A novel likely pathogenic heterozygous HECW2 missense variant in a family with variable expressivity of neurodevelopmental delay, hypotonia, and epileptiform EEG patterns

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Abstract
Pathogenic variants in HECW2 are extremely rare. So far, only 19 cases have been reported. They were associated with epilepsy, intellectual disability, absent language, hypotonia, and autism. As these cases were all de novo mutations, mostly presenting without identical variants, variable expressivity has never been investigated. Here, we describe the first family with the same novel variant in HECW2. A 19-year-old female patient presented with bursts of generalized spike–wave discharges and intellectual disability. We performed next-generation-sequencing, to detect the genetic cause. Next-generation-sequencing revealed a novel likely pathogenic variant in HECW2 (c.3571C>T; p.Arg1191Trp) in the index patient, her mother and brother. They showed some similar phenotypic patterns with intellectual disability, hypotonia and generalized epileptiform patterns. However, the mother was less severely affected and epileptiform patterns were less frequent. The brother presented with additional autistic features. In contrast to previous cases, the speech of all individuals was only mildly impaired. This is the first case report of a family with the same novel likely pathogenic variant in HECW2 and as such provides insight into the phenotypic variability of this mutation. The expressivity of symptoms may be so mild that genetic and EEG analysis are needed to disclose the correct diagnosis.

KEYWORDS
gene expression, genetic variation, genetics, phenotype

1 | BACKGROUND
HECW2 (Chr. 2q32.3, OMIM *617245) belongs to the Nedd4 family of HECT domain E3 ubiquitin ligases (Rotin & Kumar, 2009). By controlling specificity of the ubiquitin modification of proteins targeted for degradation, the E3 ligase has an effect on multiple proteins that have an essential role in neurodevelopment and neurogenesis (Killick et al., 2011).

For example, HECW2 stabilizes p73, a member of the p53 family of tumor suppressors and enhances its transcriptional activity. It also interacts with the anaphase-promoting complex/cyclosome and its activator Cdh1 (Lu et al., 2013).
Clinically, there is growing evidence that HECW2 is among the genes most intolerant to functional variation and mutations often result in neurodevelopmental disorders (Halvardson et al., 2016). Previous exome sequencing studies of neurodevelopmental disorders have identified five de novo missense variants in HECW2 that may cause intellectual disability (ID), developmental delay (DD), autism spectrum disorder, hypotonia, and seizures (Appenzeller et al., 2014; Iossifov et al., 2014; Krumm et al., 2015; Wright et al., 2015). The syndrome caused by different HECW2 variants is also referred to as “Neurodevelopmental disorder with hypotonia, seizures, and absent language” (NDHSAL; “Online Mendelian Inheritance in Man” database, number #617268).

Given the paucity of reports on affected individuals, the phenotype of pathogenic HECW2 variants is currently lacking detailed characterization and requires further investigation. As the existing cases were de novo mutations (DNM) with few identical variants, variable expressivity has not yet been investigated.

We describe the first family with the same novel likely pathogenic HECW2 variant with variable phenotypes and discuss our results in relation to previously reported cases.

## METHODS

Informed consent was obtained for clinical as well as genetic diagnostics and for publication of the data. A clinical geneticist and an adult neurologist supervised the clinical examination and the interpretation of routine electroencephalogram (EEG), long-term video-EEG and genetic findings.

The methods used for molecular analyses were established, validated and provided by CeGaT GmbH, Tübingen, Germany, which is accredited by the national accreditation body of the Federal Republic of Germany according to DIN EN ISO 15189:2014.

Sequence analysis was performed using DNA extracted from either buccal mucosa or peripheral blood cells. For exome sequence analysis of the index patient, her brother and mother, target-in-solution technology was used to enrich the coding and flanking intronic regions. Sequencing reads were then generated by the Illumina HiSeq/NovaSeq systems (Illumina, San Diego, CA 92122) and were subsequently aligned to the human reference genome with the Burrows Wheeler Aligner (BWA-mem 0.7.2, Li & Durbin, 2010).

To identify disease-causing variants, minor allele frequencies (MAF) of the detected variants were taken from an in-house database and from 1000 Genomes, dbSNP, and Genome Aggregation Database. We only evaluated variants (single nucleotide variants/small indels) in coding and flanking intronic regions (±8 bp) with a MAF <1.5% and known disease-causing variants (according to the human gene mutation database) within ±30 bp of flanking regions and up to 5% MAF.

In silico prediction software, namely FATHMM (http://fathmm.biocompute.org.uk/), SIFT (https://sift.bii.a-star.edu.sg/), Mutation Taster (http://www.mutationtaster.org/), and PROVEAN (http://provean.jcvi.org/index.php) were applied to predict the pathogenicity of the variants.

Nomenclature of the variants followed the recommendations of the American College of Genetics and Genomics (Richards et al., 2015).

Conventional Sanger sequencing was used to validate at least one of the variants. For the molecular genetic diagnostics of the father, the relevant region of the HECW2 gene (NM_020760.3) was amplified and directly sequenced via PCR using flanking and internal primer pairs.

### RESULTS

#### 3.1 Clinical report

The index patient is the first of two children born to non-consanguineous parents with European origin (Figure 1). She was born at term by vaginal delivery. At the age of 24 months, the patient started walking with an ataxic wide-based gait and a global development delay with hypotonia, speech and language impairment was diagnosed. At the last assessment, the 19-year-old patient presented with only slightly impaired speech, but with hypotonia and abnormal behavior such as hand clapping and erratic movements to express joy.

Due to intellectual disability, she attended a school for children with learning difficulties and was also working at a sheltered workshop prior to consultation at our center. A routine and long-term video-EEG revealed frequent bursts of generalized spike–wave discharges without clinically overt epileptic seizures. Magnetic resonance imaging of the brain was normal.

Her brother and mother presented a similar but variable phenotype and only had mild language impairments. However, the delayed development, hypotonia, intellectual disability and the absence of professional qualifications were remarkable. The brother also visited a school for children with learning difficulties. Interestingly, the mother’s two siblings were asymptomatic and had qualified educational backgrounds including college and university degrees. Compared to the index patient, the mother was overall less severely affected and had fewer epileptiform EEG patterns that were only evident in prolonged video-EEG recordings. Compared to the index patient, the younger 17-year-old brother had fewer epileptiform EEG patterns with bursts of delta slowing accompanied by abortive and occasional epileptiform discharges (Figure 1). He also presented with autistic features.

The patients’ clinical manifestation and those of previous cases with HECW2 variants are summarized in Figure 2.

#### 3.2 Molecular analyses

Exome and Sanger sequencing revealed a novel heterozygous HECW2 variant in the index patient, her brother and mother: c.3571C>T (Figure 1). This substitution results in an amino acid change at codon 191 (p.Arg1191Trp), and is located in the domain between the regulatory factor X-associated C-terminal binding domain and the
HECT-domain (UniProt, Q9P2P5). This variant is predicted to be deleterious and damaging to protein function by in silico prediction softwares including FATHMM (damaging, score -2.04), SIFT (damaging, score < 0.001), Mutation Taster (disease-causing, 0.9999, R1191W score: 101) and PROVEAN (deleterious, score -7.40). According to the American College of Medical Genetics and Genomics guidelines (Richards et al., 2015), the variant is classified as likely pathogenic by meeting two moderate criteria (PM2, PM5) and two supporting criteria (PP1, PP3). No other (likely) pathogenic variants were detected in known disease-causing genes associated with this phenotype. In the buccal swab sample of the mother, the c.3571C>T; p.Arg1191Trp variant in HECW2 was quantified to be present in 51.25% of the reads (184 of 359 reads).

The father of the index patient was asymptomatic and did not carry this variant. None of the maternal family members were available for genetic testing.

FIGURE 1 Novel likely pathogenic variant in HECW2. (a) Family pedigree showing the carriers of the novel likely pathogenic variant in HECW2 (c.3571C>T; p.Arg1191Trp; affected individuals are noted by shaded symbols; “+/-” indicates heterozygosity). (b) Chromatograms from Sanger sequencing of the three affected individuals including the index patient (III.1), her mother (II.4) and brother (III.2) validating heterozygosity for c.3571 cytosine (C) > (T) resulting in an amino acid change in codon 1191 of HECW2. (c) Electroencephalography samples showing bursts of delta slowing with a continuum of abortive and occasional epileptiform discharges. Compared to the index patient (III.1), the brother (III.2) had less epileptiform patterns and discharges were less frequent in the mother’s EEG (II.4). Montage is bipolar longitudinal.
It is well established that the prevalence of epilepsy is higher in patients with ID compared to the general population and a large proportion of these cases may have a genetic cause. Previous exome sequencing studies highlighted HECW2 as a new causative gene for neurodevelopmental disorders (Halvardson et al., 2016). This is the first report of a family with the same novel likely pathogenic variant in HECW2. The previous literature has reported nine cases of four different (likely) pathogenic de novo variants in HECW2 (Figure 2; Halvardson et al., 2016; Berko et al., 2017; Ullman et al., 2018). Another 10 DNM in HECW2 were detected in epilepsy, autism spectrum disorder and ID but the patients' phenotypes have not been detailed (Appenzeller et al., 2014; Iossifov et al., 2014; Krumm et al., 2015; McRae et al., 2017). The variant we identified affects the same amino acid position as a known DNM in three other patients (Berko et al., 2017; Ullman et al., 2018). Thus, arginine at this position appears to be crucial for protein function. All nine previously described patients have a common phenotype with language impairments, developmental delays, hypotonia and were mostly presenting with EEG abnormalities or seizures (Figure 2). Similarly, our three patients showed a phenotype with hypotonia, developmental delay and epilepticiform EEG patterns. Despite sharing some of the previously reported phenotypic manifestations, our patients did not present with all of these. For example, although our index patient showed some particular abnormal behavior with hand clapping and erratic movements to express joy, all of our patients had only mild language impairments with fluent and simple speech. Additionally, they did not have gastrointestinal problems and showed mostly normal motor development, as they were able to walk at the age of 2 years. Compared to previously reported cases, our patients are the oldest, which may allow the description of the phenotype in adulthood.

The mother had a relatively mild phenotype and passed on the variant to two children. Conversely, all previous variants in HECW2 were DNM (Ullman et al., 2018). Interestingly, the expression of our patients' phenotype varied in severity among the three individuals. Compared to previously reported cases, our patients are the oldest, which may allow the description of the phenotype in adulthood. The mother had a relatively mild phenotype and passed on the variant to two children. Conversely, all previous variants in HECW2 were DNM (Ullman et al., 2018). Interestingly, the expression of our patients' phenotype varied in severity among the three individuals. Compared to previously reported cases, our patients are the oldest, which may allow the description of the phenotype in adulthood. The mother had a relatively mild phenotype and passed on the variant to two children. Conversely, all previous variants in HECW2 were DNM (Ullman et al., 2018).}

| FIGURE 2 | (Likely) pathogenic variants in HECW2 and their clinical findings in the present family and individuals reported in the literature. AED, anti-epileptic drug; ASD, autism spectrum disorder; DD, developmental delay; DNM, de novo mutation; GERD, gastro-esophageal reflux disease; GI, gastro-intestinal; GTCS, generalized tonic–clonic seizures; G-tube, gastrostomy tube; ID, intellectual disability; mos, months old; NA, not available; –, absent; +, present; SWC, spike-and-wave complexes; yo, years old |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Our case 1 | Our case 2 | Our case 3 | Case 1 | Case 2 | Case 3 | Case 4 | Case 5 | Case 6 | Case 7 | Case 8 | Case 9 |
| Mutation | c.3571C>T; p.Arg1191Trp | c.3571C>T; p.Arg1191Trp | c.3571C>T; p.Arg1191Trp | c.3988C>T; p.Arg1330Trp | c.3988C>T; p.Arg1330Trp | c.3577T>G; p.Phe1193Val | c.3577T>G; p.Phe1193Val | c.4334A>G; p.Glu1445Gly | c.3572G>A; p.Arg1191Gln | c.3572G>A; p.Arg1191Gln | c.3572G>A; p.Arg1191Gln |
| DNM | + | + | + | + | + | + | + | + | + | + |
| Age at last evaluation | 19 yo | 50 yo | 17 yo | NA | 18 mos | 3 yo | 33 mos | 33 mos | 6 yo | 11 yo | 9 yo | 34 mos |
| Sex | female | female | male | NA | NA | male | female | female | female | male | male |
| Hypotonia | 9 mos | 9 mos | NA | NA | unable | unable | unable | unable | NA | NA | NA |
| Age at sitting | 13 mos | 14 mos | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| Age at crawling | 2 yo | 2 yo | NA | NA | unable | unable | unable | unable | 3 yo | 3 yo with braces |
| Age at first words | 18 mos | 18 mos | non-verbal | one word at 17 mos | non-verbal | non-verbal | non-verbal | non-verbal | non-verbal | non-verbal | 2 yo; has 15 words |
| ID | | | | | | | | | | | |
| DD | | | | | | | | | | | |
| ASB | | | | | | | | | | | |
| Stereotypies | | | | | | | | | | | |
| GI problems | | | | | | | | | | | |
| EEG abnormalities | | | | | | | | | | | |
| Epilepsy/Seizures | | | | | | | | | | | |
| Need for AEDs | yes | yes | yes | yes | yes | yes | yes | yes | yes | yes | yes |
| Brain MRI abnormalities | | | | | | | | | | | |
symptoms can be so mild that genetic and prolonged EEG recordings may be needed to unravel the phenotype–genotype relation. In the buccal swab sample of the mother, the c.3571C>T; p.Arg1191Trp variant in HECW2 was quantified to be present in 51.25% of the reads (184 of 359 reads). This indicated variable expressivity rather than mosaicism as cause for her milder phenotype. Variation in expression might be affected by yet unknown modifier genes, epigenetic factors or the environment (Marian & Roberts, 2001).

Our cases add further evidence to the causative nature of the HECW2 variants. HECW2 has shown interactions with essential genes in neurodevelopmental disorders (Halvardsson et al., 2016). It stabilizes p73 which is a mediator of neurodevelopment and neurogenesis and enhances its transcriptional activity (Miyazaki et al., 2003). Interestingly, knockout mice for this gene do not present with similar phenotypes, potentially indicating a gain of function or dominant negative role of the DNM in HECW2 (Brown & Moore, 2012).

To our knowledge, this is the first family with variable expressivity of a novel variant in HECW2. Our data provide a more complete clinical description and indicate that the phenotype may be broader and more variable than initially reported. Functional studies of the variants and studies of patient cells may contribute to a better understanding of HECW2’s role in brain development, epilepsy, and its molecular mechanisms.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Ev-Christin Heide and Niels K. Focke clinically assessed the patient; Saskia Biskup and Oliver Puk performed the molecular analyses; Ev-Christin Heide wrote the manuscript; Niels K. Focke, Saskia Biskup, Oliver Puk, Arne Krahn, Erik Rauf, Barbara A. K. Kreilkamp, and Walter Paulus reviewed the manuscript. All authors approved the final version of the manuscript.

PATIENT CONSENT STATEMENT

Patient consent was obtained for the publication of the article.

PROVENANCE AND PEER REVIEW

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

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