A Novel Genetic Syndrome Caused by Haploinsufficiency of CHD2, a Regulator of Chromatin Structure

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Introduction

Dynamic regulation of gene transcription, achieved through epigenetic modification of DNA/histones and chromatin remodeling, is essential for cell differentiation and development [1]. An emerging theme in the pathogenesis of genetic disease, including intellectual disability (ID), autism spectrum disorder (ASD) and congenital multisystem disorders, is the identification of mutations in genes involved in epigenetic modification or chromatin remodeling.

Deletion of CHD2 is linked to Neurodevelopmental Disease

There is accumulating evidence linking loss-of-function mutations in a Chromdomain helicase DNA-binding domain containing protein 2 (CHD2) to neurodevelopmental disease. Recently, we described chromosome 15q26.1 microdeletions encompassing CHD2 in a series of patients ascertained from six genetic diagnostic laboratories that perform chromosomal microarray analysis for patients broadly ascertained to have motor, speech and/or cognition delay (developmental delay), global developmental delay or ID, multiple congenital anomalies, and/or ASD [2]. Four from a total of 42,313 patients analyzed were identified with de novo CHD2 deletions, ranging in size from 78 kb to 237 kb (Table 1). The clinical findings in these four patients included developmental delays, learning difficulties or ID, and seizures were documented in 3 of the 4 patients. Although dysmorphic features were common, there was no characteristic facial gestalt identified. Brain magnetic resonance imaging (MRI) was normal when performed. Analysis of 26,826 individuals from a population-based control cohort evaluated by chromosomal microarray analysis did not reveal CHD2 deletions, suggesting that haploinsufficiency of CHD2 directly contributes to the clinical phenotype of the patients studied.

Prior to our publication, two individuals with microdeletions involving the CHD2 locus had previously been described. Capelli et al. reported a de novo deletion affecting CHD2 and the adjacent locus RGMA in a patient with speech and motor delays, ID, autistic features with attention deficit disorder, gait ataxia, and seizures which began at 24 months of age [3]. Pinto et al. reported one de novo deletion of CHD2 in a patient with mild ID and no documented seizures at the time of publication [4]. The phenotypic similarities in these unrelated patients underscore the hypothesis that CHD2 haploinsufficiency is the underlying cause of the documented clinical features.

Since our publication, two additional patients with chromosome 15q26.1 microdeletions involving CHD2 and RGMA have been reported [5]. These two adult individuals had a similar phenotype to the patients previously published, including moderate ID, behavioural problems and generalized epilepsy (Table 1). Both patients also presented with differing degrees of scoliosis, which was noted in two of our microdeletion patients [2], in addition to adolescent-onset truncal obesity. The authors speculate that haploinsufficiency of RGMA, encoding a member of the repressive guidance molecule family that functions as an axon guidance protein in the developing and adult central nervous system, may contribute to the truncal obesity phenotype, while CHD2 haploinsufficiency is responsible for the neurodevelopmental phenotype and scoliosis [5]. Given that obesity was not noted in the other two patients with deletions overlapping both CHD2 and RGMA (Table 1 and Figure 1; Patients 4 and 6), there are clearly too few cases at this point to associate deletion of RGMA with obesity or whether it is a coincidental finding.

A review of the literature also uncovered a single case describing a de novo chromosome 15q26.1 microdeletion encompassing CHD2 in a child with developmental delay, growth delay, mild dysmorphic features, and primary generalized epilepsy [6], providing additional evidence to support the requirement of CHD2 in proper neurodevelopment.

In total, 9 patients with chromosome 15q26.1 microdeletions (<1 Mb) encompassing CHD2 have been described in the literature (see Figure 1 and Table 1). Developmental delay and ID were reported in 9/9 (100%), behavioural problems were reported in 9/9 (100%), scoliosis was reported in 4/9 (44%) and seizures were reported in 7/9 (78%). Alignment of all patients’ deletions reveals that CHD2 deletions, with or without deletion of RGMA, may produce seizures indicating that deletion of RGMA is not required for manifestation of this phenotype. Larger and more complex deletions involving CHD2 have also been identified (reported deletions sizes were 3.3 Mb and 5 Mb, in addition to one patient with 6 copy number changes, including a 2 Mb chromosome 15q26.1 deletion) [7-9]. Although the clinical phenotypes are likely modified by the greater number of genes deleted in these patients, developmental delay, facial dysmorphism, and seizures were common features shared by these larger CHD2 deletion carriers. These data strongly suggest that haploinsufficiency of CHD2 contributes to the spectrum of neurodevelopmental disorders identified in these patients.

Segregating Sequence Changes within CHD2 are Associated with Neurodevelopmental Disorder

In addition to the 9 patients that have been described with CHD2 microdeletions, patients with predicted pathogenic loss-of-function mutations in CHD2 have been reported in the literature. Although it is not clear whether the reported mutations are equivalent to haplo-

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insufficiency, in our review of the literature, at least 21 individuals with unique \textit{de novo} heterozygous CHD2 mutations have thus far been described (Table 2). These individuals were ascertained from a cohort of patients with epileptic encephalopathies, ID, and ASD [10-18]. The phenotype of CHD2 mutation carriers is comparable to chromosome 15q26.1 microdeletion carriers, and includes mild-to-profound developmental delay with instances of regression, ID, ASD, behavioural problems and seizures. The presence of dysmorphic features was generally not reported. Interestingly, the seizures associated with CHD2 mutation were generally more complex than the microdeletion carriers, and were reported in over 90% of the patients identified. Furthermore, sequence variants in CHD2 were found to be over-represented in

Table 1: Clinical characteristics of patients with CHD2 microdeletions.

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<td>16</td>
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<td>21</td>
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<td>chr15 deletion coordinates [hg19]</td>
<td>93,324,047-93,515,100</td>
<td>93,286,333-93,496,391</td>
<td>93,456,168-93,534,338</td>
<td>93,563,564-93,800,894</td>
<td>93,399,003-93,482,000</td>
<td>93,412,860-93,523,856</td>
<td>93,203,932-93,619,522</td>
<td>93,545,581-93,651,582</td>
<td>92,832,405-93,563,624</td>
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<tr>
<td>Size</td>
<td>191 kb</td>
<td>210 kb</td>
<td>78 kb</td>
<td>237 kb</td>
<td>83 kb</td>
<td>511 kb</td>
<td>415 kb</td>
<td>106 kb</td>
<td>731 kb</td>
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<td>RefSeq Genes</td>
<td>CHD2, ASBP1, LOC100507217, MIR3175</td>
<td>CHD2, ASBP1, LOC100507217, MIR3175</td>
<td>CHD2</td>
<td>CHD2, RGMA, LOC100507217, MIR3175</td>
<td>CHD2, RGMA, LOC100507217, MIR3175</td>
<td>CHD2, RGMA, LOC100507217, MIR3175</td>
<td>CHD2, RGMA, LOC100507217, MIR3175</td>
<td>CHD2, RGMA, LOC100507217, MIR3175</td>
<td>CHD2, ST8IA2, c15orf32, FAM174B</td>
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<td>De novo</td>
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<td>De novo</td>
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<tr>
<td>Behaviour</td>
<td>Short attention span</td>
<td>Aggression Limited social skills</td>
<td>ADHD Limited social skills</td>
<td>Aggressive, impulsive, repetitive behaviours</td>
<td>ASD Aggression</td>
<td>ASD</td>
<td>ASD Short attention span</td>
<td>Aggression Temer tantrums</td>
<td>Behavioural problems</td>
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<tr>
<td>Seizure type (age of onset in years)</td>
<td>Jeavons syndrome (6), Absence Eyelid myoclonia</td>
<td>Absence (3)</td>
<td>None</td>
<td>Complex partial and generalized</td>
<td>None</td>
<td>Unspecified (2)</td>
<td>Generalized (2)</td>
<td>Absence (2)</td>
<td>Genorere Myoclonic</td>
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<tr>
<td>Brain MRI</td>
<td>Normal</td>
<td>Not done</td>
<td>Normal</td>
<td>Normal</td>
<td>Altered angular gyrus</td>
<td>No severe abnormalities</td>
<td>Normal</td>
<td>Not done</td>
<td>Normal</td>
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<tr>
<td>Dysmorphic Features</td>
<td>Square-shaped face, High forehead, Prominent columella, Short phalium, Fifth-finger brachydactyly, Syndactyly of toes 2 and 3</td>
<td>Triangular face, Prominent forehead, Full lips, Widely spaced central maxillary incisors, Micrognathia</td>
<td>Brachycephaly, Broad forehead, Short nose, upturned tip</td>
<td>Protruding ears, Micrognathia</td>
<td>Suggestive of Angelman syndrome, Wide mouth, Widely spaced teeth, Prognathia</td>
<td>Upplanting palpebral fissures, Long eyelashes, Short phalium, Hypoplastic alae nasi Narrow hands and feet, Tapering fingers</td>
<td>Slight upplanting palpebral fissures, Large ear lobes, Low posterior hairline, Bulbous nasal tip, with upturned nares, High palate, Small hands with tapering fingers</td>
<td>Widely set eyes, Deep pits on helix of ears bilaterally, Crowded teeth, prominent incisors, Microcephaly</td>
<td></td>
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<tr>
<td>Other features</td>
<td>Mild thoracic scoliosis, PIP joint fusion of thumbs, Mild peripheral hearing loss, Duplex kidney</td>
<td>Reduced body fat mass, Mild hypotonia, Feeding difficulties</td>
<td>Strabismus, Mild hypotonia, Feeding difficulties</td>
<td>Mild thoracic scoliosis, Tourette’s syndrome</td>
<td>Strabismus</td>
<td>Severe kyphoscoliosis, Truncal obesity, Psychiatric disorder, Neonatal hypotonia</td>
<td>Mild scoliosis, Micropenis, tests in inguinal canal, Truncal obesity, Hypotonia, Fetal hydrops, Feeding difficulties</td>
<td>Decreased fetal heart rate</td>
<td>IUGR</td>
</tr>
</tbody>
</table>

Abbreviations: ADHD, Attention-Deficit Hyperactivity Disorder; ASD, Autism Spectrum Disorder; Chr, Chromosome; ID, Intellectual Disability; IUGR, Intrauterine Growth Restriction; MRI, Magnetic Resonance Imaging; PIP, Proximal Interphalangeal; RefSeq, National Center for Biotechnology Information (NCBI) Reference Sequence Database (http://www.ncbi.nlm.nih.gov/refseq/).
### Table 1: Details of Patients

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<td>Gender</td>
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<tr>
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<td>24</td>
<td>6</td>
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<td>8</td>
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<td>29</td>
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<td>15</td>
<td>8</td>
<td>Teenager</td>
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<tr>
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<td>De novo</td>
<td>De novo</td>
<td>De novo</td>
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<td>De novo</td>
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<td>De novo</td>
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<td>De novo</td>
</tr>
<tr>
<td>Development</td>
<td>Motor delay</td>
<td>Speech delay</td>
<td>Severe learning</td>
<td>Severe learning</td>
<td>Normal</td>
<td>Slight delay</td>
<td>Global delay</td>
<td>Mild delay</td>
<td>Moderate ID</td>
<td>Global delay</td>
<td>Global delay</td>
<td>Global delay</td>
<td>Global delay</td>
</tr>
<tr>
<td>Brain MRI</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Non-specific atrophy</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Other features</td>
<td>Large mouth (small for age at birth)</td>
<td>Ataxia</td>
<td>Dysarthria</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Ossure anomaly</td>
<td>Crouch gait</td>
<td>Transient ataxia</td>
<td>Short stature</td>
<td>Stopped gait</td>
<td>Poor coordination</td>
<td>Poor balance</td>
<td>Needs help to walk</td>
</tr>
</tbody>
</table>

### Figure 1: Snapshot of the UCSC Genome browser (Human Feb. 2009 (GRCh37/hg19) Assembly) displaying all of the published chromosome 15q26.1 microdeletions involving CHD2 to date. The P# designation adjacent to each deletion (red bar) corresponds to the patient designation in Table 1.
patients with photosensitive epilepsy syndrome and associated with photosensitivity in common epiphanies suggesting that CHD2 may influence this particular trait [19]. Interestingly, partial loss of chd2 function in the zebrafish model leads to a similar phenotype of seizures [13] and photosensitivity [19].

CHD2 is Essential for Normal Neurodevelopment

While the genetic pathways regulated by CHD2 are currently not well elucidated, there is irrefutable evidence that CHD2 is required for proper development. Mice homozygous for a C-terminal truncating mutation of CHD2 are not viable, with general growth delay in late embryogenesis and perinatal death [20]. Heterozygous Chd2 mutants exhibit decreased survival rates, especially in the neonatal period, and have increased incidence of non-neoplastic lesions affecting a number of primary organs, most notably the kidneys.

A recent study has shown that CHD2 plays a role in embryonic neurogenesis [21]. CHD2, primarily expressed in Pax6 positive radial glial cells, was found to maintain the self-renewal capacity of this cell population, increasing the generation of intermediate progenitors through direct binding to the repressor element 1-silencing transcription factor (REST) genomic region. CHD2 knockdown led to a decrease in radial glial cells and an increase in intermediate progenitors, suggesting that loss of Chd2 expression during neurogenesis contributes to abnormal development of the central nervous system.

The Biochemical Function of CHD2

CHD2 is one member of a distinct family of nine evolutionarily conserved genes encoding proteins involved in chromatin remodeling. The protein domains characterizing CHD2 and this family include, but not limited to, the chromodomains (chromatin organization modifier), SNF2-related ATP-dependent helicase domain, and specific DNA-binding domains [22]. CHD2 was recently shown to function as an ATP-dependent chromatin assembly factor that has the ability to assemble periodic nucleosome arrays on a naked DNA template in vitro [23]. In vivo, more specifically in a human myoblast cell line, CHD2 was found to mediate the deposition of the H3.3 histone variant within the promoters of myogenic-specific loci, which serves as a functional epigenetic mark demarcating transcriptionally competent genes [24]. The absence of a skeletal muscle phenotype in patients with CHD2 deletions or mutations suggests that the role of CHD2 in this cell type may not be dosage-sensitive or its partial loss may be compensated for by the functional redundancy of the CHD proteins. Indeed, Siggens et al. have recently shown in K562 cells (a human erythroleukoblastoid leukemia cell line) that CHD1 and CHD2 cooperate via transcriptional-coupled recruitment to regulate the chromatin structure at transcriptionally active genes [25].

CHD Family and Genetic Disease

CHD proteins function in a variety of cellular processes through their ability to remodel chromatin and facilitate gene activation or repression of a multitude of gene targets. Therefore, it is not surprising that mutations in several CHD genes are directly linked to human disease. Most notably, haploinsufficiency of CHD7 via microdeletions or heterozygous mutations in CHD7 causes CHARGE syndrome, an acronym summarizing the six cardinal features of the multisystem disorder (Coloboma, Heart defects, choanal Atresia, mental Retardation, Genital and Ear anomalies) (26) (extensively reviewed in [27] and [28]). It is interesting to note that Chd7 mRNA is expressed in organs affected in CHARGE syndrome. As Chd7 mutations in mice block neuronal differentiation, it has been proposed that a similar mechanism exists in humans [29].

Microdeletions encompassing CHD8 were identified in patients presenting with ID and/or ASD and/or macrocephaly [30]. While the minimal deleted region contains CHD8 and the adjacent Supt16h locus, the importance of abrogated CHD8 function in the phenotype of these patients is underscored by the identification of disruptive CHD8 mutations in individuals with DD or ASD [31] and recapitulation of a subset of features of the human phenotype in the zebrafish model [32]. Although other CHD proteins have yet to be linked to human genetic disease, it has been demonstrated that CHD4 and CHD5 carry out important functions in neurogenesis [33,34]. Although beyond the scope of this communication, the contribution of CHD dysfunction in the etiology of cancer has been long recognized [35].

Conclusion

Haploinsufficiency of the chromatin remodeler CHD2 causes neurodevelopmental disease in humans. Determining the tissue-specific transcriptional targets of CHD2 and deciphering the regulatory role it plays on chromatin will provide further insight on its functions in development and may help to predict the impact of disease mutations on that function. With the significant increase in the use of chromosomal microarrays and exome sequencing technologies in genetic diagnosis, it is conceivable that, given their pivotal role in chromatin remodeling, all CHD family members will be eventually linked to human disease.

References


