Supplementary Materials for Somers et al. “A common polymorphism in the 5’ UTR of ERCC5 creates an upstream open reading frame that confers resistance to platinum-based chemotherapy”

Legends to supplementary Figures

Supplementary Figure 1.

A) Sequence of the 5’ UTR of ERCC5 showing the position of the uORFs. The first uORF (uORF1) is marked in green (with the rs751402 SNP in red), the second uORF (uORF2) in pink, the start codon of the ERCC5 ORF in brown.

B) cDNA sequence alignment of the 5’ UTR and 5’ coding region of ERCC5. The cell lines are; B-cells: GM07019 (G/G) and GM07029 (A/A); neuroblastoma: LAN5 (G/G) and CPH-212 (A/A); fibroblast: AG6558 (G/G) and GM17082 (A/A). uORF1 is marked in green (with the rs751402 SNP in red), uORF2 in pink and the ERCC5 start codon in brown. Additional known SNPs are annotated; rs2296147 (orange, 5’ UTR, invariant across the cell lines) and rs1047768 (blue, coding region, silent mutation) (Blomquist et al. 2010, Duan et al. 2012). Neither of these SNPs correlates with rs751402.

Ci) Plasmid constructs were generated to in vitro transcribe mRNA from a Luciferase control (Luc) cloned downstream of ERCC5 5’UTR variants. Construct UTR-A contains the A SNP so both uORF1 and uORF2 are present; Construct UTR-G contains the G SNP so only uORF2 is present. Constructs were also modified to remove uORF stop codons and to position uORF start codons in-frame with the luciferase coding sequence. This enables translation from these AUGs into the reporter sequence to be measured (see Cii). Construct UTR-A-inframe (UTRAinfr) has uORF1 and uORF2 AUGs in-frame with the luciferase start
codon (both stop codons mutated); UTR-G, UTR-G-inframe (UTRGinfr) lacks the uORF1 AUG and so only the uORF2 AUG is in-frame with the reporter (stop codon mutated).

Cii) The mRNAs described above were used to prime in vitro radiolabeled translation reactions. The numbers under the gel show expression relative to the amount of protein produced from luciferase RNA with no 5’ UTR. The data show that the UTR-A 5’ UTR (uORF1 and 2 present) inhibits translation of the downstream luciferase cistron 11x more than the UTR-G 5’ UTR (uORF2 only present). The reduced translation of uORF2 and luciferase in the UTR-A construct may take place following either reinitiation after translation of uORF1, or by leaky scanning of ribosomes through its AUG. To confirm that ribosomes can both recognize and translate the uORF sequences, the AUGs of the two uORFs were put in-frame with the luciferase start codon and the stop codons removed (UTRA-infr). The longest of the three products observed results from initiation at the AUG of uORF1. Since there cannot be reinitiation in the absence of stop codons, the two additional shorter products are most likely generated in this context by “leaky scanning” through uORF1 to the start codons of uORF2 and luciferase. When the AUG of uORF1 is absent and only uORF2 is placed in-frame (UTRG-infr), only the two shorter translation products are produced, reflecting initiation from uORF2 and luciferase start codons. Taken together, our data show that each uORF impacts on the number of ribosomes able to engage and translate from downstream start codons. When uORF1 is present, only some ribosomes will be able to engage with additional downstream start codons by the relatively inefficient process of reinitiation, or by leaky scanning. Of this reduced pool of ribosomes, more will subsequently engage with the AUG of uORF2 than with the more distant luciferase start
codon. The number of ribosomes able to reinitiate at the luciferase start codon after uORF2 translation would then be extremely low.

**Supplementary Figure 2.**

A) Cell lines derived from patients with neuroblastoma that were either G/G (LAN5), A/A (CHP-212) or A/G (SH-SY5Y) (i) were treated with Cisplatin and cell survival assessed by WST1 assays (ii) and annexin V/PI labeling (iii), after 24 hours.

B) B-cell lines derived from healthy individuals were either G/G (GM07019), A/A (GM07029) or A/G (GM07348) (i) were treated with Cisplatin and cell survival assessed by WST1 assays at 16 (ii) or 48 hours (iii).

C) Cell lines derived from patients with DLBCL that were either G/G (DOHH-2) or A/G (DB) (i) were treated with Cisplatin and cell survival was assessed by either WST1 assays after 24 hours (ii) or after 48 hours (iv) or the degree of apoptosis as measured by annexin V/PI labeling (iii) after 24 hours or after 48 hours.

D-E) Fibroblasts derived from healthy individuals that were either G/G (AG06558, TIG120), A/G (GM17082, TIG114) or A/A (GM17301) (Di, Ei) were treated with the doses of Cisplatin shown and survival assessed by WST1 assay after 24 hours (Dii, Eii) or 48 hours (Div) or the degree of apoptosis assessed by annexin V/PI labeling after 24 hours (Diii).

The data from both WST1 assays and Annexin/PI labeling show that there are significant differences in the survival of the cells that contain the ‘A’ allele of ERCC5 compared to those that are homozygous for the ‘G’ allele.
Supplementary Figure 3.

To investigate the role of the post-transcriptional response further, polysome analysis was carried out on two cell lines that were heterozygous for rs751402 to enable differences in polysomal association of the transcripts to be determined.

**Ai)** Primer specific PCR to show that CaLu 1 cells were heterozygous for the polymorphism (NTC= Non-template control).

**Aii)** CaLu1 were treated with Cisplatin and post-nuclear extracts were applied to 10-60% sucrose density gradients. After centrifugation and fractionation mRNA was isolated from the individual gradient fractions. This technique allows the separation of actively translating ribosomes (polysomes) from the subpolysomes (which are not actively translating) and is described in detail in Powley et al 2009 (10). The subpolysomes are comprised of the 40, 60 and 80S ribosomal subunits (marked). RT-PCR was carried out with primers that were specific for either the ‘G’ or ‘A’ allele (as shown in Ai) on the individual gradient fractions.

The data show that under control conditions the majority of ERCC5 transcripts that contain the ‘G’ allele are present in the polysomes, while in contrast a large proportion of the transcripts that contain an ‘A’ allele (uORF1) are associated with 2-3 ribosomes. This is often observed with transcripts that contain functional uORFs since the ribosomes translate the first uORF before dissociation. When treated with Cisplatin there is a relocation of the ‘G’ allele transcripts into more subpolysomal regions while the ‘A’ allele (uORF1) transcripts remain polysomal. * primer dimer band.

**B)** To assess whether there were similar differences occurring in a B-cell line, GM07348 (A/G) was used. These cells were treated with 50 µM Cisplatin and post-nuclear extracts were applied to sucrose density gradients, as described above. As expected the A254 traces
show that there is a large decrease in the amount of polysomally associated material under these conditions (compare the solid line to the dotted line) and this is consistent with the reduction in protein synthesis that occurs when these cells are treated with Cisplatin (Fig. 3Aii). To assess whether the A or G allele transcripts of ERCC5 were differentially associated with ribosomes following Cisplatin treatment, the polysome and subpolysomal regions of the gradients were pooled and RNA isolated. Then RT-PCR was performed for the ‘A’ or ‘G’ allele using the SNP genotyping primers. Raw volume intensity of each sub-polysome and polysome band was determined using ImageJ and was expressed as relative intensity = band intensity/(sub-polysome band + polysome band). The results of three independent experiments are plotted using the mean relative intensity and standard error of the mean. The data show that the ERCC5 transcripts that contain the ‘G’ allele in the 5’ UTR become less associated with the polysomes (actively translating ribosomes) after Cisplatin treatment while in contrast the A allele (uORF1) transcripts remain relatively unaffected. Hatched bars sub-polysomal, solid bars polysomal.

**Supplementary Figure 4.**

To assess whether cisplatin affects ERCC5 half life B cells were pulse labeled with 35S Methionine prior to treatment with control (DMF) or cisplatin (50 µM). ERCC5 was immunoprecipitated at 0, 4 and 8 hours after treatment. (Ai) Representative exposure of ERCC5 immunoprecipitation. (Aii) The half life of ERCC5 was not affected by cisplatin treatment. Graph shows the average and standard error of the mean of the relative turnover of ERCC5 (expressed as a percentage of time 0 (100%)) from four independent experiments.
**Supplementary Figure 5.**

**A)** The pathway downstream of bulky lesion DNA damage was inhibited either by NU7441 (DNA-PKcs inhibitor) or by overexpression of GADD34 (phosphatase of eIF2α).

**B)** To test whether pre-treatment of B cells for 30 minutes using 1 µM Nu7441 was sufficient to inhibit DNA-PKcs activity auto-phosphorylation of S2056 was monitored after 5 Gy of IR. Samples were immunoblotted for DNA-PKcs and DNA-PKcs S2056 phosphorylation antibodies (Abcam) at 1:1000. In all three B-cell lines (of each genotype) Nu7441 treatment was sufficient to inhibit DNA-PKcs S2056 auto-phosphorylation.

**Ci-Ciii)** Quantitation of western blots from B cells treated with 50 µM cisplatin in the absence and presence of 1 µM DNAPK inhibitor NU7441 (Figure 4). Measurements were made from three repeats for ERCC5 and β-tubulin, and two repeats for eIF2α Ser51 phosphorylation and total eIF2α.

**D)** To test whether eIF2-alpha phosphorylation is important for ERCC5 expression HEK293 cells (A/A cell line) were transfected with either active (V25R) or inactive (Kara) GADD34 constructs (Zhou et al. 2011, Brush and Shenolikar 2008). After 36h the cells were treated with indicated cisplatin concentrations for 18h. Samples were immunoblotted for ERCC5, eIF2α and eIF2α S51 phosphorylation antibodies. Shown is one representative repeat.
Supplementary Materials and Methods

**PCR**

rs751402 SNP genotyping PCR was performed with primers A allele (5’-ACCGAGCGGGCCATTTTTCGA-3’) or G allele (5’-ACCGAGCGGGCCATTTTTGG-3’) and ERCC5 reverse (5’-GACTCTGGCTGCAACACGTC-3’). Quantitative PCR (qPCR) was performed with sybr green (Applied Biosystems) with primers for ERCC5 (5’–GAAGCAATGCCAGAGGAG-3’, 5’-CCACTCTCCTTGACTCTACC-3’) and GAPDH (5’GGAAGGTGAAGGTCGGAGTCA-3’, 5’-GTCATTGATGGCAACAATATCCACT-3’).

**Cell survival and proliferation assays**

Measurement of cell proliferation by WST1 assays followed manufacturer’s instructions (Roche). For cell survival, Annexin C-FITC and propidium iodide (PI) staining was used together with flow cytometry.

**Immunoblotting**

Western blots were performed with Invitrogen NuPage gels and BioRad PVDF membrane. eIF2α, eIF2α phospho, β tubulin antibodies (Cell Signaling; 1:1000), β actin antibody (Sigma, 1:10000) and ERCC5 antibody (Novus Biologicals, 1:2000).

**Competitive ELISA**

The detection of platinated DNA by competitive ELISA was described (Tilby et al. 1991). This method was performed with the following modifications. A standard of platinated DNA was made from cisplatin treated B cells. The standard sample was denatured and bound overnight at 37°C in Grenier high-bind 96 well plates. Following blocking with 1% BSA, equal amounts of denatured DNA extracted from DMF or cisplatin treated B cells or neuroblastoma cells was added together with the anti-Cisplatin modified DNA antibody
[CP9/19] (Abcam) and incubated for 1 hr at 37°C. Wells were washed, and developed as described by (Arora et al. 2010). Data was normalized with competition with DMF treated DNA set as 100%.

**Protein synthesis measurement**

To determine protein synthesis rates, cells were pulse labeled with $^{35}$S methionine after treatment and treated as described (Powley et al. 2009). 10-60% sucrose density gradients were performed according to (Powley et al. 2009).

**Clinical samples**

Ependymoma samples and clinical details were obtained from Children’s Cancer Leukaemia Group (CCLG) and the study performed on behalf of CCLG Tumour Bank and Biological Studies committee. Ethical approval MREC 05/MRE04/70. French Ependymoma samples and clinical details were obtained from Société Française d'Oncologie Pédiatrique (SFOP), Paris. DNA from 55 primary intracranial Ependymomas (either snap-frozen or formalin-fixed paraffin embedded) was obtained from these sources for genotyping analysis. All patients had received primary post-operative platinum chemotherapy (Cisplatin/Carboplatin see (Grill et al. 2001; Grundy et al. 2007). Children were treated with Carboplatin as a single dose on day 1 of treatment and then at day 42 of treatment with Cisplatin for 48 hours as a continuous infusion (Grundy et al. 2007). Only 1 case was known to be metastatic at presentation. Fisher’s exact test was used to determine associations between variables in two-way frequency tables. The survival curve (Fig. 4B) was generated by the Kaplan-Meier method with significance values established by the log rank test. Progression-free survival (PFS) was defined from the date of original surgery to the date of disease recurrence, progression or death. Overall survival (OS) was calculated from the date of initial surgery to the date of
death from any cause. Patients remaining alive were censored at the date of last follow-up. Median follow-up times were estimated using the inverse Kaplan-Meier method. To assess the impact of studied factors on survival, hazard ratios were computed using Cox proportional hazard models. Variables with a significance (p) threshold below 0.25 in univariate analysis were introduced into the corresponding multivariate model as described previously. Estimators were given with their 95% confidence interval (95% CI). Survival significance in multivariate analysis was defined by p values below five percent. For discussion of any potential influence of molecular heterogeneity and genetic stability of Ependymoma patients see Supplementary Methods.

**ERCC5 half life determination**

ERCC5 half life, B cells were pulsed with [35S]-Methionine (Hartmann Analytical GmbH) in Methionine-free media for 2 hours, returned to normal media followed by DMF (vehicle control) or 50 µM Cisplatin treatment and harvested after 0, 4 and 8 hours. Samples were immunoprecipitation using the ERCC5 antibody (Novus Biologicals) and visualized by a phosphorimager screen and analyzed using ImageQuant software. Sample intensities were determined relative to time 0 control.

**In vitro translation assays**

These assays were performed with nuclease treated rabbit reticulocyte lysate (Flexi RRL, Promega) containing 60% RRL, with 0.185MBq [35S]-Cysteine (NEG022T, Perkin Elmer), 60mM added KCl, 0.5mM added Mg²⁺Cl and 10% HeLa cell high-salt postribosomal supernatant as defined (Hunt and Jackson 1999). Translation reactions were incubated at 30°C for 90 min, separated by 12.5% SDS-PAGE and visualized by a phosphorimager screen and analysed using ImageQuant software.
PCR and sequencing of cell line-derived ERCC5 cDNA

PCR was performed with primers (5’-ACCGAGCGGGCCCATTTTCTGGGTT-3’ and 5’-TCCACGGCCTTTGGAATGG-3’) from cDNA generated from isolated total RNA by SuperScript III reverse transcriptase (Invitrogen, LifeTech). The PCR products were agarose gel purified using QIAquick Gel Extraction Kit (Qiagen) and sequenced using Sanger DNA sequencing on an Applied Biosystems 3730 Genetic Analyser at the Protein Nucleic Acid Chemistry Laboratory, University of Leicester.

GADD34 Experiments

HEK293 cells were transfected with GADD34 V25R and GADD34 KARA constructs (Zhou et al. 2011, Brush and Shenolikar 2008) using Lipofectamine 2000 (Invitrogen) according to manufacturer’s protocol. After 36h transfection cells were treated with cisplatin at the specified doses for 18h before harvesting for western immunoblotting.

Molecular heterogeneity of Ependymoma

In order to assess the impact of molecular heterogeneity on Ependymoma patient survival we performed IonTorrent Sequencing using the 50 gene Ion AmpliSeq Hotspot Cancer Panel v2 (Life Technologies). Analysis was performed using Torrent Suite 3.6.2 (set to high stringency) and Ingenuity Variant Analysis software. We examined 14 G/G and 10 A/G Ependymoma patient genomes. In summary, using a call quality greater than 20 and filters to remove common variants we identified 9 variants in 8 genes as being potentially deleterious. Using filters to determine potential dominant variants within this group (occurrence in at least two G/G or one A/G samples) refined the list to two of these variants; PIK3S position 178927410 and PDGFRA position 55144534. Neither were found to show any significant statistical association (P = 0.05) with the G/G or A/G genotypes.
This finding fits in with previous research that while Ependymoma can show a degree of molecular heterogeneity, to a large extent this is determined by the age of the patient at diagnosis. It is now clear that the biology of Ependymoma arising in very young children (under 3 to 5 years of age as studied in our paper) is different from older children and markedly different from those arising in adults (Kilday et al. 2009; Dyer et al. 2002; Korshunov et al. 2010; Will et al. 2011; Johnson et al. 2010). This is also reflected at the clinical level with a strong predilection for an origin in the 4th ventricle in young children and the spinal cord in adults (Kilday et al. 2009). Some time ago we identified that Ependymoma could be separated into three groups based on copy number change identified by Comparative Genomic Hybridisation (Dyer et al. 2002). Of particular note Ependymoma arising in very young children showed very few copy number changes and in most cases had a balance genome (Dyer et al. 2002). We also showed that structural changes conferred a poor prognosis (Dyer et al. 2002). This finding was confirmed more recently in a larger cohort of patients, however, the cohort included both adult and childhood cases which has perhaps spuriously given rise to the notion that Ependymoma is genetically heterogenous (Korshunov et al. 2010). In summary, there is relatively little genetic heterogeneity in Ependymoma arising in children under 5 years of age.

Given the balanced genomic profile of Ependymoma in young children we have further investigated these cases undertaking an exome sequencing analysis with Professor Andy Futreal whilst he was at the Sangar centre. Many of the samples that were analysed as part of this paper were included in this exome sequencing analysis. In essence we have found no recurrent driver mutations in Ependymoma in very young children. A finding echoed by our colleagues at the German Cancer Foundation in Heidelberg (H Witt personal communication) and supported by our ion torrent data.
While copy number loss of chromosome 13q has been reported in CGH studies of paediatric Ependymoma (Kilday et al. 2009), this is not a common finding. Moreover, specific genetic events incorporating the ERCC5 5’UTR region have not been reported in intracranial Ependymomas from children or adults. Indeed, the most comprehensive genomic screening of 204 Ependymomas undertaken to date by ourselves in collaboration with Professor Gilbertson at St. Judes Children’s Research Hospital (Johnson et al. 2010), published in Nature, identified 175 focal amplifications and 157 focal deletions across the Ependymoma genome and included known regions of CNV, yet none incorporated the ERCC5 gene region (13q33.1:Ensembl co-ordinates 103,459,705 – 103,528,345).
Supplemental References

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Ci)

Luciferase

uORF1

uORF2

uAUG1

uAUG2

Luciferase

Luc

UTR-A

rs751402 (A)

UTR-A in frame

rs751402 (A)

UTR-G

rs751402 (G)

UTR-G in frame

rs751402 (G)

Cii)

Somers_suppl-Fig1
A) Neuroblastoma cell lines

B) B-cell lines

C) DLBCL-cell lines

D) Fibroblasts

E) Fibroblasts

[(Graphs and images of cell survival and annexin V and PI staining for various cell lines and treatments)]
Aii) Absorbance at 254 nm showing polysomes (40S, 60S, 80S).

A allele control
A allele cisplatin
G allele control
G allele cisplatin

Bii) Absorbance at 254 nm showing polysomes and sub-polysomes.

Control and Cisplatin (50 µM) relative intensity (%)

Sub-polysome Polysome Sub-polysome Polysome Sub-polysome Polysome

A allele G allele A allele G allele
Ai)  

Time (hr)  
0  4  4  8  8
-  +  -  +  50 μM Cisplatin

ERCC5

Aii)  

Relative turnover of ERCC5 (%)
0  20  40  60  80  100
0  4  8

Time (hrs)

-cisp  + cisp
A) UV → DNA-PKcs → GCN2 → elf2α → Translation inhibited

B) B cell lines

| G/G (GM07019) | A/A (GM07029) | A/G (GM07348) |
|---------------|---------------|---------------|
| - + - +       | - + - +       | - + - +       |
| - - + +       | - - + +       | - - + +       |

1 μM DNA-PKcs inhibitor (Nu7441) + 5 Gy IR

DNA-PK (P-S2056)

DNA-PK (total)

Ci) G/G (GM07019)

Cii) A/A (GM07029)

Ciii) A/G (GM07348)

D) Gadd34 V25R

Gadd34 KARA

μM cisplatin

ERCC5

β-tubulin

elf2α phos

elf2α total

Somers_suppl-Fig5
| Sample | Age (yrs) | Sex | Location | Grade | Surgery | Status | PFS (yrs) | OS (yrs) | Genotype |
|--------|-----------|-----|----------|-------|---------|--------|----------|----------|----------|
| 1P     | 0.1       | F   | ST       | III   | IR      | ADP    | 0.4      | 1.5      | G/G      |
| 2P     | 0.1       | F   | ST       | III   | CR      | ADP    | 0.4      | 0.5      | A/G      |
| 3P     | 0.6       | F   | ST       | III   | IR      | DOD    | 0.3      | 0.6      | A/G      |
| 4P     | 0.7       | M   | PF       | III   | CR      | DOD    | 0.7      | 1.2      | G/G      |
| 5P     | 0.9       | F   | PF       | II    | IR      | DOD    | 1.4      | 1.4      | A/A      |
| 6P     | 0.9       | M   | PF       | III   | CR      | DOD    | 0.8      | 4.8      | G/G      |
| 7P     | 0.9       | M   | PF       | Unknown | CR | ADF    | 0.8      | 0.8      | A/G      |
| 8P     | 1.0       | M   | PF       | III   | CR      | DOD    | 1.5      | 7.0      | G/G      |
| 9P     | 1.0       | M   | PF       | II    | CR      | DOD    | 1.4      | 2.1      | G/G      |
| 10P    | 1.1       | M   | PF       | III   | CR      | ADP    | 1.3      | 5.0      | A/G      |
| 11P    | 1.2       | M   | PF       | III   | CR      | ADP    | 1.1      | 2.7      | G/G      |
| 12P    | 1.2       | M   | PF       | III   | IR      | DOD    | 0.3      | 0.3      | G/G      |
| 13P    | 1.3       | F   | PF       | III   | IR      | DOD    | 1.5      | 1.6      | A/G      |
| 14P    | 1.3       | F   | PF       | II    | CR      | DOD    | 1.4      | 3.5      | A/G      |
| 15P    | 1.3       | M   | PF       | II    | CR      | ADF    | 3.1      | 3.1      | G/G      |
| 16P    | 1.3       | F   | ST       | III   | IR      | DOD    | 1.8      | 1.8      | G/G      |
| 17P    | 1.4       | M   | PF       | III   | CR      | DOD    | 0.4      | 0.6      | A/G      |
| 18P    | 1.4       | F   | PF       | III   | CR      | ADP    | 0.9      | 3.3      | G/G      |
| 19P    | 1.4       | F   | PF       | III   | CR      | ADF    | 0.3      | 0.9      | A/A      |
| 20P    | 1.5       | M   | ST       | III   | CR      | ADF    | 6.8      | 6.8      | G/G      |
| 21P    | 1.5       | M   | PF       | III   | CR      | DOD    | 1.0      | 2.8      | G/G      |
| 22P    | 1.6       | M   | ST       | III   | CR      | ADF    | 5.3      | 5.2      | G/G      |
| 23P    | 1.6       | F   | PF       | III   | CR      | ADF    | 0.7      | 6.1      | A/G      |
| 24P    | 1.6       | M   | PF       | III   | IR      | ADF    | 5.1      | 5.1      | G/G      |
| 25P    | 1.7       | F   | PF       | II    | IR      | ADF    | 1.2      | 13.1     | A/G      |
| 26P    | 1.8       | F   | PF       | II    | CR      | ADF    | 8.1      | 8.1      | A/G      |
| 27P    | 1.9       | M   | PF       | III   | IR      | ADF    | 7.9      | 7.9      | G/G      |
| 28P    | 1.9       | M   | PF       | III   | CR      | ADP    | 2.5      | 6.3      | G/G      |
| 29P    | 2.0       | F   | PF       | III   | CR      | DOD    | 2.3      | 6.0      | G/G      |
| 30P    | 2.0       | F   | PF       | III   | CR      | ADF    | 15.2     | 15.2     | G/G      |
| 31P    | 2.0       | M   | ST       | III   | CR      | ADP    | 1.0      | 3.8      | G/G      |
| 32P    | 2.1       | M   | PF       | II    | CR      | ADF    | 9.9      | 9.9      | G/G      |
| 33P    | 2.3       | M   | PF       | III   | IR      | ADP    | 1.3      | 6.9      | G/G      |
| 34P    | 2.3       | M   | PF       | II    | CR      | DOD    | 5.6      | 6.4      | G/G      |
| 35P    | 2.3       | M   | ST       | III   | IR      | DOD    | 0.8      | 3.6      | G/G      |
| 36P    | 2.3       | M   | ST       | III   | CR      | ADF    | 1.3      | 1.3      | G/G      |
| 37P    | 2.4       | M   | PF       | III   | IR      | ADF    | 9.5      | 9.5      | G/G      |
| 38P    | 2.4       | F   | ST       | III   | CR      | ADP    | 0.8      | 3.2      | A/G      |
| 39P    | 2.5       | M   | PF       | III   | IR      | DOD    | 2.2      | 6.9      | G/G      |
| 40P    | 2.5       | M   | PF       | III   | CR      | ADP    | 3.5      | 14.1     | G/G      |
| 41P    | 2.6       | M   | PF       | II    | IR      | DOD    | 1.8      | 1.9      | G/G      |
| 42P    | 2.6       | M   | PF       | III   | IR      | ADF    | 2.8      | 2.8      | G/G      |
| 43P    | 2.6       | M   | PF       | II    | CR      | ADF    | 6.3      | 6.3      | G/G      |
| 44P    | 2.7       | M   | PF       | II    | IR      | DOD    | 1.6      | 4.0      | G/G      |
| 45P    | 2.7       | M   | PF       | III   | CR      | DOD    | 0.4      | 2.4      | G/G      |
| 46P    | 2.8       | M   | PF       | III   | CR      | ADP    | 2.3      | 2.9      | G/G      |
| 47P    | 2.8       | M   | PF       | II    | CR      | ADF    | 1.4      | 1.4      | G/G      |
| 48P    | 3.1       | M   | PF       | III   | CR      | AFG    | 2.3      | 2.3      | G/G      |
| 49P    | 3.4       | M   | PF       | III   | CR      | DOD    | 0.4      | 4.3      | A/G      |
| 50P    | 3.5       | F   | PF       | II    | CR      | ADF    | 1.7      | 1.7      | G/G      |
| 51P    | 3.7       | M   | PF       | III   | CR      | ADF    | 0.7      | 6.8      | G/G      |
| 52P    | 4.0       | F   | PF       | II    | CR      | ADF    | 2.3      | 2.3      | A/G      |
| 53P    | 4.1       | M   | PF       | III   | CR      | DOD    | 1.4      | 10.1     | G/G      |
| 54P    | 4.1       | F   | PF       | III   | CR      | DOD    | 0.5      | 1.9      | G/G      |
| 55P    | 4.2       | M   | ST       | II    | IR      | DOD    | 2.8      | 3.5      | G/G      |

M = male, Female = female, PF = posterior fossa/infratentorial, ST = supratentorial, IR = incomplete resection, CR = complete resection, ADF = alive with no disease progression, ADP = alive with disease progression, DOD = dead of disease, PFS = progression-free survival, OS = overall survival.
### Supplemental Table 2: Comparison of reported pathological variables between genotype groups

| Variable                        | A/A or A/G genotype group (n = 15) | G/G genotype group (n = 40) | P value |
|---------------------------------|------------------------------------|----------------------------|---------|
|                                 | Number | Percentage | Number | Percentage |         |
| **Age**                         |        |            |        |            |         |
| Under three yrs                 | 14     | 93         | 37     | 93         | 1.00    |
| Over three yrs                  | 1      | 7          | 3      | 7          |         |
| **Location**                    |        |            |        |            |         |
| Supratentorial                  | 3      | 20         | 8      | 20         | 1.00    |
| Infratentorial                  | 12     | 80         | 32     | 80         |         |
| **WHO grade (1 MD)**            |        |            |        |            |         |
| II                              | 5      | 36         | 10     | 25         | 0.50    |
| III                             | 9      | 64         | 30     | 75         |         |
| **Surgical resection**          |        |            |        |            |         |
| Complete                        | 11     | 73         | 27     | 68         | 0.75    |
| Incomplete                      | 4      | 27         | 13     | 32         |         |

Probability (P) values obtained by two-tailed Fisher’s exact testing. MD = missing data