A de novo variant in the human HIST1H4J gene causes a syndrome analogous to the HIST1H4C-associated neurodevelopmental disorder

Federico Tessadori1,2 · Atteeq U. Rehman3,5 · Jacques C. Giltay2 · Fan Xia3 · Haley Streff3 · Karen Duran2 · Jeroen Bakkers1,4 · Seema R. Lalani3 · Gijs van Haaften2

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Abstract

We report here a de novo missense variant in HIST1H4J resulting in a complex syndrome combining growth delay, microcephaly and intellectual disability. Trio whole exome sequencing (WES) revealed that the proband was heterozygous for a de novo c.274 A > G p.(K91E) variant in HIST1H4J, a gene not yet associated with human disease. The patient presented with profound intellectual disability, microcephaly, and dysmorphic facial features. Functional consequences of the identified de novo missense variant were evaluated in zebrafish embryos, where they affected general development, especially resulting in defective head organs and reduced body axis length. Our results show that the monoallelic p.K91E substitution on HIST1H4J underlies a human syndrome that is genetically and phenotypically akin to the HIST1H4C-associated neurodevelopmental disorder resulting from p.K91A and p.K91Q substitutions in HIST1H4C. The highly overlapping patient phenotypes highlight functional similarities between HIST1H4J and HIST1H4C perturbations, establishing the singular importance of K91 across histone H4 genes for vertebrate development.

Introduction

The importance of chromatin regulation is reflected by the ever-growing literature on human diseases caused by genetic alterations in histone-modifying complexes or histone genes [1–4].

We recently reported monoallelic, dominant pathogenic variants affecting lysine 91 (p.K91A or p.K91Q) in HIST1H4C (RefSeq NM_003542.3) causing a severe neurodevelopmental syndrome. We pinpointed the cause of the disorder to perturbation of early developmental stages due to the accumulation of DNA damage, genomic instability, and cell cycle delay [4].

HIST1HJ (RefSeq NM_021968.3) and HIST1H4C are two of the fifteen human genes encoding histone H4. While there are differences in the coding sequences of H4 genes, they all encode an identical H4 protein [5]. Here we present data establishing that a de novo, dominant variant resulting in the substitution of lysine 91 by glutamic acid in HIST1H4J mirrors the phenotype previously reported for HIST1H4C-related disorder. The remarkable phenotypical overlap with HIST1H4C K91 patients [4] and functional data obtained in zebrafish provide compelling evidence that the mutated HIST1H4J K91 is causative for the proband’s neurodevelopmental disorder.
Materials and methods

Patient genetic investigation

The study was performed following the ethical guidelines for research involving human subjects and was approved by the Institutional Review Board at Baylor College of Medicine. Written informed consents were obtained from the participating family members. The proband was seen at Texas Children’s Hospital by SRL and was referred for clinical trio WES. Trio WES was performed at Baylor Genetics Laboratories as previously described [6, 7].

Fish lines and husbandry

Tübingen longfin zebrafish were kept in standard laboratory conditions [8]. Animal experiments were approved by the Animal Experimentation Committee of the Royal Netherlands Academy of Arts and Sciences.

Expression assay in zebrafish embryos

Capped mRNA microinjections were carried out essentially as described in [4]. Human cDNA encoding for HIST1H4J (RefSeq NM_021968.3) was used as template for single site mutagenesis with primers Hist1H4J_K91E_F: 5′-gtctacgctcgacgagcgcgtagc-3′ and Hist1H4J_K91E_R: 5′-ggccctggcgctcgagcgcgtagac-3′.

Imaging

Live phenotypical assessment of 28 hpf zebrafish embryos was carried out on a Zeiss StemiSV6 stereomicroscope (Carl Zeiss AG, Oberkochen, Germany). Imaging was performed using a Zeiss Axioplan brightfield microscope (Carl Zeiss AG) and a Leica DFC420C digital microscope camera (Leica Microsystems, Wetzlar, Germany).

Results

Patient report

The patient was a 14-year old Hispanic male with profound intellectual disability. He was the product of a full term pregnancy with limited prenatal care. His birth weight was 2.3 kg. He was born with hypospadias, which was surgically repaired. Growth parameters remained <3rd percentile throughout his medical evaluation. He was globally delayed and hypotonic. He sat unassisted at 9 months of age and walked independently around 4 years of age. He had significant language delay and was diagnosed with pervasive developmental disorder. His additional diagnoses were oculomotor apraxia (OMA) and moderate angle left esotropia. At the age of 14 years, he was nonverbal. His height was 125.3 cm (−4.64 SD), weight was 20.7 kg (−4.13 SD), and head circumference was 49.4 cm (−3.32 SD). Dysmorphic features included upslanting palpebral fissures, hypertelorism, peri-orbital fullness, arched eyebrows, flat nasal bridge, wide mouth with downturned corners, and short philtrum (Fig. 1a). He had muscle wasting involving the upper and lower extremities, slender hands, and flat feet. Echocardiogram and renal ultrasound evaluations were normal. Brain MRI showed mild prominence of supratentorial sulci and cisterns. His laboratory work-up included very long chain fatty acids, CPK, lactate, and DNA analysis for Fragile X, which were all normal. Chromosomal microarray showed a paternally inherited 207 kb gain involving KCNV1 on chromosome 8q23.2. MECP2 sequencing was normal.

Identification of the c.274 A > G p.(K91E) variant in HIST1H4J by WES

Analysis of trio WES data didn’t reveal any variant affecting or likely affecting known disease-associated genes that could explain the proband’s phenotype. Potentially disease-causing variants in maternally or paternally inherited copies of KCNV1 were not identified.

However, the proband was found to be heterozygous (110 mutant vs 138 reference reads) for a de novo variant [chr6:27792176 A > G (hg19), c.274 A > G; p.(K91E)] in HIST1H4J, a gene hitherto not associated with a human disease. The c.274 A > G variant was neither present in his biological parents nor in control databases such as ExAC or gnomAD. Moreover, the affected K91 residue is extremely well conserved across species (Fig. 1), and the effect of the variant is predicted to be deleterious (SIFT) and possibly damaging (PolyPhen-2; http://genetics.bwh.harvard.edu/pph2/). No additional de novo variants were detected in the proband.

One homolog of HIST1H4J, HIST1H4K, is located just 6.7 kb away on the short arm of chromosome 6. While the coding sequences of 13 H4 genes display substantial variation with that of HIST1H4J (Fig. S1), HIST1H4J and HIST1H4K share an identical open reading frame and differ only in their 3′UTR sequence (Fig. S2). Visual analysis of the sequencing bam files confirmed that sequence reads containing the de novo A > G variant indeed originated and mapped back exclusively to the HIST1H4J locus (Fig. S2).

Functional modeling of HIST1H4J K91E in zebrafish

We tested the HIST1H4J K91E variant for dominant effects on the development by microinjecting synthetic mRNA in zebrafish embryos (Fig. 2). A loss of function effect was not considered because of the presence of multiple loss of
function variants in a range of histone H4 genes in the healthy population (Gnomad, [9, 10]).

Analysis at 28 hpf revealed that while the expression of WT HIST1H4J had only a very mild effect on the embryonic development, the expression of HIST1H4J K91E had a clear signature on the structural development of zebrafish embryos (Fig. 2), which is reminiscent of the previously reported phenotype for HIST1H4C K91 variants [4]. Defective development of head structures such as the brain and eyes, faulty body axis growth, and a dysmorphic tail were observed, which are all features evocative of the proband’s microcephaly and short stature.

Discussion

We describe here a novel, dominant neurodevelopmental disorder associated with the substitution K91E on the HIST1H4J gene. The proband of this study presented with profound intellectual disability, microcephaly, and dysmorphic facial features. Trio WES analysis revealed that the proband was heterozygous for a de novo c.274 A > G p.(K91E) variant in HIST1H4J.

The proband’s clinical features, including his craniofacial dysmorphisms were strikingly similar to those reported previously in the patients with HIST1H4C variants (Table 1; [4]). While all patients shared general impaired neurodevelopment, growth parameters and distinctive craniofacial features, one of the features distinguishing the proband of this study was OMA, a condition characterized by defective, or absent voluntary, or attraction eye movements [11]. Since trio WES did not reveal any variants likely affecting the OMA-related genes, we concluded that this phenotype was likely related to the HIST1H4J change in the proband.

The c.274 A > G variant detected in the proband results in the substitution of a lysine (K) by glutamic acid (E) at position 91 on the HIST1H4J gene. Lysine 91 posttranslational modifications include acetylation and monoubiquitination [12, 13] which play important roles respectively in chromatin assembly and stability [13, 14] and protection against DNA-damaging agents [12]. Since the acquired glutamic acid cannot be monoubiquitinated, and given its negative charge, the HIST1H4 K91E substitution presented here is likely to result in the genomic instability as described previously for substitutions at lysine 91 on HIST1H4C [4]. In addition, similarly to HIST1H4C, HIST1H4J is relatively well expressed in early human embryos and human embryonic stem cells [15], both systems with relatively short cell cycle time and consequently sensitive to perturbation of the cell division rate.

The discovery of the HIST1H4J syndrome described here shows that K91 variants are not just specific to HIST1H4C,
Fig. 2 The K91E substitution on HIST1H4J induces early severe developmental defects in zebrafish embryos. a Phenotypes observed in zebrafish embryos at 28 h post fertilization. Wildtype HIST1H4J (WT) and K91E mRNA was microinjected at the 1-cell stage. Class 1 embryos display normal development, class 2 embryos display mild shortening of the body axis and delayed head development. Class 3 embryos have severely defective head development and a shortened AP body axis, with abnormal posterior development. In Class 4 embryos head structures and somites are largely absent.

b Histogram presenting the percentage of observed embryos in each class for each category. no inj non-injected control. The data presented were collected over three independent biological and technical experimental replicates.

Table 1 Comparison of clinical and genetic findings in patients with variants of HIST1H4C and HIST1H4J

|                | HIST1H4J | HIST1H4C (pt. 1) | HIST1H4C (pt. 2) | HIST1H4C (pt. 3) |
|----------------|----------|------------------|------------------|------------------|
| cDNA change    | c.274 A > G | c.274 A > C      | c.275 A > G      | c.275 A > G      |
| Effect         | p.K91E   | p.K91Q           | p.K91R           | p.K91R           |
| Age at last visit | 13 years | 7 year           | 13 years         | 11 days          |
| Gender         | Male     | Female           | Female           | Female           |
| Ethnicity      | Hispanic | Caucasian        | Caucasian        | Caucasian        |
| Microcephaly   | ✓         | ✓                | ✓                | ✓                |
| Hypotonia      | ✓         | ✓                | ✓                | No               |
| Developmental delay | ✓    | ✓                | ✓                | ✓                |
| Growth retardation | ✓    | ✓                | ✓                | NA               |
| Intellectual disability | ✓   | ✓                | ✓                | NA               |
| Brain MRI findings | Mild prominence of the supratentorial sulci and cisterns | Reduction in white matter bulk | Normal | NA |
| Ophthalmologic | Oculomotor apraxia, moderate angle left esotropia | Myopia, squint | Amblyopia of the left eye, small papillae, refraction anomaly, convergent strabismus of the left eye | |
| Craniofacial features | Upslanting palpebral fissures, hypertelorism, periorbital fullness, flat nasal bridge, wide mouth, short philtrum | Upslanting palpebral fissures, bifid flat nasal tip, median ridge on philtrum, wide mouth, hypertelorism, ptosis, exorbitism | Upslanting palpebral fissures, bifid flat nasal tip, median ridge on philtrum, wide mouth, hypertelorism, asymmetric eyes, periorbital fullness | Upslanting palpebral fissures, bifid flat nasal tip, retrognathia |
| Foot ray anomaly | Unknown | ✓                | ✓                | ✓                |
| Other features | Pervasive developmental disorder, hypospadias | Secundum atrial septal defect, small kidneys with lack of cortico-medullary differentiation and simple cysts, high pain threshold | Psychotic, lordosis, cutis marmorata, seizures | |

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but the substitution of K91 in other genes encoding the same H4 protein could also cause this recognizable neuro-developmental syndrome. This was an important, outstanding question after the discovery of the HIST1H4C-associated neurodevelopmental disorder [4]. Clearly, variants in epigenetic pathways underlie both developmental syndromes and oncogenesis. As the abundance and non-random presence of histone variants is becoming increasingly evident in cancer [16] (and references therein), turning our attention to histone genes could provide us with the opportunity to resolve, at the genetic level, more yet unexplained developmental syndromes.

Data availability

The genetic and phenotypical data were submitted to the Leiden Open Variation Database (LOVD; http://www.lovd.nl/3.0/home) as submission ID 00266138.

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Compliance with ethical standards

Conflict of interest

The Department of Molecular and Human Genetics at the Baylor College of Medicine derives revenue from molecular genetic testing offered at the Baylor Genetics Laboratories. The authors declare that they have no conflict of interest.

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