**ORIGINAL INVESTIGATION**

# Five years of experience in the Epigenetics and Chromatin Clinic: what have we learned and where do we go from here?

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## Abstract

The multidisciplinary Epigenetics and Chromatin Clinic at Johns Hopkins provides comprehensive medical care for individuals with rare disorders that involve disrupted epigenetics. Initially centered on classical imprinting disorders, the focus shifted to the rapidly emerging group of genetic disorders resulting from pathogenic germline variants in epigenetic machinery genes. These are collectively called the Mendelian disorders of the epigenetic machinery (MDEMs), or more broadly, Chromatinopathies. In five years, 741 clinic visits have been completed for 432 individual patients, with 153 having confirmed epigenetic diagnoses. Of these, 115 individuals have one of 26 MDEMs with every single one exhibiting global developmental delay and/or intellectual disability. This supports prior observations that intellectual disability is the most common phenotypic feature of MDEMs. Additional common phenotypes in our clinic include growth abnormalities and neurodevelopmental issues, particularly hypotonia, attention-deficit/hyperactivity disorder (ADHD), and anxiety, with seizures and autism being less common. Overall, our patient population is representative of the broader group of MDEMs and includes mostly autosomal dominant disorders impacting writers more so than erasers, readers, and remodelers of chromatin marks. There is an increased representation of dual function components with a reader and an enzymatic domain. As expected, diagnoses were made mostly by sequencing but were aided in some cases by DNA methylation profiling. Our clinic has helped to facilitate the discovery of two new disorders, and our providers are actively developing and implementing novel therapeutic strategies for MDEMs. These data and our high follow-up rate of over 60% suggest that we are achieving our mission to diagnose, learn from, and provide optimal care for our patients with disrupted epigenetics.

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# Introduction

The Epigenetics and Chromatin clinic (ECC) at Johns Hopkins was founded in 2012. At the time, there was a need to centralize care for patients with classical imprinting disorders. Imprinting disorders typically result from disruption of parent-of-origin-specific epigenetic marks and gene expression at particular genetic loci. In addition, a new group of conditions was emerging—defects in the epigenetic machinery. Mendelian disorders of the epigenetic machinery (MDEMs) (Fahrner and Bjornsson 2014)–also referred to as Chromatinopathies (Ciptasari and van Bokhoven 2020)–result from pathogenic germline variants in genes encoding components of the epigenetic and chromatin modifying machinery. At that time, only 20 genes were known to cause MDEMs. However, in the 10 years that the clinic has been operating, this number has risen drastically from 28 genes in 2014 (Fahrner and Bjornsson 2014), to 70 genes in 2019 (Fahrner and Bjornsson 2019), and now to

85 genes in 2022 (Fig. 1). The original mission statement was three-fold: (1) To diagnose and provide optimal care for patients with classical epigenetic (imprinting) disorders or MDEMs; (2) To learn fundamental truths about epigenetics that might lead to future therapeutic development; and (3) To educate healthcare providers and patients about epigenetics and MDEMs. Initially, the clinic was unique because most genetics clinics were consultative and diagnostic in nature, while this clinic structure emphasized building expertise and providing ongoing services to patients with an established diagnosis.

For the first aim of the ECC—to diagnose and provide optimal care for patients with epigenetic disorders—specialized expertise in epigenetics and epigenetic mechanisms of disease has been essential for success. Testing for imprinting disorders is relatively complicated and includes DNA methylation analysis in addition to more traditional copy number, sequencing, and deletion/duplication analyses. Centralization of care is optimal for imprinting disorders that require clinically actionable follow-up, such as tumor screening for Beckwith-Wiedemann syndrome (BWS) (Brioude et al. 2018). For the epigenetic machinery disorders, which can be thought of as genetic disorders expected to lead to genome-wide epigenetic dysregulation, specialized testing is also available. The new EpiSign test now allows for comprehensive testing of DNA methylation signatures for over 70 disorders and offers functional validation of variants of uncertain significance (Aref-Eshghi et al. 2020; Sadikovic et al. 2020).

Our second aim is to gain fundamental knowledge about epigenetics that might lead to future therapeutic development. In this regard, we have robustly defined a list of epigenetic factors and maintain a website (www.epigenetic machinery.org) that is accessible to the clinical genetics and epigenetics communities (Boukas et al. 2019). By limiting MDEMs to those where the implicated protein definitively has a writer, eraser, reader, or remodeler domain (Fahrner and Bjornsson 2019), we have learned about similarities and differences among these disorders. This has led to insights into shared pathogenic features and therapeutic strategies (Fahrner and Bjornsson 2019). Specifically, we observed that the two most common features of MDEMs are intellectual disability and growth abnormalities. In addition, disease-causing variants in genes encoding other components of histone-modifying complexes without these specific domains, such as scaffolding proteins (Imagawa et al. 2017; Machol et al. 2019) and genes encoding histones themselves (Najmabadi et al. 2011; Tatton-Brown et al. 2017; Bryant et al. 2020; Tessadori et al. 2022), have been described more recently. These disorders, encompassed by the broader term "Chromatinopathies," have overlapping phenotypes with MDEMs, including intellectual disability and growth dysregulation (defined as growth retardation or overgrowth), among others, supporting the notion that histone modification plays a key role.

Evidence that we are fulfilling the third aim of the ECC mission, which is to educate healthcare providers and patients about epigenetics and MDEMs, comes from our expanding referral base. Originally, most referrals to our clinic came from within the Johns Hopkins system, but as expertise has built in our ECC, we have observed increasing numbers of referrals from external genetics centers. Moreover, many patients learn about our clinic from patient organizations. We currently serve a multiracial population of individuals from across the U.S. as well as international patients.

Over the years, the Johns Hopkins ECC has expanded and evolved. In 2013, one year after its inception, the current director joined as a second clinical geneticist to help build the new clinical enterprise. By 2014, an additional genetic counselor had joined. Upon departure of the founding director (a clinical geneticist), and given the strong neurodevelopmental impact of many of these disorders, in 2018 the clinic became multidisciplinary, with the addition of a neurologist and neurodevelopmental specialist with expertise in this area. The current providers of ongoing care in the clinic are the director (a clinical geneticist), the neurologist and neurodevelopmental specialist, and a genetic counselor, assisted by a clinic coordinator. Since our clinic's inception, there have been similar clinics with overlapping focus established in the U.S. and internationally. Herein, we describe our demographics from the past 5 years, share some molecular and phenotypic insights and discoveries facilitated by our clinic, and discuss technological advances for diagnosis and potential future therapies.

## Methods

ECC records from our patients evaluated from July 2016 through June 2021 were examined for diagnosis, genetic workup, growth and neurodevelopmental parameters, and demographics. The study period was selected because it was the most recent five-year time period with available clinical data. Collected patient information was then de-identified. Postnatal growth abnormality refers to growth retardation or overgrowth and was defined as an absolute Z-score ($|$Z-score$|$) > 2 in length/height on CDC charts for 0–36 months-old (adjusted for gestational age when needed) or 2–20 years old. Postnatal micro-/macrocephaly was determined as an $|$Z-score$|$ > 2 on the CDC chart for 0–36 months old, or the Nellhaus chart for 2–18 years old. Growth abnormality at birth was defined as an $|$Z-score$|$ > 2 for any length, weight, or head circumference on the Fenton 2013 chart. Neurodevelopmental diagnoses (i.e., intellectual disability, attention-deficit/hyperactivity disorder, anxiety, and autism
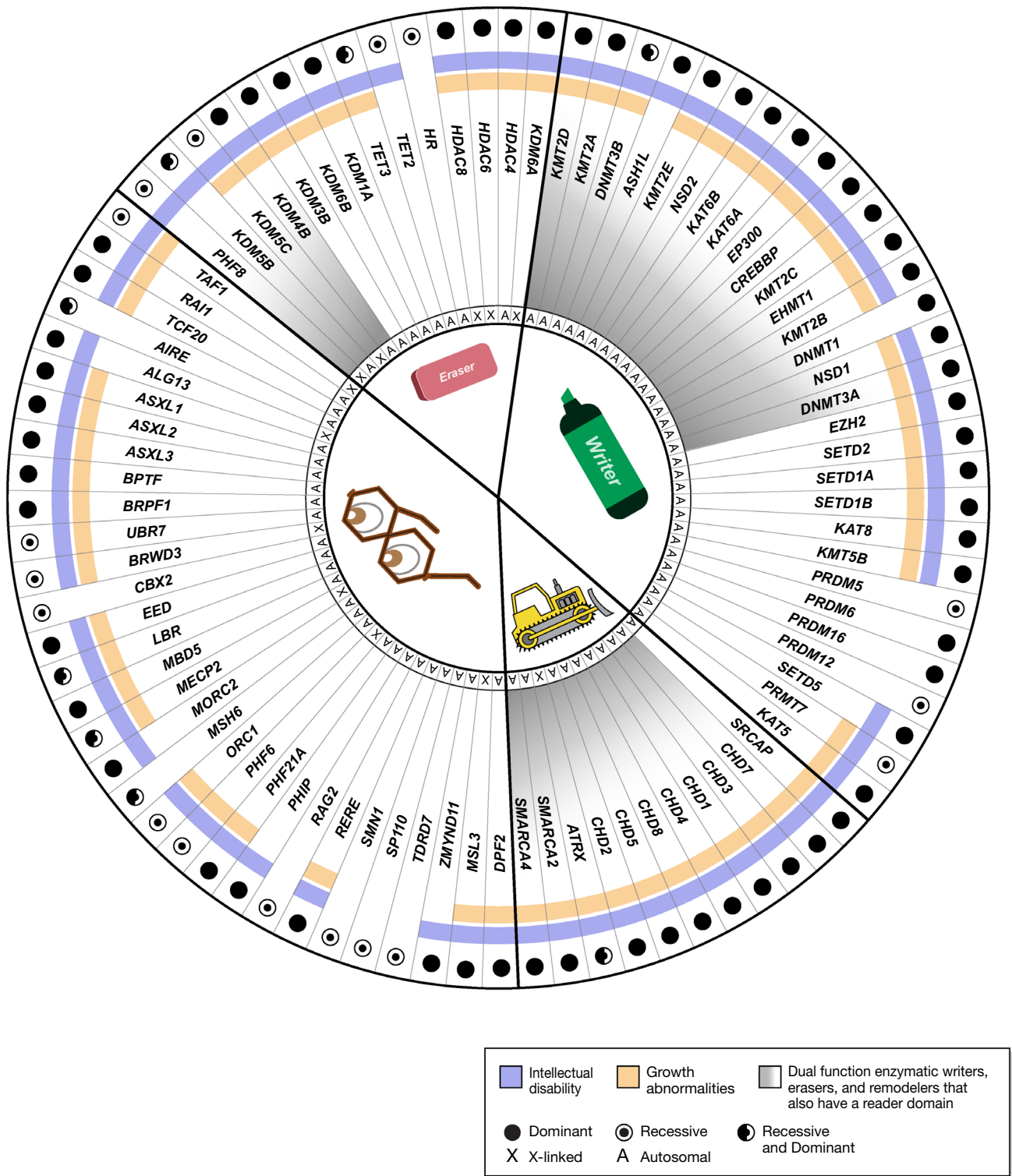
**Fig. 1** Mendelian disorders of the epigenetic machinery (MDEMs). The 85 genes known to cause MDEMs are grouped based on whether they encode enzymatic writers, erasers, or remodelers or non-enzymatic readers (middle icons). Gray shading indicates dual function components that have a reader domain in addition to one of the above enzymatic domains. The small circles around the periphery denote inheritance pattern: dominant (filled circles), recessive (dot in center of circle), or dominant and recessive inheritance reported (half dot/half-filled circle). Genes associated with intellectual disability or growth abnormalities (growth retardation or overgrowth as defined in the text) are indicated with blue or orange shading around the periphery, respectively. In the inner circle, "A" or "X" denotes a gene's location as being on an autosome or the X chromosome, respectively (adapted from Fahrner and Bjornsson 2019)

spectrum disorder) were defined as per the Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-5). Hypotonia was determined based on a physical exam of the patient during clinic visits and seizures were determined based on documented history.

Patients are referred to the ECC after being diagnosed with an epigenetic disorder or if they have suggestive features (developmental delay, intellectual disability, growth abnormality, etc.) without an alternative diagnosis; all referrals are reviewed by a genetic counselor with expertise in this area to determine appropriateness of scheduling for the clinic. Diagnosis was categorized as (1) MDEM; (2) Imprinting; (3) Epigenetic, other; (4) Non-epigenetic, (5) Undiagnosed, or (6) None. 'MDEMs' were strictly defined as resulting from disruption of genes encoding components of the epigenetic machinery, which contained writer, eraser, remodeler, and/or reader domains (Fahrner and Bjornsson 2019). 'Imprinting' were defined as disorders that disrupt parent-of-origin-specific gene expression. 'Epigenetic, other' were defined as disorders that disrupt genes encoding chromatin components that do not have an epigenetic machinery domain, for example, here, a chromatin scaffold protein and a histone. 'Non-epigenetic' was defined as a genetic disorder that does not fall within the former three categories. 'Undiagnosed' was defined as a lack of definitive diagnosis, or awaiting test results, but evaluated to have a high likelihood of genetic etiology by a medical geneticist. Patients determined to have a non-genetic etiology were categorized as 'None'.

## Results

### Epigenetics and Chromatin Clinic demographics

Over a period of five years, spanning from July 2016 through June 2021, a total of 741 visits were completed at the ECC, providing care for 432 individual patients (Table 1). Of these, 220 patients (50.9%) returned for at least one follow-up appointment, and while we continued to gain substantial numbers of new patients, follow-ups accounted for an increasing proportion of visits yearly (Fig. 2). By 2020–2021 (Year 5), 62.5% of visits were follow-ups. Genetic medicine practices typically focus on initial diagnosis, yet our experience suggests that patients and their families prefer to continue receiving care where highly specialized and multidisciplinary expertise is available for this class of rare disorders. With the departure of one medical geneticist in 2018 (Year 3), the number of appointment slots had to be reduced by approximately 50% (Fig. 2). The ECC remained fully booked throughout the COVID-19 pandemic, which reached the United States by March 2020, towards the end of Year 4. In accordance with institutional safety guidelines,

**Table 1** Epigenetic and Chromatin Clinic Demographics

| Characteristic | |
| --- | --- |
| Age (years)–all visits | |
| Mean | 8.67 |
| Range | 0 – 53 |
| Age group (%) | |
| < 1 | 182 (24.6) |
| 1–5 | 278 (37.5) |
| 6–10 | 108 (14.6) |
| 11–18 | 66 (8.9) |
| > 18 | 107 (14.4) |
| Sex–all patients (%) | |
| Male | 206 (47.7) |
| Female | 226 (52.3) |
| Race–all patients (%) | |
| Black/African American | 64 (14.8) |
| White/Caucasian | 274 (63.4) |
| Asian/Pacific Islander | 30 (6.9) |
| Other | 64 (14.8) |
| Ethnicity–all patients (%) | |
| Hispanic or Latino | 39 (9.0) |
| Non-Hispanic or Latino | 393 (91.0) |

patients were evaluated by video visit whenever possible (Fig. 2). This was especially important as certain epigenetic conditions, such as Kabuki syndrome (MIM 147920 and 300867), can be associated with immunodeficiency (Margot et al. 2020).

A notable benefit of the widespread use of telemedicine was improved access to the ECC by out-of-state patients. The ECC has always drawn patients from a wide geographic range. In these five years, patients originated from 3 countries (United States, Australia, and Brazil) and 29 states of the U.S and the District of Columbia (Fig. 3). In total, 116 patients (26.9%) resided outside of Maryland. Other top states included Virginia (30 patients, 6.9%) and Pennsylvania (18 patients, 4.2%). The patient population we encountered was mixed, consisting of 63.4% White/Caucasian, 14.8% Black/African American, 6.9% Asian or Pacific Islander, and 14.8% multiracial or other; 9.0% of all patients identified as Hispanic or Latino (Table 1). Most patients (92.8%) preferred English, with Spanish as the second most common language (4.2%). Qualified language interpretation services were present at all appointments where English was not the language of choice.

New patients are commonly referred to the ECC either because they are seeking expertise for a known epigenetic condition, or because they have a combination of a growth abnormality with developmental delay and/or intellectual disability. Altogether, 153 individuals with confirmed (clinically or molecularly) epigenetic disorders have been

**Fig. 2** Visits to the Epigenetics and Chromatin Clinic. Numbers and percentages of visits by year and type of visit. New visits are indicated with red shading, and follow-up visits are indicated with blue shading. Video visits are indicated with black diagonal lines. Year 1: July 2016-June 2017; Year 2: July 2017-June 2018; Year 3: July 2018-June 2019; Year 4: July 2019-June 2020; Year 5: July 2020-June 2021
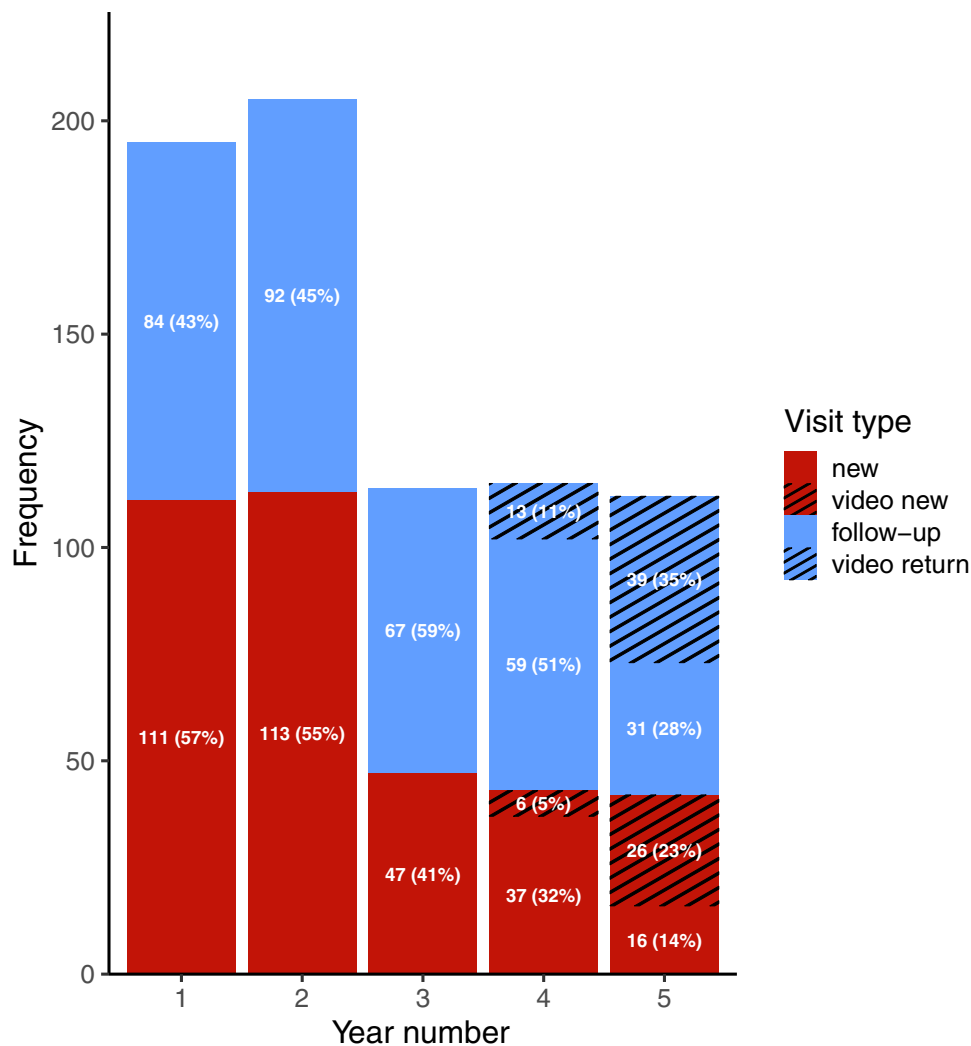
seen in the ECC over the 5-year span. 115 patients (26.6%) were diagnosed with a Mendelian disorder of the epigenetic machinery (MDEM), and 36 patients (8.3%) had an imprinting disorder (Fig. 4a). Notably, 2 patients were discovered to have pathogenic variants in *SMARCC2* (Machol et al. 2019) and *HIST1H1E* (Tatton-Brown et al. 2017), which encode for a scaffolding component of the BAF complex (He et al. 2020) and a linker histone, respectively. Though not strictly considered epigenetic machinery, these proteins nonetheless play a critical role in the proper maintenance of chromatin states and the epigenome, and therefore we classify these as epigenetic disorders (Chromatinopathies). Of the remaining patients seen in the ECC, 139 patients (32.2%) were ultimately diagnosed with a non-epigenetic disorder, 119 (27.5%) remain undiagnosed pending further genetic testing, and 21 (4.9%) were deemed to have a non-genetic etiology (Fig. 4a).

Among MDEMs, Kabuki syndrome 1 was the most common disorder seen in our clinic, accounting for 34 patients (29.6%) (Fig. 4b). This was followed by Wiedemann-Steiner

syndrome (14 patients; 12.2%; MIM 605130), Sotos syndrome (12 patients; 10.4%, MIM 117550), Arboleda-Tham syndrome, also known as KAT6A syndrome (10 patients; 8.7%; MIM 616268), and Intellectual developmental disorder, autosomal dominant 1, also known as *MRD1* (7 patients, 6.1%, MIM 156200). In total, we provided care for patients with 26 distinct MDEMs (Table 2). Among imprinting disorders, Beckwith-Wiedemann syndrome (BWS; MIM 130650) was by far the most common (20 patients; 55.6% of imprinting disorders).

## Phenotypic insights from the Epigenetics and Chromatin Clinic

Of the 153 patients with epigenetic disorders, 139 individuals have a neurodevelopmental disability of some kind, with 132 individuals exhibiting developmental delay, cognitive impairment, or intellectual disability. BWS (20 individuals) and Russell-Silver syndrome (RSS; 3 individuals) are generally reported to not cause significant neurodevelopmental
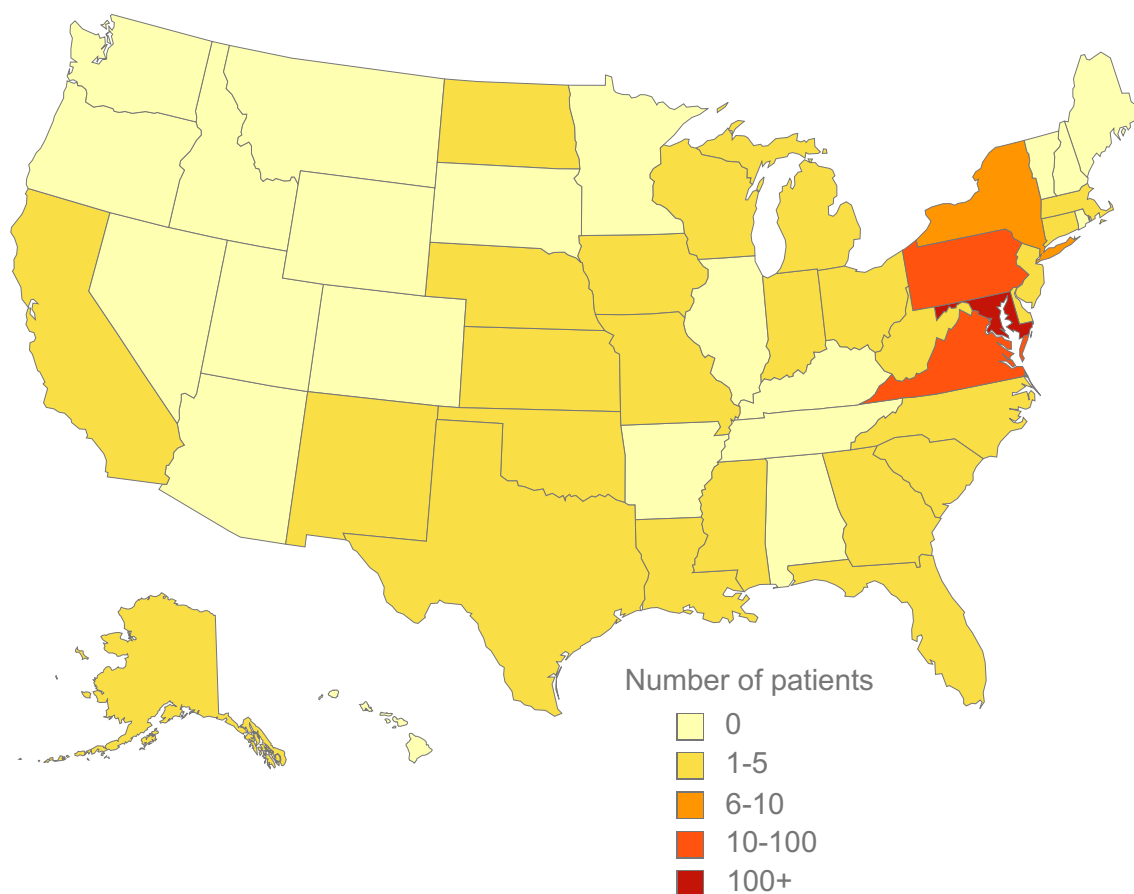
**Fig. 3** Geographic distribution of Epigenetics and Chromatin Clinic patients in the United States. Number of ECC patients residing in each state at the time of their visit(s) from July 2016 through June 2021. Colors represent the number of patients seen from the state: zero (light yellow); 1–5 (dark yellow); 6–10 (orange); 10–100 (orange red); more than 100 (dark red)

disabilities. However, we noticed that 9/20 individuals (45%) with a BWS diagnosis have attention-deficit/hyperactivity disorder (ADHD) or a language delay. This has not been previously reported in the literature, although one study from 2008 reported that these children have higher than normal behavioral and emotional problems (Kent et al. 2008). One individual with BWS had profound ID and cerebral palsy, but this was thought to be due to a severe anoxic brain injury shortly after birth as opposed to a direct result of BWS. Another individual with language delay had a confounding paternal interstitial duplication of chromosome 11. We also recognize that some of the language delay in these patients may be solely structural due to macroglossia, although with the known association of ADHD and language delay, some of this may be a true language delay (Mueller and Tomblin 2012). Of the 130 individuals with non-BWS and non-RSS epigenetic disorders, every single one (100%) has some neurodevelopmental disorder and a striking 129/130 (99.2%) had developmental delay, cognitive impairment, or intellectual disability. The single exception

is a 22-year-old with Schaaf-Yang syndrome who only has severe anxiety but no other neurodevelopmental diagnosis.

Every single one of the 115 individuals seen in the ECC with a MDEM has some neurodevelopmental disability that includes developmental delay, cognitive impairment, or intellectual disability. This ranges from profound intellectual disability to more mild but still impactful phenotypes such as specific learning disabilities or ADHD. Cognitively, 21/115 individuals (18.3%) have mild impairments that do not meet the level of intellectual disability (Fig. 5). Specifically, 39/115 individuals (33.9%) have mild intellectual disability (or a developmental quotient in that range), 34/115 (29.6%) have moderate intellectual disability (or a developmental quotient in that range), and 21/115 (18.3%) individuals have severe or profound intellectual disability (or a developmental quotient in that range; Fig. 5). Thirteen individuals (11.3%) are nonverbal (Fig. 5). In addition to cognitive impairment, the other two most commonly seen neurodevelopmental endophenotypes in the MDEM group are ADHD and anxiety. In all, 44/115 individuals (38.3%) have clinically significant

**Fig. 4** Patient diagnoses in the Epigenetics and Chromatin Clinic. **a** Broad categories of diagnoses seen in the ECC from July 2016 through June 2021 include MDEMs (red), Imprinting (blue), and Epigenetic, Other (orange), which are categorized as epigenetic, as well as Non-epigenetic (purple), Undiagnosed (yellow), and None (aqua). Diagnosis types are defined in the text. **b** Top 5 MDEMs diagnosed July 2016 through June 2021. *KS1* Kabuki syndrome 1 (red); *WSS* Wiedemann-Steiner syndrome (blue); *SS* Sotos syndrome (yellow); *ATS* Arboleda-Tham syndrome, also known as KAT6A-associated neurodevelopmental disorder or KAT6A syndrome (purple); *MRD1* Intellectual development disorder, autosomal dominant 1, formerly known as Mental retardation, autosomal dominant 1 (orange). All other MDEM diagnoses (aqua)
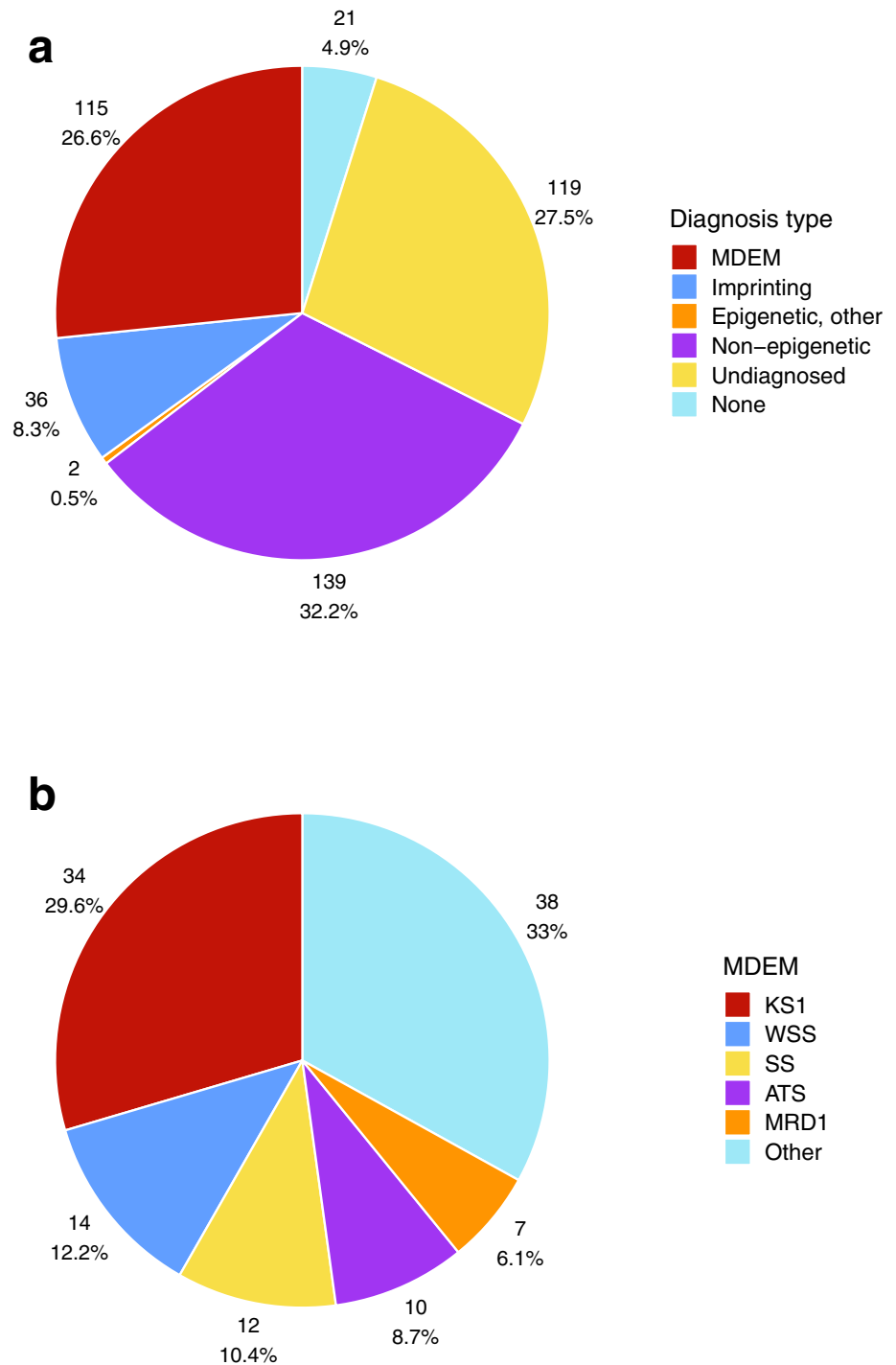


ADHD and 39/115 individuals (33.9%) have clinically significant anxiety (Fig. 5). In contrast, only 10/115 (8.7%) carry diagnoses of autism spectrum disorder (Fig. 5), which supports previous literature that many MDEM syndromes predispose individuals to high sociability (Awan et al. 2022; Chan et al. 2019; Mervis et al. 2005). Lastly, two other common neurodevelopmental features among the group of individuals with MDEMs are hypotonia and

seizures. Clinically notable hypotonia was present in 95/115 (82.6%) individuals at some point in their life. This may even be an underrepresentation given that individuals evaluated in later childhood may have poor documentation of tone in infancy and preschool ages, given that hypotonia typically improves with age. We found 24/115 (20.9%) had seizures at some point with 19 of those having a seizure disorder that required treatment (Fig. 5).

**Table 2** Mendelian disorders of the epigenetic machinery seen in the Epigenetics and Chromatin Clinic

| Disorder | Gene | EM function | Inheritance | OMIM |
|---|---|---|---|---|
| Arboleda-Tham syndrome | KAT6A | Writer* | AD | 616268 |
| Kabuki syndrome 1 | KMT2D | Writer* | AD | 147920 |
| Kleefstra syndrome 1 | EHMT1 | Writer* | AD | 610253 |
| Kleefstra syndrome 2 | KMT2C | Writer* | AD | 617768 |
| Rubinstein-Taybi syndrome 1 | CREBBP | Writer* | AD | 180849 |
| Rubinstein-Taybi syndrome 2 | EP300 | Writer* | AD | 613684 |
| Sotos syndrome | NSD1 | Writer* | AD | 117550 |
| Tatton-Brown-Rahman syndrome | DNMT3A | Writer* | AD | 615879 |
| Wiedemann-Steiner syndrome | KMT2A | Writer* | AD | 605130 |
| Wolf-Hirschhorn syndrome | NSD2 | Writer* | AD | 194190 |
| Neurodevelopmental disorder with speech impairment and dysmorphic facies (NEDSID) | SETD1A | Writer | AD | 619056 |
| Short stature, brachydactyly, intellectual developmental disability, and seizures (SBIDDS) | PRMT7 | Writer | AR | 617157 |
| Intellectual developmental disorder, autosomal recessive 65 | KDM5B | Eraser* | AR/AD | 618109 |
| Intellectual developmental disorder, X-linked, syndromic, Claes-Jensen type | KDM5C | Eraser* | XL | 300534 |
| Kabuki syndrome 2 | KDM6A | Eraser* | XL | 300867 |
| Beck-Fahrner syndrome | TET3 | Eraser | AD/AR | 618798 |
| Chromosomal 2q37 deletion syndrome | HDAC4 | Eraser | AD | 600430 |
| Neurodevelopmental disorder with coarse facies and mild distal skeletal abnormalities (NEDCFSA) | KDM6B | Eraser | AD | 618505 |
| CHARGE syndrome | CHD7 | Remodeler* | AD | 214800 |
| Developmental and epileptic encephalopathy, 94 | CHD2 | Remodeler* | AD | 615369 |
| Pilarowski-Bjornsson syndrome | CHD1 | Remodeler* | AD | 617682 |
| Borjeson-Forssman-Lehmann syndrome/CoffinSiris-like syndrome | PHF6 | Reader | XL | 301900 |
| Intellectual developmental disorder, autosomal dominant 1 (*MRD1*) | MBD5 | Reader | AD | 156200 |
| Rett syndrome, atypical | MECP2 | Reader | XLD | 312750 |
| Smith-Magenis syndrome | RAI1 | Reader | AD | 182290 |
| Kabuki-Turner syndrome | – | – | XL | – |

The disorders are ordered based on the component of the epigenetic machinery impacted and correspond to Fig. 7

*Denotes dual function component; *EM* epigenetic machinery, *OMIM* Online Mendelian Inheritance in Man, *AD* autosomal dominant, *XL* X-linked, *XLD* X-linked dominant, *AR* autosomal recessive

When we look at our 5 most common MDEM diagnoses, of 34 individuals with Kabuki syndrome type 1 (KS1), 8 (23.5%) have borderline cognitive impairment or global developmental delay above the cutoff for intellectual disability, 17 (50%) have mild intellectual disability or a developmental quotient (DQ) in that range, 7 (20.6%) have moderate intellectual disability or a DQ in that range, 2 (5.9%) have severe or profound intellectual disability (one is a child who had autoimmune encephalopathy in addition to KS1). Three (8.8%) are nonverbal. Two of these nonverbal children use assistive technology and signs to communicate, and one child has not been seen for follow up in clinic and so this data is unknown. Behaviorally, 14 (41.2%) have ADHD, 16 (47.1%) have anxiety, and 4 (11.8%) have autism. Hypotonia was present in 31 (91.2%) and 6 (17.6%) have seizures (Fig. 5). Of 14 individuals with Wiedemann-Steiner syndrome, 3 (21.4%) have borderline cognitive impairment or global developmental delay above the cutoff for intellectual disability, 8 (57.1%) have mild intellectual disability or a DQ in that range, 3 (21.4%) have moderate intellectual disability or a DQ in that range, and none have severe or profound intellectual disability. None are nonverbal. Behaviorally, 10 (71.4%) have ADHD, 9 (64.3%) have anxiety, and 1 (7.1%) has autism. Hypotonia was present in 13 (92.9%) and 1 (7.1%) has seizures (Fig. 5). Of 12 individuals with Sotos syndrome, 4 (33.3%) have borderline cognitive impairment or global developmental delay above the cutoff for intellectual disability, 4 (33.3%) have mild intellectual disability or a DQ in that range, and 4 (33.3%) have moderate intellectual disability or a DQ in that range. None have severe or profound intellectual disability, and none are nonverbal. Behaviorally, 5 (41.7%) have ADHD, 5 (41.7%) have anxiety, and none have autism. Hypotonia is present in 11 (91.7%) and 2 (16.7%) have seizures (Fig. 5). Of 10 individuals with Arboleda-Tham syndrome, 1 (10%) has borderline cognitive impairment or global developmental delay above the
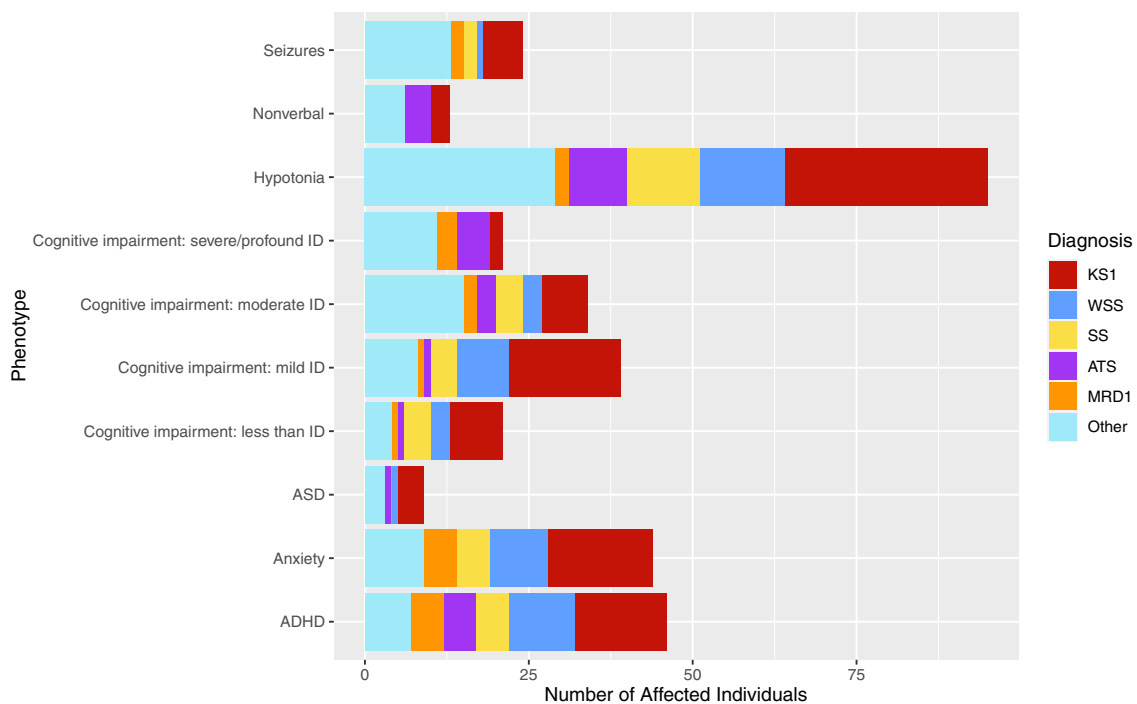
**Fig. 5** Neurodevelopmental phenotypes among Mendelian disorders of the epigenetic machinery seen in the Epigenetics and Chromatin Clinic. Number of individuals with each described neurodevelopmental phenotype among the 115 patients with MDEMs broken out by the 5 most common diagnoses and all others (aqua). *KS1* Kabuki syndrome 1 (red); *WSS* Wiedemann-Steiner syndrome (blue); *SS* Sotos syndrome (yellow); *ATS* Arboleda-Tham syndrome (also known as KAT6A-associated neurodevelopmental disorder or KAT6A syndrome; purple); *MRD1* Intellectual development disorder, autosomal dominant 1, formerly known as Mental retardation, autosomal dominant 1 (orange). It should be noted that quite a few of the individuals with the different syndromes were infants or toddlers and so their ADHD and anxiety phenotypes remain unknown

cutoff for intellectual disability, 1 (10%) has mild intellectual disability or a DQ in that range, 3 (30%) have moderate intellectual disability or a DQ in that range, and 5 (50%) have severe or profound intellectual disability. Four (40%) are nonverbal. Behaviorally, 5 (50%) have ADHD, none have anxiety, and 1 (10%) has autism. 9 (90%) have hypotonia and none have seizures (Fig. 5). Of 7 individuals with Intellectual developmental disorder, autosomal dominant 1, 1 (14%) has borderline cognitive impairment or global developmental delay above the cutoff for intellectual disability, 1 (14.3%) has mild intellectual disability or a DQ in that range, 2 (28.6%) have moderate intellectual disability or a DQ in that range, and 3 (42.9%) have severe or profound intellectual disability. None are nonverbal. Behaviorally, 5 (71.4%) have ADHD, 5 (71.4%) have anxiety, and none have autism. Two (28.6%) have hypotonia and 2 (28.6%) have seizures (Fig. 5). It should be noted that quite a few of the individuals with the different syndromes were infants or toddlers and so their ADHD and anxiety phenotypes remain unknown.

Eighty-one individuals with MDEMs (70.4%) had some type of growth abnormality, with height affected in 69 individuals (60%) and head size affected in 49 individuals (42.6%) (Fig. 6a,b). Thirty-seven individuals with MDEMs (32.2%) had growth abnormalities affecting both height and head circumference. Of those with growth abnormalities, 63 (77.8%) exhibited growth retardation–with 56 having short stature, 35 having microcephaly, and 28 with both (Fig. 6a). Eighteen individuals (22.2%) exhibited overgrowth–13 with tall stature, 14 with macrocephaly, and 9 with both (Fig. 6b). Only 18/89 individuals with MDEMs (20.2%) exhibited abnormalities in birth growth parameters; however, information was unavailable for 26 individuals.

## Molecular insights from the Epigenetics and Chromatin Clinic

MDEMs result from pathogenic variants in writers, erasers, readers, and remodelers of chromatin marks (Bjornsson 2015; Fahrner and Bjornsson 2014; Fahrner and Bjornsson 2019) (Fig. 1). Some epigenetic machinery components exhibit dual function, including both a non-enzymatic reader domain and an enzymatic writer, eraser, or remodeler domain (Boukas et al. 2019; Fahrner and Bjornsson 2019) (Fig. 1; Table 2). Of the 26 distinct MDEMs seen in our clinic in the past 5 years, 12 (46.2%) impact writers, 6 (23%) impact erasers, 4 (15.4%) impact readers without
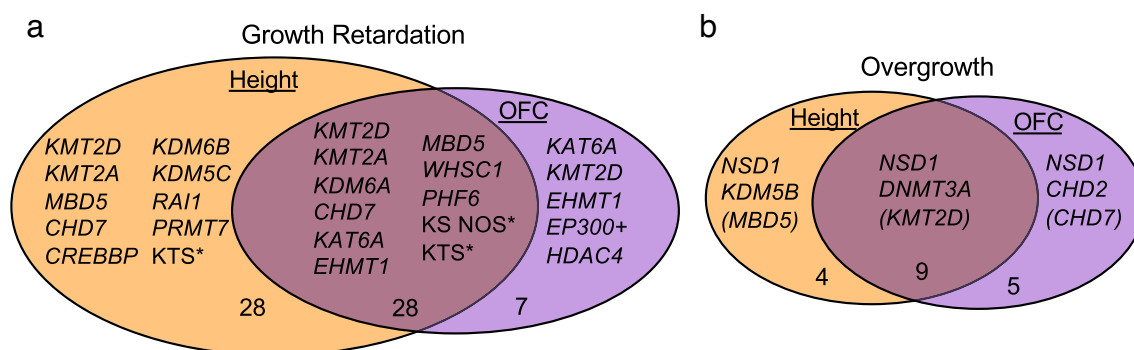
**Fig. 6** Growth abnormalities observed in patients with Mendelian disorders of the epigenetic machinery in the Epigenetics and Chromatin Clinic. Venn diagram for **a** growth retardation and **b** overgrowth based on whether height, occipital-frontal head circumference (OFC), or both were affected. Numbers indicate the total number of affected individuals with each feature; gene names indicate the epigenetic machinery component disrupted. *Indicates gene unknown. This is the case for Kabuki-Turner syndrome (KTS), as its molecular etiology is not known, and for Kabuki syndrome not otherwise specified (KS NOS), as the individual met clinical criteria for KS, but no pathogenic variant was identified in either gene that causes KS (*KMT2D* or *KDM6A*). + indicates that the affected individual had a pathogenic variant in another gene in addition to *EP300*. Parentheses indicate that for these genes, overgrowth is not typically observed. Only 18/89 individuals with MDEMs (20.2%) exhibited abnormalities in birth growth parameters; however, information was unavailable for 26 individuals

dual functions, and 3 (11.5%) impact remodelers (Fig. 7). The exact molecular etiology of one disorder, Kabuki-Turner syndrome, remains unknown (Dennis et al. 1993; Wellesley and Slaney 1994; Rodriguez et al. 2008). Sixteen MDEMs seen in our clinic (61.5%) result from pathogenic variants in dual function components (Table 2). This includes all the remodeler disorders, 10 of the 12 writer disorders, and 3 of the 6 eraser disorders (Fig. 7).

According to published literature, the vast majority of MDEMs are inherited in an autosomal dominant manner and result from de novo variants in probands; a smaller fraction of genes are located on the X chromosome or exhibit autosomal recessive inheritance (Bjornsson 2015; Fahrner and Bjornsson 2019) (Fig. 1). Of the 26 distinct MDEMs seen in the ECC, 18 (69.2%) exhibit autosomal dominant inheritance, five (19.2%) exhibit X-linked inheritance, one (3.8%) exhibits autosomal recessive inheritance, and two (7.7%) have both autosomal dominant and autosomal recessive forms described (Table 2).

Fifty-eight individuals out of 115 with MDEMs (50%) were diagnosed by whole exome sequencing (WES), and 39 (34%) were diagnosed by single gene or gene panel testing. The latter category was dominated by individuals with Kabuki, Sotos, and CHARGE (MIM 214800) syndromes, which are all well-known and recognizable to many clinical geneticists because of their well-described and specific constellations of phenotypic features. Most of the remaining 18 individuals with MDEMs who were not diagnosed using WES or gene or gene panel testing had copy number variants and thus were diagnosed by SNP microarray. This included individuals with Intellectual Developmental Disorder, Autosomal Dominant 1 due to 2q23.1 deletions or duplications, Kleefstra syndrome 1 (MIM 610253) due to 9q34.3 deletions, Sotos syndrome due to 5q35 deletions, Wolf-Hirschorn syndrome (MIM 194190) due to 4p16.3 deletion, Smith-Magenis syndrome (MIM 182290) due to 17p11.2 deletion, and Brachydactyly-mental retardation syndrome (MIM 600430) due to 2q37 deletion. Two individuals with Kabuki syndrome-like phenotypes had X chromosome abnormalities diagnosed by karyotype suggestive of Kabuki-Turner syndrome (Dennis et al. 1993; Wellesley and Slaney 1994; Rodriguez et al. 2008) with one having tested negative for mutations in both Kabuki syndrome genes. Two patients met clinical criteria for particular diagnoses (CHARGE and Kabuki syndrome) but lacked molecular confirmation. Both individuals with Chromatinopathies who did not meet criteria for a MDEM were diagnosed by exome sequencing.

## Genome-wide DNA methylation profiling as a diagnostic tool

Over the past few years, it has become clear that genome-wide DNA methylation profiling is a useful tool for the diagnosis of an increasing number of MDEMs and other neurodevelopmental disorders (Aref-Eshghi et al. 2019; Aref-Eshghi et al. 2020; Aref-Eshghi et al. 2017; Choufani et al. 2015; Choufani et al. 2020; Levy et al. 2022; Sadikovic et al. 2020; Sadikovic et al. 2021). Classically, DNA methylation occurs at cytosines followed by guanines (CpGs). Performed on DNA isolated from human whole blood, DNA methylation array analysis can identify sensitive and specific genome-wide DNA methylation profiles associated with particular genes and syndromes. These so-called "episignatures" consist of a collection of CpG sites whose DNA
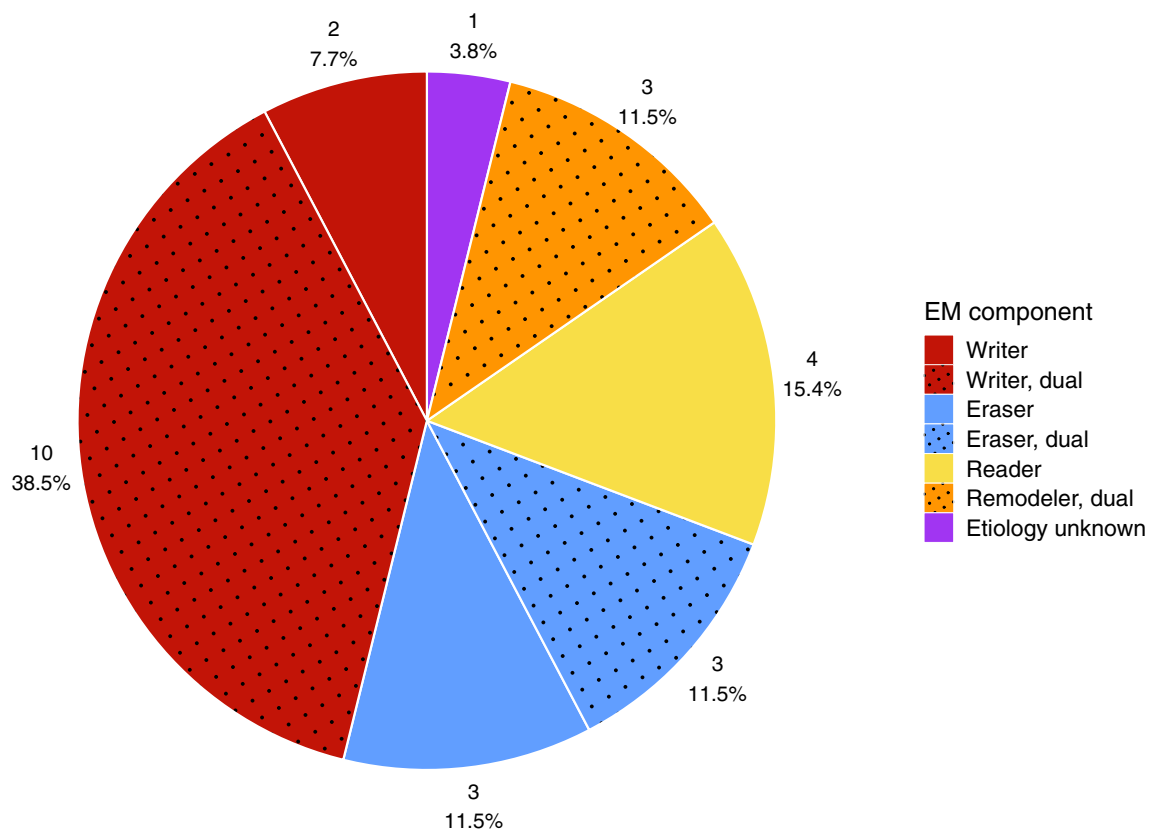
**Fig. 7** Components of the epigenetic machinery disrupted in patients from the Epigenetics and Chromatin Clinic. Functions of the epigenetic machinery implicated in the 26 Mendelian disorders of the epigenetic machinery seen in the ECC from July 2016 through June 2021. Proportion of each category: writers (red); erasers (blue); readers (yellow); and remodelers (orange). Purple indicates that the epigenetic machinery component function is unknown. The proportion of each enzymatic component (writer, eraser, remodeler) with dual function (i.e., with an accompanying reader domain) is indicated with black dots. The numbers correspond to the disorders listed in Table 2

methylation is altered similarly in a particular disease state compared to other disease states and controls. In many cases, these DNA methylation profiles can confirm or refute the pathogenicity of DNA sequence variants, aiding in diagnosis (Aref-Eshghi et al. 2019; Aref-Eshghi et al. 2020; Aref-Eshghi et al. 2017; Choufani et al. 2015; Choufani et al. 2020; Levy, McConkey et al. 2022; Sadikovic et al. 2020; Sadikovic et al. 2021). Some data suggest that differentially methylated regions (DMRs) identified by DNA methylation array profiling of blood may have biological significance as well (Levy et al. 2021; Levy, Relator, et al. 2022b). Importantly, there is now a clinical DNA methylation array test called EpiSign (Aref-Eshghi et al. 2020; Sadikovic et al. 2020) available in the United States (ggc.org/episign) and the Netherlands (genoomdiagnostiek.nl/product-tag/epi-sign/) that has been useful diagnostically in our ECC.

We have sent clinical DNA methylation profiling on six patients from our clinic since it became available in 2019. In general, clinical DNA methylation profiling is used as a variant interpretation tool (EpiSign Variant) or as part of the broader diagnostic work up (EpiSign Complete). In two

cases, EpiSign Complete was negative and non-diagnostic. In one case it revealed a diagnosis of the imprinting disorder Temple syndrome (MIM 616222) in a 7-year-old girl with reduced growth prenatally and postnatally, infantile feeding difficulties, persistent short stature, later-onset obesity, developmental delay, behavioral challenges including skin picking, hypotonia, and precocious puberty. In a second case, we successfully used EpiSign Variant to confirm pathogenicity of a variant of uncertain significance in the well-known *CHD7* gene responsible for CHARGE syndrome in a family with an atypical presentation of the disease. The proband had one major and two minor criteria for a diagnosis of CHARGE syndrome (Hale et al. 2016)–unilateral coloboma, heart defect, and mild developmental delay–and was found to have a missense variant of uncertain significance in *CHD7*. Familial testing revealed the variant in her seemingly unaffected father, who on further evaluation had a history of mild developmental delay, poor childhood growth, mild unilateral facial nerve palsy, and recurrent blocked tear ducts requiring multiple surgeries, and in her infant brother, who had cataracts, noisy breathing, and severe constipation.

Simultaneous grandparental testing revealed that the variant occurred de novo in the proband's father, supporting pathogenicity and further confirming the EpiSign results. In a third case, we used EpiSign Variant to confirm Kabuki syndrome 1 in a patient with a low-level somatic mosaic de novo nonsense variant in *KMT2D* who had severe congenital heart disease and was initially too critically ill for his features of Kabuki syndrome to be fully appreciated (Montano et al. 2022). Conversely, in addition to confirming variants of uncertain significance as pathogenic, we have used EpiSign Variant to help rule out pathogenicity. A female proband with poorly defined neurodevelopmental difficulties was found to have a missense variant of uncertain significance in *KDM5C* inherited from a mother with a history of traumatic brain injury. EpiSign Variant revealed that the DNA methylation profile did not match that of other female carriers of pathogenic *KDM5C* variants (Schenkel et al. 2018), a fraction of whom have mild ID; this helped to rule out a diagnosis of Claes-Jensen X-linked Intellectual Developmental Disorder (MIM 300534) in the family.

## Discovery and advancement of novel disorders in the Epigenetics and Chromatin Clinic

As mentioned above, there has been rapid discovery of novel MDEMs, or Chromatinopathies. The knowledge gained through our ECC has allowed our group to participate in the discovery of two such disorders. Pilarowski-Bjornsson syndrome (MIM 617682) is a rare neurodevelopmental disorder associated with speech abnormalities, macrocephaly, and developmental delay (Pilarowski et al. 2018). The original cohort involved 6 female individuals with de novo missense variants in *CHD1*. Since then, additional patients have been identified with phenotypes that overlap with other syndromes caused by the *CHD* family of genes, such as speech problems, macrocephaly, and intellectual disability (Yasin and Zahir 2020). In addition, we delineated Beck-Fahrner syndrome (BEFAHRS, MIM 618798), which results from mono- or bi-allelic variants in *TET3* and defines a new biochemical category of MDEMs impacting the DNA demethylation eraser system (Beck et al. 2020). Similar to other MDEMs, BEFAHRS has a predominantly neurodevelopmental phenotype with occasional growth abnormalities, as suggested by its mnemonic: **B**ehavioral differences, **E**pilepsy, characteristic **F**acial features, **A**utistic features, **H**ypotonia, **R**etardation of psychomotor development, and **S**ize differences (Levy et al. 2021). Moreover, we identified a genome-wide DNA methylation profile in human whole blood, which can aid in the diagnosis of individuals with this disorder, differentiate affected individuals from individuals with related conditions and non-pathogenic variants, and potentially shed light on disease pathogenesis (Levy et al. 2021).

## Current management and therapeutics on the horizon

Currently, no approved targeted therapies exist to treat the underlying etiology of any epigenetic disorder. Rather, we use supportive care to treat individual symptoms and preventive screening based on knowledge of the particular condition. We often help manage neurodevelopmental and neurobehavioral manifestations. Overall, much of the visit time, particularly with follow-up patients with MDEMs or Chromatinopathies, is spent discussing neurodevelopment and behavior. When insurance and geography allow, we universally refer all patients with MDEMs to Neuropsychology by the time they are 6-7 years old, and we have a specific neuropsychologist at the nearby Kennedy Krieger Institute who has expertise in these epigenetic conditions. We frequently refer to occupational therapy (OT), physical therapy (PT), speech and language pathology (SLP), and Behavioral Therapy. The neurologist and neurodevelopmental specialist provides specific guidance on therapies. Additionally, she provides feedback and suggestions for patients' IEPs, and in certain cases prescribes medications for ADHD, anxiety, and seizures directly from our clinic. Patients prescribed medications are often co-followed in her Neurology clinic at nearby Kennedy Krieger Institute because of the different nursing and administrative support needed for appropriate management of controlled substance prescriptions and individuals with epilepsy. This arrangement works well for us but multidisciplinary epigenetics clinics who want to incorporate the ability to prescribe medications for neurodevelopmental issues, epilepsy, or any other specialty issues will need to consider the most appropriate nursing and administrative support structure.

Excitingly, more specific target-based therapies are on the horizon for epigenetic disorders. For imprinting disorders, the discovery that topoisomerase inhibitors can reactivate the abnormally silenced allele in an Angelman syndrome mouse model (Huang et al. 2011) led to the realization that targeting an antisense transcript would be a viable therapeutic strategy in Angelman syndrome. Multiple strategies are underway to capitalize on this, including actively recruiting multi-center trials (Copping et al. 2021). For MDEMs, two independent publications showed that the memory defect identified in a mouse model of Rubinstein-Taybi syndrome (caused by haploinsufficiency of a writer of histone acetylation) could be improved with postnatal histone deacetylase (HDAC) inhibition, helping break the dogma that intellectual disability cannot be treated in postnatal life (Alarcon et al. 2004; Bourtchouladze et al. 2003). A few years later, Adrian Bird's group demonstrated the potential for postnatal malleability of MDEMs using a CRE-lox system to restore MECP2 in postnatal life and rescue postnatal lethality (Guy et al. 2007). Since then there have been a number

of examples supporting the idea that MDEMs can be treated postnatally. For instance, there have been three successful pre-clinical strategies in Kabuki syndrome using HDAC inhibition (AR-42) (Bjornsson et al. 2014), a ketogenic diet (Benjamin et al. 2017), and LSD1 inhibition (Zhang et al. 2021), and aspects of this work have been validated by other groups (Fasciani et al. 2020; Huisman et al. 2021). These basic science developments are also rapidly making their way to clinical research. Currently, the neurologist and neurodevelopmental specialist in our ECC is conducting a pilot clinical trial of the Modified Atkins diet in adults with Kabuki syndrome, and industry partners are investigating strategies such as LSD1 inhibition for larger clinical trials in this disorder.

## Discussion

The development of therapies for MDEMs/Chromatinopathies is at the cutting edge of clinical genetics and the newer field of clinical epigenetics, and our ECC stands at the forefront. Many current therapeutic strategies for MDEMs involve manipulation of epigenetic or chromatin marks to correct downstream target gene expression, which is disrupted globally due to the underlying genetic defect in the epigenetic machinery (Fahrner and Bjornsson 2019). However, target genes themselves remain fully functional, making possible the restoration of proper gene expression. Ongoing therapeutic challenges may include optimizing drug delivery to relevant tissues and cell types, identifying an optimal developmental stage at which to intervene pharmacologically, and minimizing off-target effects in affected individuals (Fahrner and Bjornsson 2019). By learning about individual disorders and the broader group of MDEMs through clinics like ours, the medical and scientific community will be in a better position to identify and optimize therapeutic interventions moving forward.

As expected, our clinic data are consistent with known genetic mechanisms and inheritance patterns in MDEMs, with the vast majority exhibiting autosomal dominant inheritance and resulting from de novo pathogenic variants in probands. Only five X-linked disorders and a single autosomal recessive disorder were seen. The preponderance of autosomal dominant inheritance in most MDEMs is unlike most other inborn errors of metabolism/enzymopathies, which often require bi-allelic pathogenic variants to manifest disease and thus exhibit autosomal recessive inheritance. This observation from our clinical epigenetics practice fits with prior observations (Fahrner and Bjornsson 2019) and highlights the high probability of loss of function intolerance (pLI) scores and extreme dosage sensitivity for epigenetic machinery genes compared to other sets of genes (Boukas et al. 2019). Dosage sensitivity is also exemplified by the

readers of DNA methylation because deletions, duplications, or single nucleotide variants in either *MECP2* or *MBD5* lead to similar phenotypes in the Rett syndrome/MECP2 spectrum (Sandweiss et al. 2020) or in the MBD5-associated neurodevelopmental disorders spectrum (Mullegama and Elsea 2016), respectively. Moreover, the observation of similar phenotypes in individuals with intragenic pathogenic variants versus microdeletions containing the corresponding epigenetic machinery genes supports haploinsufficiency as a predominant disease mechanism. Examples include Kleefstra syndrome 1 resulting from 9q34 deletions or intragenic *EHMT1* variants (Kleefstra et al. 2006), Sotos syndrome resulting from 5q35 deletions or intragenic *NSD1* variants (Kurotaki et al. 2002), and Kabuki syndrome 1, which was only recently described by our group to result from 12q13.1 deletion in addition to classic intragenic *KMT2D* variants (Luperchio et al. 2020).

When considering enzymatic functions of the epigenetic machinery components(writing, erasing, and remodeling), disorders resulting from mutations in writers predominate both in the complete group of MDEMs (Fahrner and Bjornsson 2019) (Fig. 1) and in our clinic (Fig. 7). About twice as many writer disorders compared to eraser disorders exist, and in our clinic, we saw double the number of writer disorders compared to eraser disorders (12 or 46% compared to 6 or 23%). The proportion of individual patients we saw in our clinic with writer disorders is even higher (84/115 or 73%) because our top four diagnoses—Kabuki 1 (*KMT2D*), Wiedemann-Steiner (*KMT2A*), Sotos (*NSD1*), and Arboleda-Tham (*KAT6A*) syndromes–all disrupt writers. Comparatively, we saw disproportionately few remodeler disorders in our clinic—only 3 out of 26 or 11.5% of disorders—though this percentage is similar to the complete group of MDEMs, which consists of just 13% remodelers. Readers are unique as they may function solely as readers or have one of the aforementioned enzymatic domains in addition to a reader domain (dual function components). We saw just 4 MDEMs out of 26 (15%) that impacted readers without an accompanying enzyme function. In contrast, reader-only disorders comprise more than a third of all known MDEMs. Sixteen of 26 MDEMs (61.5%) we saw in clinic impacted dual function components, which is almost double the proportion of dual function components among all known MDEMs (35%). This included all the remodeler disorders, all but two of the writer disorders, and half of the eraser disorders seen in clinic and is consistent with the MDEM literature, as all but one remodeler gene, the majority of writer genes, and close to a third of eraser genes encode dual function components.

Overall, the phenotypes observed in patients with MDEMs in the ECC support current and prior MDEM literature (Fahrner and Bjornsson 2014, 2019; Larizza and Finelli 2019). In general, on a disease-by-disease basis, the vast majority of MDEMs (up to 85%) exhibit developmental

delay and/or intellectual disability (Fahrner and Bjornsson 2019) (Fig. 1), and strikingly, all our clinic patients with MDEMs exhibit this feature. While referral bias could certainly play into the increased prevalence of developmental delay and/or intellectual disability in our clinic due to this feature being a criterion for referral of undiagnosed individuals to the ECC, many of our patients initiate care after being diagnosed with a MDEM, and we only quantified the feature in patients with MDEMs. Thus, the increased number of individuals with developmental delay and/or intellectual disability in the setting of MDEMs in the ECC compared to previous reports in the literature may be a true representation. Moreover, here we have quantified the feature on an individual basis as opposed to a disease basis in the literature; our analysis of a small slice of the MDEM population suggests prevalence may be underestimated by methods used in the literature. Alternatively, the ECC population may be skewed toward our top MDEM diagnoses, all of which exhibit developmental delay and/or intellectual disability, which could lead to our data inflating the proportion of individuals with MDEMs who have developmental delay and/or intellectual disability. We also recognize that hypotonia, which is present in the majority of our MDEM patients, contributes to early motor delay and also impacts the ability of very young children to demonstrate their cognitive skills, often making their intellectual delay appear more severe early in life. As such, some very young children in our clinic may look more delayed than they later turn out to be. Future studies of larger populations of diverse MDEMs in clinics like ours are needed to clarify.

Interestingly, 70% of ECC patients with MDEMs exhibit growth abnormalities, which is almost identical to MDEMs as a whole, in which 74% of disorders have growth abnormalities (Fig. 1). Breaking it down further, 55% of our clinic patients with MDEMs have growth retardation, similar to 58% of the complete group, and 16% of our clinic patients with MDEMs have overgrowth, slightly less than the 27% of the complete group of MDEMs (Fahrner and Bjornsson 2019). The latter may be due to incomplete height and/or head circumference data for all patients from infancy to adulthood, as it is known that anthropometric measurements in individuals with overgrowth can be more striking at an early age. For example, individuals with Sotos syndrome are more likely to exhibit increased height during toddlerhood or school age, but this may normalize in adulthood (Agwu et al. 1999; Foster et al. 2019). Interestingly, for those who exhibit reduced growth, height is more often affected than head circumference; very few of our clinic patients with MDEMs exhibit only microcephaly without height involvement. For individuals with overgrowth, height and head circumference are equally and often both affected; only about half have involvement limited to one or the other. The observations for overgrowth were mostly driven by our cohort of individuals

with Sotos syndrome, which is the most common overgrowth and intellectual disability disorder (Tatton-Brown et al. 2017).

Though intellectual disability and growth abnormalities are the most common phenotypic features described in MDEMs to date (Fahrner and Bjornsson 2019), this group of disorders can have a variety of other manifestations (Bjornsson 2015), most notably neurological and behavioral (Fahrner and Bjornsson 2019). Interestingly, our ECC data from 115 individuals with MDEMs show that hypotonia is actually the second most common feature after developmental delay/intellectual disability. This is followed by growth abnormalities, ADHD, and anxiety, with seizures and autism spectrum disorder being present less commonly. Whereas each of the above findings is non-specific in isolation, in our experience, this is a common constellation of features seen in MDEMs. We recognize that this constellation of features can also be seen in non-MDEM genetic syndromes, so it helps that some disorders additionally have quite specific findings, such as highly characteristic facial features; this is true for Kabuki (Adam et al. 2019), Sotos (Sotos et al. 1964; Tatton-Brown and Rahman 2004), and CHARGE (Hale et al. 2016) syndromes, among others. Not surprisingly, this group is easier to recognize and was more likely to have been diagnosed using targeted testing of genes or gene panels. Conditions without characteristic facial features tend to be more difficult to diagnose and to interpret variants from genome-wide studies. Fortunately, exome sequencing in combination with genome-wide DNA methylation profiling using EpiSign has the potential to improve diagnostic success rates for the latter non-specific group.

DNA methylation profiling is a useful new tool for clinical geneticists; however, relatively few in the field have taken advantage of it in practice. Its use as a variant interpretation tool is increasing globally (J.A.F., personal observation) and includes interpretation of variants of uncertain significance in the setting of non-specific phenotypic features (Levy et al. 2021; Schenkel et al. 2018) and mosaic variants (Montano et al. 2022). Our published data (Levy et al. 2021) and clinical experience suggests, however, that DNA methylation profiling is also useful as part of the initial diagnostic work up. For example, in the above vignette of Temple syndrome, the differential diagnosis also included Russell-Silver, Prader-Willi, and Fragile X syndromes, as well as multiple MDEMs with short stature and neurodevelopmental and behavioral difficulties. Testing for each of these disorders separately would have prolonged the patient's diagnostic odyssey and would have been costly to the healthcare system, requiring altogether: methylation analysis of multiple imprinted loci, *FMR1* trinucleotide repeat analysis, SNP microarray analysis, and whole exome sequencing. In this case, the use of EpiSign Complete–with its turn-around time of 4 weeks and cost of $1500—led to a much more efficient

and less costly diagnosis. This was a success story. Unfortunately, we have far more instances where we attempted to send EpiSign for similar reasons, but coverage was denied by insurance carriers, inhibiting the most efficient and (ironically) the least costly path forward. We would advocate for DNA methylation profiling to be a first-line diagnostic test along with copy number variant testing by chromosomal microarray, followed by next-generation sequencing, in clinics like ours. Consensus recommendations and clinical guidelines are needed.

It is notable that our data from the past five years show a relatively high fraction of non-epigenetic patients. We anticipate the proportion of epigenetic patients has likely increased over the past year (July 2021-June 2022), as increasing numbers of patients continue to present with confirmed molecular epigenetic diagnoses, and the proportion of follow-up visits continues to increase. It bears mentioning that although we specialize in epigenetic conditions, our clinic continues to follow certain groups of non-epigenetic disorders, which may offset our data. For example, we follow 11 patients with Malan syndrome (MIM 614753; 7.9% of non-epigenetic diagnoses), a phenotype that bears a striking physical resemblance to Sotos syndrome (Priolo et al. 2018). In addition, a prominent 'undiagnosed' population includes patients with features of BWS, who have neither received molecular confirmation of this diagnosis nor met the full clinical criteria (Brioude et al. 2018) (12 patients; 10.1% of undiagnosed). Many families in which this diagnosis is being considered elect to follow-up in the ECC for preventive tumor screening for Wilms' tumor and hepatoblastoma due to the increased risk of developing these types of tumors in the setting of BWS or other forms of hemihyperplasia (Brioude et al. 2018; Kalish et al. 2017). However, it is unclear how many may ultimately be diagnosed with BWS or another epigenetic condition.

Looking back on our mission after over 5 years of providing patient care in the ECC, we have been able to diagnose and provide high-quality care for patients with mutations of the epigenetic machinery and classical imprinting disorders. This is evidenced by the low percentage of undiagnosed individuals in our clinic—only 27.5%, in contrast to 65–75% of patients remaining undiagnosed nationwide (Marwaha et al. 2022). Furthermore, while it is an ongoing challenge for patients and families to continue follow-up in genetics clinics (Esmer et al. 2004), the ECC demonstrated a high follow-up rate of 63%. The data presented herein and elsewhere (Fahrner and Bjornsson 2014; Fahrner and Bjornsson 2019) demonstrate that we have learned from our patients some fundamental truths about epigenetic and chromatin machinery and the phenotypes that result from their disruption. Our results (Bjornsson et al. 2014; Benjamin et al. 2017; Zhang et al. 2021) and those of others (Alarcon et al. 2004; Bourtchouladze et al. 2003; Guy et al. 2007) are

on track to lead to future therapeutic development for these individuals with a clinical trial for adults with Kabuki syndrome 1 ongoing and led by the ECC neurologist and neurodevelopmental specialist. Finally, our clinic has allowed us to educate healthcare providers and patients about epigenetics and the disorders of the epigenetic machinery. This has been done through publications (Fahrner and Bjornsson 2014; Fahrner and Bjornsson 2019; Bjornsson et al. 2014; Benjamin et al. 2017; Zhang et al. 2021; Harris et al. 2019; Fahrner et al. 2019; Beck et al. 2020; Levy et al. 2021; Harris and Fahrner 2019; Montano et al. 2022; Ng, Harris, et al. 2022a; Ng, Bjornsson et al. 2022b; Ng et al. 2023a; Ng et al. 2023b), invited talks, referrals of our patients to relevant specialists outside of genetics, and referrals to our clinic from the medical genetics community at large, as well as through our interactions with patient and family support groups.

Beyond the ECC, there now are other similar clinics in the U.S., namely the EpiChroma Clinic at Boston Children's Hospital, the Epigenetics Syndromes Clinic at Cincinnati Children's Hospital, and the Chromatinopathies Clinic at University of California, Los Angeles (A. O'Donnell-Luria and L. Pais, B. Simpson, B. Russell, personal communications), in addition to other syndrome-specific clinics at various institutions. There are presumably epigenetics clinics in other countries as well. Focusing on the U.S. healthcare system, there are similarities between the chromatinopathy-focused clinics in that they are established by a clinical geneticist in an academic medical center and occur 1–2 times per month in frequency. While each clinic accepts referrals for any chromatinopathy, each clinic interestingly has a higher volume of certain diagnoses, seemingly driven by eponymous provider-named conditions as well as availability of condition-specific research. We also have learned that patient populations find these clinics through connections with support organizations and provider presentations at family events, whereas relatively fewer referrals come from other medical providers. This suggests an opportunity to further educate providers about the availability and benefit of our clinics. Furthermore, the clinics differ with respect to the distribution of in-person versus telemedicine visits, though it seems that providers agree that telemedicine is valuable for long-term follow-up after initial diagnosis, particularly for non-local patients, with goals focused on management and care coordination. The growth and increasing recognition of the ECC over time has allowed additional providers (i.e., neurologist/neurodevelopmental specialist and dedicated genetic counselors) to join and provide multidisciplinary care. The other clinics are working towards this model. The combined experiences of these clinics will inform future care for patients and hopefully lead to the creation of additional similar chromatinopathy-focused clinics to increase accessibility.

In the future, we hope to continue to spread the word about our ECC to patients and to the medical genetics and rare disease communities. In addition, we hope to expand our multidisciplinary team to include other specialists—endocrinology, orthopedics, ophthalmology, immunology, and others—as well as additional specialists in the neurodevelopmental field. We have already begun collaborating with a neuropsychologist who is performing neuropsychological phenotyping of patients with select MDEMs to advance our understanding of this aspect of the phenotype (Ng, Harris, et al. 2022a; Ng, Bjornsson, et al. 2022b; Ng et al. 2023a; Ng et al. 2023b). We strive to collaborate with other epigenetics and chromatin clinics throughout the US and worldwide to better serve individuals from diverse populations and provide the most far-reaching and optimal clinical epigenetics care possible.

**Data availability** Data sharing is not applicable to this article because no datasets were generated during the current study. Rather, this was a retrospective analysis based on prior diagnoses made using clinical tests.

## Declarations

**Conflict of interest** HTB is a consultant for Mahzi therapeutics. JRH receives research funding from Oryzon Genomics. The other authors have no known conflicts of interest to declare.

**Ethical approval** The study was reviewed by the Johns Hopkins IRB.

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