Chromosomal microarray testing identifies a 4p terminal region associated with seizures in Wolf–Hirschhorn syndrome

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ABSTRACT

Background Wolf–Hirschhorn syndrome (WHS) is a contiguous gene deletion syndrome involving variable size deletions of the 4p16.3 region. Seizures are frequently, but not always, associated with WHS. We hypothesised that the size and location of the deleted region may correlate with seizure presentation.

Methods Using chromosomal microarray analysis, we finely mapped the breakpoints of copy number variants (CNVs) in 48 individuals with WHS. Seizure phenotype data were collected through parent-reported answers to a comprehensive questionnaire and supplemented with available medical records.

Results We observed a significant correlation between the presence of an interstitial 4p deletion and lack of a seizure phenotype (Fisher’s exact test p = 3.59e-6). In our cohort, there were five individuals with interstitial deletions with a distal breakpoint at least 751 kbp proximal to the 4p terminus. Four of these individuals have never had an observable seizure, and the fifth individual had a single febrile seizure at the age of 1.5 years. All other individuals in our cohort whose deletions encompass the terminal 751 kbp region report having seizures typical of WHS. Additional examples from the literature corroborate these observations and further refine the candidate seizure susceptibility region to a region 197 kbp in size, starting 368 kbp from the terminus of chromosome 4.

Conclusions We identify a small terminal region of chromosome 4p that represents a seizure susceptibility region. Deletion of this region in the context of WHS is sufficient for seizure occurrence.

INTRODUCTION

Wolf–Hirschhorn syndrome (WHS; OMIM #194190) is a genetic disorder occurring in 1:20 000 to 1:50 000 births.1 Females are approximately twice as likely as males to be affected.2 The syndrome was first described by Hirschhorn and Cooper in a preliminary report in 1961 and later formalised with back-to-back publications by Wolf et al and Hirschhorn et al in Humangenetik in 1965.3 WHS is characterised by a specific pattern of craniofacial features including a wide nasal bridge that extends to the forehead, widely spaced eyes, distinct mouth, short philtrum, micrognathia, prenatal and postnatal growth delay, intellectual disability (ID) and seizures.2–4 Following identification of these features, WHS has historically been diagnosed by karyotype and/or FISH. Submicroscopic deletions associated with this disorder have more recently been identified by chromosomal microarray analysis (CMA).

In addition to the core features of WHS listed above, additional highly variable clinical features of WHS include, but are not limited to, feeding difficulties, congenital heart defects, hearing loss, skeletal anomalies, kidney and urinary tract malformations, and ophthalmological and dental abnormalities.2 Terminal deletion resulting in partial monosomy of chromosome 4p is the most common cause of WHS. Interstitial deletions, unbalanced translocations, ring chromosomes and other complex genetic rearrangements can also give rise to WHS.2–4 As a result, deletions associated with WHS are highly variable in size and genetic content, potentially causing or contributing to the variability in presentation of this disorder.

Two adjacent regions, located approximately 1.8–2.0 Mbp from the 4p terminus, are each proposed to be the minimal region of deletion necessary to observe the core WHS features. These regions were identified based on determination of the smallest region of overlap (SRO) of individuals with WHS. The first critical region described was a 165 kbp interval encompassing part of the WHSC1 gene and all of the WHSC2 (NELFA) gene.5 These genes play a role in the regulation of key bone differentiation genes and regulation of DNA replication and cell-cycle progression.5 The identification of two patients with the WHS phenotype who have more distal deletions led to the proposal that the critical region (designated WHSCR2) lies in an adjacent, 300–600 kbp interval that includes the 5′ end of WHSC1 and the entirety of LETM1, a candidate seizure gene6–11 (figures 1 and 2).

Epilepsy represents a major clinical challenge during early years, with significant impact on quality of life. Seizures occur in over 90% of individuals with WHS with onset typically within the first 3 years of life and are often induced by low-degree fever.12 The most frequently occurring seizure types are generalised tonic–clonic seizures, tonic spasms, complex partial seizures and clonic seizures. Unilateral/generalised clonic or tonic–clonic status epilepticus occurs in 50% of individuals with WHS.12

A significant challenge to understanding the genetics of WHS is the identification of a gene or genes that, when in hemizygous state, give rise to the core features and variable co-morbidities of
WHSC. Because WHS is a contiguous gene deletion syndrome, loss of one copy of a single gene or the synergistic effects of loss of two or more genes could give rise to the features of WHS. One such gene, \textit{LETM1}, falls within WHSCR2 and has been proposed as a candidate seizure gene due to the suggested pathogenic link between mitochondrial dysfunction and epilepsy. The protein encoded by \textit{LETM1} localises to mitochondria and functions in \textit{Ca}^{2+} homeostasis, oxidative stress prevention and ATP generation. Consistent with the hypothesis that \textit{LETM1} is a seizure susceptibility gene, heterozygous \textit{Letm1}± mice, as well as rats with a lentiviral-mediated \textit{Letm1} knockdown, demonstrate increased seizure susceptibility in response to kainic acid or pilocarpine seizure induction.

Despite this evidence, \textit{LETM1} is not likely to be the sole seizure susceptibility gene in the 4p region. In recent years, increased awareness of the diagnostic features of WHS within the medical community, coupled with the advent of high-resolution cytogenetic methods, has led to the identification and characterisation of submicroscopic 4p deletions. Some of these deletions suggest that \textit{LETM1} deletion is neither necessary nor sufficient for the expression of a seizure phenotype in individuals with WHS and have led to the proposal of alternative candidate seizure genes.

Here, we present the identification of a seizure-susceptibility region by the use of high-density microarray analysis combined with parent-reported seizure phenotypes. A relatively large, 48-individual cohort was recruited through partnership with the 4p- Support Group. Evaluation of deletion coordinates and seizure phenotypes in this cohort identified a likely seizure susceptibility region within the 751 kbp terminal region of chromosome 4p. Combining these data with cases described in the literature, we narrowed this seizure susceptibility region to a region 197 kbp in size that includes two genes and one pseudogene. We also describe the types of seizures associated with WHS observed in our cohort and the response to antiepileptic medications reported by our cohort. Our study demonstrates the potential value of using high-resolution CMA for the diagnosis and medical management of seizures associated with WHS.

METHODS

This retrospective study correlated clinical genetic testing results from high-resolution CMA with clinical traits related to WHS. Clinical feature data were collected using a comprehensive parent-completed questionnaire (provided in online supplementary materials), coupled with a review of available medical records. This study was approved by the University of Utah Institutional Review Board. Informed consent and/or parental authorisation, as appropriate, was obtained for each patient.

Patient cohort

Forty-eight individuals with a diagnosis of WHS, along with their parents, consented to this study during one of two national meetings of the 4p- Support Group held in July 2012 in Indianapolis, Indiana, and July 2014 in Harrisburg, Pennsylvania. In total, 28 females and 20 males with WHS, with an average age of 11.2 years, were recruited into this study (table 1).

Clinical and molecular cytogenetic studies

All cytogenetic analyses were performed through regular clinical services in clinical laboratory improvement amendments (CLIA)-certified laboratories. All genomic coordinates for

Figure 1  Size and relative locations of 4p deletions of 34 patients with no other clinically reportable CNV findings (henceforth designated as ‘individuals with only 4p deletions’). The deletions of individuals with seizures are shown in red. Deletions of individuals without seizures are shown in blue. The Wolf–Hirschhorn syndrome (WHS) critical regions 1 and 2 (WHSCR1 and WHSCR2) are shown in black; all patients with the exception of patient 33 have deletion encompassing both critical regions. Patient 33’s deletion partially overlaps with WHSCR2 only and excludes \textit{LETM1}. Patient 34’s deletion starts 751 kbp from the 4p terminus and is the patient deletion that lies closest to the 4p terminus. All chromosome coordinates for this patient group are given in online supplementary table S1.
CNVs are reported herein using human reference sequence hg19/GRCh37. All patients (exceptions noted below) were physician referred for clinical microarray testing to Lineagen (Salt Lake City, Utah, USA). Testing for these patients was done using Lineagen’s custom 2.8M probe SNP-based microarray. The Affymetrix Chromosome Analysis Suite (ChAS) software was used for CNV detection (Affymetrix, Santa Clara, California, USA). Exceptions to the above were as follows: a 2.7M probe Cytogenetics Array (Affymetrix) was performed by Lineagen on patients 35 and 40. Patients 12, 17 and 45 obtained prior clinical CMA from other CLIA laboratories, and these patients provided a copy of their laboratory reports for analysis.

Phenotype analysis
Phenotype data were collected through parent-reported answers to a comprehensive questionnaire developed by Battaglia et al. This questionnaire captures the health, medical profile, developmental history, and treatment responses of individuals with WHS. For the present study, we focused our attention on the presence or absence of seizures, age of seizure onset, types of seizures, antiepileptic drugs (AEDs) used and responses to these AEDs, as well as responses to the ketogenic diet. For cases with incomplete, contradictory or unclear parental responses, medical records of patients were consulted. When available medical records were also incomplete, ‘no answer’ is indicated in the relevant text and tables.

Table 1 Clinical and molecular cytogenetic findings of the study cohort

<table>
<thead>
<tr>
<th>Total participants</th>
<th>48</th>
<th>Female:Male</th>
<th>28:20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average age</td>
<td>11.2 years</td>
<td>Range</td>
<td>0.9–38 years</td>
</tr>
<tr>
<td>Initial diagnosis by karyotype/FISH</td>
<td>88% (30/34)</td>
<td>Initial diagnosis by CMA</td>
<td>12% (4/34)</td>
</tr>
<tr>
<td>Size range of 4p deletion</td>
<td>1.7–33.9 Mbp</td>
<td>Number of genes deleted</td>
<td>28–207</td>
</tr>
<tr>
<td>Individuals with a second CNV</td>
<td>29% (14/48)</td>
<td>Average size of second CNV</td>
<td>3.2 Mbp (range 51.3 kbp to 8.3 Mbp)</td>
</tr>
<tr>
<td>Individuals with only a 4p deletion by deletion type</td>
<td>Interstitial: 5</td>
<td>Terminal: 29</td>
<td></td>
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</tbody>
</table>

CMA, chromosomal microarray analysis.

The Affymetrix Chromosome Analysis Suite (ChAS) software was used for CNV detection (Affymetrix, Santa Clara, California, USA). Exceptions to the above were as follows: a 2.7M probe Cytogenetics Array (Affymetrix) was performed by Lineagen on patients 35 and 40. Patients 12, 17 and 45 obtained prior clinical CMA from other CLIA laboratories, and these patients provided a copy of their laboratory reports for analysis.

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Statistical methods
Two-tailed Fisher’s exact test was used for comparing the group of individuals with interstitial 4p deletions to the group with terminal deletions and their seizure phenotypes. Significance was defined as p ≤ 0.01.

RESULTS
Table 1 shows the age and gender characteristics of this study cohort. Prior to this study, the initial diagnosis of WHS was made by individuals’ physicians using clinical assessment and a combination of G-banded karyotyping and FISH, or CMA (table 1). Fourteen individuals did not indicate which method(s) were used in their initial diagnosis.

Physician-ordered CMA was performed on the 44 individuals comprising the cohort who had not already had chromosomal microarray testing done as part of their diagnostic work up. The array used was a custom 2.784.985-probe chromosomal micro-array to achieve high-resolution mapping of the 4p deletion breakpoints, as well as to define the breakpoints of any other clinically reportable CNVs that could be detected (see online supplementary table S1).

Twenty-nine per cent of our cohort had a second deletion or duplication involving either chromosome 4 or another chromosome. This percentage is in keeping with previous studies of chromosomal rearrangements associated with WHS (table 1). Some of the second CNVs in our cohort are pathogenic, while others are of unknown clinical significance. The pathogenic CNVs are associated with developmental delay, ID, autism spectrum disorder, dysmorphic features and seizures. The breakpoints of all patients’ 4p deletions, as well as the breakpoints of the second CNV if present, and the association of this second CNV to any clinical features are shown in online supplementary tables S1 and S2.

Consistent with previous studies,10–13 we found that 90% (43/48) of our cohort had seizures, which were of early onset (see online supplementary tables S1 and S2), were often brought on by fever (25/41 individuals reported having febrile seizures) and tended to wane in frequency during the preteen years. All seizure types surveyed (tonic–clonic, tonic, clonic, myoclonic, absence, atonic, complex partial, simple partial, atypical and status epilepticus) were detected in this cohort. The seizure types most commonly reported in our WHS cohort are shown in table 2.

Mapping a seizure susceptibility candidate region
To identify a region conferring a genetic susceptibility to seizures, we evaluated the 34 patients in our cohort with only 4p deletions. Figure 1 shows the deletions of this group aligned by size and location. All individuals in this group have deletions that encompass both critical regions WHSCR1 and WHSCR2, except for patient 33, whose deletion only overlaps WHSCR2 but not WHSCR1.

We asked whether 4p deletion size and genetic content correlate with seizure severity by first examining the records of the five individuals with the smallest terminal deletions in our cohort, patients 29–33 (figures 1 and 2). Their deletions range in size from 1.7 to 2.2 Mbp. Typically, individuals with small 4p terminal deletions less than 3.5–6 Mbp in size exhibit the mildest phenotypes, including seizure phenotypes.1 14–16 Notably, four of these five individuals (patients 29, 31, 32 and 33) reported having severe seizure phenotypes, indistinguishable in terms of seizure types, frequency or response to AEDs (see online supplementary table S1) from the rest of the cohort with larger deletion sizes. Patient 33 is noteworthy because her deletion does not remove LETM1, the purported candidate seizure gene, yet her seizures are consistent with WHS. We thus observe that in our cohort, small terminal 4p deletions including one that does not include LETM1 can result in severe seizure phenotypes.

In contrast, we identified four individuals, patients 18, 21, 24 and 34, who did not have seizures as well as one additional individual, patient 10, who is considered as not having seizures, as explained below. All of these individuals have interstitial deletions that leave, minimally, the terminal 751 kbp of chromosome 4p intact (blue bars, figure 1). Patient 10 had the largest interstitial 4p deletion, 14.6 Mb in size, who had one febrile seizure at age 1.5 years associated with a kidney infection. Having an isolated febrile seizure is an unusual presentation for WHS-associated epilepsy; in accordance with his medical records and parent answers on our survey, we scored him as not having WHS-related seizures. Taken together, these data show that deletion of the terminal 751 kbp of chromosome 4p, not monosomy of LETM1, correlates with an epileptic phenotype (p = 3.59e−6) using a two-tailed Fisher’s exact test (see online supplementary table S3).

We turned to the literature to determine if other rare interstitial deletions or small terminal deletions would support or refute the hypothesis that the deletion of the terminal region of 4p correlates with a seizure phenotype. Nine additional cases of non-related individuals with WHS and without seizures have been previously described in the literature.1 22 27 34 37 38 Their reported deletion sizes and locations are shown in figure 2, along with the deletions of patients from our cohort who lack seizures (blue bars). Also included in figure 2 are three small interstitial deletions described by Andersen et al,20 all of which encompass at least portions of the WHSCI and LETM1 genes. The three individuals with these deletions show features of WHS but do not meet the minimal diagnostic criteria for the syndrome and do not have seizures.20 Strikingly, 16 out of 17 individuals without seizures have interstitial deletions, most of which result in monosomy of LETM1 while leaving the terminal 751 kbp intact. The exception to this observed correlation was an 11-year-old girl without seizures who had a ∼3.7 Mbp terminal deletion that also removes LETM1 (Van Buggenhout 2004, patient 1) (figure 2). The corresponding chromosome coordinates for all these patients are given in online supplementary table S4.

One individual described by Van Buggenhout et al22 was a clinically normal patient with a history of multiple miscarriages and no seizures. This patient was found to have a 0.3 Mbp terminal deletion (Van Buggenhout 2004 patient 6, figure 2) using a BAC array. While the lower resolution of BAC arrays must be
Copy-number variation

taken into account, the deletion in this individual nevertheless suggests that a deletion encompassing approximately 0.3 Mbp of the 4p terminus does not contribute to the seizure phenotype or any other characteristic traits of WHS.

Next, we searched the literature for examples of individuals with seizures who had the smallest described terminal and interstitial deletions of chromosome 4p. The deletions of 12 such individuals, including five from our cohort, are shown (figure 2, red bars). Eight individuals in this group have terminal deletions and four have interstitial deletions, all of which affect at least the distal-most 500 kbp of chromosome 4p. Most notably, Zollino et al.25 have recently described two siblings, with a paternally inherited 564 kbp terminal deletion (figure 2, Zollino 2014, patients 3 and 4). Both siblings, as well as their father, have a history of seizures.

A 1.58 Mbp interstitial deletion of a 33-month-old girl overlaps with the deletions of patients 3 and 4 from Zollino et al.25 This patient, described by Izumi et al.,26 presented with a typical WHS seizure phenotype. The SRO shared by the deletions of these three patients can therefore be used to define a seizure susceptibility region 197 kbp in length, starting with the distal coordinate defined by the Izumi patient and the proximal coordinate defined by the two Zollino siblings (figures 2 and 3).

There are two genes and one pseudogene in this region: ZNF721, encoding a zinc-finger containing protein of unknown function, PIGG, a member of the phosphatidylinositol glycan anchor biosynthetic pathway, and ABCA11P, a pseudogene with sequence similarity to ATP-binding cassette, subfamily A genes (figure 3).

As our cohort and cases described in the literature have shown, individuals with interstitial 4p deletions that leave this candidate region intact (with the exception of patient 1 from Van Buggenhout et al.25) do not have seizures. Conversely, deletion of this region gives rise to seizures. These observations suggest that deletion of this region is both necessary and sufficient for the seizure phenotype in individuals with WHS.

Treatment responses

Study participants reported 19 different AEDs, as well as the ketogenic diet and homeopathic approaches, to control seizures, with varying degrees of success (table 3, see online supplementary tables S1 and S2). The responses of the four most commonly used seizure medications in this cohort are shown in table 3, with levetiracetam and valproic acid showing the most positive responses within this group. These observations are consistent with previous studies reporting that valproic acid, used alone or in combination with ethosuximide, is the effective treatment for atypical absences common to individuals with WHS.32 39

In table 3, we summarise the reported responses. AEDs were scored as positive if the patient’s parents reported without prompting that the drug gave a significant and observable increase in control over seizures. AED responses were scored as negative if the patients’ parents reported a negative reaction (allergic reaction or other) without prompting that caused them to stop using that drug, or if the drug conferred no control over seizures.

**DISCUSSION**

Genotype–phenotype correlation studies of patients with WHS have met with limited success primarily because (1) the prevalence of the disorder is low and therefore assembling a study cohort large enough to achieve statistical power to find significant correlations is difficult; (2) the phenotypic presentation of WHS is highly variable and likely influenced by a number of both genetic and environmental factors and (3) accurate breakpoint mapping has only become possible within the last decade, and the majority of individuals with a diagnosis of WHS available for such studies have not had CMA as part of their diagnostic workup. In this study, we have attempted to address these challenges by (1) partnering with the world’s largest support group for WHS, the 4p– Support Group, in order to assemble a relatively large cohort; (2) collecting phenotypic information from parents using a highly detailed questionnaire and (3) employing high-resolution clinical CMA to map deletion breakpoints as well as identify any additional CNVs that could contribute to phenotype.

Because seizures affect approximately 90% of all individuals with WHS and can greatly influence the quality of life for these individuals, we focused our analysis on seizures. By fine

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**Table 3** Responses to the four most commonly reported seizure medications

<table>
<thead>
<tr>
<th>Medication</th>
<th>Phenobarbital (n=13)</th>
<th>Levetiracetam (n=13)</th>
<th>Topiramate (n=11)</th>
<th>Valproic acid (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative reports</td>
<td>5</td>
<td>1</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Positive reports</td>
<td>0</td>
<td>4</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

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**Figure 3** Two genes and a pseudogene lie within the 197 kbp seizure candidate region, PIGG, ZNF721, and pseudogene ABCA11P.

The location of this region on Chromosome 4 is shown with the green bracket. hg19/GRCh37 coordinates for this region: chr4:367691–564593. (Screenshot is from Golden Helix GenomeBrowse visualisation tool V2.1.0 by GoldenHelix, Inc.46)
mapping the 4p deletion breakpoints of our cohort, we describe a 751 kbp terminal 4p candidate seizure region. The deletion of this region correlates strongly with the presence of seizures, and its preservation, as in cases of the interstitial WHS deletions we described, correlates with the absence of seizures. Rare interstitial and submicroscopic terminal deletions described in the literature not only support the idea that deletion of this region is necessary for seizure phenotype but also support the idea that its deletion is sufficient for predisposition to seizures. In particular, three individuals described in the literature, two of whom are siblings, allowed us to further refine the boundaries of the candidate seizure susceptibility region to a locus 197 kbp in size, starting 368 kbp from the terminal end of chromosome 4.

This 197 kbp region encompasses two genes and one pseudogene. ZNF721 encodes a zinc-finger-containing protein of unknown function, PIGG encodes a member of the phosphatidylinositol glycan anchor biosynthetic pathway and ABCA11P is a pseudogene with sequence similarity to ATP-binding cassette, subfamily A. While not much is known about the biological function of ZNF721, several intriguing lines of evidence indicate PIGG as an excellent candidate seizure susceptibility gene. PIGG encodes one of 26 members of a biosynthetic pathway involved in assembling and attaching the phosphatidylinositol glycan (GPI) anchor to a group of over 150 proteins. The GPI anchor serves to attach these proteins to the outer leaflet of the plasma membrane where they carry out various signalling and extracellular functions. Deficiencies in GPI anchor synthesis have been linked to disorders of congenital glycosylation, all of which are autosomal recessive and are associated with infantile encephalopathy, ID, and/or seizures. Further work is necessary to characterise PIGG’s role as a candidate seizure susceptibility gene. We note that if its deletion alone is sufficient to cause seizures, it would be the first description of haploinsufficiency for a GPI anchor biosynthetic gene. This may be consistent with the proposed importance of stoichiometry in the PIGG protein’s role in the biosynthetic pathway, in which it functions as a catalytic component and competes with phosphatidylinositol glycan anchor biosynthesis protein, class O (PIGO) for binding to phosphatidylinositol glycan anchor biosynthesis protein, class F (PIGF) in order to add an ethanolamine-phosphate side chain to a mannose moiety. Alternatively, deletion of one copy of PIGG always occurs in the context of the deletion of other 4p terminal genes in cases of WHS; it may be that the deletion of a combination of genes in the WHS region acts synergistically to predispose individuals to seizures.

There are significant similarities shared between the two conditions, WHS and Dravet syndrome. Dravet syndrome is characterised by early-onset seizures including febrile, afebrile, generalised/unilateral clonic, myoclonic, focal, and atypical absence seizures. These seizures can be prolonged and often are intractable to pharmacotherapies, leading to cognitive, motor and behavioural impairment. Individuals with WHS display a distinctive electroclinical pattern resembling the severe myoclonic epilepsy of infancy or Dravet syndrome. In addition, some patients with a milder presentation of WHS-related dysmorphologies are sometimes first suspected of having Dravet syndrome, as attested by published studies in which SCN1A sequencing was conducted and found to be negative in at least two cases, until the true cause, a deletion of the 4p terminus, was identified. Furthermore, carbamazepine and lamotrigine have been shown to exacerbate seizures in both individuals with WHS as well as individuals with Dravet syndrome.

In zebrafish, there is an ortholog of SCN1A that corresponds to human SCN1B that has also been linked to Dravet syndrome, designated scn1bb. The Rohon–Beard neurons of zebrafish require functional Scn1bb protein, as well as the phosphatidylinositol biosynthetic pathway, for touch sensitivity. Nakano et al showed that zebrafish mutants that lack functional members of the phosphatidylinositol biosynthetic pathway, or morpholino knockdown of members of this pathway, result in the failure of the sodium channel Scn1bb to localise correctly to the plasma membrane. This observation could provide an intriguing mechanistic link between seizures in WHS and Dravet syndrome.

Our study includes some limitations. To assess seizure phenotypes, we relied almost solely on parental answers to our questionnaire, with limited contribution from medical records. A follow-up study in which EEG recordings are analysed and correlated with genetic findings would be a valuable extension of the observations we present here. Our data on AED responses suggest that certain AEDs may be more effective than others at achieving early seizure control and warrants further study. Our interpretation of the seizure susceptibility region is based on five individuals whose lack of a seizure phenotype can change with time. Despite this fact, now that the average age of these five individuals (6.8 years) is well beyond the typical average age of seizure onset, we remain optimistic that seizure presentation in this group has already distinguished itself from typical WHS seizure presentation. Patient 1 described in Van Buggenhout et al who lacks both the candidate seizure susceptibility terminal region described here as well as LETM1 and yet who does not have seizures, highlights the complexity of this region and emphasises that the knowledge of genetic contributions to seizures is incomplete. It is highly likely that there are multiple seizure susceptibility genes in the 4p region, and that final seizure presentation is a result of the lack or presence of the unique genetic and environmental combinations that can result.

We find that the use of whole genome CMA for the genetic characterisation of individuals with WHS is valuable, since it provides a significantly higher resolution of breakpoint coordinates than does karyotyping. Additional CNVs frequently occur in this population, yet on average are smaller than would be detectable even by high-resolution karyotyping (see online supplementary table S2), and can therefore be easily missed. In addition, the presence or absence of a terminal 197 kbp deletion is most effectively detected using CMA. Further investigation of the relationship of genetics to the clinical manifestations of WHS using high-resolution mapping techniques as well as whole-genome sequencing will lead to a deeper understanding of the molecular underpinnings of this complex disorder as well as an improvement of medical treatments for these individuals.

On a final note, the identification of a relatively small candidate seizure region now affords the opportunity to create loss-of-function knockouts of candidate genes in model organisms to confirm that haploinsufficiency of such genes is sufficient to increase seizure susceptibility and also to perform functional studies that will further elucidate the mechanism of these genes’ functions in health and disease. Using such an approach, precision medicine for complex genetic disorders such as contiguous gene disorders becomes possible.

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