Comprehensive *EHMT1* variant interpretation provides insight into *EHMT1* functions

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Content

- *EHMT1* structure and functions
- *EHMT1* protein altering variant (PAV) characterization
- *EHMT1* N-terminal truncating variants
EHMT1: Structure and functions

Largely disordered region with unknown functions

Figure modified from: «Histone post-translational modifications as potential therapeutic targets for pain management», Torres-Perez et al., 2021
**EHMT1**: Structure and functions

Largely disordered region with unknown functions

Figure modified from: «Histone post-translational modifications as potential therapeutic targets for pain management», Torres-Perez et al., 2021
**EHMT1**: Structure and functions

- Largely disordered region with unknown functions

Dimer formation with EHMT2

Enzymatic function

Figure modified from: «Histone post-translational modifications as potential therapeutic targets for pain management», Torres-Perez et al., 2021
**EHMT1: Structure and functions**

- **Enzymatic function**
- **Reader function** – bringing the complex to H3K9

- Largely disordered region with unknown functions

Figure modified from: «Histone post-translational modifications as potential therapeutic targets for pain management», Torres-Perez et al., 2021
**EHMT1**: pathogenic variant spectrum

*EHMT1 / 9q34.3 deletions = Kleefstra syndrome*

- **ANK repeat**: ANK repeat [structural motif]
- **Pre-SET**: Pre-SET motif
- **SET**: SET (Su(var)3-9, Enhancer-of-zeste, Trithorax) domain
**EHMT1**: pathogenic variant spectrum

**EHMT1** / 9q34.3 deletions = Kleefstra syndrome
**EHMT1**: few known pathogenic missense

**EHMT1** / 9q34.3 deletions = Kleefstra syndrome
Many *EHMT1* protein altering variants are identified using exome sequencing.

37 individuals with NDD and 33 different *EHMT1* protein altering variants (PAVs)
We sought to classify identified PAVs using:

1. Clinical features

2. Effects on protein 3D structure

3. DNA methylational signature

4. *In vitro* functional assays

By prof. B. Sadikovic

By Ayumi Yamada & prof. Yoichi Shinkai
DNA methylation-based test

By Mike Levy and Bekim Sadikovic
Controls
Kleefstra syndrome
Negative testing cases
Positive testing cases

BY Mike Levy and Bekim Sadikovic
PAV classification results

Kleefstra syndrome episignature:
- Negative (black)
- Positive (blue)

- Gly121Ser
- Asp197Asn
- Leu250Phe
- Lys302Arg
- Met315Ile
- Gly411Ser
PAV classification results

Kleefstra syndrome episignature:
- Negative
- Positive

30/33 variants concordant results

11 are benign
19 are pathogenic
PAVs are a common cause of Kleefstra syndrome

Causes of Kleefstra syndrome at Radboudumc based using exome sequencing (2013-2022)

- Intergenic CNVs: 50%
- Intragenic Truncating: 30%
- Missense/In-frame: 20%

N=20
PAVs are a common cause of Kleefstra syndrome

Causes of Kleefstra syndrome at Radboudumc based using exome sequencing (2013-2022)

- **Intergenic CNVs**: 20%
- **Intragenic Truncating**: 50%
- **Missense/In-frame**: 30%

N=20

---

De novo EHMT1 point variants in DDD study

- **Intergenic CNVs**: 25%
- **Intragenic Truncating**: 75%

N=16

By Arianne Bouman and Martina Ruiterkamp

Kaplanis et al., 2020
Discordant PAVs

30/33 variants concordant results

11 are benign

19 are pathogenic

Kleefstra syndrome episignature:

- Negative
- Positive
- Discordant (negative)

3/33
Functional testing of the variants

1. Binding capability to EHMT2
2. Methylational activity
3. Binding capability to H3K9me1

Figures modified from Sanches et al., 2021
Functional testing of the variants

1. Binding capability to EHMT2
2. Methylation activity
3. Binding capability to H3K9me1

Figures modified from Sanches et al., 2021
Ankyrin-repeat domain variants

H3 tail

Kleefstra syndrome causative missense
Ankyrin-repeat domain variants

**p.Arg948**
**p.Trp912**
**p.Pro809**

**peptide binding assay**

**WT**
**P809L**
**W912R R948W**

- unmethyl
- me1
- me2
- me3

**ANK-repeat domain**
**H3 tail**
**Kleefstra syndrome causative missense**
Results of functional testing

<table>
<thead>
<tr>
<th>Domain</th>
<th>H3K9me2 Methylation Activity</th>
<th>EHMT2 Binding Ability</th>
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<tr>
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| ANK      |                            |                       |

Benign variant  KS-causative missense  KS non-causative missense
Results of functional testing

SET domain

ANK domain

Have no effect on EHMT2 binding or enzymatic activity
Results of functional testing

<table>
<thead>
<tr>
<th>SET domain</th>
<th>ANK domain</th>
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<td>H3K9me2 methylation activity</td>
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- Disrupt EHMT2 binding AND enzymatic activity
- Have no effect on EHMT2 binding or enzymatic activity

Benign variant: black
KS-causative missense: blue
KS non-causative missense: purple
Results of functional testing

SET domain

ANK domain

H3K9me2 methylation activity

EHMT2 binding ability

- Disrupt enzymatic activity only
- Disrupt EHMT2 binding AND enzymatic activity
- Have no effect on EHMT2 binding or enzymatic activity
Results of functional testing

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<tr>
<th>Domain</th>
<th>Variant</th>
<th>H3K9me2 methylation activity</th>
<th>EHMT2 binding ability</th>
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Neutral?
Disrupt enzymatic activity only
Disrupt EHMT2 binding AND enzymatic activity
Have no effect on EHMT2 binding or enzymatic activity
How $EHMT1$ can lose enzymatic activity but do not have Kleefstra syndrome episignature?
EHMT1 functions: summary

1. Dimer/complex formation
   \[ \text{EHMT2} + \text{EHMT1} +/\!\!/- \text{other proteins} \]
EHMT1 functions: summary

1. Dimer/complex formation

2. Reader function – bringing the complex to H3K9

Figure modified from: «Histone post-translational modifications as potential therapeutic targets for pain management», Torres-Perez et al., 2021
EHMT1 functions: summary

1. Dimer/complex formation
   \[EHMT2 + EHMT1 +/− \text{other proteins}\]

2. Reader function – bringing the complex to H3K9

3. Histone methylation??
EHMT1 functions: summary

1. Dimer/complex formation

2. Reader function – bringing the complex to H3K9

3. Histone methylation??

3. Non-Histone methylation
**EHMT1 functions: summary**

1. **Dimer/complex formation**
2. **Reader function – bringing the complex to H3K9**
3. **Histone methylation?**

---

**Classical Kleefstra syndrome**

3. **Non-Histone methylation**
EHMT1 functions: summary

1. Dimer/complex formation

2. Reader function – bringing the complex to H3K9

3. Histone methylation??

Non-Kleefstra syndrome?

3. Non-Histone methylation
Summary about *EHMT1* PAVs

1. Patogenic missense/in-frame indels are a common but underappreciated cause of Kleefstra syndrome

2. PAVs in ANK repeat domain disrupting H3 binding is sufficient to cause Kleefstra syndrome

3. PAVs in SET domain disrupting EHMT2 binding, but not enzymatic activity, is sufficient to cause classical Kleefstra syndrome
EHMT1: N-terminal truncating variants
EHMT1 / 9q34.3 deletions = Kleefstra syndrome
Characterization of a Novel Transcript of the *EHMT1* Gene Reveals Important Diagnostic Implications for Kleefstra Syndrome

Willy M. Nillesen,† Helger G. Yntema,† Marco Moscarda,2 Nienke E. Verbeek,3 Louise C. Wilson,4 Frances Cowan,5 Marga Schepens,1 Annick Raas-Rothschild,6 Orly Gafni-Weinstein,6 Marcella Zollino,2 Raymon Vijzelaar,7 Giovanni Neri,2 Marcel Nelen,1,3 Hans van Bokhoven,1 Jacques Giltay,3 and Tjitske Kleefstra1*

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**EHMT1** / 9q34.3 deletions = Kleefstra syndrome

**EHMT1** TSS and exon 1 deletion = Kleefstra syndrome
N-terminal truncating variants

[Diagram showing protein length and variants]
N-terminal truncating variants

Protein length

EHMT1: NM_024757.4

Karyotype 47, XXX
EHMT1:p.(Arg12fs*)
EHMT1:p.(Glu14fs*)

EHMT1:p.(Glu14fs*)
EHMT1:p.(Glu14fs*)
EHMT1:p.(Glu14fs*)
pathogenic ASXL3
EHMT1:p.(Ala8fs*)
EHMT1:p.(Ala8fs*)
EHMT1:p.(Glu14fs*)

EHMT1:p.(Ala8fs*)
EHMT1:p.(Glu14fs*)
EHMT1:p.(Glu14fs*)
EHMT1:p.(Arg12fs*)
EHMT1:p.(Glu14fs*)
EHMT1:p.(Glu14fs*)
EHMT1:p.(Glu14fs*)
N-terminal truncating variants

EpiSign results for Kleefstra syndrome

Inconclusive

Negative
EpiSign: N-term truncating variants

- Pathogenic truncating
- N-term truncating
- Kleefstra syndrome
- Controls
N-term truncating variants: summary

[Diagram showing protein length with variants indicated for EHMT1]
Pathogenic hypomorphic variants with incomplete penetrance vs. benign?
N-term truncating variants: summary

Pathogenic hypomorphic variants with incomplete penetrance vs. benign?
Role of N-term 31 aminoacids?

N-acetylalanine

Karyotype 47, XXX
EHMT1:p.(Arg12fs*)
EHMT1:p.(Glu4fs*)
EHMT1:p.(Glu14fs*)
EHMT1:p.(Glu14fs*)
pathogenic ASXL3
EHMT1:p.(Ala8fs*)
EHMT1:p.(Glu14fs*)
Take-home messages

Comprehensive variant characterization found among affected individuals allows to identify:
1. Main protein/gene functions
2. Disease mechanisms
3. Specific sub-phenotypes
4. Pathogenic variant spectrum

We still do not know too much about EHMT1 functions and Kleefstra syndrome pathogenesis
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Haley McConkey

Yoichi Shinkai
Ayumi Amada

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By Ayumi Yamada and Yoichi Shinkai

<table>
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<th>stability</th>
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