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Biallelic *FANCD1/BRCA2* mutations predisposing to glioblastoma multiforme with multiple oncogenic amplifications

Andrew J. Dodgshun [a,c,*], Alexandra Sexton-Oates [b,c], Richard Saffery [b,c], Michael J. Sullivan [a,b]

[a] *Children's Cancer Centre, Royal Children's Hospital, Melbourne, Victoria 3052, Australia;* [b] *Murdoch Children's Research Institute, Melbourne, Victoria 3052, Australia;* [c] *Department of Paediatrics, University of Melbourne, Melbourne, Victoria 3052, Australia*

Fanconi anaemia (FA) caused by biallelic mutation in *FANCD1/BRCA2* is rare but carries a high risk of early onset cancer. Medulloblastoma is well described in this cohort but reports of other brain tumours are uncommon. The molecular profile of tumours from FA patients is not well reported. A glioblastoma multiforme (GBM) from a 3-year-old patient with FA and confirmed biallelic *BRCA2* mutations was submitted for methylation analysis. This revealed strong clustering with the K27 mutation subgroup and copy number analysis showed gains of chromosomes 1q, 4q, part of 7q, part of 8q and 17q with resultant amplifications of *MDM4*, *CDK6*, *MET*, *MYC* and *PPM1D* (*WIP1*). We also describe for the first time the germline mutation in *BRCA2* c.8057T > C resulting in p.Leu2686Pro in our patient with confirmed FA. Biallelic *BRCA2* mutations have predisposed to an aggressive and universally fatal subtype of childhood GBM in our patient. Copy number alterations and multiple oncogenic amplifications may be secondary to inherent chromosomal instability and this raises the question of what role *BRCA2* may play in the development of GBM in children without FA.

Keywords Fanconi anaemia, BRCA2, Glioblastoma multiforme

Fanconi anaemia (FA) caused by biallelic *FANCD1/BRCA2* mutations has a phenotype which is distinct from other causes of FA and is characterised by early-onset leukaemia or solid tumours (1). CNS tumours are reported in this cohort, most often medulloblastoma (2,3). In one case report a medulloblastoma in a patient with FA caused by biallelic *BRCA2* mutations was found to be of the Sonic Hedgehog (SHH) subgroup (4). This is consistent with preclinical research indicating that cerebellar granule neuron precursor cells (the cell of origin for SHH medulloblastoma) display genomic instability and generate medulloblastoma tumours in *p53* and *BRCA2* deficient mice (5). Other brain tumours have been described in children with biallelic *BRCA2* mutations but they are mostly unbiopsied or histological confirmation cannot be obtained (6,7). There is only one previous report of a glioblastoma (GBM) in a child with biallelic *BRCA2* mutations (8).

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* Corresponding author.
E-mail address: ajdodgshun@gmail.com

Recent research has highlighted the existence of distinct biological subgroups of GBM in children and adults (9,10). Mutations in genes encoding for histone 3.1 or 3.3 at residue 27 characterise a subgroup of GBM which almost always occur in the midline and portend a rapid progression with universally fatal outcome (10). This subgroup is found exclusively in children and young adults (9,10).

Case report

Patient N was conceived naturally by non-consanguineous Western European parents in good health. There was no Ashkenazi Jewish ancestry. Antenatal ultrasound showed high likelihood of upper gastrointestinal anomaly, most likely duodenal atresia. Serial scans showed intra-uterine growth retardation and labour was induced at 36 weeks for this reason. Birth weight was 2.06 kg (4 lb, 9 oz) and side-to-side duodenostomy was performed on day 1 of life for confirmed duodenal atresia. He had neonatal jaundice and hypothermia and was treated for presumed sepsis. Further examination

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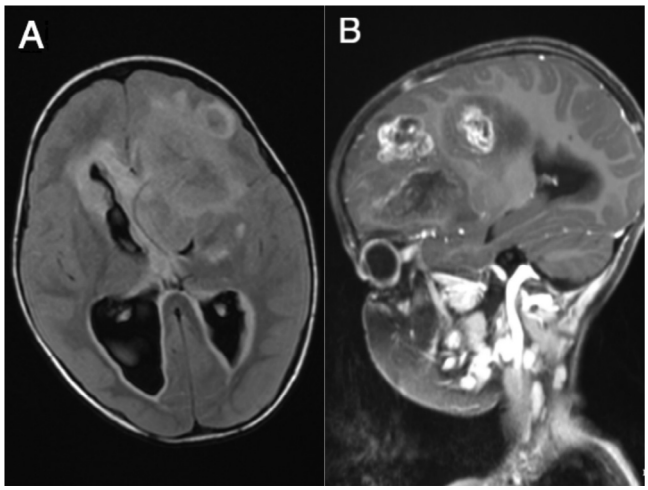

Figure 1 Axial FLAIR sequence without contrast (A) and sagittal contrast-enhanced T1 sequence (B) demonstrating the large infiltrative lesion.

revealed a patent ductus-arteriosis, developmental dysplasia of the left hip, bilateral microphthalmia, unusual facies and a mild bilateral sensorineural hearing loss. No radial ray abnormalities were present.

On the basis of this constellation of clinical features and two Mitomycin C stress tests indicating chromosomal fragility (see below) the diagnosis of FA was made at 2 months of age. There was no family history of FA, but there was a family history of cancer. A maternal great-grandmother had breast cancer diagnosed at age 60 and a uterine cancer at age 66 and a maternal great-aunt had breast cancer at age 51. A paternal great aunt had breast cancer at age 50. The patient's parents and older sibling were phenotypically normal and well.

Growth remained mildly restricted but development was within normal limits. Multiple café au lait macules became evident over the first 3 years of life. Shortly before his 4th birthday the patient began vomiting and became lethargic over a period of several weeks. When initial investigation excluded a gastrointestinal origin for symptoms, a CT scan of his brain was performed which showed a large, heterogeneously enhancing mass extensively involving both frontal lobes. MRI confirmed these findings (Figure 1) and showed evidence of metastases in the lateral ventricles, pons, medulla and down the spinal cord. The patient rapidly deteriorated and died a short time later.

A limited post-mortem examination was performed with parental consent. A large tumour measuring $90 \times 50 \times 40$ mm was seen occupying mainly the left frontal lobe with ventricular extension as well as extension into the right frontal lobe. No other areas of tumour were evident macroscopically. Histologically the lesion was highly cellular and most tumour cells showed small hyperchromatic round to oval nuclei although some larger astrocytic tumour cells were seen. Numerous mitoses were seen (up to 5/10 high powered fields). Areas of necrosis and vascular endothelial hyperplasia were noted. Morphology was consistent among samples taken from 8 different locations in the tumour. Tumour cells showed variable expression of GFAP with the smaller cells weakly expressing and the larger cells strongly expressing this marker. Ki-67 proliferation index was 60%. 15% of nuclei expressed P53 and INI-1 expression was normal. Synaptophysin expression was negative within the tumour cells.

Results

No haematological abnormalities were present on blood count and film on multiple occasions. Mitomycin C stress test was performed on two occasions in the neonatal period and showed an elevated rate of chromatid breakage compared with negative control. The number of aberrations per cell or percentage of cells with aberrations was not reported. Bone marrow morphology at 17 months of age showed mild hypocellularity but no dysplasia was present and karyotype was normal. No spontaneous chromosomal breakage or rearrangements were observed in this specimen.

Germline testing was performed. The *FANCA* gene was tested for deletions using multiplex ligation-dependent probe amplification (MLPA). The entire coding sequences of the *FANCA*, *FANCC*, *FANCE*, *FANCF*, *FANCG* and *FANCJ* genes were analysed by amplification of all exons, including splice donor and acceptor sites, by PCR followed by detection of unknown mutations by denaturing high performance liquid chromatography (dHLPC) and/or direct sequencing. No pathogenic mutations were detected. Testing by Western Blot excluded *FANCL*, *FANCM* and *FANCI* as candidates.

After the patient's death, *FANCD1/BRCA2* was then analysed by amplification of all exons (including intron/exon boundaries) using PCR followed by direct DNA sequencing. Biallelic mutations in BRCA2 were detected in the patient: c.9672dupA resulting in p.Tyr3225fs and c.8057T > C resulting in p.Leu2686Pro. Mutation c.9672dupA was detected in the patient's father and mutation c.8057T > C was detected in the patient's mother.

To characterise the tumour further, genome-wide methylation analysis was performed using the Illumina Infinium HumanMethylation450 BeadChip array according to the manufacturer's instructions. Cluster analysis against the established databank of gliomas at Deutsches Krebsforschungszentrum (DKFZ) showed the tumour clustered strongly with the K27 mutation subgroup of GBM (10). Copy number alterations were detected from the methylation data using a custom approach described previously using the sum of methylated and unmethylated signal intensity (9). Due to the high concordance of results using this methodology with more direct measurements of copy number, SNP profiling and FISH analysis were not performed. Refer to Figure S1D from Sturm et al. for further information (9). Copy number analysis showed gains of chromosomes 1q, 4q, part of 7q, part of 8q and 17q with resultant amplifications of *MDM4*, *CDK6*, *MET*, *MYC* and *PPM1D* (*WIP1*). There were losses at 1p, 3p and 10q (Figure 2). Amplifications were generally observed in the setting of larger segments of chromosomal gain although *MET* and *MYC* had signal intensities well exceeding the surrounding segments indicating a more focal amplification. (Figure 2)

Discussion

Here we report the second confirmed case of GBM in a child with FA caused by biallelic *BRCA2* mutation and the first molecular characterisation of such a tumour. We also report the first case of FA where the c.8057T > C mutation is present in the *BRCA2* gene, providing further evidence for its significance on protein function.