Abstract: OBJECTIVE Seizure disorder is one of the most relevant clinical manifestations in Wolf-Hirschhorn syndrome (WHS) and it acts as independent prognostic factor for the severity of intellectual disability (ID). LETM1, encoding a mitochondrial protein playing a role in K(+) /H(+) exchange and in Ca(2+) homeostasis, is currently considered the major candidate gene. However, whether haploinsufficiency limited to LETM1 is enough to cause epilepsy is still unclear. The main purpose of the present research is to define the 4p chromosome regions where genes for seizures reside. METHODS Comparison of our three unusual 4p16.3 deletions with 13 literature reports. Array-comparative genomic hybridization (a-CGH). Real-time polymerase chain reaction (RT-PCR) on messenger RNA (mRNA) of LETM1 and CPLX1. Direct sequencing of LETM1. RESULTS Three unusual 4p16.3 deletions were detected by array-CGH in absence of a obvious clinical diagnosis of WHS. Two of these, encompassing LETM1, were found in subjects who never had seizures. The deletions were interstitial, spanning 1.1 Mb with preservation of the terminal 1.77 Mb region in one case and 0.84 Mb with preservation of the terminal 1.07 Mb region in the other. The other deletion was terminal, affecting a 0.564 Mb segment, with preservation of LETM1, and it was associated with seizures and learning difficulties. Upon evaluating our patients along with literature reports, we noted that six of eight subjects with terminal 4p deletions preserving LETM1 had seizures, whereas seven of seven with interstitial deletions including LETM1 and preserving the terminal 1 Mb region on 4p did not. An additional chromosome region for seizures is suggested, falling within the terminal 1.5 Mb on 4p, not including LETM1. SIGNIFICANCE We consider that haploinsufficiency not limited to LETM1 but including other genes acts as a risk factor for the WHS-associated seizure disorder, according to a comorbidity model of pathogenesis. Additional candidate genes reside in the terminal 1.5 Mb region on 4p, most likely distal to LETM1. A PowerPoint slide summarizing this article is available for download in the Supporting Information section here.

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Unusual 4p16.3 deletions suggest an additional chromosome region for the Wolf-Hirschhorn syndrome–associated seizures disorder

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SUMMARY

Objective: Seizure disorder is one of the most relevant clinical manifestations in Wolf-Hirschhorn syndrome (WHS) and it acts as independent prognostic factor for the severity of intellectual disability (ID). LETM1, encoding a mitochondrial protein playing a role in K⁺/H⁺ exchange and in Ca²⁺ homeostasis, is currently considered the major candidate gene. However, whether haploinsufficiency limited to LETM1 is enough to cause epilepsy is still unclear. The main purpose of the present research is to define the 4p chromosome regions where genes for seizures reside.

Methods: Comparison of our three unusual 4p16.3 deletions with 13 literature reports. Array-comparative genomic hybridization (a-CGH). Real-time polymerase chain reaction (RT-PCR) on messenger RNA (mRNA) of LETM1 and CPLX1. Direct sequencing of LETM1.

Results: Three unusual 4p16.3 deletions were detected by array-CGH in absence of an obvious clinical diagnosis of WHS. Two of these, encompassing LETM1, were found in subjects who never had seizures. The deletions were interstitial, spanning 1.1 Mb with preservation of the terminal 1.77 Mb region in one case and 0.84 Mb with preservation of the terminal 1.07 Mb region in the other. The other deletion was terminal, affecting a 0.564 Mb segment, with preservation of LETM1, and it was associated with seizures and learning difficulties. Upon evaluating our patients along with literature reports, we noted that six of eight subjects with terminal 4p deletions preserving LETM1 had seizures, whereas seven of seven with interstitial deletions including LETM1 and preserving the terminal 1 Mb region on 4p did not. An additional chromosome region for seizures is suggested, falling within the terminal 1.5 Mb on 4p, not including LETM1.

Significance: We consider that haploinsufficiency not limited to LETM1 but including other genes acts as a risk factor for the WHS-associated seizure disorder, according to a comorbidity model of pathogenesis. Additional candidate genes reside in the terminal 1.5 Mb region on 4p, most likely distal to LETM1.

KEY WORDS: WHS pathogenesis, Candidate genes, Seizures.
Wolf-Hirschhorn syndrome (WHS, OMIM 194190) (OMIM: Online Mendelian Inheritance in Man, http://omim.org/) is a contiguous gene deletion disorder caused by partial deletion of the short arm of one chromosome 4. Several literature reports point to a great variability of the WHS phenotype, depending mostly on the variability of the underlying genomic defect.1–3 However, there is a consensus in considering the core phenotype as defined by the association of severe growth delay, including both short stature and low body weight with thin habitus, intellectual disability (ID), distinctive facial appearance (featuring the well-known Greek warrior helmet profile), and seizures (or electroencephalography [EEG] anomalies). All these signs map within the terminal 1.9 Mb region on 4p16.3, where the critical region, Wolf-Hirschhorn Syndrome Candidate Region 2 (WHSCR-2), was described.4 Typical WHS is a contiguous gene deletion disorder, since haploinsufficiency of many genes among those included in the critically deleted region was inferred to cause the core phenotype.2,3 WHSC1 is an excellent candidate gene for both facial characteristics and growth delay. LETM1 is currently considered the major gene for seizures. It encodes a protein for the inner mitochondrial membrane, playing a role in mitochondrial K+/H+ (KHE) and Ca2+/H+ exchange, in maintaining the mitochondrial membrane potential, and in the export of proteins involved in the assembly of respiratory complexes.5–8 Supporting this pathogenic link, mitochondria are currently considered to play an important role in the pathogenesis of seizures.9 However, the unique role of LETM1 in causing epilepsy appears to be questionable.2,10,11

We report on three unusual deletions on 4p16.3 that are not productive of a full WHS phenotype. WHSCR-2 (LETM1 included) was haploinsufficient in two subjects who never had seizures, whereas it was preserved, with preservation of LETM1 as well, in two siblings, in their father, and in their paternal grandmother, who all had seizures. Our observations, supported by literature data, allowed us to suggest a comorbidity model of pathogenesis for the WHS-associated seizure disorder, and to define an additional chromosome region where new candidate genes likely reside. To allow proper genetic diagnosis and a genomic-based antiepileptic surveillance, WHS is also discussed at a nosologic level. This latter point is presented as a useful tool for transferring into clinical practice the fine molecular data provided by the currently available techniques for deep genotyping.

Methods

Subjects

Subject 1

This is a 9-year-old Dutch boy who was born at term by cesarean section because of fetal distress after uneventful pregnancy. Birth weight was 2,940 g (5th percentile). Length and head circumference were not referred. Poor sucking and slight hypotonia were observed at birth. He sat unsupported at 12 months and walked independently at 19 months. Language was delayed, still limited to single words and short sentences that were ill-articulated. A moderate degree of ID of about 50 was diagnosed by formal IQ evaluation. Attention deficit/hyperactivity disorder (ADHD) was diagnosed at age 6 years, and the patient was in special education. He never had seizures and his EEG was normal. The boy was not yet toilet-trained and his motor development lag behind with stiff movements and frequent falls. He had outbursts of laughter and a tendency to overeat.

On physical examination, he appeared as a restless boy with little contact with the observer, nonfluent movements of hands, and hand-flapping. At age 6 years and 2 months, his height was 116 cm (20th percentile) and occipitofrontal circumference 48 cm (<2nd percentile). Facial characteristics were only partially consistent with WHS, including upslanting palpebral fissures, slightly protruding eyes, mild ptosis, arched eyebrows, high nasal bridge, and slightly dysplastic and protruding ears. Flat mid-face and microretrogathnia were also noted (Fig. 1). The extremities showed joint hyperlaxity, long fingers with clinodactyly of toes 4 and 5, as well as a broad hallux and short distal phalanges, bilaterally. Major malformations were not detected. At last examination, at age 9 years, seizures are still absent and EEG is normal.

Subject 2

A 4-year-old boy, born at term with birth weight of 2,600 g (3rd percentile), length 46 cm (3rd percentile), and head circumference 32 cm (2nd percentile), was referred to us because of hypotonia and mild ID. He sat unsupported at 8 months and walked independently at 24 months. Language was delayed, limited to about 20 words, but comprehension was good, and interaction with the environment was excellent. He never had seizures, and EEG was repeatedly normal. At age 3 years, weight was 10 kg (−3.5 standard deviation [SD]), height...
Figure 1. Chromosome mapping of selected 4p deletions, including present cases, with respect to Wolf-Hirschhorn syndrome critical region-2 (WHSCR-2). Clinical pictures of subject 4 and her father and of subjects 2 and 1 are shown on the top. Dark bar on the bottom shows the here-suggested chromosome region for the full WHS phenotype. Gray-shaded area highlights the deletions involving \textit{LETM1}. The associated clinical signs are summarized in Table 1.

*Epilepsia* © ILAE
was 81 cm (−4.5 SD), and head circumference was 45.6 cm (−4 SD). He presented with facial characteristics partially mimicking the WHS features, including triangular face, frontal bossing, large eyes, beaked nose with high nasal bridge, downturned mouth, and large ears (Fig. 1). He had clinodactylyous third and fourth toes on the right. His behavior was friendly and eye contact was excellent. Brain magnetic resonance imaging (MRI) and results of cardiac and kidney ultrasound examination were normal. At age 4 years EEG was normal, and the patient never experienced seizures.

Subjects 3 and 4

Subjects 3 and 4 are brother (3) and sister (4), aged 14 and 6 years, respectively, who were referred because of seizures and learning difficulties. Height, weight, and head circumference measurements had all been around the 50th centile since birth. They had no hypotonia. Facial characteristics were unremarkable (Fig. 1; photos of subject 3 are not available). Motor development was normal in the boy, whereas it was slightly delayed in the girl. The epileptic disorder consisted, in the boy, of complex febrile seizures in the first year of life and generalized febrile seizures at age 8 years. EEG examination showed a normal background activity and slow-waves with superimposed spike discharges on anterior cortical regions during wake and sleep. The girl had daily atypical absence seizures with oral automatism and eyelid myoclonus since 6 years of age. The interictal EEG was characterized by normal background activity and rhythmic 3 Hz slow-waves with superimposed spikes discharges on anterior and posterior regions. The ictal EEG was characterized by irregular, generalized at 2.5–3 Hz spike-wave discharge with asynchronous onset, clinically associated with loss of contact and eyelid myoclonus or oral automatism. Both children underwent molecular testing for mutation in SCN1A gene, with normal results. Of note, the father had febrile seizures and learning difficulties as a child, although he is currently healthy and a skilled worker. He underwent phenotypic evaluation once he was diagnosed to carry the 4p deletion.

Genetics

Genetic investigations included array–comparative genomic hybridization (array-CGH), locus-specific fluorescence in situ hybridization (FISH) analysis, real-time polymerase chain reaction (real-time-PCR) on messenger RNA (mRNA), and direct gene sequencing.

Array-CGH

Array-CGH was performed on genomic DNA from uncultured peripheral blood cells at an average resolution of 35 kb (244K kit; Agilent Technologies, Santa Clara, CA, U.S.A.), following the manufacturer’s instructions. Probe alignments were referred to NCBI 37 (NCBI: National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov/), UCSC (UCSC: University of California, Santa Cruz, http://genome.ucsc.edu/) hg19 build.

Locus-specific FISH experiments

FISH were carried out on metaphase chromosomes according to standardized procedures on both parents in each occasion, with the purpose to look for the presence of 4p deletion as in their child or for balanced chromosome rearrangements. Molecular probes were selected on the basis of individual extent of the 4p deletion.

Real-time PCR

Real-time PCR was performed to measure levels of LETM1 and CPLXI mRNA extracted from peripheral blood cells by TaqMan Gene Expression Assay (Hs00360061_m1 LETM1, Hs00362510_m1 CPLXI from Life Technologies, Carlsbad, CA, U.S.A.). Experimental data were obtained with the SDS v.2.2.2 Software (Applied Biosystems Carlsbad, CA, U.S.A.) and the comparative threshold cycle (Ct) method (with the calculation of the $2^{-\Delta\Delta CT}$) was used for subsequent analysis, comparing LETM1 and CPLXI versus glyceraldehyde-3-phosphate dehydrogenase ($\text{GAPDH}$, Hs99999905_m1; Applied Biosystems). Real-time PCR reactions of each sample were performed in triplicate, and experiments were repeated three times.

Direct gene sequencing

Sanger sequencing of coding regions of LETM1 (exons and intron–exon boundaries) was performed on samples from subjects 3 and 4, their father, and their paternal grandmother. Primer sequences are available on request.

RESULTS

Array-CGH and FISH

Subject 1

An interstitial deletion spanning about 1.1 Mb on chromosome region 4p16.3 was detected by array-CGH, with breakpoints at 1.77 Mb from the 4p telomere, distally, and at 2.90 Mb from the 4p telomere, proximally.
defining the following karyotype: arr 4p16.3(1,778,765–2,909,499)x1. Included in the deletion interval were WHSC1 and LETM1 and additional 17 transcribed genes (FGFR3, WHSC2, SCARNA22, C4orf48, NAT8L, POLN, HAUS3, MXD4, ZFYVE28, RNF4, FAM193A, TNIP2, SH3BP2, ADD1, MFSD10, NOP14, and C4orf10). Both parents were analyzed with use of FISH with the WHSC1-specific 190b4 cosmid probe, and with a 4p subtelomeric probe (VYSIS, ToTel Vision; Abbott Laboratories, Abbott Park, IL, U.S.A.), with normal results. Accordingly, a de novo occurrence of the deletion was established in the child.

Subject 2

The 4p16.3 deletion detected by array-CGH spanned about 0.840 Mb, with breakpoints at 1.07 Mb from the 4p telomere distally, and at 1.91 Mb from the 4p telomere proximally. The deletion encompassed WHSC1, LETM1, and additional 15 transcribed genes (RNF212, TMED11P, SPON2, CTBP1, MAEA, KIAA1530, UVSSA, CRIPAK, FLJ34443, DNTNP, FAM53A, SLBP, TMEM129, TACC3, and FGFR3). The karyotype was defined as: arr 4p16.3(1,079,721–1,919,855)x1. The rearrangement was assessed to be de novo, with both parents having normal results on FISH analysis with the WHSC1-specific 190b4 cosmid probe.

Subjects 3 and 4

These siblings were found to carry a 0.564 Mb terminal deletion on 4p16.3, preserving both the candidate genes WHSC1 and LETM1, with karyotype defined as arr 4p16.3(71,552–564,593)x1. The deletion included eight transcribed genes (ZNF718, ZNF595, ZNF876P, ZNF732, ZNF141, ABCA11P, ZNF721, and PIGG). The 4p subtelomeric FISH probe was found to be deleted in both the father and the paternal grandmother. With use of array-CGH, the father’s 4p deletion appeared to be identical to that of his children and mother.

Results and partial gene content of individual deletions are shown in Figure 1.

Real-time PCR

Semiquantitative real-time PCR on LETM1 mRNAs was performed on patient 2 (LETM1 deleted), and on patient 4 and her father (LETM1 preserved). As expected, LETM1 expression was significantly reduced in patient 2, whereas in patient 4 and in her father no diminished dose of LETM1 mRNA was noted in comparison to controls. Therefore, transcription impairment of LETM1 due to positional effects was ruled out in patients who had LETM1 preserved. The limited level of expression of CPLX1 in peripheral blood cells hampered the correct assessment of mRNA level both in patients and in control subjects; however, a slightly decreased expression was observed in patient 4 and in her father, who both had CPLX1 preserved (Fig. 2).

Sanger sequencing

No variants of the coding sequence of LETM1 were detected, apart from one single nucleotide polymorphism (SNP; rs116753949), which shows a minor allele frequency of 5% in a control population of European ancestry (1,000 genomes data: http://www.1000genomes.org).

Discussion

We discuss genotype–phenotype correlations in two patients and a family with unusual 4p16.3 deletions that were detected during the application of molecular karyotyping to the diagnosis of ID in the absence of obvious clinical evidence of WHS. The main purpose of this report is an attempt to elucidate the pathogenic mechanism underlying epilepsy in WHS.

Figure 2.
Graphs of semiquantitative real-time PCR results on subjects 2 and 4, and on the father of subjects 3 and 4. Data represent average fold changes (2−ΔΔCt) ± SD. (A) LETM1 mRNA levels. (B) CPLX1 mRNA levels. The high standard deviation (and the resulting low significance of the results) is likely due to the low expression levels of the CPLX1 transcript in peripheral blood (i.e., threshold cycles were around 35).

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<table>
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<th>SUBJECTS</th>
<th>FNM367&lt;sup&gt;15&lt;/sup&gt;</th>
<th>C3&lt;sup&gt;15&lt;/sup&gt;</th>
<th>C4&lt;sup&gt;15&lt;/sup&gt;</th>
<th>C6&lt;sup&gt;16&lt;/sup&gt;</th>
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<th>South et al.</th>
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<td>1.2–2.5</td>
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<td>0–1.3</td>
<td>0–1.27/1.46</td>
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<td>0–1.7</td>
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<td>M (13)</td>
<td>M (13)&lt;sup&gt;15&lt;/sup&gt;</td>
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<td>F (56)</td>
<td>M (2)&lt;sup&gt;15&lt;/sup&gt;</td>
<td>M (11)&lt;sup&gt;10&lt;/sup&gt;</td>
<td>F (5&lt;sup&gt;12&lt;/sup&gt;)</td>
<td>F (22&lt;sup&gt;12&lt;/sup&gt;)</td>
<td>F (8)&lt;sup&gt;12&lt;/sup&gt;</td>
<td>M (2)&lt;sup&gt;15&lt;/sup&gt;</td>
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<sup>1</sup> present; <sup>2</sup> absent; LD, learning disabilities; DD, developmental delay.
<sup>a</sup> Not included among the pathogenic deletions.
WHS is a contiguous gene syndrome characterized by great variability of the basic genomic defect. Although the associated clinical phenotype varies greatly in individual patients, depending mainly on the size of the 4p deletion, there is a consensus in considering the core WHS phenotype as defined by the association of severe growth delay, ID, typical facial appearance featuring the well-known Greek warrior helmet profile, and seizures (or EEG anomalies). All these signs were mapped within the terminal 1.9 Mb region on 4p16.3, where the critical region, WHSCR-2, was described. Of importance, seizures represent one of the greatest problems in the clinical management of WHS and they act as an independent prognostic factor for the final degree of ID. Characterizing the pathogenic genes for seizures could allow a better elucidation of disease mechanisms that in turn should facilitate development of targeted therapy.

Wide evidence exists that the core WHS phenotype is caused by haploinsufficiency of many genes. Among those included in the critically deleted region, WHSC1 is the major candidate gene for growth delay and facial dysmorphisms and LETM1 the major gene for seizures. LETM1, deleted in nearly the totality of WHS patients with full clinical presentation, encodes a mitochondrial protein with two putative Ca\(^{2+}\) binding EF hands, which is involved in mitochondrial K\(^+\)/H\(^+\) (KHE) and Ca\(^{2+}\)/H\(^+\) exchange, in maintaining the mitochondrial membrane potential and in the export of proteins of the respiratory complexes. The likely pathogenic role of LETM1 in causing seizures has been supported by the evidence that mitochondria play a relevant role in the pathogenesis of epilepsy. However, there is no proven direct-link between haploinsufficiency of LETM1, mitochondrial dysfunction, and epilepsy in humans. On the other hand, it was observed that knockout of Letm1 in mice results in early embryonic lethality in homozygous carriers, whereas half of the heterozygotes survive, showing increased susceptibility to seizures after kainic acid application, with a seizure score about 1.5-fold higher than in wild-type mice. Moreover, high frequency and prolonged duration of seizures was noted after Letm1 knock-down in rats exposed to pilocarpine. Intrahippocampal injection of a lentivirus bearing LV-Letm1-sh. Although no spontaneous seizures were noted, these data suggest that Letm1 can play a role in predisposing to epilepsy.

Two of the 4p16.3 unusual deletions we report here encompassed a 1.1 Mb interstitial interval, with preservation of the terminal 1.77 Mb region in one subject, and a 0.84 Mb interstitial interval, with preservation of the terminal 1.07 Mb region in another. Of note, WHSCR-2, LETM1 included, was haploinsufficient in both subjects. Clinically, we found that some features were consistent with WHS, with particular respect to growth delay in subject 2 and several facial dysmorphisms in both, but the whole clinical phenotype was not. More importantly, they never had clinically evident seizures. It was previously shown that onset of seizures (or EEG anomalies) in WHS occur within the first 3 years of life, with a peak incidence at around 6–12 months of age. Thus we would not expect an age effect as a likely explanation for the absence of seizures in our patients 1 and 2, who were 9 and 4 years of age at last examination, respectively.

The remaining deletion, affecting the terminal 0.564 Mb segment on 4p, was detected in two siblings with normal growth and unremarkable facial appearance, who were referred because of learning disabilities and seizures. WHSCR-2, LETM1 included, was fully preserved in both. The same deletion was detected in the father and in the paternal grandmother. The father had febrile seizures and very mild learning difficulties as a child, but he is currently healthy and a skilled worker. The maternal grandmother had a single prolonged generalized tonic–clonic seizure episode with fever at age 2 years, and she had borderline ID. Although on clinical examination they both presented with large eyes, arched eyebrows, and high nasal bridge, it is worth noting that the father was enrolled in genetic testing with the only purpose to assess inheritance of the chromosome rearrangement detected in his children, and the maternal grandmother with the purpose to look for cosegregation of the seizure disorder and the 4p deletion in the family. Considering that subjects 3 and 4 in this family shared common features including generalized epilepsy with febrile seizures plus, they were first analyzed for the presence of SCN1A mutations, with normal results. Because impairment of gene transcription can be caused not only by gene deletion but also by positional effects, we tested this hypothesis in two subjects (subject 4 and her father), who had seizures and LETM1 preserved, but LETM1 mRNA levels were normal. We also investigated whether a loss-of-function heterozygous mutation on LETM1 segregates in subjects 3 and 4 and in their affected relatives, but direct gene sequencing yielded normal results in all. Although a common genetic cause for seizures, other than the 4p deletion, can still be hypothesized in this family, a pathogenic link between the genomic defect and the seizure phenotype is likely.

We compared our data with those of several literature reports dealing with either terminal 4p deletions preserving LETM1, and with interstitial 4p deletions encompassing LETM1. Looking at our patients and at literature patients as well, we noted that six of eight subjects with terminal 4p deletions preserving LETM1 had seizures, whereas seven of seven with interstitial deletions including LETM1, but preserving a relatively large terminal 4p segment did not (Fig. 1). It was previously shown that seizures (or EEG anomalies) occur in about 95% of patients with a diagnosis, leading us to consider that incomplete penetrance cannot explain absence of seizures in these subjects. Of importance, the present genotype–phenotype correlation analysis suggests that haploinsufficiency of LETM1 alone
may not be sufficient in causing seizures. We found that loss of the terminal 1.5 Mb region with breakpoint at about 300 kb from the \textit{LETM1} locus, preserving \textit{LETM1}, is critical for the seizure disorder (Fig. 1 and Table 1). Monosomy of the terminal 0.4 Mb region on 4p was suggested to be a normal population variant.\textsuperscript{15,16} Although no compelling evidence exists about this assumption, we can tentatively infer that additional genes for seizures reside in an about 1.1 Mb interval, between 0.4 and 1.5 Mb from the 4p telomere. A comorbidity model for the WHS-associated seizure disorder can be inferred, implying haploinsufficiency of \textit{LETM1} and of other genes as well.

A total of 23 known annotated genes reside within this newly characterized interval. Some of them, in particular \textit{CTBP1}\textsuperscript{17} and \textit{PIGG},\textsuperscript{18} have already been proposed as candidate genes for seizures. We hypothesize that \textit{CPLX1}, encoding complexin 1, may be a good additional candidate gene for epilepsy in WHS. Complexin 1 is a member of complexin/synaphtin gene family; it is largely expressed in the mouse brain and primarily in the inhibitory neurons of the cerebellar cortex, the deep cerebellar nuclei, and the thalamus.\textsuperscript{19} Complexins are presynaptic regulatory proteins that bind reversibly to the soluble NFS attachment protein receptor complex, playing an important role in the modulation of neurotransmitters release.\textsuperscript{20} Dysregulation of complexin expression was demonstrated to occur in neurodegenerative diseases and neuropsychiatric disorders, such as schizophrenia and depressive illness. Furthermore, \textit{Cplx1}\textsuperscript{−/−} mice displayed marked deficit in social behaviors, motor impairment, and brain morphologic changes.\textsuperscript{21,22} Because \textit{Cplx1}\textsuperscript{−/−} mice do not present seizures, a comorbidity model with haploinsufficiency of several genes could be considered for \textit{CPLX1} as well.

Further considerations are in order. From a nosologic point of view, the WHS critical region was tentatively assigned to a 300–600 Kb region, which was referred to as WHSCR-2.\textsuperscript{4} However, we are rather inclined to map the whole WHS core phenotype within a much larger segment, ranging from 1.9 to 0.4 Mb from the 4p telomere. Notably, one subject with a full WHS phenotype, including seizures, was recently reported to carry an interstitial 4p16.3 deletion overlapping the same 1.5 Mb interval.\textsuperscript{24} On the other hand, it is worth noting that a WHS clinical diagnosis was not hypothesized by different clinicians with expertise in this field in subjects 1 and 2, who both had WHRCS-2 deleted and the terminal 1.77 and 1.07 Mb segment on 4p preserved, respectively.

Additional observations can be helpful in supporting the present genotype–phenotype correlations.

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**Disclosure**

None of the authors has any conflicts of interest in relation to this work to disclose. We confirm that we have read the Journal’s position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

**References**


