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MBD4-associated neoplasia syndrome associated with a homozygous missense variant in *MBD4* – expansion of an emerging phenotype

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Authorship contributions

PB conceived of the project and designed the study and provided clinical care; PB, GR, AR, ERT, ZS, MW, SG analyzed genomic data; PB, AJ, LF, SP, FK provided clinical care to the patient; and all authors reviewed the data and contributed to critical revision of the manuscript

Germline biallelic loss-of-function variants in *MBD4* have recently been associated with a syndrome characterized by early onset hematological malignancy and colonic polyposis (provisionally termed MBD4-associated neoplasia syndrome [MANS])(Palles*, et al* 2021, Sanders*, et al* 2018). This clinical phenotype is caused by a base excision repair (BER) defect as a result of loss of MBD4 function and the subsequent accumulation of C>T transitions at CpG dinucleotides after spontaneous 5-methylcytosine deamination(Sanders*, et al* 2018). To date, four patients (from three families) have been identified with MANS harboring deleterious variants in *MBD4* (NM_003925.2) including an in-frame deletion (p.(His567del)), canonical splice site (c.1562-1G>T) and truncating variants (p.(Glu314Argfs*13) and p.(Ser205Thrfs*9))(Palles*, et al* 2021, Sanders*, et al* 2018). Herein we describe a patient with MANS as a result of a homozygous missense germline variant in *MBD4* providing novel insights into the clinicobiological features of this emerging phenotype.

A 37-year-old man of Middle Eastern ethnicity and consanguineous parents presented with symptomatic anemia and pancytopenia. A diagnosis of myelodysplastic syndrome with ring sideroblasts and multi-lineage dysplasia (MDS-RS-MLD) was made on bone marrow biopsy. His history was significant for the presence of multiple (75) colonic polyps which had led to total colectomy five years previously. No variants were identified in *MUTYH* or *APC* at that time. He also had a right-sided vestibular schwannoma resected three years before diagnosis with MDS. There was no other relevant personal or family history.

The patient was transfusion dependent as a result of MDS-RS-MLD and he underwent a matched unrelated donor ASCT. Despite initial engraftment at day 28 he became transfusion dependent again at 3 months post-ASCT and a bone marrow biopsy showed relapsed MDS (without blast excess) at that time. He was commenced on lenalidomide and azacytidine and had a further bone marrow biopsy (9 months post-ASCT) which showed disease progression with a blast excess of 11% and karyotyping

demonstrating a monosomy 7 and del(5q). He then progressed rapidly to acute myeloid leukemia (AML) and despite the addition of venetoclax to azacytidine he had no disease response and care was redirected towards palliation.

WGS was first performed on DNA extracted from hair follicles in order to investigate possible germline causes for the patient's phenotype (Supplementary methods). A homozygous missense variant in the catalytic domain of *MBD4* was detected - c.1535G>A, p.(Arg512Gln). A different amino acid substitution (Arg512Trp) at this critical residue has been shown experimentally to result in reduced catalytic activity with the loss of an arginine predicted to disrupt protein structure(Alekseeva, et al 2021). The Arg512Trp and Arg512Gln show a similar degree of predicted deleterious protein effect by commonly accepted *in silico* predictors (REVEL scores for the Trp and Gln substitution of 0.88 and 0.82, respectively). The Arg512Gln has an allele count of seven in the Genome Aggregation Database (gnomAD) (v2.1.1) with no homozygotes present. The variant was classified as likely pathogenic using the American College of Medical Genetics guidelines for classification of germline variants (Richards*, et al* 2015). The patient's parents and sibling were heterozygous carriers of the Arg512Gln variant (Figure 1A).

Paired WGS was then performed using DNA extracted from hair as a germline sample and from peripheral blood at time of diagnosis of MDS-RS-MLD as a tumor sample (Supplementary methods). The global mutation profile from the hematopoietic compartment showed a markedly elevated tumor mutation burden (18,938 somatic single nucleotide variants, 16.88 mutations/Mb) and the vast majority of mutations being C>T transitions occurring at CpG dinucleotides (highest in the context of ACG triplet followed by CCG, GCG then TCG) (Figure 1B and Figure 1C). Assessment of recurrently mutated genes in hematological malignancy was also performed using UMI-based targeted sequencing to detect variants in the MDS diagnostic blood sample (Supplementary methods) and revealed mutations in *TET2* and *SF3B1* at a variant allele frequency (VAF) of approximately 25% (Figure 2). In addition, low VAF mutations (0.5-2%) in *DNMT3A*, *IDH2* and *TP53* were detected at this

timepoint. Sequencing of longitudinal samples from 3, 9 and 12 months post-ASCT revealed continued clonal evolution across all time points with emergence of a dominant clone containing *TP53*, *IDH1, CBL* and *DNMT3A* mutations which was then replaced by a tumor compartment dominated by *TP53* mutations and wild-type for *IDH1* and *IDH2* (Figure 2).

Three out of the four patients described to date have presented with AML and harbored mutations in *DNMT3A* and *IDH1*/*IDH2*. It is therefore notable that our patient presented in an MDS phase with dominant *SF3B1* and *TET2* mutations, particularly as variants in *SF3B1* are central to the pathogenesis of MDS with ring sideroblasts and the specific variant in our patient (c.1873C>T; p.(Arg625Cys)) is the result of a C>T transition at a CpG dinucleotide. However, low level mutations in *DNMT3A* and *IDH2* were detected at this diagnostic timepoint before ASCT, possibly representing an early transformation event. Interestingly, during overt transformation of his disease to a more aggressive phase characterized by blast excess post-ASCT he acquired dominant mutations in *DNMT3A* and *IDH1* (9 months post-ASCT, Figure 2) further supporting the hypothesis that this may be a relatively stereotyped pathway to leukemia in patients with MANS(Sanders*, et al* 2018).

Targeted sequencing (TSO500) was then performed on DNA extracted from the vestibular schwannoma (Supplementary methods) which showed the same mutational signature that was observed in the hematopoietic compartment as well as two nonsense mutations in *NF2* (NM_000268.3) c.169C>T;p.(Arg57*) and c.1021C>T;p.(Arg341*), both C>T transitions at CpG dinucleotides. Biallelic loss-of-function mutations in *NF2* are ubiquitous early molecular events in sporadic vestibular schwannoma(Carlson*, et al* 2018).

In summary, we have a described a patient with MANS as a result of a homozygous germline variant in *MBD4* with multiple novel features including (i) the first missense variant in *MBD4* described in this condition (ii) expansion of the phenotype to include vestibular schwannoma as part of the potential neoplastic spectrum and (iii) the observation of a *DNMT3A*/*IDH1*/*IDH2*-independent development of

hematological malignancy prior to leukemic transformation. Descriptions of more patients with this novel syndrome will contribute to understanding the full clinical spectrum of this emerging phenotype.

Figure 1 - Pedigree and genomic features of MDS in a patient with homozygous *MBD4* variant (p.(Arg512Gln)). (A) Pedigree depicting proband (arrow). Phenotypic features are indicated by the colored squares. Genotype for *MBD4* p.(Arg512Gln) is indicated for each individual with + (for detected) (B) Circos plot depicting somatic mutation distribution across the genome in the MDS-RS-MLD tumor. Each single nucleotide variant is indicated by a dot and colored by the base change, with C>T substitutions indicated by red dots (C) Riverplot of base substitutions and surrounding sequence context demonstrating the dominance of C>T transitions at CpG dinucleotides

Figure 2 – Variants detected with targeted sequencing from a patient with homozygous MBD4 variant p.(Arg512Gln) from diagnosis to 12 months post allogeneic stem cell transplant (ASCT). Asterisks indicate three variants present at MDS diagnostic timepoint with variant allele frequencies (VAF) under 2%. Possible clonal relationships, inferred from VAFs, are illustrated in the hierarchy diagram.

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FIGURE 1

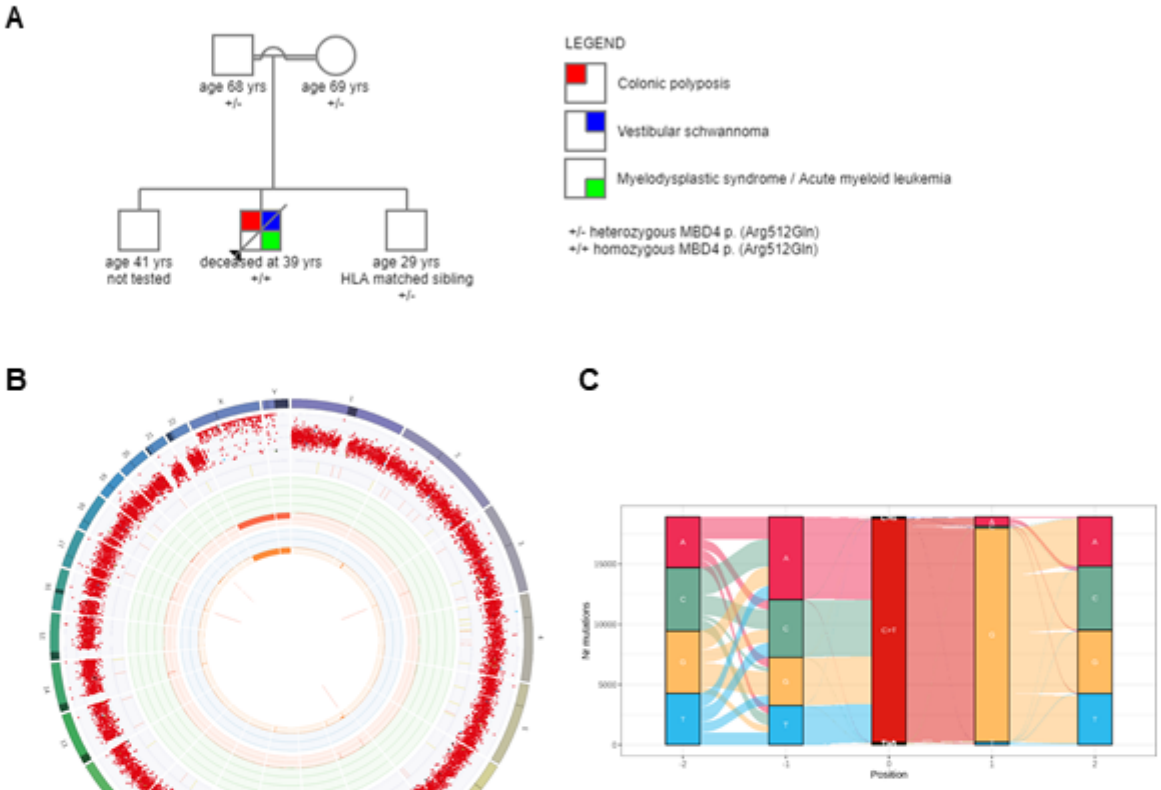

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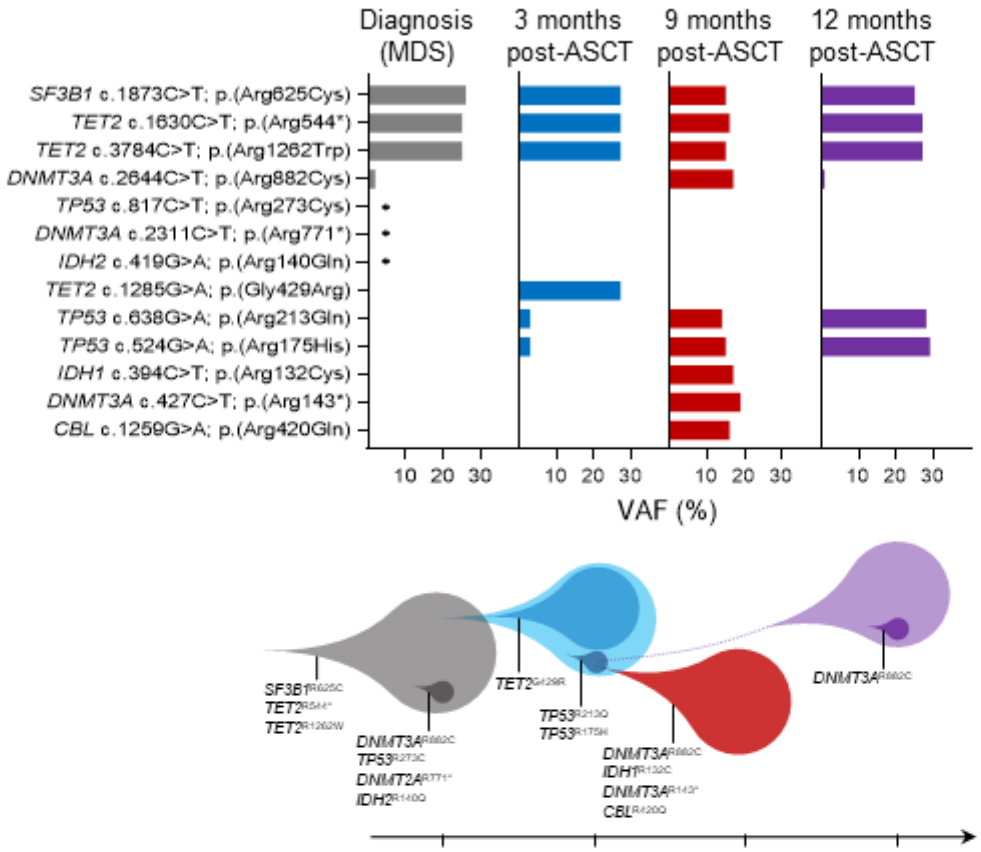
FIGURE 2



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