DNA Methylation as a Potential Molecular Mechanism in X-linked Dystonia-Parkinsonism

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ABSTRACT: **Background:** X-linked dystonia-parkinsonism is a neurodegenerative movement disorder. The underlying molecular basis has still not been completely elucidated, but likely involves dysregulation of TAF1 expression. In X-linked dystonia-parkinsonism, 3 disease-specific single-nucleotide changes (DSCs) introduce (DSC12) or abolish (DSC2 and DSC3) CpG dinucleotides and consequently sites of putative DNA methylation. Because transcriptional regulation tightly correlates with specific epigenetic marks, we investigated the role of DNA methylation in the pathogenesis of X-linked dystonia-parkinsonism.

**Methods:** DNA methylation at DSC12, DSC3, and DSC2 was quantified by bisulfite pyrosequencing in DNA from peripheral blood leukocytes, fibroblasts, induced pluripotent stem cell-derived cortical neurons and brain tissue from X-linked dystonia-parkinsonism patients and age- and sex-matched healthy Filipino controls in a prospective study.

**Results:** Compared with controls, X-linked dystonia-parkinsonism patients showed striking differences in DNA methylation at the 3 investigated CpG sites. Using methylation-sensitive luciferase reporter gene assays and immunoprecipitation, we demonstrated (1) that lack of DNA methylation because of DSC2 and DSC3 affects gene promoter activity and (2) that methylation at all 3 investigated CpG sites alters DNA–protein interaction. Interestingly, DSC3 decreased promoter activity per se compared with wild type, and promoter activity further decreased when methylation was present. Moreover, we identified specific binding of proteins to the investigated DSCs that are associated with splicing and RNA and DNA binding.

**Conclusions:** We identified altered DNA methylation in X-linked dystonia-parkinsonism patients as a possible additional mechanism modulating TAF1 expression and putative novel targets for future therapies using DNA

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X-linked dystonia-parkinsonism (XDP) is a severe movement disorder resulting from progressive loss of striatal medium spiny neurons, manifesting as adult-onset dystonia combined with parkinsonism. All currently known XDP patients are of Filipino origin, sharing the same disease-relevant haplotype because of a genetic founder effect.

The molecular basis of XDP is still not completely understood. A minimal XDP-linked region on the X chromosome has been narrowed to 202 kb containing 5 disease-specific single-nucleotide changes (DSC1, DSC2, DSC3, DSC10, DSC12), a 48-bp deletion, and a SINE-VNTR-Alu (SVA) retrotransposon insertion that is associated with the TAF1 gene (Fig. 1). None of these variations are within coding regions except for DSC3, which lies in the alternative exon d4 of the TAF1/IDT3 multiple transcript system sharing exons with other TAF1 isoforms. Moreover, reduced expression of the neuron-specific isoform N-TAF1 was observed in brain samples of XDP patients. Indeed, recent studies confirmed this initial finding and converged on transcriptional dysregulation as the molecular basis of XDP, given the identified downregulation of TAF1 and TAF1-dependently transcribed genes in cells derived from XDP patients compared with cells of healthy controls. Because CRISPR/Cas9 excision of the SVA retrotransposon rescues TAF1 expression in cells of XDP patients, this insertion is currently considered the major culprit in XDP. In addition, variable length of a polymorphic hexanucleotide repeat within this SVA accounts for ~50% of the variance in age at onset and disease severity in XDP. Nevertheless, other transcription-influencing mechanisms may contribute to the pathogenesis of XDP and modify TAF1 expression, given that transcripts harboring DSC3 have been shown to affect the transcription of genes involved in central vesicular transport and dopamine metabolism. As epigenetic mechanisms including DNA methylation are potent regulators of gene expression, we hypothesized that aberrant DNA methylation contributes in a coregulatory manner to the above described mechanisms to the observed TAF1 dysregulation in XDP.

**Methods**

A detailed description of the methods can be found in the supplemental material.

**Subjects and Samples**

Ninety-five individuals carrying the XDP haplotype were included in this prospective study (Table S1). Our patient cohort consisted of 85 male hemizygous patients manifesting with XDP, 6 male nonmanifesting carriers, 2 heterozygous female patients, and 1 homozygous female patient. In addition, we included 1 female XDP patient with mosaic Turner syndrome (karyotype: mos45,X/46, XX). Age at onset (AAO) of the 85 male XDP patients ranged from 20 to 67 years, with a mean AAO ± standard deviation (SD) of 38.9 ± 24.1 years. The control cohort

![FIG. 1. XDP haplotype overview. The disease-causing XDP haplotype is associated with a 202-kb locus on chromosome X (dashed lines) containing 5 disease-specific single-nucleotide changes (DSC1, DSC2, DSC3, DSC10, DSC12), a 48-bp deletion, and a SINE-VNTR-Alu (SVA) retrotransposon insertion within the TATA-box binding protein associated factor 1 (TAF1) gene and a nontranslated multiple transcript system (MTS). The exon 34' (gray) is an alternative exon that is incorporated during splicing of the neuronal isoform nTAF1. Only DSC3 is within the coding region of alternative exon d4 of the MTS. Regions with high CpG density (CpG island, GGI) are indicated in dashed boxes.](image-url)
consisted of 57 healthy male ethnically and age-matched individuals without the XDP haplotype (wild type). Furthermore, 2 wild-type male relatives of female XDP patients were studied as well as brain samples from 1 male wild-type individual of white origin. After obtaining written informed consent, genomic DNA was extracted from peripheral blood leukocytes using standard procedures. In addition, samples from several brain regions of 2 deceased XDP patients and 2 deceased controls were available for analysis, as were DNA samples from fibroblasts and induced pluripotent stem cell (iPSC)–derived cortical neurons of 2 patients and 3 controls. The study was approved by the ethics committees of the University of Lübeck, Germany, and of the Metropolitan Medical Center, Manila, Philippines.

Analysis of 5-Methylcytosine

Genomic DNA was treated with sodium-bisulfite using an EpiTect Fast kit (Qiagen, Hilden, Germany) and analyzed by pyrosequencing on a PyroMark Q48 Autoprep (Qiagen, Hilden, Germany) using a PyroMark Q48 advanced CpG kit (Qiagen, Hilden, Germany). For each set of primers, quality control was performed including analysis of control DNA with known methylation status (0%, 50%, and 100% DNA methylation) and sequence analysis of control DNA with known methylation status. Primer sequences and polymerase chain reaction (PCR) conditions are listed in Table 1.

Analysis of 5-Hydroxymethylcytosine

5-hydroxymethylcytosine (5hmC) was analyzed in brain samples of 2 XDP patients and 2 white controls. One microgram of genomic DNA was divided into 2 aliquots of 500 ng and processed through either bisulfite-only (BS) or the oxidative bisulfite (oxBS) conversion workflow according to the manufacturer’s manual (TrueMethyl Seq Kit, Cambridge Epigenetix, Cambridge, England). All samples were bisulfite-converted using the condition and reagents provided by the TrueMethyl kit. Converted DNA was purified and analyzed by pyrosequencing on a PyroMark Q48 Autoprep (Qiagen, Hilden, Germany). The content of 5hmC was calculated by values derived from the oxBS-reaction (represent 5mC) subtracted from values derived by the BS-only reaction (representing 5mC and 5hmC).

Luciferase Reporter Gene Assays

The CpG-free plasmid pCpG-CMV containing a universal CMV promoter and luciferase was a kind gift from Prof. Rehli. PCR products covering DSC2, DSC3, and DSC12 were generated by PCR using human genomic DNA of XDP patients or control subjects (PCR primers indicated in Table 1). Methylation or mock-methylated plasmids were transfected into SH-SY5Y cells using lipofectamine2000 (Life Technologies, Carlsbad, CA). To control for transfection efficiency, cells were cotransfected with a Renilla-SV40 plasmid (pRL-SV40; Promega) at a ratio of 1:10. Luminescence was measured 24 hours after transfection in 3 replicates per gene and condition.

Immunoprecipitation and Mass Spectrometry

Protein preparation and immunoprecipitation (IP) were performed according to the protocol of the Pierce MS-compatible Magnetic IP kit (Thermo Fisher). Biotinylated double-stranded oligonucleotides of DSC2, DSC3, and DSC12 (see supplemental methods) were incubated with HepG2 protein lysate overnight. The IP was performed with magnetic beads covered with

| TABLE 1. Chromosomal position, nucleotide variation, and PCR conditions of the n-shore region of the TAF1 gene and 3 XDP-specific variants analyzed for changes in DNA methylation |
|---------------------------------------------------------------|---------------------------------------------------------------|---------------------------------------------------------------|
| SNP or region ID     | Chromosomal position (GRCh38)     | Variant in context | Primer sequences | PCR condition |
|----------------------|-----------------------------------|-------------------|-----------------|---------------|
| n-shore TAF1         | X:71365666-71365765 8 CpG sites | F+S: 5’-GTG TAT GGA TGG AGT-3’ | R: 5’-biotin-ACA TTT TCC AAT AAC AAT TAA TTT TTT AAC-3’ | 45 × 54°C + 35 × 54°C |
| DSC12                | X:71391195 CTA > GGA          | F+S: 5’-AAG TGG GAT GAT AGG TAT-3’ | R: 5’-biotin-TAA ATA CCA ACT ACA CAC TAC TCC-3’ | Touchdown |
| DSC3 (rs397509359)   | X:71529785 TCG > TTG          | F: 5’-GGT TTA TGG TGG TGG TGG -3’ | R: 5’-biotin-CAA TCC TCA TAA TCC TCC TCC TAA CT-3’ | 60°C–50°C |
| DSC2                 | X:71531433 CGA > CAA          | F+S: 5’-AGG TTA ATT TTT TAA TAA-3’ | R: 5’-biotin-CAT CTC ACA ATT AAA TAA CAT-3’ | Touchdown |
| rs41438158           | X:71633571 GGG > GTG          | F: 5’-TTT TTA TTA GGG GGG TGG TGG TGG -3’ | R: 5’-biotin-CAC TAA CCC ATC TAA AAT AAC TAC T-3’ | 45 × 54°C |

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streptavidin. DNA–protein complexes were eluted for protein identification by mass spectrometry using an Orbitrap Fusion Lumos mass spectrometer (Thermo Scientific, Waltham, MA). Proteins were annotated with Swiss-Prot13 and characterized by PANTHER14 data bases.

**Statistical Analyses**

Methylation data are represented as mean ± SEM. Correlation between DNA methylation and AAO or DNA methylation and age at blood draw was performed with Pearson’s correlation, with the significance level set at \( P < 0.05 \) (2-tailed). In addition, the occurrence of nonlinear effects was examined. Differences for luciferase reporter gene assays were tested with 2-way analysis of variance and Tukey’s multiple-comparisons test.

**Data Availability**

The authors confirm that the data supporting the findings of this study are available within the article and its supplemental material.

**Results**

**DNA Methylation Within the XDP-linked Region Differs Greatly Between Patients and Controls**

To study if DNA methylation is potentially involved in regulating TAF1 expression, we investigated whether any of the 5 XDP-related DSCs introduce or abolish a methylation (CpG) site. Moreover, we examined 8 CpG sites located in the shore region of the TAF1 promoter-associated CpG island (Table 1) because variation between samples is expected to be high in CpG island shores.15 However, this region of the TAF1 promoter was only weakly methylated (<10%) and did not differ between XDP patients and controls (Fig. 2A). Thus, DNA methylation in the analyzed TAF1 promoter region did not correlate with decreased TAF1 gene expression in patients with XDP.11

Of the 5 DSCs, 3 created (DSC12: CT>CG) or erased (DSC2: CG>CA, and DSC3: CG>TG) a CpG site (Table 1 and Fig. 1), and therefore methylation levels may differ between patients and controls. Based on these findings, theoretically, DNA methylation could range from 0% to 100% at DSC12, whereas the expected methylation at the CpG-erasing DSC2 and DSC3 would be 0% in XDP patients. Correspondingly, in controls, the wild-type sequence (CT) at a position of DSC12 (wtDSC12) is not predicted to be methylated, whereas the wild-type CpG sites at a position of DSC2 and DSC3 (wtDSC2 and wtDSC3) may be methylated.

We found a high percentage of DNA methylation at DSC12 in XDP patients. Mean DNA methylation at DSC12 in leukocyte DNA was 62% (41%–71%), in fibroblasts was 68% (67%–68%), in DNA from iPSC-derived cortical neurons was 68% (67%–69%), and in striatum from XDP patients was 60% (59%–62%); see Figure 2B. In controls, the analyzed cytosine also exists but it is followed by a thymine (CpT site). Because non-CpG methylation (CpN) may occur, we evaluated the methylation status of this cytosine in controls as well and detected only very little methylation, ranging from 1% (0.9%–1.7%) in fibroblasts, 1% (1.0%–1.1%) in iPSC-derived cortical neurons, 2% (2%–3%) in brain tissue to 4% (4.3%–4.9%) in leukocytes. DNA methylation at DSC12 was compared with TAF1 gene expression in leukocytes from a subset of patients of whom RNA was available.11 However, no significant correlation was found (\( n = 26, r = 0.3122, P = 0.1205 \)).

Given that DSC2 and DSC3 abolished CpG sites, as anticipated, almost no methylation (<5%) at these 2 loci was detected in XDP patients. Control individuals of the same ethnicity, however, showed hypermethylation at the wtDSC2 (>80%) and wtDSC3 (>90%) across all investigated tissues (Fig. 2C,D). Similar to DSC12, the DSC2 change did not affect the cytosine itself, but the following nucleotide and the measured methylation at DSC2 might be attributed to CpA methylation. In summary, all 3 investigated DSCs introduced striking differences in DNA methylation between XDP patients and controls.

We next studied leukocyte DNA methylation at DSC12 in 3 affected women carrying the disease-specific haplotype on at least 1 X chromosome and their family members (Fig. 2E–G). The first female patient suffered from an atypical Turner syndrome.16 The detected methylation at DSC12 was 38% in this patient and >1.5 times higher in her affected brothers (ie, 61%–65%) (see Fig. 2E). The second investigated female patient was a homozygous carrier of the XDP haplotype, and her DNA methylation at DSC12 was 51%, and thus slightly lower than in her 2 affected sons (55% and 69%) (see Fig. 2F). Finally, DNA methylation was studied in an unaffected woman who was a heterozygous carrier of the XDP haplotype and had skewed inactivation of the X chromosome toward preferential expression of the affected X chromosome.17 In this patient, DNA methylation at DSC12 was 26% (Fig. 2G). Interestingly, among the investigated family members, 2 individuals not carrying the XDP haplotype exhibited relatively high non-CpG methylation at wtDSC12, that is, 9% and 14% (Fig. 2E,G).

**DNA Methylation at DSC12 Is Stable and Not Associated With Age and AAO**

Given the progressive course of XDP, which involves gradually increasing neurodegeneration and severity of the symptoms over time, we assessed the stability of methylation at DSC12 in 6 patients whose blood was...
FIG. 2. DNA methylation at the TAF1 promoter and in the XDP-linked region. (A) DNA methylation of 8 CpG sites in the shore region of the TAF1 promoter-associated CpG island is not changed (data represented as mean ± SEM). (B–D) DNA methylation at DSC12, DSC3, and DSC2 in DNA derived from leukocytes, fibroblasts, and iPSC-derived cortical neurons and brain tissue of XDP patients and controls. leuko, Leukocyte DNA; fibro, fibroblast DNA; c neuron, DNA extracted from iPSC-derived cortical neurons. (E–G) Pedigrees of 3 female XDP patients and the percentages of DNA methylation at DSC12 in DNA derived from leukocytes of these patients and their investigated family members. The index patients are indicated by arrows. Sex chromosome symbols and methylation percentages are shown only for the investigated individuals. Circles, females; squares, males; black symbols, affected members; white symbols, unaffected individuals; black X, wild-type X chromosome; red X, X chromosome with XDP-specific changes.
collected at consecutive points. This analysis showed that DSC12-induced DNA methylation does not change over a time course of several years (Fig. 3A). Furthermore, in our complete cohort of male XDP patients, DNA methylation in leukocytes at DSC12 did not correlate with age at examination (Fig. 3B), AAO (Fig. 3C), or with years of disease duration \((r = -0.0170, P = 0.8785)\). To identify a possible disease-causing association between DNA methylation and the SVA insertion, we investigated the relationship between percentage of DNA methylation at DSC12, the number of hexanucleotide repeats within the SVA insertion,\(^{11}\) and AAO in 83 male XDP patients for whom AAO data were available. Pertaining to merely AAO and SVA repeat number, AAO showed a significant and inverse correlation with repeat length (Pearson’s product-moment correlation: \(r = -0.76, P < 0.0001\); Fig. S1A) in our cohort, as previously reported.\(^{10}\) However, repeat number had no effect on the percentage of DNA methylation at DSC12 (Fig. S1B).

Moreover, the percentage of methylation had no effect on AAO (Fig. S1C).

Next, we investigated the stability of DNA methylation at DSC12 in the brains of 2 male XDP patients and 1 male white control. In the process of demethylation, 5-hydroxymethylation (5hmC) is generated and is therefore considered a mark of dynamic DNA methylation and is frequently detected in neurons. The 5hmC in brain samples from the 2 XDP patients was 20% and therefore higher compared with the white control sample (3%; Fig. 3D).

**DNA Methylation at DSC2, DSC3, and DSC12 Affects Promoter Activity and DNA–Protein Interaction**

To test whether the methylation status at the 3 investigated DSCs affected gene expression, we performed luciferase reporter gene assays in neuronal SH-SY5Y

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**FIG. 3.** Stability of altered DNA methylation at DSC12. (A) Time course of leukocyte DNA methylation at DSC12 in 6 XDP patients indicated with 1 colored line per patient. (B) Association between leukocyte DNA methylation at DSC12 in XDP patients and age at blood collection. (C) Correlation of leukocyte DNA methylation at DSC12 and AAO. (D) DNA hydroxymethylation at DSC12 in brain regions form 2 XDP patients and 1 individual without the XDP haplotype. cort neuro, iPSC-derived cortical neurons; FL, frontal lobe; Ce, cerebellum.
cells. To control for the possibility that DSC2, DSC3, and DSC12 have an effect on gene expression independently of the methylation status, we compared the expression of plasmids containing DSC2, DSC3, or DSC12 versus wild-type sequences (wtDSC2, wtDSC3, and wtDSC12), which were either fully methylated or nonmethylated (mock-methylated) in vitro.

Complete DNA methylation (100%) versus mock-methylation (0%) of the CpG site created in XDP by DSC12 had no influence on luciferase activity. In addition, the sequence variation created by DSC12 had no effect on luciferase activity compared with the wild-type sequence wtDSC12 (data not shown).

Methylation of the wild-type CpG site at the DSC2 locus significantly increased luciferase activity compared with the unmethylated wtDSC2 construct and compared with the DSC2 constructs (Fig. 4A). Because the wtDSC2 sequence was highly methylated in our control cohort (Fig. 2D), loss of methylation at DSC2 as observed in XDP could have contributed to reduced expression of TAF1. Deletion of this CpG site as found in XDP patients had no effect on gene expression per se, as luciferase activity remained similar in the DSC2 and wild type in the unmethylated state (mock).

DNA methylation of the wild-type CpG site at the DSC3 locus had a significant effect on gene expression in wtDSC3 plasmids (Fig. 4B). Promoter activity was dramatically increased in the unmethylated wtDSC3 plasmid compared with all other conditions. DSC3 abolished DNA methylation in XDP, and thus, no difference in luciferase activity should have been seen between methylated and nonmethylated plasmids containing DSC3. The slight but not significant difference seen in methylated and unmethylated DSC3 plasmids could most likely be attributed to the 2 additional CpG sites in close proximity to the DSC3 position that are present in XDP patients and controls and could not be isolated from DSC3 for the reporter gene assay. Interestingly, in addition to DNA methylation, the genotype at DSC3 has an effect on promoter activity, as luciferase activity was highest in the mock-treated wild-type condition and significantly lower in the mock-methylated DSC3 condition.

To test whether DNA methylation at DSC2, DSC3, and DSC12 affects binding of specific proteins (eg, transcription factors), we performed immunoprecipitation (IP) and subsequent mass spectrometry (MS) analysis to identify proteins attached to the respective oligonucleotides. DNA sequences including each DSC were incubated with whole-cell HepG2 lysate to ensure enough protein for binding. We identified several proteins specifically binding to the DSCs, which are involved in either splicing DNA or RNA binding such as heterogeneous nuclear ribonucleoproteins or nuclear factor transcription factors (Table S2).

**Discussion**

Our study investigated for the first time the role of epigenetic modifications in the pathogenesis of XDP by analyzing DNA methylation at 3 CpG sites that are either created or abolished by XDP-specific changes (DSC12 vs DSC2 and DSC3). Compared with controls, XDP patients showed striking differences in DNA methylation at all 3 sites. These differences were to a lesser extent also present in female carriers of the haplotype and were stable over time. Interestingly, the high
levels of DNA methylation at DSC12 in XDP patients were uniformly present across all investigated tissues and were preserved or reestablished in iPSC-derived cortical neurons, which are subjected to genome-wide erasure of DNA methylation during the reprogramming process. Also in the disease-relevant brain tissue methylation, differences exist and DNA methylation might be more dynamic in brains of patients compared with controls, as indicated by higher hydroxymethylation. Because we had access to only 2 patients and 1 control brain, no firm conclusions could be drawn from the brain-derived data. However, as indicated by the level of hydroxymethylation, it is tempting to speculate that DNA methylation may change dynamically according to the disease state in brains of XDP patients. 5-Fluorouracil is derived from active DNA demethylation and may be a stable epigenetic mark that inhibits binding of the methyl CpG-binding protein 2 (MeCP2) specifically in the brain. 18 Inhibition of MeCP2 binding to gene bodies could lead to increased gene expression. 18

For the DNA–protein binding analysis, we used nuclear extract derived from HepG2 cells because sufficient amounts of protein for the IP analysis could not be derived from neuronal cells. Previously, HepG2 cells have been successfully employed to study ubiquitously expressed transcription factors and, therefore, were used for our analyses. 19–23

DNA methylation within gene bodies is generally high and characteristic for transcribed genes. 24 As TAF1 is ubiquitously expressed, it seems conceivable that any de novo–created CpG site will eventually become methylated to facilitate TAF1 transcription. This could explain the high methylation of the DSC12-induced CpG site that we observed across tissues from XDP patients. To understand the consequence of changed DNA methylation in XDP patients, we analyzed the effect of methylated and unmethylated DSCs versus wrDSCs in luciferase reporter gene assays. We concluded that with respect to the DSC12-created CpG site, neither DSC12 sequence nor DNA methylation status influences luciferase activity. At the DSC2-deleted CpG site, lack of methylation and not variation in the DSC2 sequence decreases gene expression, whereas at the DSC3-erased CpG site, both DSC3 sequence variation and full DNA methylation decrease luciferase activity.

XDP is a movement disorder with a seemingly homogenous genetic cause, and transcriptional dysregulation is the likely molecular basis. 5–8 Recently, the SVA retrotransposon insertion has been linked to the decreased expression of TAF1 that could be rescued by excision of this insertion in patient-derived cells. 8,9 Nevertheless, at least 1 additional XDP-specific change, DSC3, affects gene expression, implying its potential functional importance in the pathogenesis or expressivity of XDP. 5 Gene body DNA methylation defines exon boundaries and alters mRNA splicing 25 by mediating the activity of CTCF, 26 MECP2, 27 and HP1 28 among others. As DSC3 is located in the alternative exon d4 of the TAF1/DYT3 transcript, 5 DSC3-induced sequence and DNA methylation changes could be involved in regulating the expression of exon d4. These data are supported by our reporter gene assays showing that both sequence changes induced by DSC3 as well as methylation changes because of CpG deletion significantly reduce promoter activity.

MS analysis of immunoprecipitated proteins indicated specific binding of proteins to DSC2, DSC3, and DSC12 independently of the haplotype (DSC vs wild type).

We identified proteins binding to the DSC3 region that are involved in splicing and also RNA and DNA binding. MS analysis revealed that DSC2 appears to mainly interact with heterogeneous nuclear ribonucleoproteins (HNRC1–4, HNRPC), which are involved in DNA methylation-sensitive splicing and spliceosome assembly. 29 DSC12 also shows the formation of protein–DNA complexes with heterogeneous nuclear ribonucleoprotein G-like 1 (RMLXL1), which is involved in pre-mRNA splicing. SVA-mediated aberrant splicing of TAF1 in XDP was previously reported. 8 Thus, it is tempting to speculate that the already known splice variants of TAF1 could also be influenced by DNA methylation of the DSCs via these methylation-sensitive ribonucleoproteins. DSC2 is located distal and DSC12 proximal to the SVA retrotransposon, and their putative effect on alternative splicing or exon retention has to be elucidated in future experiments. In addition to splicing, DSC12 might also form DNA–protein complexes with members of the nuclear factor 1 transcription factor family (NFIA, NFIB, NFIC, and NFIX), which are known to be involved in neurogenesis without neurological disease context 30 and are shown to be sensitive to DNA methylation. 31

Because of the limited availability of brain tissues from sufficient affected individuals, expression and DNA methylation of the ubiquitously expressed TAF1 gene were investigated in leukocytes of patients and controls. It is known that testing DNA methylation in cells purified from blood samples is affected by cell type composition and differences in DNA methylation levels between individuals might only be caused by a different composition of blood cell types. 32 Moreover, DNA methylation is highly tissue specific, and leukocytes might not accurately reflect DNA methylation of disease-affected tissues. Therefore, we analyzed DNA methylation in the brains of 2 deceased XDP patients and concluded that TAF1 DNA methylation is comparable between tissues. Nevertheless, the small sample size and lack of ethnically matched controls limit the interpretation of these data. Another limitation is the small sample size of female subjects with XDP. Given that XDP is an X-linked disease, only a few women
with skewed X-chromosome inactivation or only 1 functional X chromosome are affected.

In summary, we determined that DNA methylation is a possible mechanism by which TAF1 expression or splicing could be modulated, even if the implicated variations are located in noncoding regions. Interestingly, both effects on promoter activity and on protein binding at DSC2 are independent of the sequence variation and are solely from changes in DNA methylation levels as promoter activity and protein binding were unchanged when comparing mutated with wild-type sequences in the unmethylated state. At DSC3, sequence variation and DNA methylation have an effect on promoter activity and protein binding. Our findings therefore offer new insights into molecular mechanisms regulating TAF1 expression relevant for the understanding of XDP pathogenesis. Therapeutic approaches to modulate DNA methylation are already used in the treatment of different types of cancer and may also provide new perspectives for the treatment of XDP patients.

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Supporting Data

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