Novel *HIVEP2* Variants in Patients with Intellectual Disability

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Abstract

Intellectual disability (ID) occurs in approximately 1% of the population. Over the last years, broad sequencing approaches such as whole exome sequencing (WES) substantially contributed to the definition of the molecular defects underlying nonsyndromic ID. Pathogenic variants in *HIVEP2*, which encodes the human immunodeficiency virus type I enhancer binding protein 2, have recently been reported as a cause of ID, developmental delay, behavioral disorders, and dysmorphic features. *HIVEP2* serves as a transcriptional factor regulating NF- κ B and diverse genes that are essential in neural development. To date, only 8 patients with pathogenic de novo nonsense or frameshift variants and 1 patient with a pathogenic missense variant in *HIVEP2* have been reported. By WES, we identified 2 novel truncating *HIVEP2* variants, c.6609_6616delTGAGGGTC (p.Glu2204*) and c.6667C>T (p.Arg2223*), in 2 young adults presenting with developmental delay and mild ID without any dysmorphic features, systemic malformations, or behavioral issues.

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Intellectual disability (ID) occurs in approximately 1% of the population [Polder et al., 2002; Maulik et al., 2011] and represents a major socioeconomic problem. Family members can be burdened by a variety of factors that are highly dependent on the social abilities and medical presentation of the patients. Advances in sequencing technologies enabled clinicians to identify rare genetic causes for ID in isolated cases [Ropers, 2010; Rauch et al., 2012]. Especially the application of trio-whole exome sequencing (WES) led to the identification of numerous nonsynonymous de novo mutations that have been associated with autism, ID, and schizophrenia. These observations lend support to the “de novo hypothesis,” i.e., that under consideration of evolutionary conservation, protein function and mutation type, these unique events may play a significant role in the pathogenesis of a number of rare developmental disorders [Vissers et al., 2010; Kong et al., 2012; Veltman and Brunner, 2012].

HIVEP2 (OMIM 143054) encodes the human immunodeficiency virus type I enhancer binding protein 2, a member of the large zinc-finger transcriptional proteins family (ZnF-C2H2 type) that is involved in immunological responses, adipogenesis, bone formations, and brain development [Dörflinger et al., 1999; Hong et al., 2003;

Fig. 1. Patient 1. **a** 15 months. **b** 18 months. **c** 2 years. **d** 7 years. **e** 27 years. **f** The *HIVEP2* frameshift variant c.6609_6616delTGAGGGTC was confirmed in patient 1 by Sanger sequencing (arrow) but was absent in both parents.

Takagi et al., 2006; Imamura et al., 2014]. Recently, de novo variants in *HIVEP2* have been suggested to cause autosomal dominant mental retardation type 43 (MRD43, OMIM 616977) in a small number of patients. This is consistent with evidence that *HIVEP2* is expressed in several brain regions and plays an important role in neural maturation and brain development [Takao et al., 2013; Srivastava et al., 2016]. *Hivep2* knock-out mice displayed anxiety and hyperactivity that have also been observed in the majority of the reported patients [Takagi et al., 2006]. Here, we provide detailed information on the clinical presentation of 2 individuals carrying predictively truncating de novo *HIVEP2* variants over a disease course of 23 and 27 years, respectively.

Clinical Reports

Patient 1 is the first child of healthy unrelated German parents. The girl was born after a normal pregnancy at 40 weeks of gestation by spontaneous vaginal delivery with normal length (53 cm; 72nd centile) and weight (3,570 g; 59th centile). The first clinical presentation was orofacial hypotonia with an open mouth posture in infancy. Considerable global developmental delay became apparent approximately at the age of 2 years. Motor development was slightly delayed, but she walked independently at 18 months of age. Some neurological signs such as general muscular hypotonia, poor posture, and notable difficulties in gross and fine motor coordination were noted. Language development was severely affected as she spoke first words at the age of 4 years. A nonverbal intelligence test at 9 years of age revealed mild ID with an IQ of 58. Her behavior has generally been friendly, amiable, and cooperative. Her medical history is unremarkable; there were neither organic malformations, complicated hospitalizations nor any evidence of seizures. She does not show any distinctive dysmorphic features, but a marginally broader nose, narrow chin, and thin upper lip could be conceived (Fig. 1). Her height, weight, and head circumference have always been within the normal range. Currently, at the age of 27 years, she works at a sheltered workshop and does not have any difficulties socializing with other workmates. Prior to WES, conventional chromosomal and SNP array analyses revealed normal results, and Fragile X syndrome was excluded via trinucleotide repeat expansion analysis. Screening for congenital metabolic diseases and congenital disorders of glycosylation yielded negative results.

Patient 2, a female, was the only child of a healthy 29-year-old woman. There is no family medical history regarding ID or any other neurological disorders. Pregnancy was unremarkable and prenatal sonographic scans were normal. She was born at 37 weeks of gestation by uncomplicated vaginal delivery with a birthweight of 2,450 g (19th centile), birth length of 49 cm (72nd centile), and head circumference of 31 cm (9th centile). The patient demonstrated mild motor developmental delay: she started rolling over at 7 months, could sit up and stand with support at 11 months, and learned to walk independently at approximately 20–24 months. She spoke first words at the age of 24 months. She has an elongat-

ed and mildly narrow face as well as small ears, but no specific dysmorphic features. Neurological examination was unremarkable without signs of muscular hypotonia, seizures, or autism. EEG was normal. She was diagnosed with mild ID with an IQ of 52. Until the age of 16 years, she attended special schools for ID students. Now, she is 23 years old and has slow speech and limited vocabulary. She tends to use short and repetitive sentences and is often difficult to understand for others, with the exception of her parents. Although she presents irritability and impulsivity, she rarely shows aggressive behavior. Repetitive interests and limited food preference were seen as characteristic features within normal range for a patient with ID. Predominant autistic features were excluded. Regarding medical problems, the patient frequently suffered from upper airway infections, constipation, and oligomenorrhea. Otherwise, no other complicated medical issues have occurred so far. Chromosomal abnormalities, including microdeletions, microduplications, and other copy-number variants (CNVs) have been excluded by conventional karyotyping and array CGH.

Methods and Results

For patient 1, diagnostic WES was performed on genomic DNA extracted from peripheral blood. Coding regions were enriched using a SureSelect XT Human All Exon kit v6 (Agilent Technologies, Santa Clara, CA, USA) for subsequent sequencing as 2 × 125-bp paired-end reads on a HiSeq2500 system (Illumina, San Diego, CA, USA) as published previously [Fritzen et al., 2018]. Generated sequences were analyzed using the megSAP pipeline (<https://github.com/imgag/megSAP>). Clinical variant prioritization included different filtering steps including a search for rare (MAF <0.1% in ExAC, gnomAD, 3,000 in-house exome datasets) variants in genes that have been associated with the patient's phenotype according to an in-house standard operating procedure.

For patient 2, WES was performed on genomic DNA isolated from the proband's EDTA blood using a TruSeq Exome Enrichment kit (Illumina) on an Illumina HiSeq 2500 sequencer. Approximately 100 million paired reads per sample were generated. The average sequencing depth was 70.2× with over 80% of the target sequence covered at ≥20×. Genomic variants were filtered and prioritized according to the following criteria: the gene reported is to be involved in neurological or mental disorders, its frequency in publicly available variant databases <0.01%, and in silico predicted pathogenic effect of the variant. Bidirectional Sanger sequencing was performed to confirm the variants in both patients and their de novo status.

Mutational Analysis

WES revealed a heterozygous frameshift variant NM_006734.3:c.6609_6616delTGAGGGTC (p.Glu2204*) in patient 1 and a heterozygous nonsense variant c.6667C>T (p.Arg2223*) in patient 2. Both variants are located in the last exon of *HIVEP2*. Sanger sequencing confirmed that the variants were absent from either of the patients' parental DNAs extracted from peripheral blood, suggesting a de novo status of the variants in both subjects. These variants were absent from the 1000 Genomes database, the Genome Aggregation Database (gnomAD), and the Exome Aggregation Consortium (ExAC) database (last consultation: 1 September 2018).

Discussion

Six years ago, in a cohort study of 51 patients, Rauch et al. [2012] reported 1 de novo heterozygous truncating variant in *HIVEP2* as a possible cause of nonsyndromic ID in a female patient. Only limited clinical information of the patient was presented. Srivastava et al. [2016] reported further clinical details for this patient who exhibited not only severe ID, but also dysmorphic features, congenital malformations, ataxic gait, and aggressive behavior. In addition, the same authors identified 2 further unrelated children with de novo heterozygous *HIVEP2* truncating variants presenting with moderate ID, developmental delay, mild facial dysmorphism, and, in one of them, behavioral problems, suggesting loss of function and haploinsufficiency as the pathomechanism [Rauch et al., 2012]. More recently, Steinfeld et al. [2016] identified 5 de novo truncating variants and 1 de novo missense variant in *HIVEP2* in 6 unrelated children with ID. Last, in 2 cohort studies, 1 de novo missense variant and 1 de novo nonsense variant have been identified among patients affected by bipolar disorder and ID, respectively [Kataoka et al., 2016; Hamdan et al., 2017]. However, both publications did not provide any clinical phenotype details.

HIVEP2 variants identified so far in ID patients are shown in Figure 2. Clinical details are given in Table 1. Overall, only 8 patients with pathogenic nonsense or frameshift variants and 1 patient with a pathogenic missense variant in *HIVEP2* have been reported together with clinical information. All of them, 5 females and 4 males, showed mild to moderate ID. Motor development was markedly delayed in all. Seven out of 9 patients were able to walk independently at the age of ~2–3 years and 1 of them needed a walker until the age of 4–5 years. Apart from general muscular hypotonia which was present in 7 out of 9 patients, other overlapping neurological features such as ataxia, dystonia, dyspraxia, dysphagia, gait difficulties, spasticity, and progressive Parkinsonism and quadriplegia have been registered in 7 cases. All of them had remarkable language development delay and 7 showed pronounced behavioral abnormalities, such as hyperactivity, impulsivity, lack of concentration, but also anxiety, aggressive and/or hand flapping behavior. Less frequently reported clinical features include microcephaly (3 cases) or seizures (2 cases). Some of them also presented with nonspecific dysmorphic features, which were not predominant in our patients. The majority of reported cases suffered from minor medical problems, mostly gastrointestinal symptoms, such as abdominal

Fig. 2. Previously reported de novo variants in *HIVEP2* identified in ID patients and predicted to be pathogenic. Patient 1 had a frameshift truncating variant c.6609_6616del/TGAGGGTC (p.Glu2204*), and patient 2 had a nonsense variant c.6667C>T (p.Arg2223*), both located in the last exon of *HIVEP2*.

pain, constipation or reflux. Congenital anomalies are uncommon. Brain MRI did not reveal any specific pathological findings. In comparison to the previously reported cases, our 2 patients did not show any complex neurological findings and had an uncomplicated medical history without predominant behavioral abnormalities. The variants identified in our patients are located in the last exon of *HIVEP2* and are predicted to result in the generation of a truncated protein. This could possibly lead to some residual function or a dominant-negative effect and might also explain the milder phenotype of these patients.

HIVEP2 operates as a transcription factor for various genes involved in brain development, such as the somatostatin receptor type II (*SSTR-2*) that is known to show expression in the frontal cortex and hippocampus during embryonic brain and nerve formation [Takagi et al., 2006]. *HIVEP2* also binds to nuclear factor- κ B (NF- κ B) reducing the transcription of NF- κ B-dependent genes that play an important role in immune response. *Hivep2* knock-out mice displayed chronic inflammation signs in several brain areas due to upregulation of NF- κ B target gene proposing a paradigm that alterations in immune

response can influence neurodevelopmental disorders [Takao et al., 2013; Choi et al., 2015]. RNA expression analysis in hippocampus and amygdala illustrated a downregulation of some early genes that are involved in stress response and neuroplasticity [Takagi et al., 2006]. *Hivep2* knock-out mice showed not only severe cognitive impairments but also behavioral abnormalities, which could be compared with those of 7 of the reported patients. No remarkable deficits in health condition, physical appearance, or motor functions were observed in the mutant mice. Administration of haloperidol and anti-inflammatory drugs to murine models reduced inflammation markers in the brain and improved social interactions and memory abilities [Takao et al., 2013]. Although behavioral problems have not been observed in our patients, this type of pharmacological therapy has been suggested as a possible treatment for such symptoms [Takao et al., 2013].

This report supports the growing evidence that WES can be a useful approach to elucidate the underlying molecular cause of ID [Helsmoortel et al., 2015; Vrijenhoek et al., 2018]. Patients with heterozygous pathogenic variants in *HIVEP2* share a nonspecific phenotype of devel-

Table 1. Patients with *HIVEP2* variants

	This publication		Srivastava et al. [2016]		
	patient 1	patient 2	patient 1	patient 2	patient 3
Gender	Female	Female	Female	Male	Female
Age at the time of publication	27 ys	23 ys	4 ys 7 mo	3 ys 9 mo	21 ys
Mutation	Frameshift	Nonsense	Nonsense	Nonsense	Frameshift
Variant	c.6609_6616delTGAGGGTC, p.Glu2204*	c.6667C>T, p.Arg2223*	c.2827C>T, p.Arg943*	c.3556C>T, p.Gln1186*	c.5737delG, p.Asp1913Metfs*15
Inherital status	De novo	De novo	De novo	De novo	De novo
Birth					
Head circumference	Unknown	31 cm (9th)	(10th)	(50th)	(3rd)
Height	53 cm (72th)	49 cm (72th)	(70th)	(25th)	(25–50th)
Weight	3,570 g (59th)	2,450 g (19th)	(85th)	(25–50th)	(25–50th)
Current measurements					
Head circumference	54.5 cm; Z = -0.6	48 cm; Z = -1	48 cm; Z = -1.5	48 cm; Z = -1.4	52.2 cm; Z = -2
Height	Unknown	111 cm (75th)	100 cm (70th)	80 cm (25th)	164 cm (25–50th)
Weight	Unknown	15.3 kg (15th)	17.1 kg (85th)	11 kg (25–50 th)	59 kg (25–50th)
Developmental delay					
Age at walking	18 mo	20–24 mo	36 mo	30 mo	36 mo
First words	4 ys	24 mo	24 mo	24 mo	7.5 ys
Language capability at the time of publication	Short sentences, limited vocabulary, partly inadequate to situation	Slow speech, difficult to understand, short and repetitive sentences, limited vocabulary	Language age equivalent of 15 mo	Unknown	Partially slurred articulation
Neurological features					
Intellectual disability	Mild delay	Mild delay	Severe delay	Moderate delay	Severe delay
IQ	58	52	Unknown	Unknown	Unknown
Behavioral disorders	No	No	Hyperactivity, impulsivity, distractibility	No	Aggressivity, impulsivity, self-stimulation, hyperactivity
Muscular hypotonia	Yes	No	No	Yes	Yes
Other neurological features	No	No	Dysphagia, frequent head tilt and leftward eye movements with preserved ability to fix/follow objects	Clumsy gait	Ataxia, dystonia
Brain CT/MRI	Not done	Not done	Slightly thin corpus callosum	Hypoplasia of corpus callosum	Mild frontal atrophy
Seizures	No	No	No	No	No
Other features					
Dysmorphic features	Slightly broader nose, narrow chin, thin upper lip	Elongated and mildly narrow face, small ears	Widely set eyes, broad nasal root, slightly upturned nose, high-arched palate	High forehead, medial eyebrow flare, widely set eyes, broad nasal root, small mouth, slightly tapering fingers, flat feet	Upslanting palpebral fissures, mild synophrys, small ears with attached earlobes, prominent nose with high nasal bridge and columella extending below the alae nasi, short philtrum, thin upper lip, overbite, hypertrophic gingiva, tapering fingers, radial deviation of 4th fingers, wide feet with short toes, hirsutism
Other medical issues	No	Constipation, oligo-menorrhea, frequent upper airways infections	Asymmetric cry, gastroparesis and projectile vomiting, unilateral hip dysplasia	Significant salivation, hypermetropia	Chronic constipation, gingival swelling and bleeding, high TSH levels, umbilical hernia, minor pulmonary stenosis

opmental delay/ID and other variable clinical features. Our 2 cases are the oldest reported patients with pathogenic *HIVEP2* variants that have been clinically characterized so far. Both reached maturity with mild ID but without predominant behavioral difficulties or other serious medical problems. Clinical follow-up of additional

patients will outline the development of the mental phenotype and further define the clinical spectrum of *HIVEP2*-associated ID. Functional studies are needed to shed light on the molecular mechanisms underlying the pathological effect of the variants on the central nervous system.

Table 1 (continued)

	Steinfeld et al. [2016]					
	patient 1	patient 2	patient 3	patient 4	patient 5	patient 6
Gender	Female	Female	Male	Male	Female	Male
Age at the time of publication	7 ys	14 ys	10 ys	2 ys	11 ys	6 ys
Mutation	Nonsense	Nonsense	Frameshift	Missense	Frameshift	Frameshift
Variant	c.6475G>T, p.Gly2159*	c.2857G>T, p.Glu953*	c.5614dupG, p.Glu1872Glyfs*16	c.1189G>T, p.Asp397Tyr	c.6625dupT, p.Tyr2209Leufs*53	c.3434delC, p.Pro1145Argfs*2
Inherital status	De novo	De novo	De novo	De novo	De novo	De novo
Birth						
Head circumference	Unknown	32.4 cm	Unknown	Unknown	Unknown	Unknown
Height	53.34 cm (90th)	48.26 cm (25–50th)	53.3 cm (90th)	Unknown	Unknown	Unknown
Weight	3,997 g (90th)	3,062 g (25th)	3,856 g (75th)	3,770 g (50–75th)	3,880 g (75–90th)	4,100 g (90th)
Current measurements						
Head circumference	48.5 cm; Z = −2.4	51 cm; Z = −2.6	48.7 cm; Z = −3.2	46.7 cm; Z = −1.7	50.8 cm; Z = 0.7	52 cm; Z = 0.3
Height	125 cm (75th)	149.5 cm (5th)	140.9 cm (50–75th)	80.8 cm (<3rd)	152 cm (50–75th)	120 cm (50th)
Weight	22.8 kg (50th)	47.5 kg (25–50th)	31.4 kg (25–50th)	10.6 kg (<5th)	44 kg (50–75th)	24 kg (50–75th)
Developmental delay						
Age at walking	24 mo	36 mo	With walker, 30–36 mo; without walker 4–5 ys	Not yet	30 mo	22 mo
First words	18 mo	Unknown	Unknown	24 mo	10 mo	18 mo
Language capability at the time of publication	Speaks in short sentences; had 100 words by 3 ys	No verbal speech	No verbal speech	3 words	Full sentences and imitation; difficult to understand	Sentences with 3–4 words
Neurological features						
Intellectual disability	Mild delay	Delayed	Delayed	Unknown	Mild delay	Mild delay
IQ	75	Unknown	Unknown	Unknown	50	50–70
Behavioral disorders	Hand-wringing, oppositional/defiant behaviors	Hands in hair, hands up like puppet, wringing of hands	Tic-like head jerking at one point, impulsive, distractible	No	Sensitive to stimuli, requires structure	Hyperactivity, concentration problems, anxiety
Muscular hypotonia	Yes	Yes	Yes	Yes	Yes	No
Other neurological features	Static encephalopathy, dystonia, exercise intolerance/easily fatigued	Ataxia, spasticity, cerebral palsy, quadriplegia, muscle weakness, dystonia	Spasticity, muscle weakness, tremors, progressive parkinsonism, right hemiparesis, tongue fasciculations	No	Dyspraxia	No
Brain CT/MRI	Normal	Mild volume loss	Normal MRI abnormal MR spectroscopy	Normal	Incomplete myelination at age 4 ys	Normal
Seizures	Possible	Yes	Yes	No	No	No
Other Features						
Dysmorphic features	Mild retrognathia	Elongated and narrow face	Low anterior hairline, hirsute, prominent eyebrows, synophrys, epicanthal folds, mildly thickened helices and simple antihelices in ears, mild dental crowding of lower jaw	Small hands and feet	Somewhat high nasal bridge, broad mouth, rather flat philtrum, mild bifrontal narrowing, mildly broad halluces, sacral dimple, broad thorax, mild finger webbing	Square face, high/broad forehead, unilateral strabismus, high nasal bridge, columella under alae nasi, small square ears with transverse crease, small square teeth, microretro-gnathia
Other medical issues	Temperature instability, abdominal pain, GERD	Iron deficiency anemia, nephrocalcinosis on renal US, hip dysplasia, irregular menstrual periods, syncope, dyspnea, constipation, GERD, strabismus	GERD, milk protein intolerance as infant, frequent tonsillitis, strabismus (esotropia)	Reactive airway disease; AFOs primarily for ankles rolling due to hypotonia, short stature	Hypermobility of fingers, increased inversion of feet and decreased eversion	Bronchial hyper-reactivity, breath holding spells, amblyopia and strabismus, high hyper-metropia

AFO, ankle-foot orthoses; GERD, gastroesophageal reflux disease; mo, months; TSH, thyroid-stimulating hormone; US, ultrasound; ys, years; Z, Z-score. Percentiles are given in parentheses.

Statement of Ethics

Informed consent was obtained prior to investigation.

Disclosure Statement

The authors have no conflicts of interest to declare.

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Author Contributions

J. Park collected and analyzed the data and drafted the manuscript. R. Colombo and L. Janiri kindly provided the clinical and molecular information of the second patient. In addition, R. Colombo contributed to the conception of the work, reviewed for accurate interpretation of molecular data, and revised the work thoroughly for scientific content. K. Schäferhoff and M. Grimm were involved in WES data analysis and provided the molecular information. M. Sturm developed and applied the bioinformatics data analysis pipeline. T. Haack reviewed the work critically for appropriate content and supervised execution of the project. M. Kehrer, U. Grasshoff, and A. Dufke contributed as clinicians to the phenotypic analysis and cared for close communication with patient 1. M. Kehrer initiated and supervised the project.

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