Mutations in the Chromatin Regulator Gene BRPF1 Cause Syndromic Intellectual Disability and Deficient Histone Acetylation

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Identification of over 500 epigenetic regulators in humans raises an interesting question regarding how chromatin dysregulation contributes to different diseases. Bromodomain and PHD finger-containing protein 1 (BRPF1) is a multivalent chromatin regulator possessing three histone-binding domains, one non-specific DNA-binding module, and several motifs for interacting with and activating three lysine acetyltransferases. Genetic analyses of fish brpf1 and mouse Brpf1 have uncovered an important role in skeletal, hematopoietic, and brain development, but it remains unclear how BRPF1 is linked to human development and disease. Here, we describe an intellectual disability disorder in ten individuals with inherited or de novo monoallelic BRPF1 mutations. Symptoms include infantile hypotonia, global developmental delay, intellectual disability, expressive language impairment, and facial dysmorphisms. Central nervous system and spinal abnormalities are also seen in some individuals. These clinical features overlap with but are not identical to those reported for persons with KAT6A or KAT6B mutations, suggesting that BRPF1 targets these two acetyltransferases and additional partners in humans. Functional assays showed that the resulting BRPF1 variants are pathogenic and impair acetylation of histone H3 at lysine 23, an abundant but poorly characterized epigenetic mark. We also found a similar deficiency in different lines of Brpf1-knockout mice. These data indicate that aberrations in the chromatin regulator gene BRPF1 cause histone H3 acetylation deficiency and a previously unrecognized intellectual disability syndrome.

Introduction

Epigenetic regulation is essential for human development and becomes aberrant in different diseases. The human genome encodes over 500 epigenetic regulators.1–3 To date, ~30 chromatin modifiers have been associated with human diseases, including the ATP-dependent helicases CHD4, CHD7, CHD8, SMARCA2, SMARCA4, and ATRX;4–6 the DNA methyltransferases DNMT1 and DNMT3b;4 the histone methyltransferases EZH2 and KMT3B;4 the lysine acetyltransferases CREBBP, EP300, KAT6A, KAT6B, and ESCO2;7–14 and the histone deacetylases HDAC4 and HDAC8.15,16 By contrast, disease association remains much less clear for hundreds of chromatin readers that utilize structural modules to sense DNA and histone modification states for chromatin-based signaling.17,18 Thus, it is important to identify disease links for such chromatin readers. Bromodomain and PHD finger-containing protein 1 (BRPF1) is a multivalent chromatin reader composed of multiple nucleosome-binding modules, including double PHD fingers (linked with a C2HC zinc knuckle), a bromodomain, and a PWWP domain.19–21 The PHD fingers and zinc knuckle form a bivalent nucleosome-binding unit, known as the PZP (PHD finger–zinc knuckle–PHD finger) domain.22,23 In addition, BRPF1 possesses two enhancer of polycomb (EPC)-like motifs flanking the PZP domain.19–21 Through one of these EPC-like motifs and its N-terminal region, BRPF1 interacts with and activates three histone acetyltransferases, KAT6A, KAT6B, and KAT7.19–21 Thus, BRPF1 is a unique chromatin reader that also possesses...
sequence motifs mediating interaction with histone acetyltransferases. Zebrfish and mouse genetic studies have shown that Brpf1 is essential for embryo survival, hematopoiesis, head patterning, and brain development. Moreover, mutations in KAT6A (MIM: 601408) and KAT6B (MIM: 605880) have recently been discovered in multiple individuals with several developmental disorders characterized by intellectual disability and other abnormalities. Thus, an interesting question is whether mutations of human Brpf1 (MIM: 602410) cause similar developmental anomalies. Here, we report that heterozygous missense, nonsense, and reading frameshift mutations in this gene cause a neurodevelopmental disorder characterized by congenital hypotonia, global developmental delay, intellectual disability, and facial dysmorphism. We show that these mutations cause deficiency of histone H3K23 acetylation and provide evidence that BRPF1 acts through KAT6A and KAT6B to govern this chromatin modification in vitro and in vivo. Together, this study and the recent reports on individuals with KAT6A and KAT6B mutations indicate an emerging group of developmental disorders caused by aberrant histone H3 acetylation.

Subjects and Methods

Human Subjects

Families gave consent for studies approved by the CHU Sainte-Justine Institutional Review Board or by the institutional review boards of the DDD (Decipher Developmental Disorder) Study, the CAUSES (Clinical Assessment of the Utility of Sequencing and Evaluation as a Service) Study, or the Leiden University Medical Center and Maastricht University Medical Center. Written informed consent was obtained for all ten individuals involved in this study.

Exome and Sanger Sequencing

Individual P1’s exome sequencing was performed as a part of the CAUSES study. Clinical exome sequencing of individual P2 was carried out at Baylor Genetics. For individual P3 (sibling of individual P2), only Sanger sequencing of a candidate mutation was done at Baylor Genetics. Clinical exome sequencing for individuals P4 and P5 was performed at GeneDx. Exome sequencing of individuals P6 and P10 was carried out at Leiden University Medical Center and Maastricht University Medical Center, respectively. Individuals P7, P8, and P9 were subject to research exome sequencing as a part of the DDD study. After exome sequencing, PCR fragments were amplified from genomic DNA or reversely transcribed cDNA to confirm BRPF1 mutations.

Lymphoblastoid Cell Line Preparation

Lymphoblastoid cell lines were established and cultured as described previously.

Animal Study Approval

Mice used were in the C57BL/6j genetic background. The Brpf1 allele contains two loxP sites flanking exons 4–6 of Brpf1. Ella-Cre-mediated total knockouts, as well as forebrain-specific and hematopoietic-specific knockouts, have been described elsewhere. To generate epiblast-specific knockouts, Brpf1 mice were mated with the Mox2-Cre strain (Jackson Laboratory, 003755; Bar Harbor). Knockout efficiency was verified by genomic PCR and/or quantitative reverse-transcriptase PCR (qRT-PCR) as previously described. Mouse-related procedures were performed according to the Animal Use Protocol 5786, which was reviewed and approved by the Facility Animal Care Committee of McGill University.

Immunoprecipitation and Histone Acetylation Assays

An expression plasmid for FLAG-tagged KAT6A was transfected into HEK293 cells, along with expression vectors for HA-tagged BRPF1, ING5, and MEAF6, as specified. About 48 hr after transfection, cells were washed twice with PBS, and soluble protein extracts were prepared for affinity purification on anti-FLAG M2 agarose (Sigma) as previously described. The FLAG peptide was used to elute bound proteins for immunoblotting with anti-FLAG and -HA antibodies or for acetyltransferase activity determination. Acetylation of HeLa oligonucleosomes was performed as previously reported. After acetylation reactions, immunoblotting was carried out to detect histone H3 or its site-specific acetylation and was performed with anti-histone H3 (Abcam, ab1791), anti-H3K9ac (Abcam, ab10812), anti-H3K14ac (EMD Millipore, 07-353), anti-H3K18ac (EMD Millipore, 07-354), and anti-H3K23ac (EMD Millipore, 07-355) antibodies.

Fluorescence Microscopy

For analysis of subcellular localization, expression plasmids for EGFP-BRPF1 and its mutants were transiently transfected into HEK293 cells with or without those for KAT6A, ING5, and MEAF6, as specified in Figure S4. About 16 hr after transfection, live green fluorescence signals were examined under a fluorescence microscope, and fluorescence images were captured for further processing as previously described.

qRT-PCR and Regular PCR

For analysis of nonsense-mediated mRNA decay (NMD), control and mutant lymphoblastoid cells and fibroblasts were prepared for RNA extraction. RNA was reversely transcribed with the QuantiTect Reverse Transcription Kit (QIAGEN). qRT-PCR was performed on Realplex2 (Eppendorf) with the Green-2-Go qPCR Mastermix (BioBasic). Primers were designed on the web-based IDT real-time PCR primer designer and synthesized by IDT Biotechnology. BR27 (5’-TTT CCC GAA TTC AGG GCT-3’) and BR20 (5’-GGG CCC AAG CTT AGCT GTG CAG CCT-3’) were for amplification of the region corresponding to the N-terminal part of BRPF1, whereas BR11 (5’-GG CCA CGG ACA ATG TGG CAG AGA-3’) and BR10 (5’-TTT CCC AAG CTT AGT GGT GGC CAG CCT-3’) were for a region encoding the C-terminal part of BRPF1. To determine whether NMD definitely degrades the mutated BRPF1 transcript, cDNA prepared from fibroblasts cultured from a skin biopsy of individual P5 was used for PCR amplification with primers BR419 (5’-TTG AGC ACA TCC TGG CAG AGA-3’) and BR421 (5’-TTG TCT TTA AGG GCC CAG GTT-3’). The amplified fragment was gel purified for Sanger sequencing. To verify her heterozygous mutation, genomic DNA from this individual and her parents was used for PCR amplification with primers BR419 and BR420 (5’-TCT TTA AGG GCC CAG GTT-3’). DNA fragments were gel purified for sequencing.
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