Bioinformatics Analysis**

Forty RNA samples were hybridized to GeneChip® Human Exon 1.0ST gene expression microarrays (Affymetrix, Santa Clara, USA), and 36 DNA samples were hybridized to Illumina® 450K Infinium methylation bead arrays (Illumina, San Diego, USA) as per manufacturer’s instructions. An initial set of 55 tumor DNA samples was hybridized to Genome-Wide Human SNP Array 6.0 (Affymetrix), while an independent set of 20 tumor DNA samples was hybridized to Affymetrix OncoScan™ FFPE Express 2.0 arrays. One technical replicate was included in both genotyping platforms and analyzed for copy number call consistency.

Partek® Genomics Suite™ 6.5 (PGS) (Partek Inc. St. Louis, USA) and BioDiscovery Nexus Copy Number software (Discovery Edition 7.0, BioDiscovery, Hawthorne, USA) were used for copy number analysis as previously described(5) (see Appendix). Copy number changes encompassing more than 75% of the chromosome were called whole-
chromosome aberrations. Allele-specific copy number analysis of tumors (ASCAT) was performed in R as previously described(10) and verified by Nexus software. Tumor ploidy, where hypodiploid <1.90 and hyperdiploid >2.10, heterogeneity, and allelic imbalances were inferred from the output. ASCAT failed to resolve the ploidy of two samples with very low aberrant fraction, so these were excluded from further analysis. Clustering of gene expression and methylation data was investigated in R (version 3.0.1) by unsupervised hierarchical clustering (uHCL), non-negative matrix factorization (NMF), and PVCLUST algorithms. Differential gene expression analysis was conducted with PGS 6.5 (see Appendix). Gene set enrichment analysis (GSEA) was performed as previously described(11) and its visualization was obtained by Cytoscape and Enrichment Map using an in-house curated database containing freely available NCI, KEGG, PFAM, Biocarta and GO databases as described in Witt et. al. (12) Differences in DNA methylation status were analyzed with the Illumina® GenomeStudio software (see Appendix). All probesets were annotated according to the human genome build hg19 (GRCh37). Microarray data can be accessed from GEO (GSE####, GSE####, GSE#### and GSE####).

Statistical analysis

Statistical analyses of copy number and gene expression were performed in PGS 6.5, whereas methylation was analyzed in Genome Studio. Patient survival was calculated in StataSE (version 12), while other statistical analyses were conducted in R (version 3.0.1) (see Appendix). Survival estimates for tumor subgroups, and for CPCs by TP53 and ploidy status were generated using the Kaplan-Meier method and curves were compared
using a log-rank test. Overall survival (OS) measured time from initial diagnosis to death from any cause or last follow-up as of December 1, 2013. Event-free survival (EFS) measured time from initial diagnosis to tumor progression, recurrence or death from any cause.

RESULTS

Genomic, Transcriptomic and DNA Methylation Profiling of CPTs Reveal Significant Segregation of CPCs from CPPs and aCPPs

Unsupervised clustering analyses performed with gene expression and methylation data revealed clear segregation of CPCs from CPPs and aCPPs (Fig. 1). NMF analysis of gene expression (Fig. 1 A) and methylation (Fig. 1 B) data demonstrated greatest difference between two subgroups (FDR-corrected p=2.54x10^{-7} and p=1.02x10^{-34}, respectively), segregating CPCs from CPPs and aCPPs. This significant molecular stratification was also observed using a smaller number of probesets for gene expression and methylation differences analyzed by PVCLUST (Supplementary Fig. S2). The concordance between tumors stratified by gene expression and methylation was significant (Rand index=0.73, p<1.0x10^{-4}) and revealed consistent molecular segregation of CPTs into unique molecular subgroups.

Although copy number analysis revealed widespread chromosomal instability in all tumor subgroups (Fig. 2), a distinct signature characterized by increased frequency of chromosome-wide gains and losses was observed in CPCs (average 5.43 chromosomes gained and 5.65 lost per CPC), compared to increased frequency of chromosome-wide gains but very few losses in CPPs and aCPPs (average 6.68 chromosomes gained and
0.32 lost per CPP, and 10.00 versus 0.25 per aCPP) (Fig. 2). The frequency of chromosome-wide losses in CPCs was significantly greater than in CPPs and aCPPs (Two-tailed t-test p<1.00 x10^{-4}). Allele-specific copy number analysis allowed us to investigate the allelic ratios in our samples. This technique revealed a striking pattern of copy number-neutral loss of heterozygosity. This phenomenon is commonly observed in cancer cells and may also be referred to as acquired uniparental disomy (aUPD), wherein a chromosome pair is homozygous, thus having two copies of the same allele (13). CPCs exhibited frequent aUPD events with an average of 2.31 aUPD events per sample while the phenomenon occurred less frequently in CPPs and aCPPs (average 0.32 and 1 events per sample, respectively).

Analysis of clinical variables between the three histological subgroups revealed no significant difference of age at diagnosis (Kruskal-Wallis test p=0.30) or ratio of males to females (Two-way ANOVA p=0.26) (Table S2). Survival outcomes for CPCs were significantly worse than for CPPs and aCPPs. Five-year OS for CPCs was 56.3% (95% CI 36.5%-72.0%), compared to 92.9% (59.1%-99.0%) and 100% for CPPs and aCPPs, respectively (p=0.03) (Fig. 3B). Only one patient with CPP died due to complications from a concurrent diagnosis of ependymoma. Five-year EFS for CPCs was 39.7% (95% CI 22.8%-56.2%), compared to 87.4% (58.1%-91.9%) and 70.0% (22.5%-91.8%) for CPPs and aCPPs, respectively (p=4.90x10^{-3}) (Fig. 3C)

**CPPs and aCPPs Share Similar Molecular Signatures Which Correlate With Favorable Survival Outcomes**
Analyzing CPPs and aCPPs independently from CPCs revealed a striking molecular similarity between the papilloma subgroups (Fig. 4). Unsupervised clustering analysis demonstrated that CPPs and aCPP did not segregate according to differences in gene expression or methylation (Fig. 4 A&B). Supervised analysis using the Wilcoxon rank-sum (WRS) test between CPPs and aCPPs revealed no significant differences in gene expression or methylation (Fig. 4 C&D). Additionally, signatures of chromosomal instability characterized by recurrent chromosome-wide gains and very few losses were observed in both CPPs and aCPPs; no significant differences in the frequency of chromosome-wide gains and losses were observed (Two-tailed t-test p=0.32 and p=0.49, respectively) (Fig. 4E). There were no differences in age at diagnosis (Mann-Whitney test p=0.45) or ratio of males to females (Fisher’s Exact test p=0.31) between CPPs and aCPPs. Moreover, survival outcomes for CPP and aCPP patients were not significantly different (Log-Rank test, OS p=0.51, EFS p=0.30).

**Ploidy Analysis Reveals Novel CPC Subgroups With Unique Molecular Alterations**

Ploidy analysis revealed the presence of aneuploidy in 87% of tumors (Supplementary Fig. S3). CPPs and aCPPs exhibited ploidy greater than 2 (hyperdiploidy); however, CPCs exhibited a wide distribution of ploidy values, with two significantly distinct subgroups observed: hyperdiploid CPCs (average ploidy 2.76, range: 2.21-3.34) and hypodiploid CPCs (average ploidy 1.45, range: 1.25-1.71) (Mann-Whitney test, p<1.00x10^-4). Only three of 36 CPCs were diploid. Hypodiploid CPCs exhibited recurrent chromosome-wide losses and very few gains with an average of 12.70 chromosomes lost and 0.10 gained per tumor. Chromosome 3 was lost in all hypodiploid CPCs, with loss of 3.
chromosomes 6, 9, and 22 observed in 90% of tumors (Fig. 3A). Hyperdiploid CPCs exhibited a high frequency of chromosome-wide gains and almost no losses (average 12.22 and 0.22 chromosomes, respectively). Chromosomes 12, 7 and 1 were gained in more than 80% of hyperdiploid CPCs (Fig. 3A). In addition to a high frequency of chromosomal gains, hyperdiploid CPCs also exhibited aUPD more frequently than hypodiploid CPCs (average of 4.93 affected chromosomes per tumor compared to 0.33 chromosomes in hypodiploid CPCs) (Fisher’s Exact test p<0.0001) (Fig. 3A). Moreover, significant enrichment in aUPD was observed in TP53 mutant hyperdiploid CPCs compared to hyperdiploid CPCs with wild-type TP53 (Fisher’s Exact test p<0.0001). aUPD was most frequently observed in chromosome 17, affecting 30% (10/33) of CPCs.

We conducted GSEA to identify biological pathways and processes that are differentially expressed between hypodiploid and hyperdiploid CPCs. GSEA revealed enrichment in RNA processing, DNA replication and repair, and chromosome segregation in hyperdiploid CPCs. Hypodiploid CPCs exhibited enrichment in cellular metabolism, signaling and cell migration pathways, as well as leukocyte activation and proliferation (5% FDR, p<0.05) (Supplementary Fig. S4). The patterns of enrichment observed suggest hyperdiploids are more proliferative than hypodiploid CPCs, and that the latter tumors are undergoing a significant immune response. A greater understanding of these distinct enrichment patterns will elucidate the mechanisms underlying the progression of these molecularly distinct CPC subgroups (Supplementary Fig. S4). There were no significant differences in DNA methylation between subgroups, although this may be due to the low number of samples in the comparison groups (hypodiploid n=4, hyperdiploid n=9).
Increased Number of Copies of Mutant TP53 is Associated With Tumor Aggressiveness and Unfavorable Survival Outcomes

Mutations in TP53 were assessed in our cohort by Sanger sequencing. Sixty percent of CPCs (35/58) were mutant for TP53. Fifteen (15/58, 26%) of these samples belonged to 12 LFS patients carrying a germline mutation in TP53. Mutations in TP53 were observed in both hypodiploid and hyperdiploid CPCs, however, in our cohort, the frequency of TP53 mutations was significantly greater in hypodiploid CPCs (16/18, 89%) than hyperdiploid CPCs (7/15, 47%) (Fisher’s Exact test p=0.02). Diploid CPCs (n=3) were TP53 wild-type (Supplementary Table S3). No significant enrichment for LFS patients was observed in either hypo- or hyperdiploid subgroups.

Unsupervised clustering using gene expression and methylation data segregated CPCs into two significantly distinct clusters (p=0.05) (Fig. 5A & B). Although CPCs did not segregate according to ploidy status, we observed two clusters, which were significantly distinct according to TP53 status using DNA methylation data (Fisher’s Exact test, p=0.007), but did not reach significance using gene expression data (Fisher’s Exact test, p=0.089). LFS-CPCs did not segregate from the spontaneous CPCs, suggesting no unique aberrations were present in tumors arising from patients with an inherited TP53 mutation. An in-depth analysis of the type of TP53 mutations revealed that a few samples, which appeared to be miscategorized by unsupervised clustering, had an uncharacterized intronic alteration (c.28-28G>A/wt) and mutations outside the DNA-binding domain (ie. c.290T>A in the SH3-like/Proline-rich domain), which may account for differences in the transcriptomic and epigenomic signature of these samples (Fig. 5).
Combining $TP53$ sequencing results with allele-specific copy number status of chromosome 17 in 33 CPCs, we estimated the number of mutated copies of $TP53$. We found that 36.4% of CPCs (12/33) had 2 copies of mutant p53, 30.3% (10/33) had 1 copy of mutant p53 and 33.3% (11/33) had zero copies of mutant p53 (wildtype). CPCs with 2 mutant copies of p53 exhibited a homozygous $TP53$ mutation status in all but one tumor sample with a low aberrant cell fraction (46%), suggesting this sample was largely contaminated with normal cells. Seventy-five percent of samples with 2 copies of mutated p53 (9/12) exhibited aUPD in chromosome 17. Eighty-three percent of CPCs with 2 copies of mutated p53 (10/12) had missense mutations in the DNA binding domain, while 1 sample had a missense mutation in the SH3-like/Proline-rich domain and the other sample, a splicing mutation. CPCs with 1 copy of mutated p53 had missense mutations in the DNA binding (9/10) and tetramerization (1/10) domains, and carried a single copy of chromosome 17, exhibiting LOH of the entire chromosome. Three samples exhibited a heterozygous $TP53$ mutation status by sequencing, which may be a result of normal cell contamination. Gene expression and methylation analyses revealed no significant differences among CPCs carrying 1 or 2 mutated copies of p53 because of the limited sample sizes (gene expression: 3 and 6 samples, respectively; methylation: 1 and 6 samples, respectively).

Examining clinical variables among CPCs revealed no differences in the age of diagnosis (Mann-Whitney t-test $p=0.80$, and $p=0.59$) or the ratio of males to females (Fisher’s Exact test $p=1.0$, and $p=1.0$) according to ploidy nor p53 status, respectively (Table S2). No significant differences in OS or EFS estimates were observed between CPC patients exhibiting a hyper- or hypodiploid tumor genome ($p=0.82$, $p=0.94$, respectively).
TP53 status had a significant effect on the OS of our CPC cohort (Log-rank test $p=3.8 \times 10^{-3}$), however EFS was not significantly different between TP53 mutant and wildtype CPCs ($p=0.07$). (Fig. 5C & D).

Investigating survival differences according to the number of mutant copies of TP53 in CPCs revealed a significant reduction in OS (Log-rank test 2 copies vs. 1 copy, $p=0.04$; 2 copies vs. 0 copies, $p<1.0 \times 10^{-4}$), and EFS (Log-rank test 2 copies vs. 1 copy $p=0.03$; 2 copies vs. 0 copies $p=0.003$) in patients harboring a greater number of mutant TP53 copies. The estimated OS of patients with CPCs harboring wildtype TP53 (zero copies) was 88.9% (95% CI 43.3%-98.4%) and EFS, 66.5% (32.9%-86.1%). Patients with CPCs harboring a single copy of mutant TP53 exhibited an estimated OS of 66.7% (28.2%-87.8%) and EFS of 44.4% (13.6%-71.9%), while patients with CPCs harboring two copies of mutant TP53 showed an OS of 14.3% (0.71%-46.5%) and EFS of 0%. (Fig. 6).

DISCUSSION

Our study is the first and largest comprehensive investigation of the molecular alterations found in CPTs, demonstrating that the molecular profile of CPCs is significantly distinct from that of CPPs and aCPPs, and that the papillomas are not significantly distinct from each other. In addition, using an innovative allele-specific approach in combination with TP53 sequencing, we identified a particularly poor prognostic subgroup in TP53 mutant CPC patients exhibiting aUPD in chromosome 17, and who as a result had an elevated number of mutated copies of p53. This study provides evidence for the crucial role of molecular stratification as a tool to improve the clinical management of patients with CPT.
Atypical CPPs are currently distinguished from CPPs by histopathology, where aCPPs exhibit increased mitotic activity (14); yet, survival outcomes for both CPPs and aCPPs are comparably favorable. Standard of care for these tumors consists of surgical resection with very few aCPP cases requiring adjuvant chemotherapy. In our cohort, all aCPP patients for which we had clinical history (6/11), were treated with surgical resection alone, yet demonstrated favorable survival comparable to CPPs. We suggest that the benign phenotype of aCPPs may reflect the molecular characteristics it shares with CPPs, including very few chromosome-wide losses, and similar gene enrichment patterns and methylation signatures. The data lend support for the conservative management of aCPP patients with surgical resection followed by observation.

Our findings also revealed that copy number, gene expression and methylation profiles were significantly distinct between the papillomas and CPCs, indicating the unlikelihood that CPPs or aCPPs progress unto CPCs by the acquisition of a few additional aberrations. Although a few studies have reported on progression from papillomas to CPCs (9,15), we believe this unlikely scenario may have been the result of a heterogeneous tumor sample harboring co-existing CPC and papilloma cells. Analyzing tumor heterogeneity in CPTs, will be necessary in order to identify benign tumors more likely to recur with an aggressive phenotype.

Wide variability in clinical outcome has been observed among CPC patients despite the use of similar treatment protocols (2,16,17). Our findings demonstrate that the molecular heterogeneity of CPCs may be driving this clinical variability.
Extensive chromosomal alterations were recurrent in CPCs. An allele-specific copy number approach allowed us to identify aneuploidy in 91% of CPCs, and distinguish between hypodiploid CPCs, exhibiting numerous chromosomal losses, and hyperdiploid CPCs, exhibiting numerous chromosomal gains and concurrent aUPD. Our findings uncovered that chromosomal instability is a common mechanism involved in CPC development; however, further examination of the molecular differences driving hypodiploid and hyperdiploid development will identify distinct mechanisms responsible for tumor progression. Ploidy was not significantly associated with age at diagnosis, or patient survival; nonetheless, we identified that hyperdiploid CPCs were significantly enriched in chromosomes exhibiting aUPD. A recent study reported similar subgroupings in CPCs, where a higher frequency of chromosomal losses were observed in younger children and chromosomal gains in older children, and loss of 12q was associated with shorter survival(18). In our cohort we found no significant correlations between patient age and CPC subgroups or TP53 status. Moreover, survival differences were identified only when TP53 copy number and mutation status were examined concomitantly.

Arising from somatic recombination errors during mitosis, aUPD is an important mechanism leading to loss of heterozygosity with an unaffected copy number, and is therefore associated with the enrichment of chromosomes or regions harboring preexisting mutations, specific promoter methylation patterns, and focal deletion of genes (13). In our study, we observed aUPD affect entire chromosomes in all tumor subgroups, however aUPD was most frequent in CPCs harboring TP53 mutations. We identified chromosome 17 to be the most frequent site affected by aUPD, and that 90% of CPCs
exhibiting aUPD of chromosome 17 harbored a mutation in TP53, increasing the number of mutant p53 copies to 2 in these tumors. Our findings suggest aUPD is a mechanism by which CPCs accumulate deleterious aberrations, such as TP53 mutations, while retaining the normal function of other genes due to an unaffected chromosome copy number.

Focusing on the known association between p53 mutations and CPCs, we identified that the number of mutated copies of p53 was significantly associated with patient survival. Our findings support the concept that in addition to the loss of tumor-suppressive activity of p53, mutant TP53 also acquires oncogenic activities that promote CPC development. The gain of function (GOF) properties of mutant p53 include cellular invasion, proliferation, genomic instability, and polyploidy, among others (Reviewed in (19)). Because of increased GOF activity, in addition to a complete loss of the tumor suppressor functions of p53, an elevated number of mutant p53 copies could result in an aggressive phenotype associated with decreased survival as we observed in our high risk CPC patient cohort. Since we did not assess the mutation status of other cancer genes in chromosome 17, we cannot infer that the number of mutant copies of p53 is the only aberration on this locus driving CPC development and tumor aggressiveness in the high risk CPC patient cohort. However, the significant dose-dependent correlation observed with overall and event-free survival in CPC patients indicates a role for p53 GOF activity in CPC aggressiveness.

TP53 mutations alone do not drive chromosomal instability in CPCs as TP53 wild-type tumors also exhibit high levels of chromosome-wide gains and losses. However, we have demonstrated that TP53 mutations are associated with changes in gene expression and methylation patterns that may result in increased tumor aggressiveness and may elucidate
the different clinical outcomes observed. Alterations affecting the p53 pathway, either upstream or downstream of p53, may generate the molecular background necessary for CPC development, and should be investigated further.

Recurrent lesions, such as the chromosome-wide gains of chromosome 1, which was not only recurrently gained but also the least frequently lost in CPCs, chromosome 12, and chromosome-wide loss of chromosome 3, may also be contributing to CPC’s unique genotype and would need to be further investigated in order to identify unique targets for effective therapies.

Our study demonstrates that investigating the molecular characteristics of CPTs is crucial to further refine the molecular stratification of patients in order to improve patient care. We suggest that the prognostic significance of TP53 mutation and copy number status in CPCs be validated prospectively in future cooperative clinical trials. Validation of these data in future prospective studies will inform risk stratification of CPC patients, and set the framework for future treatment intensification for high-risk patients.
References:


Figure 1: Unsupervised clustering of (A) gene expression normalized intensities and (B) methylation Beta-values by non-negative matrix factorization (NMF) demonstrate significant segregation of CPCs (red) from CPPs (yellow) and aCPPs (light blue). No segregation was observed between CPPs and aCPPs. NMF was conducted using 5000 probesets with the largest median absolute deviation (MAD). This clustering algorithm identified the most significant measures of similarity (cophenetic coefficient) when the data was at k=2 (2 clusters). In the matrix, red represents the highest measure of similarity (1), while blue/purple represents the lowest measure of similarity (0). Any other colors within the matrix represent a spectrum of changing measures of similarity, from red to blue/purple. Colors: TP53 mutation status: Black: TP53 mutant, White: TP53 wildtype.

Figure 2: Characterization of recurrent chromosome-wide gains (n>2, grey) and losses (n<2, black) for each tumor subtype. (CPC: n=37, CPP: n=25, aCPP: n=11). P-values were calculated using the frequency of chromosome-wide copy number alterations per subgroup and the non-parametric Mann-Whitney test.

Figure 3: (A) Genome-wide characterization of chromosome-wide gains (red), losses (blue), and aUPD (teal) in 71 unique CPC samples. White squares represent unchanged chromosome-wide copy number status. Chromosome-wide aberrations were defined as aberrations that encompass more than 75% of the chromosome. Ploidy: green: hyperdiploid, blue: hypodiploid, tan: diploid. TP53 status: black: mutant, white: wildtype. Kaplan-Meier curves depicting (B) overall and (C) event-free survival estimates of CPT patients by diagnosis. Statistical values were obtained with the Log-rank (Mantel-Cox) test.

Figure 4: Unsupervised clustering of (A) gene expression normalized intensities and (B) methylation Beta values demonstrate no segregation between CPPs (yellow) and aCPPs (light blue). Volcano plot comparing the number of significant differentially expressed genes (C) and significantly methylated regions (D) reveals no significant gene expression or methylation differences between the two subgroups (after FDR adjustment). Signatures of chromosomal instability (E), as measured by the number of chromosome-wide gains and losses in CPPs and aCPPs, were similar between the two subgroups and characterized by extensive chromosomal gains and very few losses. Black squares represent chromosome-wide aberrations, while white squares represent unchanged chromosome copy number. Gender was also depicted (pink: female, purple: male).

Figure 5: Unsupervised hierarchical clustering of (A) gene expression normalized intensities and (B) methylation Beta values using the PVCLUST algorithm demonstrates significant segregation among CPCs, where subgroups are characterized by differences in TP53 status (mutant=black, wild-type=white). Red rectangles delineate statistically significant different groups (p=0.05). PVCLUST algorithm was conducted using 1000 probesets with the largest median absolute deviation (MAD). * shows sample with an uncharacterized intronic alteration, # shows samples with mutation in SH3-like/Proline-rich TP53 domain. Colors: Diagnosis: Red: CPC, Yellow: CPP, Light blue: aCPP, TP53
status: Black: \textit{TP53} mutant, White: \textit{TP53} wild-type, Ploidy: Green: hyperdiploid, Blue: hypodiploid, Tan: Diploid, Grey: unknown. Kaplan-Meier curves depicting (C) overall and (D) event-free survival estimates of CPC patients by \textit{TP53} mutation status. Statistical values were obtained with the Log-rank (Mantel-Cox) test.

\textbf{Figure 6:} Kaplan-Meier curves depicting (A) overall and (B) event-free survival estimates of CPC patients by number of mutated copies of p53, as estimated by Sanger sequencing and allele-specific copy number analysis. Statistical values were obtained with the Log-rank (Mantel-Cox) test.
Fig. 2

CPC
Frequency of chromosome-wide
Losses Gains

%100 80 60 40 20 0 0 20 40 60 80 100%

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22

X

n=35

CPP
Frequency of chromosome-wide
Losses Gains

%100 80 60 40 20 0 0 20 40 60 80 100%

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22

n=22

aCPP
Frequency of chromosome-wide
Losses Gains

%100 80 60 40 20 0 0 20 40 60 80 100%

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22

n=11

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<td>CPC vs. CPP</td>
<td>&lt;1.0E-4</td>
<td>CPC vs. CPP</td>
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<td>CPC vs. aCPP</td>
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Molecular characterization of choroid plexus tumors reveals novel clinically relevant subgroups

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