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CONFERENCE REPORT

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# International meeting on Wolf-Hirschhorn syndrome: Update on the nosology and new insights on the pathogenic mechanisms for seizures and growth delay

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## Abstract

"An International Meeting on Wolf-Hirschhorn Syndrome (WHS)" was held at The University Hospital La Paz in Madrid, Spain (October 13–14, 2017). One hundred and twenty-five people, including physicians, scientists and affected families, attended the meeting. Parent and patient advocates from the Spanish Association of WHS opened the meeting with a panel discussion to set the stage regarding their hopes and expectations for therapeutic advances. In keeping with the theme on therapeutic development, the sessions followed a progression from description of the phenotype and definition of therapeutic endpoints, to definition of genomic changes. These proceedings will review the major points of discussion.

**KEYWORDS**
4p-, antiepileptic drugs, hepatoadenomas, seizures, WHS

## 1 | INTRODUCTION: WOLF-HIRSCHHORN SYNDROME

Wolf-Hirschhorn syndrome (WHS) (OMIM 194190) is a contiguous gene syndrome characterized by intellectual disability and congenital anomalies, caused by partial deletion of the distal short arm of Chromosome 4. WHS was first described in 1965 by Wolf et al., and by Hirschhorn et al., and was associated with large chromosome deletions, as detected by conventional cytogenetics of the time. Accordingly, the first nosologic description of this condition refers to a severe phenotype, with clinical manifestations including a characteristic facial profile, severe growth delay of prenatal onset, hypotonia, epilepsy, and major malformations. The latter includes congenital heart defects, midline defects, such as cleft palate and hypospadias, ocular colobomas, renal abnormalities, and skeletal anomalies. Recent technical advances and clinical availability of molecular cytogenetics testing, above all chromosomal microarrays (CMAs), has allowed for the detection of an increasing number of small atypical deletions that are usually associated with milder or atypical phenotypes. These rarer, smaller, high-resolution deletions are contributing significantly to deciphering the contribution of different genes to the characteristic phenotype of this syndrome.

### 1.1 | Session 1. Family hope, needs, and expectations

Mrs Celia Pérez, former President of Asociación Española del Síndrome de Wolf-Hirschhorn (AESWH), opened the meeting by presenting a perspective on the problems, expectations, and needs that a family with WHS experiences in Spain on a daily basis. She highlighted the value of becoming a member of the AESWH, in sharing experiences and receiving support. She also summarized the achievements of the AESWH over the last 7 years, including the ability to promote research agendas on WHS through raising money and garnering legal recognition for rare diseases.

While significant strides have been made in Spain for genetic diagnosis of WHS, the situation is not the same for all regions. In the United States as well as other countries, there are now published medical guidelines that recommend microarray technology as a first tier test for patients with intellectual disabilities, congenital malformations, or autism spectrum disorder in either a postnatal context or a prenatal context, or both (Miller et al., 2010; Vermeesch et al., 2007, Cigudosa & Lapunzina, 2012, Battaglia et al., 2013). In Spain, however, only 16.28% of the public network of hospitals completely follow these recommendations (a recent survey elaborated by the Spanish Human Genetics' Association, AEGH; 2017, data not published). Thus, rare diseases, such as WHS are probably misdiagnosed, or missed altogether, because classical cytogenetics and Multiplex ligand probe amplification (MLPA) only detect about 50% of the cases (Battaglia, Carey, & South, 2015).

This first session was concluded by another parent's point of view; Mr Damien Douglas (Wolf-Hirschhorn Syndrome Trust, UK and Ireland) presented his family's experience with their 22-year-old twins, Una and Alis, who were diagnosed in Ireland two decades ago when very little was known about this syndrome. He showed selected pictures of the twins' development and their medical challenges, noting their love of music, which is the characteristic of many WHS affected individuals. He concluded by sharing how his family values improved with the affected twins.

## 1.2 | Session 2. Social managements of the patients

### 1.2.1 | Sensory integration concerns in the child with WHS

Dr I.B.B. (CTOPBB, Oviedo, Spain) discussed the role of sensory integration dysfunction in affecting development and the capacity to participate in daily life occupations. Simple activities such as eating, dressing, bathing, or playing can become difficult challenges to overcome, and in many cases, the causes of the difficulty are attributed to other reasons. A sensory integration dysfunction occurs when the central nervous system is not able to interpret and adequately organize sensory information captured by the various sense organs (Ayres, 1964, 1966), and is frequently associated with neurodevelopmental conditions such as autism, cerebral palsy or Down syndrome (Ben-Sasson et al., 2008; Bruni, Cameron, Dua, & Noy, 2010; Goble, Hurvitz, & Brown, 2009), little is known about this disorder in persons with WHS.

She presented a preliminary exploration of sensory integration issues in children with WHS carried out in collaboration with Tania Moriyon-Iglesias. Twenty-four parents whose children were between 3 and 10 years old were invited to complete a questionnaire about their children's reactions to everyday sensory experiences (short sensory profile; McIntosh, Miller, Shyu, & Dunn, 1999). A low score on this questionnaire reflects greater difficulty in processing sensory input. Interestingly, in all of the sensory areas, the group of children with WHS obtained mean results well below one *SD* of the mean of children with typical development (Figure 1). Most of them, but one, had difficulties in two or more areas of sensory processing. The most prominent areas of difficulty were obtained in the low energy/weak and under-responsive/seeks sensation sections of the short sensory profile. People with these types of difficulties often have low muscle tone or problems of postural control. On the other hand, an atypical score in the under-responsive/seeks sensation area may be a manifestation of difficulties in registering sensory information. People with these kinds of difficulties often have a need to experience sensations at more intense levels than most individuals in order to become aware of sensory information. Dr I.B.B. offered a preliminary view on the presence of sensory integration concerns in children with WHS, highlighting the need to take into account sensory integration issues as a possible contributing factor to the developmental and participation difficulties experienced by these patients. An individualized assessment, carried out by an occupational therapist with advanced training in the assessment of sensory integration problems, is therefore recommended as part of the medical care of these children.

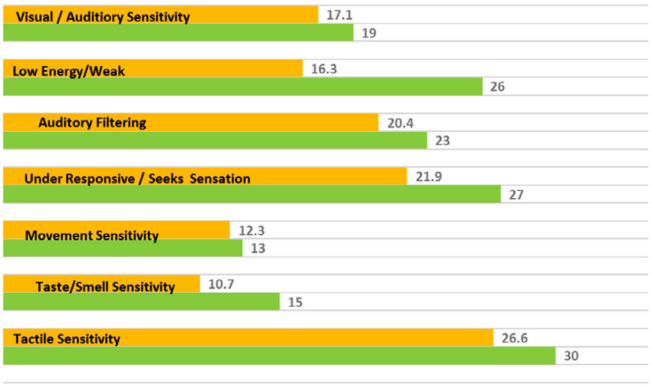


**FIGURE 1** Results of the short sensory profile questionnaire: In yellow, the mean score of children in the Wolf-Hirschhorn syndrome (WHS) group. In green one, *SD* below the mean score of the normative sample of typically developing children [Color figure can be viewed at wileyonlinelibrary.com]

### 1.2.2 | A comparative study of the efficacy of a tele-assistance intervention program for caregivers of children and teenagers with rare diseases

Dr S.B. (Neuro-e-Motion Research Team—University of Deusto, Bilbao, Spain) aimed to assess the effectiveness of an online psychosocial support program to improve health-related quality of life levels and reduce adverse symptoms through social interactions with other affected individuals. This was a unique study, as there is a lack of current research assessing the value of tele-assistance for caregivers of people with rare diseases.

A chronic disease involves a process with a long time extension and often entails high costs in physical, mental, and economic resources, restriction of social activities as well. Frequently burnout symptoms, anxiety, depression, and stress appear in the primary caregiver, which in turn impacts the entire family's quality of life (Davies, Rixon, & Newman, 2013; Drummond Soares de Moura et al., 2015). Family structure and social roles have been shown to change after a rare disease diagnosis in a family member. Moreover, the different coping strategies in parents could cause misunderstanding of feelings, emotional isolation, and communication difficulties (Jometón et al., 2015). There are few studies that conduct a comprehensive analysis of the emotional charge and coping strategies in caregivers of children with rare diseases (Picci et al., 2015). As well, there are no studies to our knowledge focused on the psychosocial aspects in caregivers of individuals with WHS.

The project aimed to demonstrate the use efficacy of a tele-assistance-based program in quality of life improvement. The first phase of the study included a comprehensive evaluation of the caregiver's experience, and evaluated different domains such as quality of life, burnout symptoms, coping strategies, quality and quantity of social network, spiritual, religious, and personal beliefs. The recruited sample for the first stage of the research included 42 participants. The second phase included a psychosocial intervention program, which includes the following intervention approaches: cognitive-based therapy, problem solving therapy, and acceptance and commitment therapy. The study is ongoing, for more information about this project, please visit: http://neuroemotion.deusto.es/en/i-care/.

## 1.3 | Session 3. Clinical and genomic approaches

Dr M.Z.'s (Catholic University of Rome, Italy) presentation reviewed genomic and clinical aspects of this syndrome:

### 1.3.1 | The basic genomic defect

There is a great variability of the extent of the 4p deletion in individual WHS patients, ranging from less than 2 Mb up to 30 Mb. Rearrangements are mostly de novo in origin; they segregate from a balanced parental translocation affecting the 4p region and different chromosome arms in 10–15% of cases. Interestingly, these de novo rearrangements are heterogeneous in nature; including isolated deletions (70% of these cases), unbalanced de novo translocations, especially t(4p;8p), but also t(4p;7p), t(4p;11p), t(4p;20q), t(4p;21q), t (4p;12p), and t(4p;Dp/Gp) (22% of cases); inverted duplications associated with terminal deletions on the same 4p arm or unbalanced pericentric inversions in the remaining 8% (Maas et al., 2007; South, Whitby, Battaglia, Carey, & Brothman, 2008; Zollino et al., 2008).

Among the heterogeneous categories of the WHS-associated rearrangements, t(4p;8p) translocations that account for about 10–15% of cases, could represent a distinct genetic entity. They usually arise in the maternal meiosis as a result of homologous nonallelic recombination mediated by segmental duplications on both 4p and 8p, and, in addition, can be associated with a maternal inversion polymorphism on 4p16. On the contrary, no recurrent breakpoints nor an association with a parental inversion polymorphism are observed in the remaining WHS-associated rearrangements, both de novo and inherited. De novo rearrangements are usually paternal in origin.

CMA techniques are currently allowing for the diagnosis of atypical, and in several occasions, very small 4p deletions, either interstitial or terminal, not associated with typical WHS. Atypical deletions are essential tools for discovering new pathogenic genes in WHS. However, whether a diagnosis of WHS can be given in many of these instances is questionable. Along with genomic data, clinical diagnostic criteria for WHS are needed.

### 1.3.2 | About the clinical phenotype

The constellation of morphogenic anomalies and major malformations and the severity of intellectual disability mostly depends on the extent of the 4p deletion and on the complexity of the basic genomic defect, as well. Both these genomic features make the resulting phenotype highly variable in individual patients. Large 4p deletions, above 9 Mb in length, are associated with a more severe phenotype, including the typical facial dysmorphism, hypotonia, severe growth delay, significant neurodevelopmental impairment, major malformations, and seizures. However, small deletions, of less than 4 Mb in length, are usually associated with a milder phenotype, with clinical manifestations limited to the typical facial dysmorphism, growth delay, mild intellectual disability, and seizures. For the benefit of precision genetic diagnosis, the WHS phenotype in now distinguished in at least two major categories, referring to the classical and the mild phenotype, respectively. It must be specified that seizures act as independent prognostic factor for the severity of ID. Accordingly, the pattern of seizures and the basic genomic defect are the most important prognostic factors for the neurodevelopmental issues. Of importance, strict clinical diagnostic criteria for WHS are needed in the current era of whole genomic investigations by CMA techniques, by which several very small or atypical deletion on 4p have been detected in the absence of a clinical suspicion of WHS. There is a consensus in considering the core WHS phenotype defined by the association of the typical facial dysmorphism, growth delay, intellectual disability, and seizures (or electroencephalogram (EEG) anomalies). All these signs together justify giving a clinical diagnosis of WHS to the families. On the other hand, atypical deletions represent an important tool for new candidate genes to be investigated. In fact, the core WHS phenotype is multigenic in origin, and different genes are pathogenically related to the different features of the core phenotype. Based upon this evidence, the definition of the WHS critical region is worthy of revision.

### 1.3.3 | The WHS critical region

The first WHS critical region was described in Wright et al. (1997); WHSCR, limited to a 165 kb interval at about 2 Mb from the telomere, defined by the loci D4S166 and D4S3327. Two genes, *WHSC1* (Stec et al., 1998) (OMIM 602952), two thirds of which map within the distal half of the WHSCR, and *WHSC2* (Wright, Costa, Naranjo, Francis-West, & Altherr, 1999) (OMIM 606026), falling entirely within WHSCR, were described as candidate genes. *LETM1* (leucine zipper/EF-hand containing transmembrane (OMIM604407), which is involved in $Ca^{2+}$signaling, was the canonical candidate gene for seizures. However, it maps outside the WHSCR, distally to it. In fact, most WHS-associated deletions are much larger than WHSCR, usually including all the candidate genes. Rauch et al. (2001) reported a small interstitial deletion case restricted to WHSCR and in whom the *LETM1* gene was preserved, presenting an atypical WHS phenotype, with no seizures. Indeed, this and other reports such as the detection of a 1.9 Mb deletion productive of the full WHS core phenotype, including seizures, but not including the whole WHSCR, allowed redefinition of the critical region within an interval of 300–600 kb between the loci D4S3327 (at 1.9 Mb from the telomere) and D4S98-D4S168 (at 1.6–1.3 Mb from the telomere) (Zollino et al., 2003). The newly described region was referred to as WHSCR-2. It is contiguous, distally, to the previous one, sharing with it the *WHSC1* gene. *LETM1* lies within this region. However, there is convincing evidence that heterozygous loss-of-function of *LETM1* alone is not sufficient in causing seizures, but haploinsufficiency of other more telomeric genes seem to be required, as discussed later.

The facial dysmorphism is multigenic in pathogenesis as well, with additional candidate genes residing outside both the WHSCR and WHSCR2, distally to them. Of relevance, definition of the WHS critical region has the purpose of elucidating all the major genes for the

core phenotype. Based on the present observations, the WHS critical region has to be broadened to encompass the interstitial 1.5 Mb region between 1.9 and 0.4 Mb from the p-telomere of Chromosome 4 (Zollino et al., 2014), and pathogenesis of WHS is largely considered to be multigenic.

### 1.3.4 | Pathogenesis of the WHS phenotype and candidate genes

Among the many OMIM genes residing within this region proposed above, *WHSC1*, the most proximal one, is the major candidate gene for growth delay. It appears to be a very important gene for the facial dysmorphism as well, but synergistic haploinsufficiency of additional genes, distal to it, is the underlying mechanism for the full WHS facial phenotype, most likely, as observed in different cohort of patients, and as suggested by Battaglia et al. (2015).

The *WHSC1* gene (OMIM *602952, also known as *NSD2* or *MMSET*) codes for a H3K36me3-specific histone methyltransferase. It has been demonstrated that the protein is involved in transcriptional regulation, mainly acting as a histone methyltransferases, and is expressed ubiquitously in early development. It appears to play an important role in normal development, but it is also involved in various neoplastic conditions. *LETM1* is another important OMIM gene residing within the recently suggested 1.5 Mb critical region on 4p. *LETM1* is the canonical candidate gene for seizures. It encodes a protein for the inner mitochondrial membrane, playing a role in mitochondrial $K^+/H^+$ (KHE) and $Ca^{2+}/H^+$ exchange, in maintaining the mitochondrial membrane potential, and in the export of proteins involved in the assembly of respiratory complexes. However, the unique role of *LETM1* in causing epilepsy is questionable. Among the new genes for seizures, *CPLX1* was suggested as a strong candidate gene. *CPLX1* (Complexin 1), a member of complexin/synaphin gene family, is largely expressed in the mouse brain. Complexins are presynaptic regulatory proteins, playing an important role in the modulation of neurotransmitters release. Dysregulation of complexin expression was demonstrated to occur in neurodegenerative diseases and neuropsychiatric disorders, such as schizophrenia and depressive illness. Furthermore, *Cplx1* mutant mice display marked deficit in social behaviors, motor impairment, and brain morphologic changes. Because *Cplx1*−/− mice do not present seizures, a comorbidity model with haploinsufficiency of several genes could be considered for *CPLX1* as well (Zollino et al., 2014).

### 1.3.5 | Seizure genetics and seizure management in WHS

Dr K.H. (Lineagen, Inc., Salt lake City, UT) also discussed seizures and genetics in WHS. In her presentation, she described the fine mapping of a seizure susceptibility region within the terminal WHS 4p region (Ho et al., 2016; Ho & Wassman, 2017). This candidate region contained two genes encoding zinc-finger-containing proteins of unknown function and *PIGG* (OMIM *606918) residing very distally to *LETM1*. PIGG functions as a member of the phosphatidylinositol (PI) glycan anchor biosynthetic pathway. In humans, homozygous loss of function mutations in *PIGG* lead to a congenital disorder of glycosylation associated with seizures (Makrythanasis et al., 2016). In zebrafish, loss of PI-glycan anchor pathway function leads to cytoplasmic mislocalization of the membrane-associated sodium channel, *SCN1B* (Nakano et al., 2010). This insight together with the observation of the similarity of the electroclinical picture (Battaglia, Filippi, South, & Carey, 2009), potentially connects WHS to the pediatric seizure syndrome known as Dravet syndrome, which is caused by loss of function mutations in *SCN1A* and *SCN1B*. The potential connection to Dravet syndrome led to the proposal that new therapies proving to be effective for Dravet syndrome seizures may also be effective for Wolf-Hirschhorn-related seizures. For all these reasons, Ho et al. (2016) proposed *PIGG* gene as a candidate seizure susceptibility gene in WHS and presented evidence that haploinsufficiency of *LETM1* alone is not sufficient in causing the WHS-associated seizures disorder. Taken together, Ho et al., supported a synergistic haploinsufficiency of additional genes and hypothesized a multigenic model of pathogenesis.

Dr K.S.H. also presented the results of a study that used caregiver reports to capture the comparative efficacies of commonly used antiepileptic medications in a large U.S. population of individuals (N-141) with WHS. Adverse events for each drug were also cataloged. Using the Early Childhood Epilepsy Severity Scale, WHS-associated seizures are demonstrably severe regardless of deletion size. The best-performing antiepileptic drugs for controlling seizures in this cohort were broad spectrum drugs clobazam, levetiracetam, and lamotrigine, whereas, the three commonly used carboxamide class drugs: carbamazepine, phenytoin, and oxcarbazepine, were reported to have little effect on, or even exacerbate, seizures. The carboxamide class drugs, along with phenobarbital and topiramate, were also associated with the highest rate of intolerance due to cooccurrence of adverse events. Levetiracetam, clobazam, and clonazepam demonstrated higher tolerability and comparatively less severe adverse events (Wilcoxon rank sum comparison between performance of levetiracetam and carboxamide class drugs gives a $p < .0001$ after multiple comparison adjustment). The results suggested once more that the genetic etiology of seizures and an accurate electroclinical delineation, are important components of drug selection, even in contiguous gene syndromes, which may have complex seizure etiologies (Battaglia et al., 2009; Ho et al., 2018).

## 1.4 | Session 4. Spanish-based Wolf-Hirschhorn's cohort

Drs R.B. (HUCA, Oviedo, Spain) and J.N. (INGEMM, Madrid, Spain and Co-Organizer of the Meeting) briefly described the Spanish-based cohort of WHS recruited in the last 4 years of work as a collaboration agreement to AESWH and contact to Argentinean group of WHS (as a part, described in Blanco-Lago et al. (2017)). This cohort is

composed of 102 members, and can be split into two populations; the Spanish (with 60 patients from Spain) and the Latino-American (mainly Argentinians; 42 individuals; Table 1). This cohort is one of the largest and well-characterized population of WHS (partially published; Blanco-Lago et al., 2013; 2017).

In a first approach, Dr R.B. (HUCA) talked about the study proposed in this cohort and its data collection, which constituted her PhD thesis at the beginning of 2016 (https://wolfhirschhorn.com/guia-del-swh/). The data came from a parent's questionnaire and medical reports and includes: motor and cognitive skills (six and five items, respectively), epilepsy-based, drugs used and its combination, comorbidity aspects, growth features, epidemiological data, and other general clinical issues. Genetics data were incorporated after included most of the cases (90/102) under a single nucleotide polymorphism (SNP)-array (CytoSNP 850K; Illumina, San Diego, CA). In other cases, an array comparative genomic hybridization (CGH) was previously done.

In a second approach, Dr J.N. (INGEMM, HULP) described this cohort genetically, (see Table 1). These 102 individuals included 55 with terminal deletions, three with interstitial deletions, and 44 with additional duplications (14 with dup 8pter; 3 with dup 10qter; 3 with 11pter). In fact, around 45% have additional terminal duplications in other chromosome arms, (numbers which are quite similar in both Latin-American and Spanish populations). Without CMA testing, most of the additional gains could be not detected. A first question that emerged from this data was the unexpectedly high number of cases with additional duplications in WHS (previously described as around 20–40%, South et al., 2008). He posed the question: Are those cases similar among them or could we establish different cohorts in terms of the genomic rearrangement found. In fact, historically, the cases with 4p−/8p+ were considered a different entity to WHS.

Dr J.N. established a comparison between Latin-American and Spanish populations in terms of gender, age at diagnosis, age of onset of epilepsy, and so forth. Age of onset for seizures (around 9 months in both cases) and polymorphic epilepsy types were similar in both populations. Interestingly, the Latin-American cohort presented a shorter diagnosis time than Spanish one (more than 20 months; L-A: 10 months of average, Sp: 30 months of average; $p < .05$. Student's *t* test). A possible reason for that is a larger size of deletions in the Latin-American cohort, which may have more severe clinical features, and which was diagnosed by karyotype, because CMA is not available routinely in Argentina. In fact, average deletion size for Latin-American cohort was higher by 2.5 Mb compared to the average Spanish deletion size. In addition, the significant differences between these two cohorts are more critical in terms of pharmacological drugs used for seizures control, suggesting a different management of drugs between Spain and overseas in this aspect. The main aim of the project presented for Drs J.N./R.B. was focused in possible genotype–phenotype correlationships using lineal regression data analysis in a functional classified cohort of WHS corrected by age. This approach under a preliminary data analysis, suggest the existence of a clear relationship between the size of the deletion in the patient, the degree of developmental delay, the presence of status epilepticus and the number of drugs used to achieve adequate control of epilepsy.

**TABLE 1** Type of genomic rearrangements in the Spanish-based cohort

| Cohort | Terminal deletions (cases) | Interstitial deletions (cases) | Deletions with additional rearrangments |
|---|---|---|---|
| Spanish (63 cases) | 36 | 02 | 26 |
| | | | (9 cases 4p−/8p+) |
| Latino-American (38 cases) | 19 | 01 | 18 |
| | | | (5 cases 4p−/8p+) |
| Total (102 cases) | 55 | 03 | 44 |
| | | | (14 cases 4p−/8p+) |

## 1.5 | Session 5. Preliminary experimental and therapeutic approaches

Dr M.Z.'s group is currently focusing on the pathogenic mechanisms for growth delay and seizure in WHS, with particular regard to *LETM1* and *WHSC1*genes (see recent Zollino & Doronzio, 2018). Molecular and biological investigations are carried out on fibroblast and myoblast cell lines derived from three WHS patients with different extent of the 4p deletion. With respect to *LETM1*, they found that it undergoes haploinsufficiency in both fibroblasts and myoblasts, with mRNA amount of about 50% with respect to controls. In *Saccharomyces cerevisiae* and Caenorhabditis *elegans*, reduced levels of orthologous genes transcripts led to mitochondrial swelling and growth delay (Hasegawa et al, 2007). Using electron microscopy, mitochondria appeared to be enlarged and elongated with fragmented cristae in different specimens, leading to the speculation that haploinsufficiency of *LETM1* causes mitochondrial dysfunction also in humans. Regarding *WHSC1*, they investigated whether (a) it exhibits haploinsufficiency; (b) its heterozygous deletion affects the cellular mitotic activity; and (c) WHS cells are able to react to clastogenic agents in vitro by increasing their WHSC1 protein level. They found that *WHSC1* mRNA is transcribed at about 50–70% normal levels in both fibroblasts and myoblasts in basal conditions, confirming that it acts as a dose-sensitive gene. Cellular proliferation and cell viability was observed to be reduced in skin fibroblasts from all WHS patients with respect to similar primary cultures from two healthy controls (MTT assay; Sigma Aldrich, according to the manufacturer's instructions). Based on these experiments, they tentatively hypothesized that *WHSC1* haploinsufficiency gives rise to growth delay by reducing the compartment of proliferating cells. Whether growth delay results also from increased cell death due to reduced ability to restore the DNA damage triggered by environmental agents is questionable, and it needs further investigation.

Dr C.C. (CBM, Madrid. Spain) discussed a specific role for the *WHSC1* gene in the immune defects found in WHS patients. In general, the functions of the members of this gene family in normal hematopoiesis had not been investigated (Hu & Shilatifard, 2016), even

though they are recurrently involved in hematopoietic malignancies. Dr C.C. presented a new animal model for the study of the hematopoietic deficiencies linked to WHS. His group studied hematopoietic development in *Whsc1*$^{+/-}$ mice and, since the homozygous loss of *Whsc1* is early lethal in mice, they performed fetal liver transplantations into irradiated adult recipient animals to study hematopoietic development in the total absence of *Whsc1*. In this way, they could provide (Campos-Sanchez et al., 2017) in vivo genetic evidence showing that *Whsc1* deficiency impairs normal hematopoietic development at several stages and cellular lineages, and particularly affects B cell differentiation, mature B cell function and hematopoietic stem cell (HSC) survival. These findings reveal the role of Whsc1 as a player in hematopoietic development and indicate that many of the immune defects associated to WHS can be directly attributed to the reduced levels of *Whsc1*. There is an impairment in the development of *Whsc1*$^{+/-}$ lymphocytes. This finding could have serious implications for the long-term prognosis of immunodeficiency in WHS patients (hemizygous for *WHSC1*) because it implies that, their immune response at late stages of life, might be seriously impaired.

Total loss of *Whsc1* in KO mice affects the development and function of different blood cell types; in B cells, at several developmental stages. (i.1) In the absence of *Whsc1*, the early stages of B cell development are seriously compromised, leading to very reduced numbers of B cells in the peripheral organs. (i.2) Regarding later stages of B cell development, there is an impairment in the class switch recombination reaction, related to an aberrant DNA replication and altered cellular proliferation of *Whsc1*-deficient cells. This leads to an inefficient generation of switched immunoglobulins. In HSCs, (ii.1) there is a decrease in the percentages of HSCs cells in the absence of *Whsc1*. (ii.2) Serial transplantation experiments lead to bone marrow failure and death in tertiary recipients of *Whsc1*-KO cells, a paradigmatic demonstration of stem cell failure. ii.3) *Whsc1*$^{-/-}$ HSCs present cell cycle problems associated with impaired DNA replication and accumulation of DNA damage. This leads to a progressive reduction in their numbers with time.

Altogether, the results presented by Dr Cobaleda et al. show that Whsc1 is involved in hematopoietic development at several stages implicating different cellular lineages. Furthermore, it participates in the regulation of different molecular mechanisms throughout these different stages and cell types, from HSC function to B cell lineage specification and commitment, fitness or cellular proliferation. These findings provide a framework for the understanding, prognosis, and potential future treatment of immunodeficiency in WHS. In addition, it is remarkable the fact that we now have an ideal preclinical model for the testing of new potential therapeutic approaches to revert or ameliorate the patient's conditions.

In the same way, Dr E.L.G. (Immunological Service at HULP, Madrid) showed a research project proposal to be tested about the role of B-lymphocyte in the antibody responsive role in WHS's patients.

Dr R.B. (HUCA, Asturias. Spain) showed the preliminary results of a preclinical trial using Coenzyme-Q10 (ubiquinone). It is a molecule that naturally forms part of the mitochondrial respiratory chain. It functions as a powerful antioxidant to allow the flow of electrons in the respiratory chain, and is often used in patients affected by mitochondrial diseases with improvement of muscle, cardiac, or neurological multifunction activity in some cases. Given its safety and easy management, currently its indications have been expanded to many entities whose origin appears due to a mitochondrial dysfunction (ataxia, myopathies, pigmentary retinopathy, cardiomyopathies, rhabdomyolysis associated with encephalopathy cortico-resistant, Friedreich ataxia, nephrotic syndrome, or migraine). A preliminary study from Hart, Rauch, Carr, Vermeesch, and O'Driscoll (2014) showed a potential role of the *LETM1* gene with distinct mitochondrial phenotypes, including altered intracellular [$Ca^{2+}$] levels, dysfunctional mitochondrial transition-pore opening, hyperpolarization, and superoxide leakage from resting mitochondria in WHS's patients. They also found that those phenotypes segregated with seizures in their WHS cohort. A recent work by Durigon et al. (2018) seemed to support these findings.

The clinical WHS, with a predominance of psychomotor disability, seizures, hypotonia, developmental delay, sensory disturbances, poor tolerance to the increase in body temperature (febrile seizures), and multiorgan involvement mimics mitochondrial diseases. Altogether, Dr R.B. proposed the following working hypothesis: could CoenzQ10 improve the quality of life of patients with WHS, and/or restore his seemingly impaired, mitochondrial function? Dr Blanco et al. (neuropediatricians) designed a protocol for record of use of CoenzQ10, a preclinical trial, in children affected with WHS. To end this, they planned to complete three questionnaires: one before starting the treatment, one at 4 months and another at the eighth month of treatment, collecting the following data: basic epidemiological (age, sex, weight, height, OFC), as well as Coenzyme administered dose, the presence of epilepsy and its characteristics (level of control), and a series of questions about the degree of psychomotor development (13 items on level of motor activity reached, attitude and ability to communicate) (Table 2). The last part of the questionnaire includes the presence of adverse effects and subjective comments from parents/caregivers or a primary care physician. Written informed consent was obtained from all the families who agreed to participate in the project. Each doctor decided whether to offer CoenzQ10 to his/her patient with WHS and if so, scored in the form changes that were observed.

At the end, 28 families agreed to participate in the project. The characteristics of the sample are listed in Table 2. The average age of the sample was 9.2 years and the degree of psychomotor disability was moderate or severe in 70% of cases. The mean dose of CoenzQ10 managed was 7.8 mg/kg/day. Data were collected in only 18 of the 28 participants, showing a qualitative and quantitative improvement in the muscle tone, as well as in connection with the environment and in the child's communicative skills. Regarding the improvement in the muscle tone, this was inferred by both data extracted for motor items and by free/subjective comments on the parent's questionnaire. In fact, families indicated an improvement in stability in walking, as they compared previous to the treatment (without significant changes in the physical therapies received at the same

**TABLE 2** Items (13) collected for the estimation of the degree of psychomotor development of each child (corrected by age) at the start of the study and in the further revisions to the 4 and 8 months after CoenzQ10 supplement

| | | |
|---|---|---|
| 1. The child holds the head | YES | NO |
| 2. The child is able to remain seated | YES | NO |
| 3. The child is capable of sitting him alone, without help | YES | NO |
| 4. The child is able to walk with help | YES | NO |
| 5. The child is able to walk without any help | YES | NO |
| 6. The child is able to eat without help | YES | NO |
| 7.The child is capable to get up if he dropped, without the child help | YES | NO |
| 8. The child catches objects spontaneously with the hands | YES | NO |
| 9. The child uses diapers | YES | NO |
| 10. The child interacts with its environment, smiles and is interested by what happens | YES | NO |
| 11. The child communicates by gestures and pictograms | YES | NO |
| 12. The child says some words | YES | NO |
| 13. The child speaks with short phrases, but understandable | YES | NO |

time). Positive data were observed in 43% of children, whereas 25% did not show any improvement. Unfortunately, other 32% of the cases did not report any data. On the communication domain, families reported an improvement in nonverbal communication skills. In some cases, they reported an increased number of words accompanied with a more open, active and participatory attitude. The data collected on epilepsy were obtained from 20 of the 28 participants showing an improvement on the degree of seizures control in 4 (20%). In addition, all patients showed an increase in weight and height during the study period. Of the 28 children who initiated the trial, 32% continues to take CoenzQ10. Then, 28% discontinued treatment at some point, and 40% did not fill out the questionnaires. Also 50% of the patients reported no side effects; 25% described mild side effects, mostly gastrointestinal (such as diarrhea), and 25% did not fill out the questionnaires. Currently, a new evaluation of the data is ongoing. As a summary, Dr R.B. highlighted positively that, some parents have appreciated an improvement in the quality of life of children and therefore in their own families, despite using a low dose of CoenzQ10. These improvements are mainly focused on higher interest in the communicative skills and better muscle tone with no significant adverse effects. The present work could constitute a basis for the design of a double-blind controlled clinical trial.

Dr C.G. (IGBMC, Illkirch-Graffenstaden, France) showed how the use of structural surrogate phenotypes in zebrafish embryos can identify the major genes responsible for copy number variants (CNVs)-associated phenotypes. CNVs are frequent rearrangements involved in both rare and complex disorders, such as WHS. She analyzed functionally five genes present in a CNV on 4p16.1. Existing cases from both DECIPHER and AChro-Puce databases indicated that the 4p16.1 deletion was associated with micrognathia (small jaw) and microcephaly whereas the duplication was associated with an abnormal facial shape and macrocephaly. They thus sought to determine the contribution of these genes to brain and face development. To mimic the duplication, they expressed each of the five human transcripts in zebrafish embryos. She found discrete drivers for the two major anatomical features tested. Overexpression of either *SLC2A9* or *ZNF518B* was sufficient to induce macrocephaly. However, scoring for the possible drivers of the craniofacial defect neither gene induced appreciable pathology. In contrast, expression of *CLNK* and *WDR1* led to macrognathia and abnormal U-shaped Meckel's cartilage, respectively.

Finally, Dr Golzio et al. asked whether the same transcripts might be relevant to the deletion by inducing deletions in each of *WDR1* and *CLNK* orthologues by CRISPR/Cas9; in contrast to the duplication experiment, only the loss of *Wdr1* led to micrognathia. Taken together, these preliminary data suggest that the craniofacial and neuroanatomical phenotypes are due to the dose imbalance of several genes present in the 4p16.1 following a cis-interaction complex model rather than the effect of a major gene driver. The cytogenetic short armband primarily affected in WHS is 4p16.3, but many deletions extend to 4p16.1. A similar model could be used to establish a role of several genes within WHSCR 1 and 2 at 4p16.3.

## 2 | CONCLUSIONS

This meeting provides an overview and updating on the nosology of WHS, covering the current state of the field; mainly on seizures and growth delay issues, nuclear aspects in this syndrome. In addition, this meeting showed the need for future collaborative efforts among scientists working on different aspects (research, clinical routine), other clinical specialists (therapists, etc.), patients, and families to design new strategies for revealing critical issues for this contiguous gene syndrome.

## A FURTHER UPDATE

Since meeting was due, a few scientific articles and The 2018 U.S. National Conference on 4p minus syndrome have been taken, complementing some of the results presented herein. They can be summarized in the following works:

(*i*) *Highlighting a major role of WHSC1* (*NSD2*) *gene in the pathogenesis of the WHS main features* (*ID*, *characteristic facies*, *growth and developmental delay*), *in fact all these works suggest; that truncating variants on NSD2 gene will lead to atypical clinical manifestations of WHS, not including the epilepsy* (Barrie et al., 2019; Boczek et al., 2018; Cammarata-Scalisi et al., 2019; Corrêa et al., 2018; Hirschhorn, 2008; Jiang et al., 2019):

Zollino, M. & Doronzio P. N. (2018). Dissecting the Wolf-Hirschhorn syndrome phenotype: WHSC1 is a neurodevelopmental gene contributing to growth delay, intellectual disability, and to the facial dysmorphism. *Journal of Human Genetics*, *63*(8), 859–861.

Bernardini, L., et al. (2018). Small 4p16.3 deletions: Three additional patients and review of the literature. *American Journal Medical Genetics Part A*, *176*(11), 2501–2508.

Lozier, E. R., et al. (2018). De novo nonsense mutation in WHSC1 (NSD2) in patient with intellectual disability and dysmorphic features. *Journal of Human Genetics*, *63*(8), 919–922.

Derar, N., et al. (2019). De novo truncating variants in WHSC1 recapitulate the Wolf-Hirschhorn (4p16.3 microdeletion) syndrome phenotype. *Genet. Med.*, *21*(1), 185–188.

Corrêa, T., et al. (2018). Cytogenomic Integrative Network Analysis of the Critical Region Associated with Wolf-Hirschhorn Syndrome. *Biomed. Res. Int.*, *12*, 5436187.

Boczek, N. J., et al. (2018). Developmental delay and failure to thrive associated with a loss-of-function variant in WHSC1 (NSD2). *Am. J. Med. Genet. A*, *176*(12), 2798–2802.

Barrie, E. S., et al. (2019). De novo loss-of-function variants in NSD2 (WHSC1) associate with a subset of Wolf- Hirschhorn syndrome. *Cold Spring Harb. Mol. Case Stud. pii: mcs.a004044*. doi: 10.1101/mcs.a004044.

Jiang, Y., et al. (2019). De novo truncating variant in NSD2 gene leading to atypical Wolf-Hirschhorn syndrome phenotype. *BMC Medical Genetics*, *20*, 134–138.

(*ii*) *Suggesting a putative association between hepatocellular neoplasias/hepatoblastomas and WHS*:

Battaglia, A., et al. (2018). Risk of hepatic neoplasms in Wolf-Hirschhorn syndrome (4p): Four new cases and review of the literature. *Am. J. Med. Genet. A*, *176*(11), 2389–2394.

Bayhan, T., et al. (2017). Hepatoblastoma and Wolf-Hirschhorn syndrome: Coincidence or a new feature of a rare disease? *Pediatr Int.*, *59*(9), 1028–1029.

(*iii*) *Showing comparative efficacies of the commonly used antiepileptic medications in a large population of individuals with WHS*:

Ho, K. S., et al. (2018). A survey of antiepileptic drug responses identifies drugs with potential efficacy for seizure control in Wolf-Hirschhorn syndrome. *Epilepsy Behaviour*, *81*, 55–61.

(*iv*) *Suggesting a putative role of epigenetic changes in primary immunodeficiencies observed in patients with different syndromes, including Wolf-Hirschhorn*:

Campos-Sanchez, E., et al. (2019). Epigenetic Deregulation in Human Primary Immunodeficiencies. *Trends Immunology*, *40*(1), 49–65.

(*v*) *Oral manifestations of Wolf-Hirschhorn syndrome* (*SWH*) *objective and methodology*.

Dr J.L. from University of Santiago de Compostela (Galicia, Spain), and colleagues (in several cities of Spain) have initiated a systematic description of the oral manifestations in a group of patients (32 participants, 12 boys and 20 girls; were aged between 1.5 and 18 years old) diagnosed of SWH, under a collaboration with the Spanish Association of SWH. To end this, patients were derived to undergo a standardized dental scan that included: filiation, odontogram and evaluation of the oral cavity and complementary tests of image data. The obtained variables were grouped into eight sections: assessment of the degree of collaboration of the patient, odontogram, description of dental anomalies, basic periodontal examination, occlusal, complementary exploratory analysis, bad habits, and imaging techniques. As we know, this is the first and the most complete oral-dental analysis in Wolf-Hirschhorn's population. Preliminary results indicate that almost half (46%) of the individuals require moderate to severe physical restriction for a complete examination and the most common finding was the dental attrition (68.7%) secondary to bruxism and microdontia. In a remarkable number of patients had disorders of the dental form ($n$ = 12) and only seven patients had cavities. Most of the group (71.8%) showed eruptive delay with regard to the chronological age, and the predominant occlusal pattern was the angle's classification of malocclusion Type II (62.5%). Image analysis, only available in half of them (11 patients) yielded a 54% of tooth agenesis. In summary, the SWH does not have a specific pathological findings; however, a series of oral–dental diseases manifest with greater prevalence and severity than in the general population.

(*vi*) *New studies in Spanish patients with WHS*:

Blanco-Lago, R., et al. (2017). Wolf-Hirschhorn syndrome. Description of a Spanish cohort of 51 cases and a literature review. *Review Neurology*, *64*(9), 393–400. Review. Spanish.

Cammarata-Scalisi F, et al. (2019). Wolf-Hirschhorn syndrome. Description of five cases characterized by means of single nucleotide polymorphism microarrays. *Arch. Argentinian. Pediatrics*, *117*(4), e406–e412. Spanish.

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## CONFLICT OF INTEREST

The authors declare no conflict of interests.

## DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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