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RESEARCH REVIEW

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# The delineation of the Wolf-Hirschhorn syndrome over six decades: Illustration of the ongoing advances in phenotype analysis and cytogenomic technology

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## Abstract

Since Hirschhorn's description in 1961, the history and chronology of the clinical, cytogenetic, and molecular characterization of Wolf-Hirschhorn syndrome (WHS) elegantly demonstrates the remarkable advances in genetic technology over the last six decades that have paralleled the delineation of the phenotype. After mention in the *Human Chromosome Newsletter* of a child with a visible deletion of the top of a B chromosome group, 4–5, Hirschhorn and colleagues companioned their report with that of Wolf et al. in *Humangenetik* in 1965, and the condition was recognized and named. The 1960–1970s witnessed the description of many of the now classic chromosome disorders, including WHS, while HRB allowed for the recognition of chromosome syndromes with smaller deletions/duplications. FISH probes, developed in the next two decades, enabled the characterization of the critical region of WHS and improved clinical diagnosis with subtelomeric probes. Cytogenomic microarray in the early-mid 2000s led to both improved diagnosis of WHS patients and documentation of microdeletions of <5 megabases, helping to characterize the critical regions for specific component phenotypes (e.g., seizures, face). Recently exome sequencing technology has led to the discovery of WHS patients with *WHSC1* loss of function variants, displaying some cardinal features of the phenotype (face, growth, and developmental delay).

**KEYWORDS**
4p-syndrome, advances in Cytogenomic technology, deletion 4p, monosomy 4p, Wolf-Hirschhorn syndrome

## 1 | INTRODUCTION

The Wolf-Hirschhorn syndrome (WHS) is the first human chromosome partial deletion syndrome described in humans. The now well characterized contiguous gene syndrome is due to deletions encompassing the distal 4p16 (4p-) where the critical genes underlying the recognizable phenotype lie. In this review we will highlight the landmarks in the history of this important syndrome illustrating the remarkable advances in genetic technology over the last six decades that have paralleled the clinical delineation of the phenotype. We describe specific periods of technological advances within two "Eras" of clinical genetics.

## 2 | THE "PHENOTYPE-FIRST" ERA

### 2.1 | Prebanding period

In 1961, just 5 years after the characterization of human cells having 46 chromosomes (Hsu, 1952; Hsu & Pomerat, 1953; Ford &

Hamerton, 1956; Tjio JH & Levan A, 1956), and 2 years after the description of the chromosomal basis of Down syndrome (Lejeune, 1959), Cooper and Hirschhorn published a case report in the Mammalian Chromosome Newsletter (later the Human Chromosome Newsletter) of a child with midline defects and an obvious deletion of the short arm of one of the B group chromosomes (Cooper & Hirschhorn, 1961). The partial monosomy in this patient represented the first example of such an observation in humans and consisted of a deletion of more than half of the short arm of the chromosome. In 1964, after the description of three individuals with a deletion of a B group chromosome and a distinctive syndrome involving a characteristic cry (eventually known as the "cri du chat syndrome"), German, Lejeune & Macintire 1964 employed chromosomal autoradiography to determine that such deletions were likely on two different B group chromosomes. Hence, the separation of chromosomes 4 and 5 in the B group. A year later, Wolf et al. (1965) submitted a report of a patient with 4p deletion to *Humangenetik* (now called *Human Genetics*). The editor, Arno Motulsky, a pioneer in the field of medical genetics, recalled Cooper and Hirschhorn's report in the Newsletter of 1961 and requested them to submit their case so that both reports could appear simultaneously in the journal. These two reports by Wolf et al. (1965) and Hirschhorn et al. (1965) brought Wolf-Hirschhorn syndrome (WHS) to the attention of the genetics community and confirmed the existence of human deletion syndromes (Figure 1). As indicated, the chromosomal basis of WHS consists of a deletion of the terminal portion of the short arm of chromosome 4. Early on in the history of the syndrome, the deleted segment of reported individuals represented about one half of the short arm, occurring distal to the bands now called 4p15.1–15.2.

## 2.2 | Banding period

The 1960–1970s witnessed the description of many of the now classic chromosome disorders, including WHS, while high resolution banding (HRB) techniques allowed for the recognition of chromosome syndromes with smaller deletions/duplications. Between 1965 and 1971, 18 additional individuals with WHS were documented in the literature, mostly in the US pediatric literature, and the syndrome was introduced to the pediatric community (Guthrie et al., 1971). With the development of G-banding techniques in 1971 (Drets & Shaw, 1971), smaller deletions could be detected, leading to the determination that the core WHS phenotype (pre and postnatal growth delay, typical craniofacial features, cognitive disability, and seizure disorder) was caused by monosomy of material within 4p16. By 1975, just over 40 individuals with 4p deletion had been reported in the literature (Johnson et al., 1976). By 1981, the concept that the critical deletion of WHS was located within band 4p16 had become widely accepted. During the 1980s, individuals with the WHS phenotype and apparently normal chromosomes were described (Pitt et al., 1984). By the

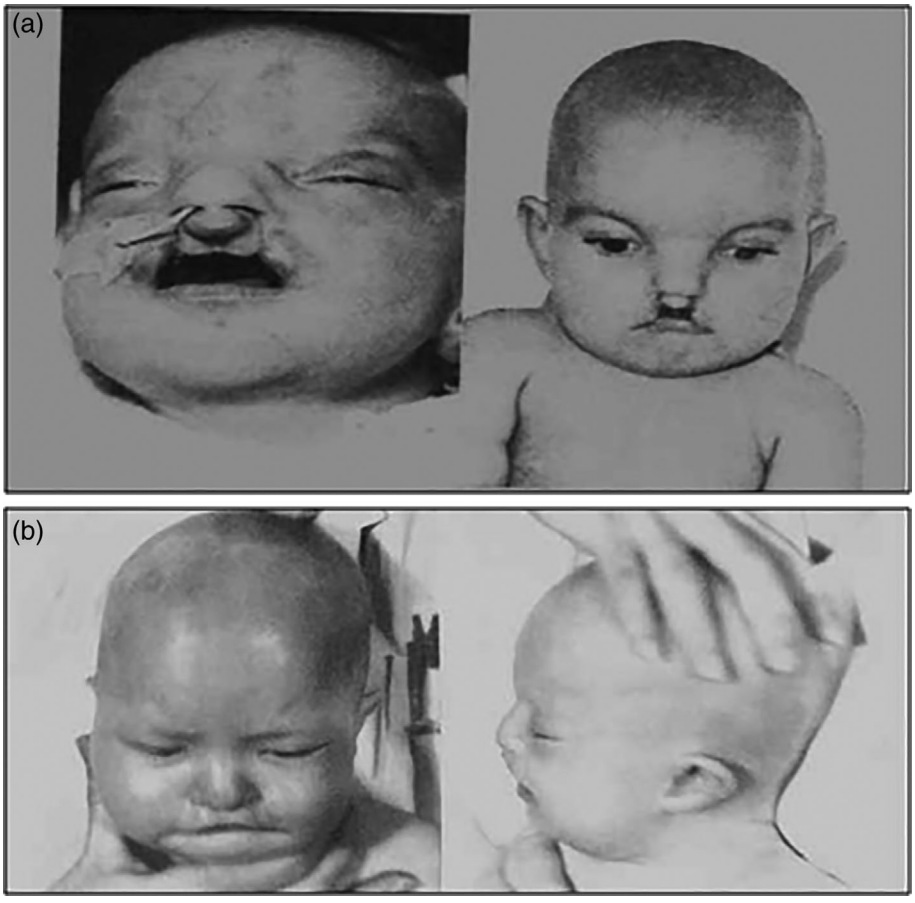



**FIGURE 1** The photos depict the original patients of Wolf et al. (1965) (a) and Hirschhorn et al. (1965) (b), (republished with permission from Hirschhorn, 2008)

end of that decade, advances in molecular techniques, including cytogenetic techniques, allowed for the ability to recognize that these individuals had submicroscopic deletions. In 1982 Curry and colleagues documented a patient with a small deletion of distal 4p who showed the typical WHS phenotype but was not diagnosed until the better HRB techniques emerged. The patient had a microdeletion of distal 4p due to an unbalanced translocation of 4p;22q showing one of the first individuals with the deletion due to a familial rearrangement (Curry et al., 1982) (Figure 2).

### 2.3 | FISH/Subtelomeric FISH period

Subsequently FISH probes, developed in the next two decades, enabled the characterization of the critical region of the WHS phenotype and improved clinical diagnosis with subtelomeric probe panels. Then in the early 1990s, continued progression in molecular techniques led to the description of the smallest region of overlap of the microdeletions in a number of reported individuals with the core WHS phenotype, and, consequently, to the boundaries of what is now known as the WHS critical region (WHSCR) (Estabrooks et al., 1995). The proximal boundary of the WHSCR was defined by the identification of two individuals with all four components of the WHS core phenotype and a 1.9 megabase terminal deletion of 4p16.3 that includes the proposed candidate genes *WHSC1* (*NSD2*) and *LETM1* (Zollino et al., 2003; Rodriguez et al., 2005). The distal boundary of the WHSCR was established through the analysis of persons with an interstitial 4p16 deletion and a full WHS phentotype (Wright et al., 1997) and persons with a terminal 4p deletion without the craniofacial features of the WHS phenotype (South, Hannes, et al., 2008). Due to the identification of individuals with components of the core phenotype (seizures or growth delay or craniofacial features) with more distal deletions, the current hypothesis is that WHS represents a true contiguous gene syndrome with contribution of genes within a 1.5–1.6 Mb region in the ~0.4–1.9 Mb terminal 4p16.3 (Hammond et al., 2012; South et al., 2007; Van Buggenhout et al., 2004). Routine and high resolution chromosome analysis detects about 50%–60% of the deletions, whereas FISH, using a WHSCR probe, detects more than 95%. By the mid-1990s a critical region that included two genes hypothesized to account for the phenotype (*WHSC1* and *WHSC2*) was delimited (Wright et al., 1997; Zollino et al., 2003). In the last two decades of the 20th century, a number of notable series of individuals with WHS have been published, and these have helped in the delineation and definition of this important condition (Battaglia et al., 1999; Preus et al., 1985; Rauch et al., 2001; Wilson et al., 1981; Zollino et al., 2000). Through these reports, the phenotypic spectrum of WHS and the critical regions underlying the phenotype had been mostly characterized (Figure 3).

## 3 | THE "GENOTYPE FIRST" ERA

### 3.1 | Cytogenomic microarray period

The introduction of cytogenomic hybridization microarray in both the research and clinical domains in the early 2000s led to both improved diagnosis of patients with WHS and documentation of microdeletions of less than five megabases; eventually even smaller (<1 Mb), were recognized, helping to characterize the critical regions for specific component phenotypes (e.g.: seizures, face) (Rodriguez et al., 2005; Battaglia et al., 2008; Zollino et al., 2008; South, Hannes, et al., 2008; Izumi et al., 2010; Shimizu et al., 2014; Ho et al., 2016) (see Figure 4). Ho et al., (2016) reviewed the relevant patients with microdeletions of distal 4p16 and proposed that *PIGG* was a second candidate gene besides *LETM1* underlying seizures in WHS. Cytogenomic microarray (CMA) was also helpful in demonstrating the high prevalence of translocations, even cryptic, not detected by a previous karyotype combined with WHS-specific FISH (South, Whitby, et al., 2008).CMA

**FIGURE 2** The two siblings with 4p deletion due to an unbalanced 4p;22q translocation detected by HRB banding (Curry et al., 1982) (photo courtesy of Dr Cynthia Curry)

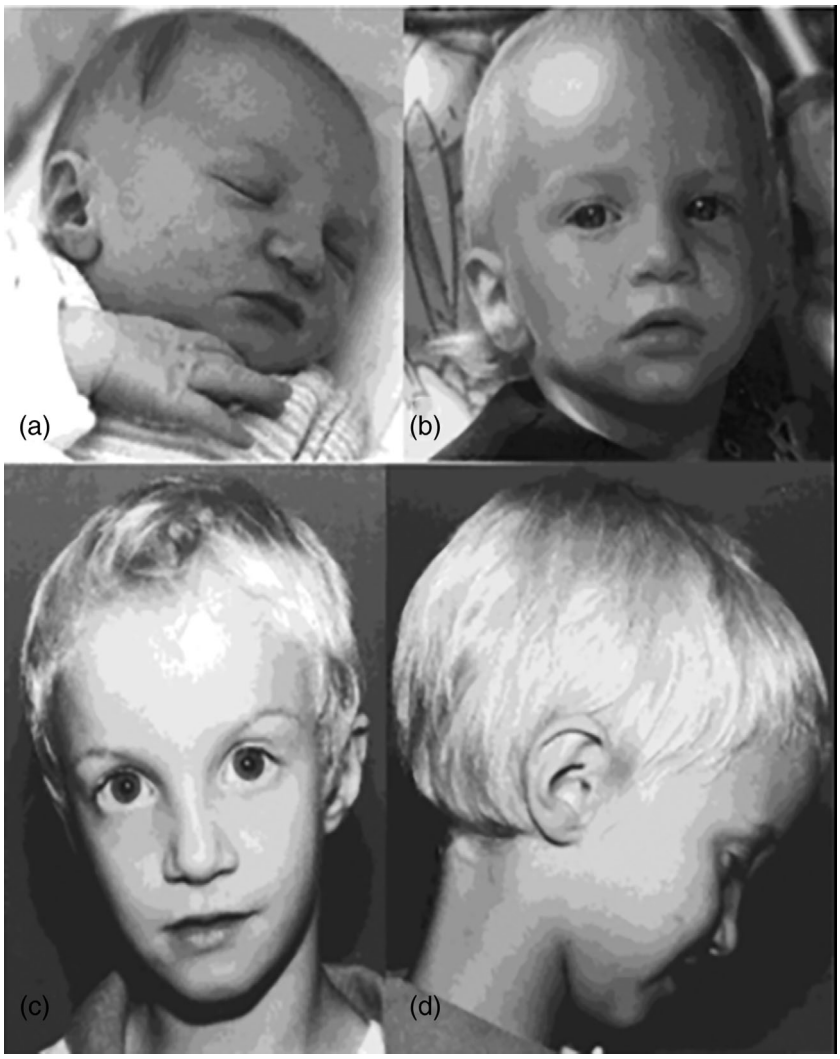



**FIGURE 3** This boy with a subtle WHS phenotype had the smallest microdeletion reported up to that time (Rauch et al., 2001) (reprinted with permission from John Wiley & Sons)

detects all currently known deletions of the WHSCR and can determine if the deletion is pure or part of a more complex imbalance, more accurately than either FISH or standard cytogenetics alone. Standard cytogenetics, however, may accompany CMA to characterize any complex aberration if present. Almost 55% of WHS individuals have a deletion with no other cytogenetic aberration; the remaining have a more complex cytogenetic aberration, such as ring 4 chromosome, 4p- mosaicism, or a derivative chromosome 4 resulting from an unbalanced translocation (South, Whitby, et al., 2008; Zollino et al., 2008). The unbalanced translocations can be de novo or inherited from a parent with a balanced rearrangement. The most commonly observed translocation involves a rearrangement of distal 4p and 8p. The breakpoints on the 4p and 8p vary on both chromosomes but usually cluster around the loci of olfactory receptor gene clusters located on both chromosomes (Zollino et al., 2008).

## 3.2 | Next generation sequencing period

A quantum leap in genetic testing occurred with the development of next generation sequencing (NGS) whereby DNA from individuals could be screened for sequence variants in days to weeks rather than months to years (Rehm et al., 2013). Not only did this technique increase sensitivity for detection of pathogenic mutations in single genes, it enabled laboratories to develop panels to screen many genes for a given phenotype. Genetic syndromes with overlapping manifestations could be easily screened simultaneously. The convenience of NGS led to the development of wider and wider testing panels, ultimately making exome sequence analysis a reality in clinical care, considered by many to be the first tier test in the genetic assessment of individuals with neurodevelopmental disabilities (Srivastava et al., 2019).

## 3.3 | Clinical exome sequencing period

The availability of exome sequencing (ES) at lower costs and increased availability has led to the emergence of a "genotype first" approach when a genetic syndrome is not diagnosed clinically. This is particularly the case when studying individuals with milder manifestations that may not be easily recognized. In addition, a broader molecular screening approach may lead to the diagnosis of

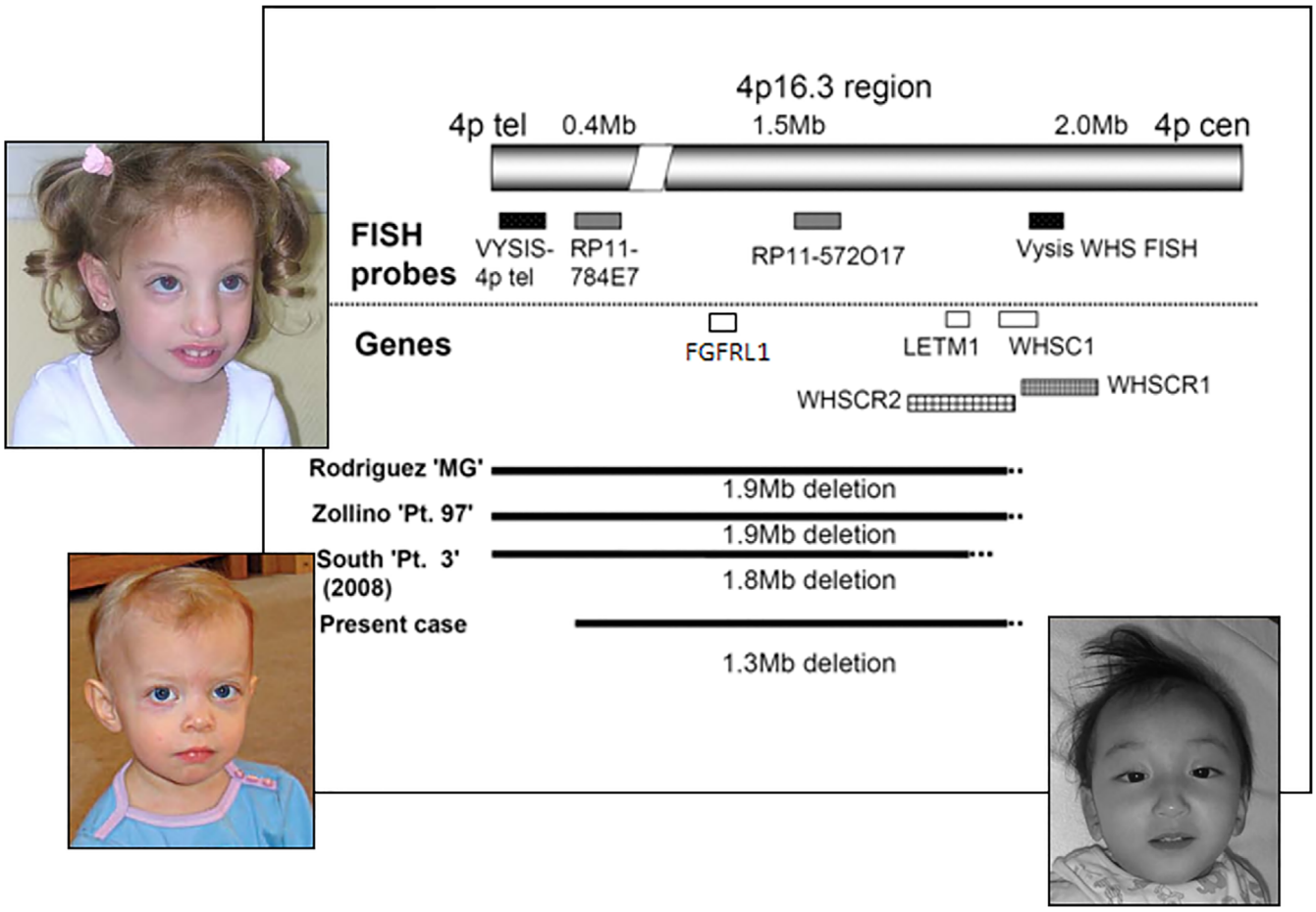



**FIGURE 4** The important genes and critical regions of 4p16.3 are shown with three elucidative patients with microdeletions of the terminal 2 Mb region of 4p16; the upper left thumbnail is the patient reported by Rodriguez et al., 2005 with typical WHS encompassing the proposed critical region; the lower left is the patient reported by South, Hannes, et al., 2008, who does not show the typical facial features and has a deletion distal to the critical regions; and the child in the lower right is the Japanese patient reported by Izumi et al., 2010 with WHS and a microdeletion with the displayed region (all reprinted with permission from John Wiley & Sons)

individuals on the milder end of a given syndrome spectrum. This application of "genotype first" spawned the recognition of phenotype expansion for some of the disorders in clinical genetics, which may provide a better understanding of specific management issues on a genotype–phenotype basis (Srivastava et al., 2019). The methodologies for genetic testing have been optimized for many of the syndromes.

There is convincing clinical evidence that the pathogenesis of the WHS phenotype is multigenic. The WHS core phenotype (pre and postnatal growth delay, distinctive craniofacial features, intellectual disability, and seizures) is due to haploinsufficiency of several closely linked genes as opposed to a single gene (Battaglia, 2021; Maas et al., 2008; Zollino et al., 2008). This view is now clearly supported by the recognition of a number of individuals carrying a pathogenic variant of *WHSC1 (NSD2)* (loss of function, missense, truncating), or *CTBP1*, showing much of the core phenotype of the syndrome (developmental delay and failure to thrive; or intellectual disability and subtle dysmorphic features; or prenatal growth delay and intellectual disability) (Beck et al., 2016; Boczek et al., 2018; Derar et al., 2019; Hu et al., 2020; Jiang et al., 2019; Lozier et al., 2018) (Figures 5 and 6). The observation of such individuals has become possible with the wider employment of ES in the genetic testing of persons with neurodevelopmental disorders. Indeed, based on scientific data and on our own experience, we would propose to consider ES as a first line test for such disorders.

Currently, more than 300 individuals with WHS have been reported in varying degrees of detail, and only in recent years has a more complete continuum of the phenotype evolved (Battaglia et al., 2015, Battaglia et al., 2021; Carey et al., 2021). Of note, until the end of the twentieth century, some families were still told by the doctors to "take their baby/child home to die" or "take him/her home, not to get attached to him/her, he/she will never be able to do anything, and he/she will be dead by the time he/she will be 3-4 years old".

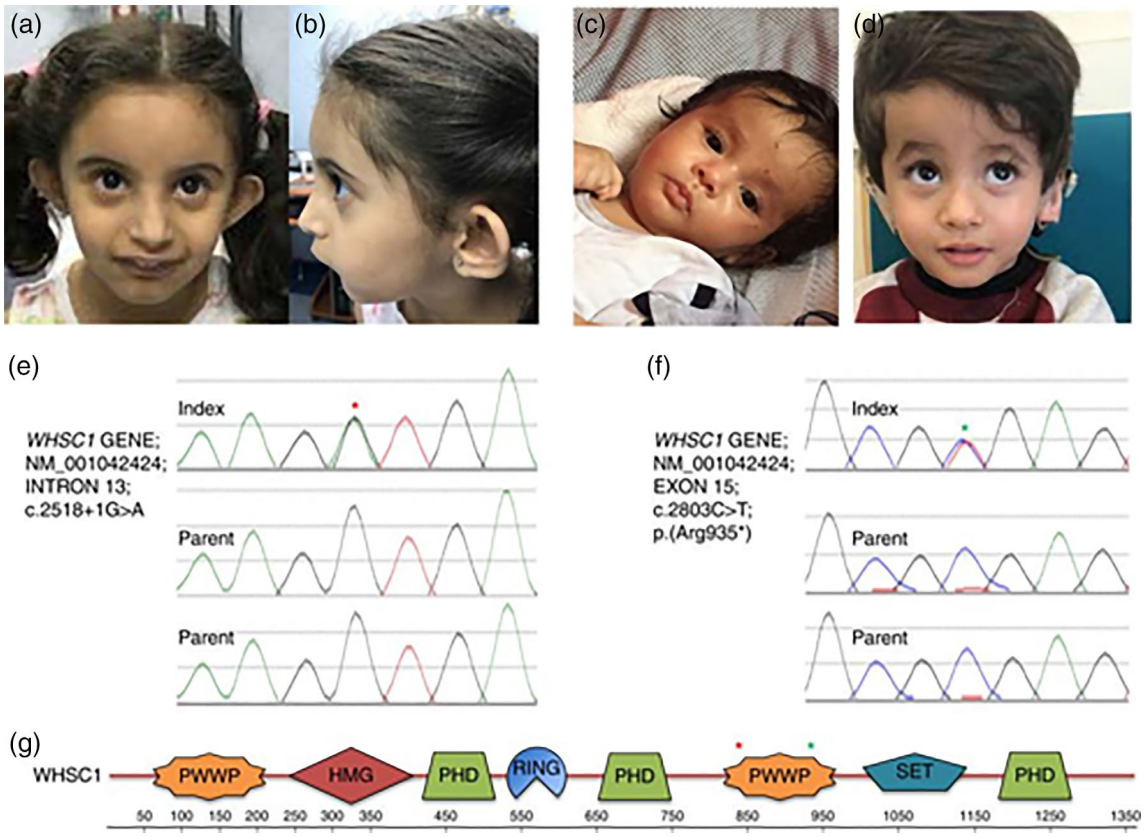
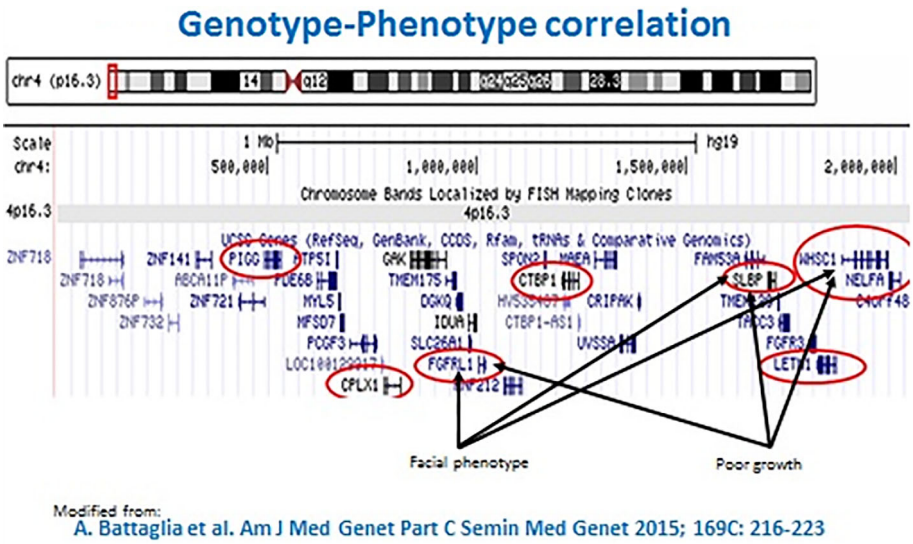



**FIGURE 5** The two patients reported by Derar et al., 2019 with loss of function variants of *WHSC1*; the typical facial features of WHS are subtle but consistent with the syndrome. The children displayed the core phenotype of WHS except for seizures, which would not be expected since the two candidate genes underlying seizures (*LETMI* and *PIGG*) (Ho et al., 2016) are not involved (reprinted with permission)

**FIGURE 6** The diagram displays the genes in the distal 2 Mb of 4p16 and indicates the candidate genes underlying two of the core features of the WHS phenotype, the face and growth delay (from Battaglia et al., 2015 and Battaglia, 2021) (reprinted with permission from John Wiley & Sons)




## 4 | CONCLUDING REMARKS

In the six decades since the original description, knowledge of the clinical phenotype, natural history, and cytogenetics of WHS has greatly increased, particularly in the last 10 years. The combination of case reports and series of well characterized individuals with WHS, and technological developments in molecular cytogenetics defined what we now know is a prototypic contiguous gene syndrome with compelling candidate genes underlying the core phenotype. Most recent work has led to the documentation of a detailed natural history

into adulthood of WHS individuals (Battaglia et al., 2021; Carey et al., 2021), allowing the emergence of a more rational and comprehensive plan for routine health supervision and anticipatory guidance to assist the primary care practitioner in the care of WHS at all ages.

The model of historical narrative presented here could be applied to other now established chromosome syndromes where one or more genes within the copy number variant underlie most or much of the phenotype, e.g., 1p36 deletion syndrome, Koolen-deVries syndrome.

Until 2010, the practice of clinical genetics was based on a "phenotype-first approach" (the observable constitution of an organism). Syndromes were described and delineated by grouping patients with similar and overlapping phenotypic manifestations together (e.g., Rubinstein-Taybi syndrome, Kabuki syndrome). As ES testing has become more readily available and expansive, there has been a trend toward the early genotyping of patients, the so-called "genotype-first approach". Indeed, based on scientific data and on our own experience, we propose to consider ES as a first tier test for neurodevelopmental disorders. In the next few years, genome sequencing (WGS) may supplant ES as the most comprehensive clinical genomic diagnostic test. However, the interpretation of and counseling for the many variants of uncertain significance that can be found when performing ES/WGS constitutes an enormous challenge. This has spurred a renewed interest in phenotype, and the notion of "deep phenotyping" has emerged, and has highlighted the paramount importance of the partnership between clinicians and laboratory geneticists (Carey, 2017).

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## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

## DATA AVAILABILITY STATEMENT

There are no data in this manuscript.

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