Research Articles

From a Single Gene Defect Towards a Cross Species Neurocognitive Phenotype: The EHMT1 Disruption Example (Kleefstra Syndrome)

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Abstract

Aim: Neuropsychiatric disorders comprise a clinically heterogeneous group of conditions that share underlying molecular and pathological mechanisms. Knowledge about how molecular pathways affect cognition and emotion is highly fragmentary. The Euchromatin Histone Methyl Transferase 1 (EHMT1) gene encodes a protein involved in chromatin modification and is presumed to have an important role in the etiology of cognitive and emotional dysfunctions. While animal studies with EHMT1 mutant species showed deviations in learning, attention and social cognition, systematic studies investigating behavioral characteristics in humans have been hardly conducted. Adult case studies showed behavioral disturbances with a sudden decline in functioning together with sleep problems. The focus of this report is to describe symptoms and developmental aspects in children with EHMT1 defects and compare these two previous studies in adults and animal models.

Methods: Four children with EHMT1 defects were investigated on adaptive functioning, psychiatric symptoms and temperament.

Results: All subjects have a severe developmental delay and meet the criteria for autism spectrum disorders, irrespective of severity of their intellectual disability. Three subjects have extreme sleep problems, while two subjects also have anxiety and mood disorders. Temperament is characterized by deficits in attentional focusing and inhibition.

Conclusion: These results show that EHMT1 defects severely affect cognitive processing, behavior and emotional functioning in children. This is in agreement with previous observations in animal studies and adult patients. The important role of EHMT1 in cognitive processing suggests that disruptions in a single gene can result in a broad spectrum of psychiatric pathology in human.

Keywords: Kleefstra syndrome; EHMT1 gen; Intellectual disability; Autism; Sleep; Behavior

Introduction

Twin, adoption and molecular genetic studies have produced evidence that genetic factors are significant contributors to the etiology of several neuropsychiatric disorders and related traits. Although the genetic architecture of these disorders is complex, it has become evident that rare, moderate risk variants such as de novo Copy Number Variants (CNVs) are an important contributor to the disease risk [1-5]. Identification of harmful CNVs may be challenging, but even more difficult is defining how such genomic changes affect biological pathways and result in disease.

Known disruptions in a specific gene, well described in terms of biological functioning and associated phenotype will be of vital importance to understand the role of CNVs in neuropsychiatric disorders. Disruptions of EHMT1 are a clear example of a specific genetic abnormality that results in psychiatric symptoms. In a recent study using Next Generation Sequencing in humans with various cognitive dysfunctions, EHMT1 was identified as the only common mutated gene in an overlapping group of cognitive disorders: Intellectual Disability (ID), autism and schizophrenia [6]. The EHMT1 gene is located on 9q34 at the subtelomeric end. Either 9q34 micro deletions or intragenic EHMT1 mutations can similarly lead to impaired cognitive functioning [7]. The gene is expressed widely in neural tissue strongly suggesting that the EHMT1 gene plays an important role in neurodevelopment. The enzyme, for which it encodes, is capable to methylate lysine 9 of histone 3 (K9H3 methylase). Consequently, changes in chromatin modulation occur, thereby influencing epigenetic processes of gene regulation [8,9].

Importantly, the EHMT1 gene is studied cross species. Behavioral studies in Drosophila melanogaster and mice with EHMT1 defects have been performed [10,11] and show strong associations between EHMT defects and neurocognitive abnormalities. For example, in Drosophila, neurodevelopment and behavioral analyses identified EHMT as a regulator of larval loco motor behavior, non-associative learning, and courtship (complex) memory. Moreover, memory dysfunction was repaired by EHMT1 re-expression during adulthood.
indicating that cognitive defects are reversible in EHMT Drosophila mutants [11]. Additionally, the study of heterozygous EHMT1 knock-out mice demonstrated difficulties in the processing of novel stimuli, higher levels of anxiety and a deviation in social reaction to strangers [10].

In humans, mutations of EHMT1 result in a clinical syndrome, known as Kleefstra Syndrome (KS) or chromosome 9q sub telomere deletion syndrome [OMIM 610253]. Worldwide about 150 cases of this syndrome are reported so far [12-14]. The clinical picture of this syndrome is dominated by intellectual disability, hypotonia and other genes (3.2 Mb). Patient 3 had a 9q34.3 micro deletion encompassing entire 9q34.3 deletion syndrome [OMIM 610253]. Worldwide about 150 cases of this syndrome are reported so far [12-14]. Patient 1, 2 and 4 had an intragenic EHMT1 mutation; patient 8 respectively in [7, 14], patient 4 was reported as patient 21 in [14] . Patient 2 and patient 3 were reported as patient 4 in short in previous clinical genetic studies; patient 1 was reported as patient characteristics and developmental aspects are listed in table 1. Detailed descriptions of the subjects are added in the appendix. The medical and genetic characteristics of all cases have been published in short in previous clinical genetic studies; patient 1 was reported as patient 21 in [14] . Patient 2 and patient 3 were reported as patient 4 and patient 8 respectively in [7, 14], patient 4 was reported as patient 27 in [14]. Patient 1, 2 and 4 had an intragenic EHMT1 mutation; patient 3 had a 9q34.3 micro deletion encompassing entire EHMT1 and other genes (3.2 Mb).

### Patient Characteristics

<table>
<thead>
<tr>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age/ gender</strong></td>
<td><strong>Genetic defect</strong></td>
<td><strong>Developmental characteristics</strong></td>
<td><strong>Genetic defect</strong></td>
</tr>
<tr>
<td>5 years, male</td>
<td>EHMT1 mutation c.2785_2788del (p.Ser929fs)</td>
<td>Floppy baby</td>
<td>11, female</td>
</tr>
<tr>
<td>10, male</td>
<td>EHMT1 mutation c.3409C=T (p.R1137*)</td>
<td>Floppy baby</td>
<td><strong>Developmental characteristics</strong></td>
</tr>
<tr>
<td>32</td>
<td>Blood loss and minimal fetal movements during pregnancy.</td>
<td>Floppy baby</td>
<td>41</td>
</tr>
<tr>
<td>32</td>
<td>Meconium in amniotic fluid</td>
<td>Floppy baby</td>
<td>41</td>
</tr>
<tr>
<td>2950 g</td>
<td></td>
<td>Frogs baby</td>
<td>3,2 Mb (137,04-140,21 MB, UCSC, NCBI build 36.1)</td>
</tr>
</tbody>
</table>

#### Table 1: Patient Characteristics.
Cognitive and behavioral measures

(Mal) Adaptive functioning. VABS, the dutch adaptation of the vineland adaptive behavior scale (VABS); [17]: This is a widely used clinical interview to determine the level of adaptive functioning of people with an intellectual disability. The VABS consists of 3 domains: communication skills, daily living skills and social skills. This instrument has a good reliability and validity in this specific population [18]. Primary caregivers were interviewed about the participants.

The mini psychiatric assessment schedules for adults with developmental disabilities (mini PAS-ADD); [19]; in dutch translation [20]: This is used to determine behavioral problems and psychiatric disease in subjects with an intellectual disability by interviewing the proxy. It consists of 86 items on a 4-point scale: 0 (symptom not present) - 3 (symptom is severe). The interview is divided into seven subscales: Depression, Anxiety, Obsessive/ Compulsive disorder, Hypomania/Mania, Psychosis, Unspecified disorder and Autism. All criteria are based on the International Classification of Diseases (ICD-10). This instrument has proven psychometric qualities in this specific ID adult population, but not yet in a population sample with children [20,21].

Clinical assessment: The assessment started with observation of the participant, while (s) he was playing/interacting with his (her) mother. Subsequently, the observer took over the play and tested reactions to several toys; a toy telephone, a doll with a feeding bottle, building-blocks, a picture book, a plastic tea set and a song. The interviews and assessments were conducted in the same order by the first author (KV). All the assessments were videotaped.

Child behavior checklist (CBCL/1,5-5) [22]; dutch version [23]: This questionnaire measures problem behavior on a 3-point scale: 0=symptom is absent, 1=sometimes present, 2=often present. It is intended for children between 1, 5 and 5 years of (developmental) age and is completed by the parent(s). It consists of 100 items, which reflect problem behaviors. The psychometric properties of this questionnaire were proved in a population sample of Dutch normally developing children [23] as well as in a sample of children with an intellectual disability [24].

Children’s behavior questionnaire (CBQ) [25]: Measures temperament on 16 subscales: activity level, anger/frustration, approach/anticipation, attentional focusing, attentional shifting, discomfort, falling reactivity and soothability, fear, high pleasure, impulsivity, inhibitory control, low pleasure, perceptual sensitivity, sadness, shyness and smiling and laughter. The questionnaire should be completed by parents on a 7-point rating scale (1=not applicable till 7=totally applicable). It is intended for children with a developmental age between 3 and 8 year. The psychometric properties are proved in a Dutch sample [26].

Social communication questionnaire: (SCQ); [27]; in Dutch: [28]) detects problems in social behavior and features of autism spectrum disorders. It is based on items of the Autism Diagnostic Interview-Revised(ADI-R) [29]. Its subcategories correspond to the three domains of autism in the DSM-diagnosis. It supplies a 40 item binary scale, which is completed by the primary caregiver(s).

The data are presented in the next section, subdivided into the following categories: adaptive behavior, temperament and maladaptive behavior.

Results

The components of adaptive behavior (VABS) are graphically represented in figure 1 as deciles scores, based on the norm group of people with a severe intellectual disability. All subjects had a severe developmental delay. A common deficit in social skills is shown for all subjects. The raw scores are presented in Table 2.

Results on temperament (CBQ) are shown in (Figure 2), where the mean scores of the children with KS were plotted against the mean of toddlers from the general population [25]. CBQ scores of the children with KS were remarkably low on the ‘attentional focusing’ and ‘inhibitory control’ categories and relatively high on the categories ‘low intensity pleasure’ and ‘smiling and laughter’.

Table 2: Results on the VABS, CBCL and SCQ.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vineland score (developmental age in years; months)</td>
<td>110 (1; 7)</td>
<td>46 (11m)</td>
<td>52 (1;0)</td>
</tr>
<tr>
<td>Communication skills</td>
<td>41 (2;2)</td>
<td>10 (0;1)</td>
<td>15 (1;1)</td>
</tr>
<tr>
<td>Daily life skills</td>
<td>44 (2;0)</td>
<td>24 (1;4)</td>
<td>17 (1;1)</td>
</tr>
<tr>
<td>Social skills</td>
<td>25 (1;3 )</td>
<td>12(&lt;0;11)</td>
<td>20 (0;11)</td>
</tr>
</tbody>
</table>

CBCL: categories in the clinical range (t-score):

Emotionally Reactive x (80) 0 x(87.5) x(70)
Anxious-Depressed 0 0 x (75) x (75)
Somatic Complaints 0 0 x (87.5) x (75)
Withdrawn x(70) x (85) x (95) x (75)
Sleep problems 0 0 x (75) 0
Attention Problems x (85) 0 x (75) x (80)
Aggressive behavior x (90) 0 x (70) 0
SCQ (total score) 24 22 23 26

Vineland: higher scores represent more abilities (comparable to developmental age in years; months)

CBCL: clinical scores: x= present, 0=absent, (t-scores)

SCQ: cut-off >11
Symptom categories of maladaptive behavior are measured by using the CBCL. All subjects scored in the clinical range for withdrawn behavior. Emotional reactivity scores and attention problems scores were also in the clinical range for patients 1, 3 & 4. The results within the clinical range are presented in Table 2, together with the raw scores. Results on the Mini PAS-ADD and clinical assessment are presented in Table 3. They show a heterogeneous but psychiatric picture. All the children fulfilled the mini PAS-ADD criteria for an Autism Spectrum Disorder, accompanied by extreme avoidance of contact in two of them and aggressive behavior in one of them. The majority of the subjects fulfilled the mini PAS-ADD criteria for Anxiety Disorder, Sleep Disorder and Mood Disorders. Psychotic symptoms in the past are reported in patient 3. All subjects scored well above the cut-off (11) on the SCQ [30].

Discussion

This first study on the cognitive and neurobehavioral characteristics of children with KS strongly suggests that deficits in attention, cognitive processing and sleep are associated with a severe neuropsychiatric outcome, characterized by autism spectrum traits and mood and anxiety problems. The combined data presented in this study form a unique picture of neuro-behavioral abnormalities associated with a single but well described genetic abnormality. While the genetic abnormality of the EHMT1 locus is rare, the present study may serve as a proof of principle and template. Indeed, a diagnosis of ASD in the presence of severe developmental delays should only be made when impairments in social skills outweigh the expected loss of social functioning due to Intellectual Disabilities (ID) [31]. The impairments of the patients described here, are clearly seen in social skills, especially in comparison with other genetic syndromes, like Down syndrome, Prader-Willi syndrome and Angel man syndrome [32]. Clinically, it is relevant to distinguish between intellectually disability with and without autism spectrum disorder [33]. An accurate description and diagnosis of the pattern of behavioral skills and difficulties enables professionals to optimize treatment approaches in a personalized treatment.

Besides ASD and developmental delay, sleep problems as well as attention problems are present in our cases. Sleep disturbances have been observed previously in the adult patients with EHMT1 mutations [15,16]. Three of the children described in our report, experience severe problems in falling asleep, often taking several hours. Also, they wake up several hours earlier than expected. While there may be some resemblance with the sleep problems in Smith-Magenis syndrome, which are due to alterations in the melatonin metabolism [34], no information is yet available on the metabolism of melatonin in Kleefstra syndrome.

EHMT1 heterozygous knock-out mice showed hypo activity and autistics like features – less reaction to social cues, reduced exploration and higher levels of anxiety-, in comparison to wild type littermates [10]. These knock-out mice revealed reduced activity and exploration, increased anxiety while exposed to novel environments and diminished social play. In our subjects we demonstrated social withdrawal and high levels of emotional reactivity and anxiety/depression. The attentional and social problems observed during the clinical assessments have also been reported in adults with EHMT1 defects. A study in Drosophila identified the EHMT gene as a key regulator of cognition that orchestrates an epigenetic program featuring classic learning and memory genes [11]. The effects of this mutation on cognition were found to be reversible. This provides a challenge to develop therapeutic opportunities.
Our results should be considered in a broader perspective with genetic research in other conditions. In a large cohort of patients with schizophrenia de novo 9q34.3 deletions encompassing EHMT1 were found in three patients [35]. This indicates that deletions of EHMT1 in humans might result in highly penetrant phenotypes comprising developmental disabilities and several congenital anomalies. In addition, the EHMT1 locus is suspected to contribute to the development of psychopathology through disturbances in epigenetic mechanisms. Our findings as well as the results in adults [15,16] suggest that this is not only the case for schizophrenia, but also for a larger range of psychopathology. This supports the hypothesis that several psychiatric syndromes such as schizophrenia, autism and intellectual disability have common genetic underpinning and share common pathways in the pathogenesis [5,36].

A limitation of this case study is the relatively small number of participants. However, even with this small number of subjects, specific neurobehavioral abnormalities such as deficits in social reciprocity and cognition, sleep disturbances and attention problems stand out. Also, these abnormalities seem to be present in EHMT1 mutant animal models.

We have several recommendations for future research. At first, prospective continuation of the present study can help to further elucidate the behavioral phenotype of the Kleefstra Syndrome. The question is whether these children also experience a loss of functioning later in life, like it was described in adult cases [15], and what is the course of the neurocognitive deficits? Eventually, prospective research may clarify the role of EHMT1 in neurodegenerative processes. Secondly, replication of these findings is needed in a larger cohort of participants. We recommend comparison of results of the animal studies with those in human subjects. Finally, identification of other gene disruptions that operate in the same pathway as EHMT may be highly relevant. Especially when these are combined with cognitive and social data descriptions across species.

In conclusion, our study provides a first overview of the development and behavior in four children with the KS. Their behavioral and developmental profile is characterized by a developmental delay, autism, sleep disturbances and attention problems. Deletion of the EHMT1 gene increases the risk for psychopathology in childhood, followed by a decline in functioning in adulthood. This suggests an important role in epigenetic regulation in the pathophysiological pathway of several psychiatric syndromes. Further clinical as well as fundamental research in this area may help to elucidate the precise role of EHMT1 and its clinical implications.

**Acknowledgement**

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**References**


