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SHORT REPORT

Refining the 9q34.3 microduplication syndrome reveals mild neurodevelopmental features associated with a distinct global DNA methylation profile

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Abstract

Precise regulation of gene expression is important for correct neurodevelopment. 9q34.3 deletions affecting the *EHMT1* gene result in a syndromic neurodevelopmental disorder named Kleefstra syndrome. In contrast, duplications of the 9q34.3 locus encompassing *EHMT1* have been suggested to cause developmental disorders, but only limited information has been available. We have identified 15 individuals from

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10 unrelated families, with 9q34.3 duplications <1.5 Mb in size, encompassing *EHMT1* entirely. Clinical features included mild developmental delay, mild intellectual disability or learning problems, autism spectrum disorder, and behavior problems. The individuals did not consistently display dysmorphic features, congenital anomalies, or growth abnormalities. DNA methylation analysis revealed a weak DNAm profile for the cases with 9q34.3 duplication encompassing *EHMT1*, which could segregate the majority of the affected cases from controls. This study shows that individuals with 9q34.3 duplications including *EHMT1* gene present with mild non-syndromic neurodevelopmental disorders and DNA methylation changes different from Kleefstra syndrome.

KEYWORDS
9q34.3 duplication, DNA methylation, *EHMT1*, neurodevelopmental disorder

1 | INTRODUCTION

Brain and neuronal development are complicated processes requiring precise regulation of gene expression.[1,2] Therefore, disrupted genes encoding factors that modify chromatin (so-called epigenetic machinery), are a common cause of monogenic neurodevelopmental disorders (NDDs).[2]

EHMT1 is a member of the epigenetic machinery. Through the complex with EHMT2 and other proteins, it is involved in gene expression and chromatin structure regulation by histone-3 lysine-9 methylation.[3,4] *EHMT1* haploinsufficiency results in syndromic NDD named Kleefstra syndrome (KS), previously known as 9q34.3 deletion syndrome (OMIM:#610253).[4,5] KS is mainly characterized by moderate–severe intellectual disability, recognizable facial features, hypotonia, microcephaly, short stature, and congenital anomalies.[5] Additionally, individuals with KS have specific DNA methylation changes.[6,7]

Recently, a large cohort study shown that the majority of haploinsufficient genes are predicted to also be triplosensitive, including *EHMT1*.[8] While haploinsufficiency of *EHMT1* is well-known, only several individuals with 9q34.3 duplications of variable size have been described.[9] Therefore, in this study, we aimed to describe clinical, molecular and DNA methylation features of individuals with small 9q34.3 microduplications entirely encompassing *EHMT1*.

2 | METHODS

For this study, we have focused on individuals with small 9q34.3 microduplications (<1.5 Megabases in size) entirely encompassing *EHMT1* without other known haploinsufficient or triplosensitive genes. We have collected clinical and molecular data of 15 cases from 10 unrelated families via the Radboudumc expertise center, international collaborations, and literature. All individuals consented for the study. Two of the cases have been published previously by Bonati et al.[9] The duplications were identified in diagnostic settings using chromosomal microarrays or exome sequencing. To confirm breakpoints, PCR-free genome sequencing was performed for two individuals, as described before.[10]

For 11/15 included cases, blood-derived DNA was available for methylation analysis (Table S1). The analysis of global DNA methylation profile was performed based on our laboratory's previously described methods (Supplementary methods).[6,11]

3 | RESULTS

To define the clinical and molecular spectrum of individuals with 9q34.3 duplications, we have recruited 15 individuals from 10 families (Tables 1 and S1). In 4/15 cases, the duplication has been confirmed to occur *de novo*, while in 8/15 cases, it was inherited from a mildly affected parent and in 3/15, the inheritance was unknown.

Most of the identified duplications (8/10) were <1 Mb in size (~0.3–1.4 Mb), containing from 3 to 53 protein-coding genes. The duplication positions with genic content are depicted in Figure 1 and provided in Table S1. Only *EHMT1* and *ARRDC1* genes overlap all duplications. To confirm and specify the (de novo) duplications in two cases (P2 and P1 from Bonati et al.), genome sequencing was performed which confirmed the duplications contain full-length *EHMT1* and are in tandem.

The most prevalent feature among the individuals was mild developmental delay (DD) (present in 92%) and mild intellectual disability (ID) or learning problems (77%). Additionally, these individuals commonly presented with autism spectrum disorder (38%) and/or other behavior problems (57%) like aggression, anxiety etc. Similar to KS, sleeping issues were commonly reported (40%), but were not associated with psychoses (Tables 1 and S1).

These individuals did not display recognizable or prominent dysmorphic features, nor had any congenital anomalies. One adult individual (P8, brother of P7), was reported to have no neurodevelopmental or neurological symptoms, suggesting incomplete penetrance, but we cannot exclude presence of mild symptoms.

TABLE 1 Main clinical features among individuals with 9q34.3 copy number variants.

Feature	Frequency among 9q34.3 duplication cohort $N = 15$ (%)
Inheritance	4/15 *de novo* (27%) (6 paternal/2 maternal/3 unknown)
Sex (males/total)	10/15 (67%)
Growth	
Low birth weight	1/9 (11%)
Overweight or obesity[KS]	2/13 (15%)
Short stature[KS]	1/13 (8%)
Abnormal head circumference[KS]	0/10 (0%)
Neurodevelopmental and psychiatric issues	
Language/speech delay[KS]	12/13 (92%)
Motor delay[KS]	8/12 (67%)
Intellectual disability[KS] or learning problems	10/13 (77%)
Autism spectrum disorder[KS]	5/13 (38%)
Behavior problems, not autism spectrum	8/14 (57%)
Psychoses or Schizophrenia[KS]	0/15 (0%)
Neurological issues	
Seizures[KS]	2/15 (13%)
Hypotonia[KS]	2/14 (14%)
Sleep disturbances[KS]	4/10 (40%)
Congenital anomalies	
Congenital heart disease[KS]	0/15 (0%)
Cleft lip or palate	0/15 (0%)
Genitourinary abnormalities[KS]	0/8 (0%)

Abbreviations: KS, features typical for Kleefstra syndrome.

To determine whether 9q34.3 duplications encompassing *EHMT1* (further named "EHMT1dup" for simplicity) would cause DNA methylation changes, we compared 10 affected cases against controls. We identified 261 differentially methylated probes (Table S2) associated with EHMT1dup, but did not identify any significant DMRs. We demonstrated that the selected DMPs were capable of segregating the majority of the affected EHMT1dup cases from controls using unsupervised clustering (Figure 2A,B). Using the constructed SVM classifier (Figure 2C), all EHMT1dup positive cases showed a methylation variant pathogenicity (MVP) score close to 1 compared with the negative cases close to 0. We observed no elevation in MVP score for any KS cases. One case (P1 from Bonati et al.) did not share the same DNA methylation changes and was classified as "negative" (Figure 2). Additionally, leave-one-out cross validation suggested low sensitivity of the identified methylation profile (Figure S1).

Additionally, the classifier did not show complete specificity for the 9q34.3 duplications, as two of the ADCADN (OMIM:# 604121) training, and two testing samples, and one case each from the CSS9 (OMIM:#615866), MRXSCJ (OMIM:#300534) and WHS (OMIM:#194190) cohorts, had elevated MVP scores. When compared EHMT1dup separately with the ADCADN episignature, we observed that the methylation profiling can distinguish between the two conditions (Figure S2A,B). Therefore, the identified DNAm changes are mild and not fully sensitive and specific, so we call them as EHMT1dup DNA methylation "profile" rather than "episignature."

Finally, we compared the EHMT1dup samples with KS samples[6] (Figure S2C,D). We observed that the KS cases do not overlap with EHMT1dup. We observed that the EHMT1dup DNA methylation profile contained 162 (162/261, 62%) hypomethylated CpGs compared with 132 (132/136, 97%) in the KS profile. Therefore, both cohorts' DMPs are predominantly hypomethylated, with no DMPs overlap between the two cohorts but different DMPs overlapping the same gene: *TRAPPC9* (OMIM:#611966). *TRAPPC9* is associated with autosomal recessive intellectual developmental disorder (OMIM:#613192).

4 | DISCUSSION

In this study, we further define that individuals with 9q34.3 duplications encompassing *EHMT1* share non-syndromic NDD with mild DD/ID, autism spectrum disorder and behavioral problems, as well as a common DNA methylation profile. Importantly, 9q34.3 duplications involving *EHMT1* and nearby genes are not found in healthy control cohorts[8,13] and contain several genes predicted to be triplosensitive (pTriplo >0.9), notably, including *EHMT1*.[8] While the clinical features are non-specific and variable, common genetic findings and DNA methylation profile likely confirm the 9q34.3 microduplications as the main cause of the NDD among the described individuals.

Though *EHMT1* is a compelling candidate for being the main 9q34.3 triplosensitivity driver,[9] it is almost impossible to have an isolated duplication of a single gene in this region because of the high gene density in 9q34.3. All duplications described here include at least one additional gene that is predicted to be triplosensitive, e.g. *ZMYND19* (pTriplo = 0.91) or *CACNA1B* (pTriplo = 1).[8] *ZMYND19* has not been associated with a human phenotype, but *CACNA1B* is associated with a recessive NDD with epilepsy.[14] To reduce the bias and possible contribution by other genes, individuals with large duplications were not included in the study.

Clinical features, as well as DNA methylation changes associated with the duplications are distinct from those found among KS individuals.[5] Cellular effects of the *EHMT1* overexpression vs. loss are also different: for example, *EHMT1* is overexpressed in many cancers, and it induces cell proliferation and resistance to treatment, while *EHMT1* loss results in cell apoptosis or reduced proliferation.[15,16] In *Drosophila*, both the *EHMT1* ortholog overexpression and loss resulted in reduced learning and memory but were more prominent for loss.[17] This indicates that *EHMT1* expression is highly dosage sensitive for correct neurodevelopment.

FIGURE 1 Genomic view of the 9q34.3 regions with microduplications.[12] Genes are colored based on the pTriplo (from 0 to 1; light blue to dark blue, accordingly) and pHaplo scores (from 0 to 1; light gray to black, accordingly). pTriplo or pHaplo score values are shown after the gene names with higher score representing higher level of predicted dosage sensitivity. [Colour figure can be viewed at wileyonlinelibrary.com]

Interestingly, the 9q34.3 duplication DNA methylation profile is not "mirroring" or overlapping the KS episignature, as has been demonstrated for some reciprocal microdeletion/microduplication syndromes.[18] It is possible that the difference in cellular effects of the overexpression vs. loss results in different DNA methylation changes. It is also possible that the profile identified among the 9q34.3 microduplication cases results from overexpression of several genes or another gene (e.g., *ZMYND19*), as has been shown for 22q13.3 deletion.[19] Noticeably, the individual who was classified negatively on the DNA methylation profile (P1 from Bonati et al.), has the most distal duplication with the least genes included (besides *EHMT1*, includes only *CACNA1B* and *ARRDC1*), which might explain the negative results. By using genome sequencing the duplication in this case was confirmed to include the entire *EHMT1* gene, as well as previously was shown to result in *EHMT1* overexpression.[9]

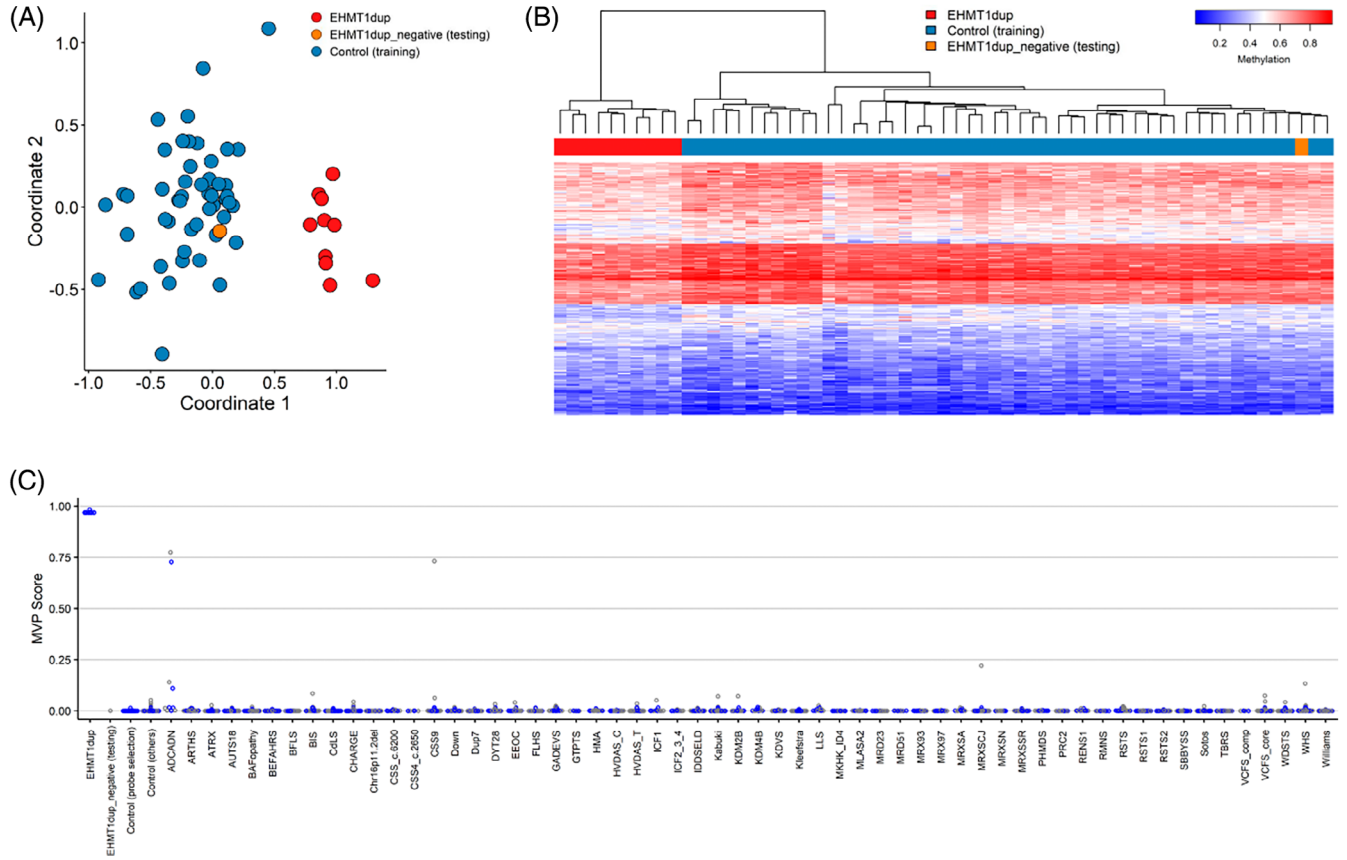

FIGURE 2 9q34.3 duplication encompassing *EHMT1* (EHMT1dup) DNA methylation profile. (A) Multidimensional scaling plot shows clustering of the EHMT1dup cases (red) together and away from controls (blue) and one negative case (orange); (B) Heatmap indicates clear separation of the EHMT1dup cases from controls using the identified 261 DMPs; (C) The classification results using EHMT1dup SVM model. [Colour figure can be viewed at wileyonlinelibrary.com]

While *EHMT1* is one of the main 9q34.3 triplosensitive genes, a combination of duplicated genes likely contributes to the 9q34.3 microduplication phenotype and, possibly, to the DNA methylation changes. The identified DNA methylation profile is mild and more cases with different breakpoints are necessary to clarify the main drivers of the methylation changes and triplosensitivity of the 9q34 region.

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CONFLICT OF INTEREST STATEMENT

B.S. is a shareholder in EpiSign Inc., a biotechnology company involved in commercialization of EpiSign™ technology.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/cge.14498.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article. All identified duplications are submitted to ClinVar (accession numbers: SCV004027852-SCV004027862). Raw DNA methylation data are not available due to institutional and ethics restrictions.

ETHICS STATEMENT

This study was conducted in accordance with the regulations of the Western University Research Ethics Board (REB116108; REB106302) and the ethics committee of Arnhem-Nijmegen (Nr.2018-4540).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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