J Clin Immunol
DOI 10.1007/s10875-016-0317-y

ORIGINAL ARTICLE

Fatal Lymphoproliferative Disease in Two Siblings Lacking Functional FAAP24

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Received: 5 April 2016 / Accepted: 13 July 2016

Abstract Hereditary defects in several genes have been shown to disturb the normal immune response to EBV and to give rise to severe EBV-induced lymphoproliferation in the recent years. Nevertheless, in many patients, the molecular basis of fatal EBV infection still remains unclear. The Fanconi anemia-associated protein 24 (FAAP24) plays a dual role in DNA repair. By association with FANCM as component of the FA core complex, it recruits the FA core complex to damaged DNA. Additionally, FAAP24 has been shown to evoke ATR-mediated checkpoint responses independently of the FA core complex. By whole exome sequencing, we identified a homozygous missense mutation in the *FAAP24* gene (cC635T, pT212M) in two siblings of a consanguineous Turkish family who died from an EBV-associated lymphoproliferative disease after infection with a variant EBV strain, expressing a previously unknown EBNA2 allele.

In order to analyze the functionality of the variant FAAP24 allele, we used herpes virus saimiri-transformed patient T cells to test endogenous cellular FAAP24 functions that are known to be important in DNA damage control. We saw an impaired FANCD2 monoubiquitination as well as delayed checkpoint responses, especially affecting CHK1 phosphorylation in patient samples in comparison to healthy controls. The phenotype of this FAAP24 mutation might have been further accelerated by an EBV strain that harbors an EBNA2 allele with enhanced activities compared to the prototype laboratory strain B95.8. This is the first report of an FAAP24 loss of function mutation found in human patients with EBV-associated lymphoproliferation.

Keywords EBV-associated lymphoproliferation · FAAP24 deficiency · EBV · immunodeficiency · DNA repair defects

Introduction

Epstein Barr virus (EBV) is a latent γ-herpesvirus that infects more than 95 % of the adult population worldwide. Primary

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Electronic supplementary material The online version of this article (doi:10.1007/s10875-016-0317-y) contains supplementary material, which is available to authorized users.

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Published online: 29 July 2016

EBV infection in young children is often asymptomatic and infectious mononucleosis (IM) usually affects those who have primary EBV infection during or after the second decade of life. During primary infection, immunocompetent people mount a vigorous immune response consisting of natural killer (NK) cells and EBV-specific cytotoxic $CD8^{+}$T (CTL), which control both primary infection and the periodic reactivations that occur in all EBV-seropositive persons. After clearance of primary infection, EBV persists in infected memory B cells, establishing latent infection characterized by the expression of only a limited set of EBV antigens. There are, however, several primary immunodeficiencies in which the delicate, tightly controlled anti-EBV immune response is severely disturbed. Examples include diseases like XLP 1 and 2, ITK-, CD27-, LCK-, coronin1A-, MAGT1-, and CTPS1 deficiency [1–10].

We analyzed a consanguineous Turkish family in which two siblings (boy and girl) died from a similar progressive EBV-associated lymphoproliferative disease induced by an EBNA2 mutant EBV strain. After immunological and genetic exclusion of known primary immunodeficiencies that may be present with EBV-driven lymphoproliferation, we performed whole exome sequencing and identified a homozygous missense mutation in the *Fanconi anemia-associated protein 24* (*FAAP24*) gene (cC635T, pT212M, rs148106526) in both siblings as the only possible disease-causing homozygous variant that perfectly segregated with the disease in this family. A human disease with loss-of-function mutations within FAAP24 has not been reported yet.

FAAP24 plays a crucial role in two interacting DNA repair systems, the Fanconi-anemia (FA) signaling pathway activated by interstrand cross-links and the ATR/Chk1 signaling pathway coordinating the cellular response to different kinds of replication stress. FAAP24 is part of the FA core complex [11]. It has been shown to interact specifically with the C-terminal part of FANCM and is thought to promote the targeting of FANCM/FAAP24 dimers and other components of the FA core complex to damaged DNA. Once recruited, the FA core complex catalyzes the monoubiquitination of FANCD2, which is essential for lesion repair [12]. A defect in FANCD2 monoubiquitination is a common feature of cells from individuals with FA due to a mutant core complex component. Ciccia et al. showed that downregulation of FAAP24 by small interfering RNA also resulted in reduced levels of monoubiquitinated FANCD2 after exposure to DNA cross-linking agents [11]. Moreover, it has been shown that FAAP24 and FANCM are able to activate the ATR signaling pathway under replication stress independently from the FA core complex [11–13]. Knockdown of either FANCM or FAAP24 compromised ATR/CHK1-mediated checkpoint signaling, leading to increased endogenous DNA damage and failure to efficiently invoke cell cycle checkpoint responses [12].

We analyzed the functionality of the mutant FAAP24 protein in the male patient's T cells and discuss the possible association of the loss of function of FAAP24 with the patients' clinical phenotype, also with regard to the unusual EBV variant harbored by both siblings.

Methods

Patients

The material from patients and healthy donors was obtained upon informed consent in accordance with the Declaration of Helsinki. The family was analyzed in Düsseldorf, Germany. The study was approved by the respective institutional review boards.

The siblings were treated at the University Children's Hospital, Würzburg, Germany, in the 1980s. A detailed summary of their clinical phenotype as well as the results of an extensive immunological and virological workup was published by Schuster et al. [14, 15]. The children were born to healthy consanguineous Turkish parents. Four older siblings were healthy, whereas four other siblings had died of unknown cause in Turkey. At the age of 15 (patient 1, girl) and 30 months (patient 2, boy), the children became symptomatic with fever, generalized lymphadenopathy, hepatosple nomegaly, and encephalopathy (patient 1). EBV DNA was detected in the bone marrow and lymph node biopsies. Despite treatment with acyclovir, corticosteroids, and cyclophosphamide, the children died from progressive lymphoproliferative disease 25 and 16 weeks after admission. Both patients did not have a syndromal appearance and did not show any characteristic phenotypic features of known DNA repair defects nor hematologic abnormalities as found, e.g., in Fanconi anemia.

Immunological studies are available from patient 2. He was admitted for recurrent abscesses of his left thigh at the age of 15 months. At that time, immunoglobulin levels (IgG, IgA, and IgM), the response to polio vaccine, B- and T cells counts, T cell subsets (CD4+, CD8+), and the capacity of lymphocytes to respond to unspecific mitogens (PAH and PWM) measured by 3H thymidine incorporation were normal, and there was no evidence of a phagocytic disorder. EBV early antibodies were positive at that time without clinical evidence of an active EBV infection. When admitted at the age of 30 months with EBV-induced lymphoproliferation, he had secondary hypogammaglobulinemia and a moderately low CD4+ T cell count, elevated CD8+ T cells, and a high expression of HLA DR on T cells.

T cell lines derived from a fresh lymph node and freshly isolated PBMCs were tested by a 51Cr release assay against autologous EBV-transformed lymphoblastoid B cell lines and natural killer-sensitive K562 cells. PBMCs as well as lymph

node-derived T cells failed to kill autologous EBV-transformed B cells. PBMCs showed normal natural killer cell activity.

EBV-specific memory T cell activity was measured by regression tests using PBMCs from patient 2 as well as from EBV-seronegative and EBV-seropositive healthy donors. PBMCs from the patient and from negative donors failed to limit outgrowth of EBV-transformed B cells, whereas PBMCs of EBV-positive donors achieved a 50 % incidence of regression at a low cell number. It may be concluded that the patient was unable to generate or maintain an EBV-directed effector and memory CTL response in vivo. As the cells were obtained at the time of a highly replicative EBV infection that was ongoing for some time, the results, however, may have been influenced by T cell exhaustion.

Several lymph node biopsies in both patients were interpreted as atypical EBV-induced B cell proliferation, some with clonal rearrangements of Ig heavy chain. A transition to a malignant lymphoblastic lymphoma or Burkitt's lymphoma was discussed but could not clearly be demonstrated nor ruled out in the different specimen obtained and analyzed 30 years ago. Molecular characterization of EBV isolates from both patients revealed an identical variant with a 51-bp deletion and six nucleotide changes within EBNA2 [15].

After excluding mutations in ITK, which predispose for EBV-associated lymphoproliferative diseases [3], by capillary sequencing, we analyzed the DNA samples of both children and the two healthy siblings (brother and sister) by whole exome sequencing (WES).

Exome Sequencing and Data Analysis

Whole exome sequencing was performed by a 100-bp single-read sequencing strategy on an Illumina HiSeq2000 Sequencer running on HiSeq Control Software (HCS) 1.4.8. For WES, DNA was diluted in 4 μg in 130 μl EB buffer and the sample preparation was carried out using the SPRIworks Fragment Library System I (Beckman Coulter) for library preparation and NimbleGen SeqCap EZ Exome Library SR (Roche) for exome enrichment. Raw image data conversion was performed using Consensus Assessment of Sequence and Variation version 1.8 (CASAVA). FASTQ files were generated by using BcltoFastq 1.8.4 (Illumina). BWA version 0.7.4 [16] was used to align sequence data to the human reference genome (GRCh37). Conversion steps were carried out using Samtools [17] followed by removal of duplicate reads [18]. Local realignment around indels, single nucleotide polymorphism (SNP) calling, annotation, and recalibration was facilitated by GATK 2.4.9 [19]. Resulting variation calls were annotated by Variant Effect Predictor [20] using the Ensembl database (v70) and imported into an in-house MySQL database to facilitate automatic and manual annotations, reconciliation, and data analysis by complex database queries. Loss of function prediction scores for PolyPhen2 [21] and SIFT [22] were extracted from this Ensembl release.

Induction of FAAP24 After TCR Stimulation

In order to analyze a possible increase of FAAP24 expression after TCR stimulation, we treated primary T cells with 10 μg/ml phytohemagglutinin (PHA) or with 0.1 μg/ml anti-CD3 and 0.5 μg/ml anti-CD28 for 0, 8, 24, 48, and 72 h. Subsequently, the expression level was measured using qRT-PCR.

Immortalization and Cultivation of Lymph Node Cells and PBMCs of the Male Patient

Cryoconserved lymph node cells and PBMCs from the male patient were used for the generation of HVS transformed, immortalized T cells (HVS T cells) as described elsewhere [23, 24]. These T cell lines carry the original FAAP24 point mutation and could infinitely be expanded to perform in-depth experiments (Supplementary Figure S1). Immortalized T cells were cultivated with RPMI 1640 (Life Technologies) and Panserin 401 (PAN Biotech) in a ratio 1:1, supplemented with 15 % FCS (PAN Biotech), 100 μg/ml gentamicin, and 10 U/ml IL-2 (Roche).

Detection of Monoubiquitinated FANCD2

HVS cells either harboring the homozygous FAAP24 T212M mutation or HVS cells from a healthy control were treated with mitomycin C (MMC; Sigma-Aldrich) to elucidate the capability of FANCD2 monoubiquitination. Cells (3×10^6) were cultured with medium containing 1.5 μM MMC for 2 or 4 h. Cells were lysed in 50 mM Tris-Cl pH 8.0, 150 mM NaCl, 1 % Triton, 0.75 % deoxycholate, 0.1 % SDS, and ×1 protease inhibitor cocktail (Roche Complete) for 15 min on ice, briefly sonicated, followed again by 15 min on ice. Lysates were cleared at 10,000*g* for 10 min at 2 °C. Lysates from 1×10^6 cells were supplemented with 0.5 M urea and 1 % SDS in addition to standard Laemmli sample buffer before subjecting to SDS PAGE on 7.5 % gels. For immunoblot analyses, the proteins were transferred to PVDF membranes after separation and stained with a monoclonal antibody against FANCD2 (FI17, sc-20022, Santa Cruz Biotechnology).

Cell Cycle Control

Two million HVS T cells of the patient and of the two healthy control cell lines were incubated with 5 mM hydroxyurea, 10 mM doxorubicin, and 10 μM etoposide, respectively, and harvested after 2 and 18 h. For Western blot analysis, T cell

pellets were resuspended in 150 μl lysis buffer (20 mM Tris-HCl (pH 7.5), 150 mM KCl, 2 mM EDTA, 1 mM NaF, 0.5 % NP40, 0.5 mM EDTA-free protease inhibitor cocktail (Roche Diagnostics)), and 25 μg protein of patient and healthy control T cell lysates was loaded on a 10 % SDS-PAGE. Proteins were transferred to PVDF membranes by semidry blotting for 2 h at 80 mA. Membranes were blocked with 5 % milk powder (Bio-Rad) and incubated with the primary phosphorylation-specific antibodies, pp53 (Ser15; product # 9284), pChk1 (Ser345; 133D3; rabbit mAb; product # 2348), and pChk2 (Thr68; product # 2661) (Cell Signaling Technologies) in a ratio 1:1000 in 5 % BSA at 4 °C overnight. The membranes were incubated with the secondary HRP-linked antibody (Cell Signaling Technologies) in a ratio 1:2000 in 5 % milk powder for 1 h at room temperature. Antibodies against β-actin (D6A8; rabbit mAb; product # 8457) served as protein loading controls.

Sister Chromatid Exchange Rate

Fifty-micromolar BrdU substituting for thymidine was incorporated into cellular DNA during two replication rounds resulting in differentially labelled sister chromatids. Colcemid-arrested metaphases were fixed, dropped onto glass microscopic slides, and air dried. Sister chromatid differentiation staining was performed according to standard protocols. The mean number of sister chromatid exchanges (SCEs) was determined from 25 cells.

Plasmids

The DNA fragments encoding the open reading frames of EBNA2 of EBV strain B95.8 or the strain variant (designated EBNA2 FLD for fatal lymphoproliferative disease) as described in [15] were produced by gene synthesis and subcloned into the expression vector pSG5. All constructs were confirmed by sequencing.

Luciferase Assays

DG75 [25] cells (5×10^6) were transfected by electroporation with a mixture of firefly luciferase reporter plasmid pGa981-6 [26], 0.5 μg Renilla luciferase reporter plasmid (Promega), and either EBNA2 expression plasmids of strain B95.8 or of the EBNA2 variant described in [15] or empty vector controls (pSG5). The Renilla and firefly luciferase activity was determined using the dual luciferase assay kit (Promega) following the manufacturer's instructions. Data were presented as the mean ratio of the firefly luciferase activity normalized to the Renilla luciferase activity.

Results

Whole Exome Sequencing Identifies a Homozygous Mutation in *FAAP24*

We analyzed the DNA samples of both children with EBV-LPD and the two healthy siblings (brother and sister) by WES. As both parents were healthy, an autosomal recessive inheritance seemed likely. Given the consanguinity of the parents, we assumed a homozygous mutation. We therefore filtered for non-synonymous SNPs and insertion/deletion variants (indels) located in the coding regions with a minor allele frequency (MAF) of <0.01, which were homozygous in both patients but not in the healthy siblings.

By applying these criteria, only one variant (rs148106526) was identified, namely the missense variant (cC635T) in exon 5 of the *Fanconi anemia-associated protein*, *24 kDa* gene (*FAAP24*, alias *C19orf40*), leading to the amino acid substitution T212M (Supplementary Figure S2). The homozygous mutation was validated by capillary sequencing in both patients. The healthy parents and all four healthy siblings were found to be heterozygous (Fig. 1a).

According to the *1000 Genomes Project*, a T at this position occurs with an allele frequency of 0.0014. The T-allele is reported only as part of a heterozygous genotype C/T, which was observed in 0.3 % of 2504 genotypes (genotype frequency 0.003). The homozygous genotype T/T identified in our patients has not been reported so far (www.1000genomes.org).

The mutation is located in the second helix-hairpin-helix (HhH) domain in the C-terminal part of FAAP24 (Supplementary Figure S2). This domain has been shown to be indispensable for the binding of FAAP24 to damaged DNA as well as for the interaction with the Fanconi anemia core complex protein FANCM [27]. The affected amino acid residue is not highly conserved between different species; however, the mutation is predicted to be "possibly damaging" and "deleterious" by the prediction tools Polyphen2 and SIFT strongly indicating a negative effect on protein structure or function.

FAAP24 Seems Not to be Involved in T Cell Receptor Signaling

The inherited predisposition to develop EBV-associated lymphoproliferative disorders is a clinically and genetically heterogeneous group of primary immunodeficiencies. In recent years, several defects affecting T cell signaling have been identified, including mutations in ITK, CD27, MAGT1, coronin1A, or CPTS1 [3, 4, 7–10]. A role for FAAP24 in T cell signaling pathways has not been described in the literature. To analyze if FAAP24 expression

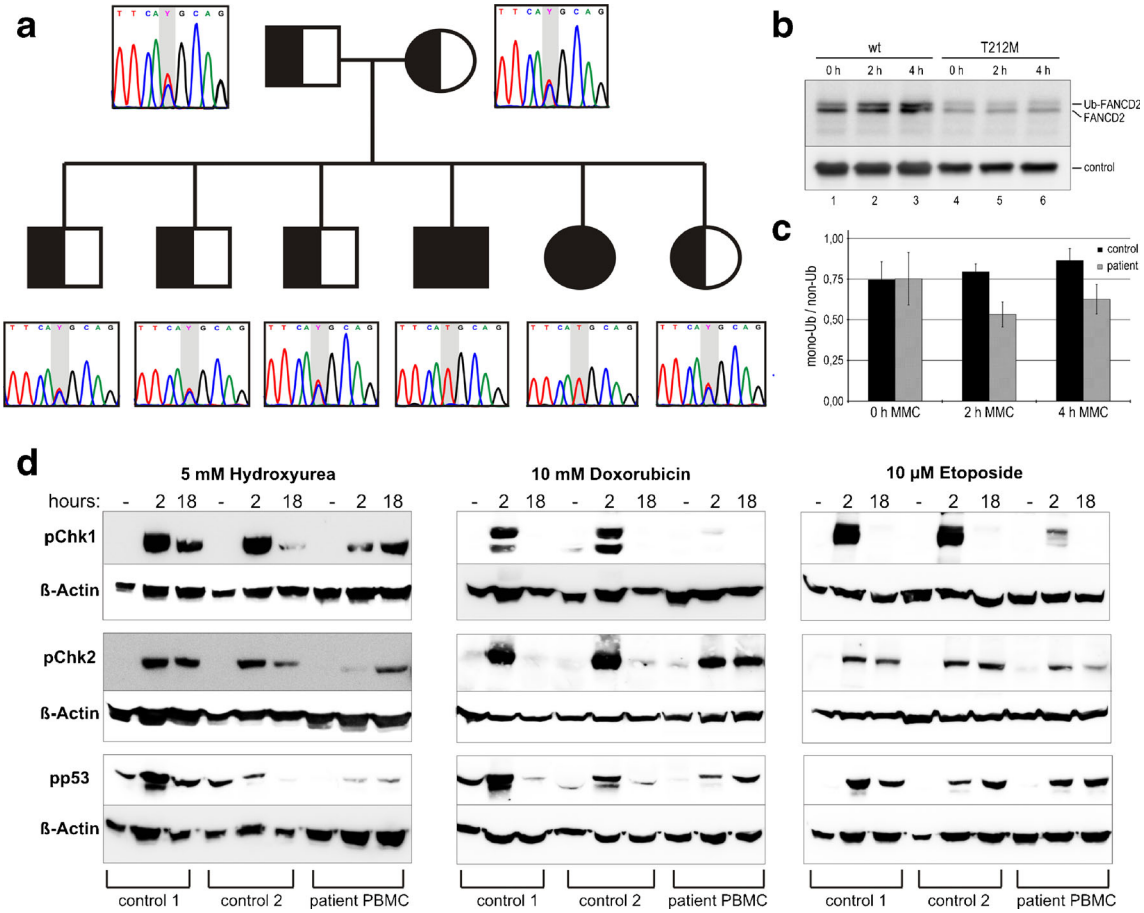

Fig. 1 **a** Validation of the missense mutation cC635T (highlighted in *gray*) in the *Fanconi anemia-associated protein*, *24 kDa* gene (*FAAP24*, alias *C19orf40*) by capillary sequencing. Both patients were found to be homozygous (T/T, *solid black symbols*), whereas the healthy parents and all four healthy siblings were found to be heterozygous (C/T, *bicolored symbols*). **b** Immunoblot analysis of FANCD2 monoubiquitination in mutant patient HVS T cells after MMC treatment. Lysates of whole cells from a healthy control (lanes *1–3*) or cells harboring the FAAP24 cC635T/pT212M mutation (lanes *4–6*) were subjected to SDS PAGE and Western blotting after treatment with MMC for 0, 2, or 4 h. Blots were stained with FANCD2 antibodies (*top*) or antibodies against NCL (*bottom*) as a loading control. Positions of FANCD2 and its monoubiquitinated form are indicated. **c** Ratio of monoubiquitinated FANCD2 to non-ubiquitinated protein from three independent experiments. Standard deviations are indicated as *error bars*. **d** Comparison of the Chk1/2 and p53 activation ability of the mutant patient HVS T cells and the two healthy control HVS cell lines after DNA damage. DNA strand breaks were produced by incubation with hydroxyurea, doxorubicin, and etoposide for 2 and 18 h. β-Actin was used as loading control

is induced after T cell receptor stimulation, which would argue for a role of the protein in downstream TCR signaling, we treated primary T cells with PHA or anti-CD3/anti-CD28 for 0, 8, 24, 48, and 72 h (Supplementary Figure S3). On the transcriptomic level, we found a slight decrease in *FAAP24*-mRNA expression between 0 and 48 h after PHA treatment and a more distinct decrease after the incubation with CD3/28. Only after 72 h of incubation, an increase of *FAAP24*-mRNA expression was detectable, whereas no increase in FAAP24 protein expression could be shown by Western blot analysis. Consequently, a role of FAAP24 in T cell signaling pathways could not clearly be revealed.

We then asked if the T212M mutation impaired the known protein functions of FAAP24 as part of the DNA repair machinery. Therefore, FANCD2 monoubiquitination and ATR/CHK1-mediated checkpoint signaling were analyzed in HVS T cell lines of the male patient and healthy controls.

Impaired Monoubiquitination of FANCD2

It has been shown that FAAP24 is required for the monoubiquitination of FANCD2 [11]. To assess the influence of our FAAP24 mutant on the ubiquitination of FANCD2, we treated patient and control cell lines with MMC followed by immunoblot analyses of the non-ubiquitinated and the monoubiquitinated forms of FANCD2, 155 or 162 kD, respectively [28]. In the mutant patient T cells, we found less FANCD2 monoubiquitination as compared to the control without prior treatment with MMC as well as after the generation of DNA damage by MMC treatment (Fig. 1b, c).

Delay in Cell Cycle Activation of Immortalized Patient T Cells After DNA Damage

A wide variety of DNA damaging agents can activate the FA core complex [28, 29] and thus evoke cell cycle checkpoint responses. The ability to activate the checkpoint kinases Chk1 and Chk2 as well as p53 after DNA damage was analyzed in HVS T cells of the patient and the two healthy controls. DNA strand breaks were generated by treatment with hydroxyurea, doxorubicin, and etoposide followed by Western blot detection using antibodies recognizing the phosphorylated forms of the proteins. After doxorubicin treatment, the mutant patient T cells were not able to activate Chk1, whereas the activation of Chk2 and p53 was delayed as compared to the healthy controls. Treatment with hydroxyurea showed a strong delay in Chk1/2 and a clear impact on p53 activation, whereas the treatment with etoposide affected primarily the activation of Chk1 (Fig. 1d).

FAAP24 T212M Shows No Difference in Sister Chromatid Exchange Rate

FA and Bloom syndrome (BS), a DNA repair disorder caused by homozygous mutations in the *BLM* gene which is a component of the BS complex, share overlapping phenotypes in terms of defective DNA repair and cancer predisposition. DNA cross-linking agents promote the association of the FA core complex and the BS complex in a supercomplex in which FANCM functions as the bridge [30]. Like BLM-deficient cells, cells completely deficient of FANCM [30] and FAAP24 [31] show a high rate of spontaneous SCEs, whereas cells with mutations in other FA core complex components have no increased SCE. We asked if also the FAAP24 mutation in our patients resulted in an elevated SCE rate, but no difference could be shown in the amount of SCEs between healthy control and patient T cells (data not shown).

The EBNA2 Variant Is a Gain of Function Variant

DG75 B cells were co-transfected with different amounts of expression plasmids (0 to 8 μg) either for EBNA2 of strain B95.8 (E2 B95.8; wild type) or for EBNA2 of the FLD variant strain (E2 FLD) detected in both siblings and 5 μg of the CBF1 firefly luciferase reporter plasmid (Ga981-6). Cells were harvested 2 days post transfection and tested for firefly luciferase activity. EBNA2 of the FLD variant strain shows a higher transactivation capacity compared to the EBNA2 protein of the wild-type strain B95.8 (Fig. 2) indicating a gain of function potentially leading to a higher biological activity of the variant EBV strain.

Discussion

We identified a homozygous missense variant of FAAP24 in the two siblings who died from a fatal EBV-associated lymphoproliferation in early childhood. A delay in cell cycle activation after DNA damage as well as an impaired FANCD2 monoubiquitination clearly shows that the mutation leads to a functional defect of FAAP24 in immortalized patient T cells. FAAP24 has been shown to execute its function in DNA repair either alone, i.e., induction of ATR-dependent checkpoint responses, or in a complex with FANCM, i.e., FA pathway activation and suppression of sister chromatid exchange [31]. The T212M mutation harbored by our patients is localized in the second HhH domain of FAAP24, which is essential for the association with FANCM as well as for the DNA binding activity. By testing different mutants which affect either HhH domain residues relevant for FANCM association or

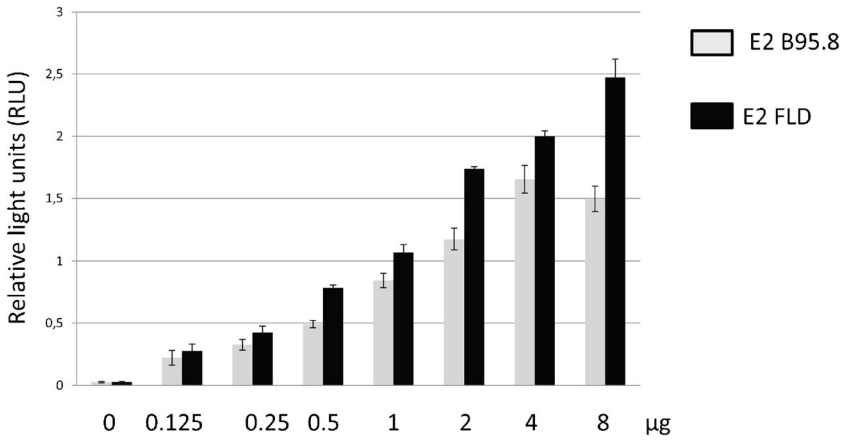

Fig. 2 DG75 B cells were co-transfected with the indicated amounts of expression plasmids either for EBNA2 of strain B95.8 (E2 B95.8) or for EBNA2 of the FLD variant strain (E2 FLD) and 5 μg of the CBF1 firefly luciferase reporter plasmid (Ga981-6). Cells were harvested 2 days post transfection and tested for firefly luciferase activity. The data are shown as the mean value of triplicates normalized to Renilla luciferase expressed as relative light units (RLU). Standard deviations are indicated as *error bars*

DNA binding, Wang et al. demonstrated that mutants with either impaired FANCM association or DNA binding activity partially restored MMC hypersensitivity and defective FANCD2 monoubiquitination in FAAP24−/− cells, mutations affecting both functions showed profound MMC hypersensitivity and little rescue of defective FANCD2 monoubiquitination, whereas only the DNA binding activity was found to be essential for ATR-dependent phosphorylation of CHK1 [27]. In the mutant T cells of our patient, we found a moderately impaired FANCD2 monoubiquitination and delayed checkpoint responses, especially affecting CHK1 phosphorylation. These results may hint towards an impairment of the DNA binding ability rather than FANCM association in our patient's T cells.

There are several possible explanations for a functional relationship between the lack of functional FAAP24 and the clinical picture of a chronic EBV infection with EBV-associated lymphoproliferation.

Mutations in more than 15 different Fanconi anemia genes lead to the clinical picture of FA, characterized by developmental defects, progressive bone marrow failure, and propensity to malignancies. Only one patient with biallelic loss of function mutations in FANCM, which is functionally most closely related to FAAP24, has been described so far [32]. After complementation with FANCM which did not restore the defective FANCD2 monoubiquitination in the patient's cells, further studies revealed that she also carried biallelic mutations in FANCA [33]. On the other hand, after complementation with FANCA, the FANCM-deficient cells still were hypersensitive to cross-linking agents and showed reduced but not absent FANCD2 monoubiquitination. Interestingly, the FANCA and FANCM-deficient patient did not have clinical symptoms of FA but was diagnosed by a chromosomal breakage assay after FA had been diagnosed in her brother. The brother, who displayed typical features of FA, carried the same mutations in FANCA but only a heterozygous FANCM mutation. Singh et al. suggested that FANCM-deficient patients present with a different phenotype than FA and that the FANCM defect may have overruled the FANCA deficiency and changed the clinical phenotype in this unique patient. Horejsi et al. already hypothesized that FANCM and FAAP24 might be causal for human disorders outside the FA complex [34]. Our report supports this view, as mutations in FAAP24 have not been reported in patients with FA or any other human disease, but now in the two siblings with EBV-triggered fatal lymphoproliferation.

There is a recognized albeit not completely understood association of different genetically defined human DNA repair disorders and infections with oncogenic viruses. FA patients have an increased risk to human papillomavirus infections which are detected in 85 % of the head and neck squamous cell carcinoma of these patients. It has been suggested that the extension of G2 phase in FA cells from unrepaired interstrand cross-links increases the susceptibility to human papillomavirus infection [35, 36].

Patients with ataxia-telangiektasia (AT; defect in ATM) and Nijmegen breakage syndrome (NBS; mutations in NBS1) often suffer from chronic herpes virus infections [37–39]. In an AT mouse model, it could be shown that the expression of ATM is necessary for an optimal adaptive immune response to EBV [38]. For the rare Bloom syndrome (mutation in BLM), an association to EBV has not been reported clinically. However, there are hints for a defective immune response towards EBV in vitro [40]. Patients with AT, NBS, and Bloom syndrome suffer from a variable T cell or combined immunodeficiency. Furthermore, the interaction of EBV latent proteins and FAAP24-deficient B cells may contribute to the patients' phenotype. The EBV nuclear antigens (EBNA) 1 and 3C are capable of inducing genomic instability [41].

EBNA2, one of the six viral nuclear proteins expressed in latently infected B lymphocytes, is essential for EBV-mediated growth transformation and immortalization of B cells [42]. The clinical significance of the EBNA2 variant detected in both children has been discussed before and still remains unclear [15]. Reviewing the literature, an association of EBNA2 variant EBV strains with specific EBV-induced diseases has not been consistently demonstrated so far [43], nor—to our knowledge—have virus isolates been genotyped in other patients with immundeficiencies predisposed to EBV-associated disorders. The variant does not contain immunodominant T cell epitopes or relevant MHC class I and II peptide motifs with regard to patient 2's HLA type, which could prevent an adequate T cell response [15]. We synthesized an identical EBNA2 variant. B cells transfected with the EBNA2 variant and wild-type EBNA2 of laboratory strain B95.8 indicated. The EBNA2 variant exhibited significantly stronger biological activities. While it has been assumed earlier that the deleted region was not relevant for the efficacy of B cell transformation [15], these results now may be interpreted as a hint towards a higher transformation capability of this specific EBNA2 variant. Possibly, the elevated transactivation capacity has an additive effect on the induction and maintenance of B cell proliferation in our FAAP24-deficient patients.

Acknowledgments We thank Monika Schmidt, Clinical and Molecular Virology, Friedrich-Alexander-University Erlangen-Nürnberg, for the technical assistance with HVS transformation of primary patient T cells and Conny Kuklik-Roos for performing the luciferase assays.

Compliance with Ethical Standards

Ethical Approval All procedures involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Authorship SD, KB, and AB designed the study. VS and HWK treated the patients and provided patient samples as well as clinical, immunological, and virological information. SD, RML, AH, GF, CJ, BF, and BK performed the research. MG and SG developed the in-house exome sequencing and data analysis pipeline. SD and KB drafted the manuscript.

Disclosures No author has any financial or other potential conflict of interest to disclose.

The project was funded by a grant from the German Research Foundation (Deutsche Forschungsgemeinschaft) to KB and a grant from the Deutsche Krebshilfe to BK.

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