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When deafness meets blindness: cognitive, clinical and molecular characterization of Usher Syndrome in pediatric age

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WHEN DEAFNESS MEETS BLINDNESS: COGNITIVE, CLINICAL AND MOLECULAR CHARACTERIZATION OF USHER SYNDROME IN PEDIATRIC AGE

Original Article

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Abbreviations

CDC HP-CHUC – Centro de Desenvolvimento da Criança – Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra

DFNB2 – Deafness, Autosomal Recessive 2

FSIQ – Full Scale Intelligence Quotient

GJB2 – Gap Junction Beta 2

GMDS – The Griffiths Mental Developmental Scales

IQR – Interquartile Range

Mdn – Median

PIQ – Performance Intelligence Quotient

RP – Retinitis Pigmentosa

SD – Standard Deviation

SNHL – Sensorineural Hearing Loss

USH – Usher Syndrome

USH1 – Usher Syndrome Type 1

VIQ – Verbal Intelligence Quotient

WISC-III – Wechsler Intelligence Scale for Children—third edition

Resumo

Introdução: A Síndrome de Usher (USH) é a condição genética mais comumente associada a surdo-cegueira progressiva. É por vezes incorretamente diagnosticada como surdez não-sindrómica, uma vez que a perda de audição ocorre antes dos achados oftalmológicos. Para além disso, o diagnóstico molecular constitui ainda um desafio devido à significativa heterogeneidade clínica e genética da doença.

Objetivos: O presente estudo pretende comparar o perfil cognitivo e desenvolvimental de um grupo de crianças com USH e um grupo de crianças com surdez não-sindrómica. Pretende também efetuar uma caracterização clínica e molecular do grupo com USH.

Métodos: Foi realizado um estudo retrospetivo observacional onde se comparou a idade de aquisição de marcos do desenvolvimento psicomotor e os resultados em testes de avaliação neuropsicológica de um grupo de 10 crianças com USH de famílias independentes e um grupo de 18 crianças com surdez não sindrómica. As diferenças foram avaliadas através de testes não paramétricos (Teste de Mann-Whitney *U*).

Resultados: Os dois grupos diferem de forma estatisticamente significativa na idade de aquisição da marcha ($p = 0.001$), de sentar sem apoio ($p = 0.001$) e das primeiras palavras ($p = 0.013$), com o grupo de USH a adquirir mais tarde todas as competências. Nas Escalas de Desenvolvimento Mental de Griffiths os dois grupos têm um perfil cognitivo semelhante, exceto na subescala "Raciocínio Prático", onde as crianças com USH têm uma pontuação significativamente mais baixa ($p = 0.034$). Na WISC-III, o grupo com USH tem uma pontuação significativamente mais baixa nos subtestes "Código" ($p = 0.032$), "Disposição de Gravuras" ($p = 0.039$) e "Pesquisa de Símbolos" ($p = 0.011$). A caracterização do grupo com USH revelou uma heterogeneidade clínica e molecular, com a maioria dos casos a apresentar variantes que podem também estar associadas a surdez não-sindrómica.

Conclusão: Crianças com indícios de atraso motor e surdez neurossensorial devem ser rastreadas para USH, independentemente de não apresentarem envolvimento oftalmológico. As crianças com USH parecem ter dificuldades em tarefas relacionadas com velocidade de processamento e adaptação social/ambiental, ao contrário do grupo em que só a audição está afetada. A identificação destes achados em conjunto com os dados clínicos e moleculares podem ser úteis na deteção precoce da doença e na implementação de estratégias mais adaptadas às suas dificuldades.

Palavras-chave: Síndrome de Usher; surdez neurossensorial; retinite pigmentosa; surdocegueira; avaliação do desenvolvimento neuropsicológico; perfil genético.

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Abstract

Introduction: Usher Syndrome (USH) is the most common genetic condition responsible for progressive deafblindness. It is sometimes misdiagnosed as non-syndromic deafness, as hearing loss typically occurs before ophthalmological findings. Furthermore, molecular diagnosis remains a challenge due to the significant clinical and genetic heterogeneity of the disease.

Objectives: The present study aims to compare the developmental and cognitive profile of a USH pediatric group to a group of non-syndromic deaf children. Additionally, we aim to perform a clinical and molecular characterization of the USH group.

Methods: A retrospective observational study was performed comparing the age of acquisition of early developmental milestones and results in neuropsychological tests of 10 unrelated children with USH to a control group of 18 non-syndromic deaf children. Differences between groups were assessed through non-parametric tests (Mann-Whitney *U* Test).

Results: A statistically significant difference between groups was found in the age of acquisition of gait ($p = 0.001$), sitting alone without support ($p = 0.001$) and first words ($p = 0.013$) with the USH group showing later onset in all of three. At Griffiths Mental Developmental Scales evaluation, the two groups have a similar cognitive profile, except for in the "Practical Reasoning" subscale where USH children significantly score lower ($p = 0.034$). At WISC-III evaluation, USH group significantly score lower in "Coding" ($p = 0.032$), "Picture Arrangement" ($p = 0.039$) and "Symbol Search" ($p = 0.011$) subtests. The characterization of USH group revealed a clinical and molecular heterogeneity, with most cases having variants that may also be associated with non-syndromic deafness.

Conclusion: Children with delayed motor milestones and hearing loss should be evaluated for USH, regardless of normal ophthalmologic findings. USH children may struggle with tests related to processing speed and social/environmental adaptation, differing from those where only hearing is impaired. Identifying these factors, along with clinical and molecular data, can aid in early detection of the disease and implementation of more tailored intervention strategies.

Keywords: Usher Syndrome; sensorineural hearing loss; retinitis pigmentosa; deafblindness; developmental neuropsychological assessment; genetic profile.

1. Introduction

Human communication and environmental interaction heavily rely on our vision and hearing senses. But how do we manage to thrive when we know that we are going to lose both?

Hearing loss is one of the most common sensory disabilities affecting 432 million adults and 34 million children across the world. [1] In develop countries, approximately 50% to 60% of hearing loss cases have a genetic origin with the remaining percentage being explained by age and multiple environmental factors. [2,3] The genetic causes are very heterogeneous and can be divided into syndromic and non-syndromic. The non-syndromic disorders are those where the inner ear is the only organ affected, accounting for 70% of genetic hearing loss. [2] It is predominantly transmitted as an autosomal recessive trait and mutations in the Gap Junction Beta 2 (*GJB2*) gene are a major cause. [4,5] In the remaining 30%, the syndromic disorders, hearing loss is accompanied by the involvement of other organ systems, such as retinitis pigmentosa (RP) in Usher Syndrome (USH). [6]

USH is an autosomal recessive disease characterized by the association of sensorineural hearing loss (SNHL), RP, and variable vestibular dysfunction. Although rare, with an estimated prevalence of 4 to 17 cases per 1000 individuals, it is the most common hereditary condition responsible for combined hearing and vision loss. [7,8]

Since USH is a clinically and genetically heterogenous syndrome, it is classified into three distinct subtypes according to symptom severity, progression, and the presence of vestibular dysfunction. USH Type 1 (USH1) is the most severe subtype with patients presenting profound bilateral congenital SNHL, lack of bilateral vestibular function (areflexia), and early onset of RP. It has been associated with the causative genes *MYO7A* (MIM #276900), *PCDH15* (MIM #602083), *USH1C* (MIM #276904)*, CDH23* (MIM #601067)*, USH1G* (MIM #606943) and *CIB2* (MIM #614869). USH Type 2, the most prevalent form, is characterized by moderate to severe congenital SNHL, later onset of RP, and normal vestibular function. Variants in the *USH2A* (MIM #276901), *ADGRV1* (MIM #605472) and *WHRN* (MIM #611383) genes have been associated with this subtype. For its part, USH type 3 is the least severe and least common of the subtypes. It is characterized by a delayed onset and progressive hearing loss, variable onset of RP, and variable presence of vestibular dysfunction. Variants on the *CLRN1* (MIM #276902) gene have been identified in these patients. [7–10] Moreover, studies report the identification of several 'atypical' USH genes, which cause phenotypes that do not align with the clinical classification mentioned above. [11]

It is worth noting that pathogenic variants on the USH causative genes may also be associated with non-syndromic recessive deafness. [12] Adding complexity, for some of these genes, both

autosomal recessive and autosomal dominant inheritance have been reported (e.g. *MYO7A* is implicated in both Deafness, autosomal dominant 11, DFNA11, MIM #601317 and Deafness, autosomal recessive 2, DFNB2, MIM #600060, besides USH type 1B, MIM #276900). [13] Moreover, in the three types of USH, hearing loss is typically identified before any ophthalmological symptoms, being commonly misdiagnosed as non-syndromic hearing loss. [8] This highlights the importance of a detailed ophthalmological assessment and follow-up, and other clinical findings, for an accurate diagnosis.

Because of neonatal screening program, early intervention with cochlear implants can mitigate the impact of the deficit and potentiate the development of auditory function and speech. [14] On the other hand, RP is normally identified after the onset of deafness. Initially, it is characterized by impaired night vision, progressive narrowing of the visual field, and loss of peripheral vision. It ends up evolving into blindness within a period of one or more decades, making it the most debilitating symptom of USH. This progressive vision loss leads to a constant change in the communication mode. It may even compromise the use of lip reading and visual sign language, making it only a temporary solution and leaving the patient without the means to communicate. [8,15,16]

The dual sensory loss experienced by patients with USH creates a unique entity that cannot be approached as the coexistence of two different and isolated impaired senses. The combined vision and hearing deficit make it difficult for the impaired senses to compensate for each other. This is why progressive deafblindness must be considered a distinct disability, which impacts communication, orientation, access to information, participation in society, and the capacity to move freely and safely. [17,18]

With this study, we aim to characterize the clinical and molecular profile of USH in a pediatric population and improve our knowledge of its cognitive and developmental impact compared to a group of non-syndromic deaf children. We hypothesize that the concomitant limitations in hearing, vision, and balance could result in a neurodevelopment profile that differs from those in which only hearing is impaired. Characterizing these specificities can potentiate a correct diagnosis and an earlier differentiation from non-syndromic deafness. This timely identification of USH will be crucial to best meet the early intervention needs particular to this syndrome, to prevent co-morbidities associated with progressive deafblindness, and to manage the prognosis and expectations of these children and their families.

2. Methods

2.1. Study design

A retrospective observational study was performed comparing a population of children with established or highly suspected USH diagnosis to a control group of non-syndromic deaf children. Patients were identified by searching the etiological diagnosis in the internal database of the Centro de Desenvolvimento da Criança - Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra (CDC HP-CHUC). Further review of the electronic clinical record was performed for those who met the inclusion criteria.

This study was conducted according to the ethical standards of the Declaration of Helsinki and was approved by the ethics committee of the Centro Hospitalar e Universitário de Coimbra (OBS.SF.175-2022).

2.2. Participants

To be included in this study, participants were required to have their diagnosis confirmed or highly suspected by a specialized neurodevelopmental and genetics multidisciplinary team and undergo a formal assessment of their psychomotor and intellectual development. Children who had a severe intellectual disability that precluded psychomotor and intellectual assessment or those who had a low socio-economic status that could hinder their normal development were excluded.

A total of 28 children were included in the study and were divided into two main groups. The clinical group consisted of 10 subjects diagnosed with or highly suspected to have USH. This accounts for all the USH population followed in a tertiary hospital, except for one subject who was excluded due to severe intellectual disabilities. The control group comprised 18 children with non-syndromic hearing loss with a causative variant in *GJB2* (molecularly-confirmed Deafness, autosomal recessive 1, DFNB1, cases).

2.3. Data sources and variables

Clinical data, including demographic variables (gender, age at last medical visit, cochlear implant and psychomotor evaluation), early developmental milestones (age at sitting without support, walking three steps alone - ‘gait’, speaking first words, and sphincter control), global assessment of psychomotor and intellectual development scores, clinical features and genetic information, were retrospectively collected from the electronic clinical records and internal databases of the CDC HP-CHUC. These variables were routinely registered during multidisciplinary assessments by experienced pediatric doctors, medical geneticists, and psychologists.

2.4. Measures

Global assessment of psychomotor and intellectual development was obtained by using the Portuguese-adapted version of The Griffiths Mental Developmental Scales (GMDS) – second edition [19,20] or the Portuguese-adapted version of the Wechsler Intelligence Scale for Children—third edition (WISC-III) [21,22] according to the children's age.

2.4.1. The Griffiths Mental Developmental Scales (GMDS)

The GMDS is a widely used tool for assessing child development. It has two separate development scales: one for infants and toddlers aged 0-2 years and the extended scale for children aged 2-8 years. The child's abilities are measured by six subscales: subscale A, “Locomotor”, measuring gross motor skills important for upright posture and walking; subscale B, “Personal-Social”, measures early adaptative behavior and social skills; subscale C, “Hearing and Language”, evaluates the development of language and communication; subscale D, “Eye and Hand Coordination”, assess fine-motor skills and visual ability; subscale E, “Performance”, which evaluates visual perception awareness, manipulation, working speed and precision of work; and subscale F, “Practical Reasoning”, which is used from the 24 months onward and assesses problem-solving skills, math concepts, and moral issues. [23–25] The raw scores obtained in each scale can be used to calculate standardized scores for each domain and a general development quotient (Mean = 100, standard deviation [SD] = 15).

2.4.2. Wechsler Intelligence Scale for Children — third edition (WISC-III)

The WISC-III is a standardized intelligence test designed for children and adolescents between the ages of 6 and 16 years and 11 months. It consists of 13 subtests (Mean = 10, SD = 3) that assess intelligence in both verbal and nonverbal (performance) components. The verbal scale includes six subtests – “Information”, “Similarities”, “Arithmetic”, “Vocabulary”, “Comprehension”, and “Digit span” – which evaluate the child's language-related abilities such as comprehension, answering questions, and language processing. The performance scale plays a critical role in evaluating deaf children and consists of seven subtests – “Picture completion”, “Coding”, “Picture arrangement”, “Block design”, “Object assembly”, “Symbol search”, and “Mazes” (which was not used in this study). It assesses visual processing, planning and organizing skills, non-verbal learning, and memory. Further information about each subtest will be provided in the "Discussion" section as relevant. Based on the subject’s performance in the various subtests we can calculate three composite results (Mean = 100; SD = 15) – verbal intelligence quotient (VIQ), performance intelligence quotient (PIQ), and full-scale intelligence quotient (FSIQ). [26]

2.5. Statistical analysis

Statistical analysis was conducted using the IBM Statistical Package for the Social Sciences (SPSS) software, version 28.0 for Microsoft Windows.

Descriptive statistics were employed to describe the demographic and developmental characteristics of both study groups. Continuous variables were expressed as median (Mdn) and interquartile range (IQR), while categorical variables were presented as frequency and percentages.

Since the sample size was small, non-parametric statistical tests for independent samples (Mann-Whitney's *U* Test) were used to compare demographic and clinical data between groups. For comparisons within each group a Wilcoxon Signed Rank Test was used. A significance level (α) = 0.05 was considered.

3. Results

3.1. Demographics

The study sample consisted of 28 participants divided in two main groups: the USH (n = 10; Mdn age at last medical visit = 13.50 years, age at last medical visit range = 2–18 years; 70.0% male) and the GJB2 group (n = 18; Mdn age at last medical visit = 11 years, age at last medical visit range = 5 –18 years; 72.2% male), respectively. No statistically significant difference was found in age at last medical visit between groups (U = 79.500, Z = -0.506, p = 0.613).

Considering the age at etiologic diagnosis, i.e., the age at which the molecular study was completed, our analysis showed that the diagnosis of USH occurred at a later age (Mdn = 103 months) compared to GJB2 group (Mdn = 53 months). However, the difference was not statistically significant (U = 59.000, Z = -1.488, p = 0.137). Additionally, no statistically significant difference was found in the age of cochlear implantation between the USH group (n = 9; Mdn = 24 months) and GJB2 group (n = 16; Mdn = 24.50 months), U = 71.500, Z = -0.028, p = 0.977, ensuring that the two groups had equal opportunities for deafness intervention.

To account for the progressive deterioration of USH disease, an initial analysis was also conducted to ensure that the two groups do not differ in terms of the age at which psychomotor development was assessed. No statistically significant differences were found between this variable across groups (GMDS evaluation age: U = 26.500, Z = -1.087, p = 0.277; WISC-III evaluation age: U = 10.500, Z = -1.507, p = 0.132). For more detailed information please refer to the respective sections dedicated to each test.

3.2. Early development milestones

In the analysis of the age at which early development milestones were attained, we observed a statistically significant difference between groups with respect to the "Gait", with the USH group showing later onset (Mdn = 22 months) compared to children with *GJB2* mutation (Mdn = 12 months), U = 11.500, Z = -3.337, p = 0.001. Similar statistically significant differences were found in the age of "Sitting without support" variable, with the USH group (Mdn = 9 months) showing a later onset than the GJB2 group (Mdn = 7 months), U = 4.500, Z = -3.367, p = 0.001. The same trend was observed for the age of the "First words" variable with the USH group (Mdn = 39 months) speaking their first words later than the GJB2 group (Mdn = 25 months), U = 5.000, Z = -2.475, p = 0.013. These results and differences between groups are summarized in **Table 1.**

TABLE 1 AGE OF ACQUISITION OF EARLY DEVELOPMENTAL MILESTONES (USH VS. GJB2)

	USH		GJB2		Differences between groups		
	n	**Median (IQR; min-max)**	**n**	**Median (IQR; min-max)**	***U***	***Z***	***p***
Sitting without support	7	9.00 (4.00; 9 – 24)	10	7.00 (2.00; 5 – 10)	4.500	-3.367	0.001*
Daytime sphincter control	5	36.00 (9.00; 24 – 36)	17	28.00 (12.00; 14 – 36)	27.500	-1.222	0.222
Night-time sphincter control	5	36.00 (8.00; 24 – 40)	16	33.00 (9.00; 16 – 60)	38.000	-0.167	0.867
First Words	4	39.00 (6.00; 36 – 42)	14	25.00 (8.50; 12 – 42)	5.000	-2.475	0.013*
Gait	8	22.00 (8.75; 12 –30)	17	12.00 (3.50; 11 – 18)	11.500	-3.337	0.001*

USH, Usher Syndrome; GJB2, Gap junction beta 2 protein; IQR, Interquartile range; min, minimum; max, maximum.
Comparisons signaled with * represent statistically significant p values (Mann–Whitney U, $p < 0.05$).
All age data is presented in months.

3.3. Psychomotor and intellectual development assessment

3.3.1. GMDS standard scores

In the analysis of GMDS standard scores **(Table 2)** most subscales did not reveal statistically significant differences between the two groups (Mann-Whitney U, $p \geq 0.05$), except for the "Practical Reasoning" subscale where the USH group (Mdn = 36.50) scored significantly lower than the GJB2 group (Mdn = 81), U = 4.500, Z = -2.089, p = 0.034. Of the 18 children assessed with the GMDS, 13 (72.2%) were able to respond to this subscale and two of them scored zero

in this domain. Also, both USH (Mdn = 58.50) and GJB2 group (Mdn = 63.50) showed lower scores in the "Hearing and Language" subscale compared to other subscales. The subscale "Performance" had the highest scores for the USH group (Mdn = 100), whereas "Eye and Hand Coordination" had the highest scores for the GJB2 group (Mdn = 100).

TABLE 2 GMDS STANDARD SCORES (USH VS. GJB2)

	USH		GJB2		Differences between groups		
	n	**Median (IQR; min-max)**	**n**	**Median (IQR; min-max)**	***U***	***Z***	***p***
Evaluation Age	7	23.00 (54; 13 – 125)	11	58.00 (32; 18 – 87)	26.500	-1.087	0.277
General Quotient	6	85.00 (21.5; 68 – 109)	10	92.00 (8.50; 70 – 99)	16.000	-1.522	0.128
Locomotor	7	81.00 (24.00; 24 –126)	11	100.00 (24.00; 70 –128)	19.000	-1.767	0.077
Personal-Social	7	82.00 (40.00; 16 –117)	10	91.00 (16.00; 70 – 114)	34.000	-0.409	0.683
Hearing and Language	6	58.50 (33.25; 17 – 84)	11	63.50 (40.00; 34 – 98)	25.000	-0.542	0.588
Eye and Hand Coordination	7	89.00 (54.00; 22 – 133)	11	100.00 (24.00; 70 – 122)	34.500	-0.362	0.717
Performance	7	100.00 (58.00; 24 – 133)	11	95.00 (19.00; 70 – 117)	35.000	-0.317	0.751
Practical Reasoning	4	36.50 (51.25; 0 – 60)	9	81.00 (23.00; 0 – 103)	4.500	-2.089	0.034*

GMDS, The Griffiths Mental Developmental Scales; USH, Usher Syndrome; GJB2, Gap junction beta 2 protein; IQR, Interquartile range; min, minimum; max, maximum.
Comparisons signaled with * represent statistically significant *p* values (Mann–Whitney *U, p* < 0.05).
All age data is presented in months.

Note "Practical Reasoning" subscale cannot be administered to an age below 24 months.

Except for the "Practical Reasoning" subscale, the two groups subscales profile are nearly identical as seen in **Fig. 1.**

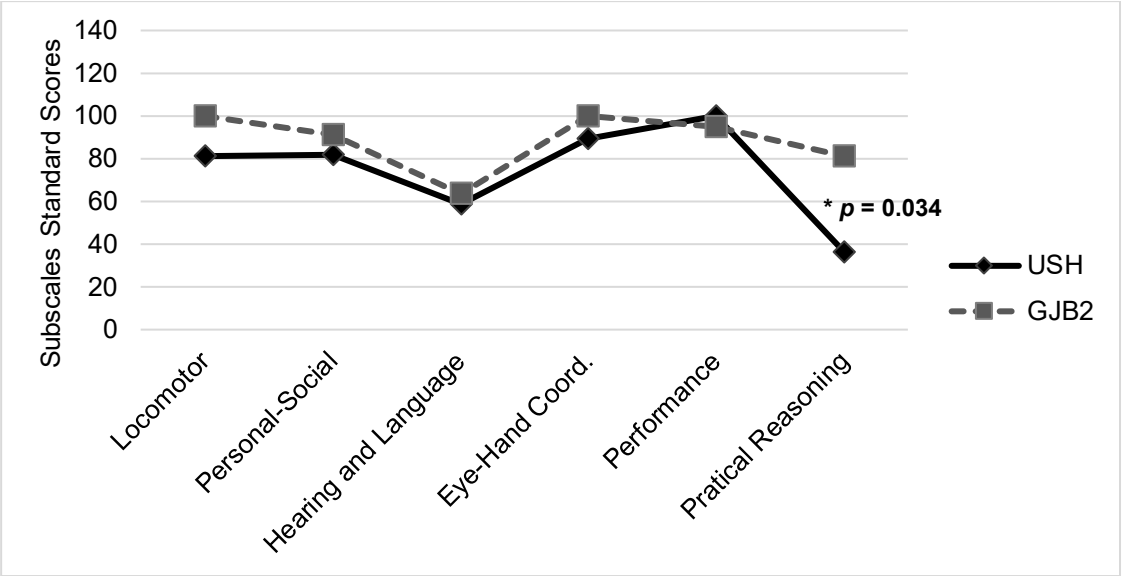

FIG. 1 GMDS SUBSCALES STANDARD SCORES PROFILE FOR USH AND GJB2 GROUPS.
Comparisons signaled with * represent statistically significant p values (Mann–Whitney *U*, $p < 0.05$).

3.3.2. *WISC-III standard scores*

In the analysis of WISC-III standard scores **(Table 3)** no statistically significant differences were found in FSIQ, VIQ and PIQ between USH and GJB2 groups (FSIQ: $U = 16.500$, $Z = -0.496$, $p = 0.620$; VIQ: $U = 15.000$, $Z = -0.709$, $p = 0.478$; PIQ: $U = 9.500$, $Z = -1.641$, $p = 0.101$).

To investigate whether there was a difference between PIQ and VIQ for both groups, we performed a Wilcoxon Signed Rank Test. In the USH group, PIQ was found to be greater than VIQ in most cases, although this difference was not statistically significant ($Z = -1.095$, $p = 0.273$). In contrast, the GJB2 group showed a significant difference in which PIQ was greater than VIQ ($Z = -2.701$, $p = 0.007$). To perform this analysis one subject from the GJB2 group was excluded for not having any registered score in the verbal scale subtests, making it impossible to calculate their VIQ.

Among the subtests score, the two groups differed with statistically significance in three of them: “Coding” ($U = 5.000$, $Z = -2.145$, $p = 0.032$), “Picture Arrangement” ($U = 0.000$, $Z = -2.067$, $p = 0.039$) and “Symbol Search” ($U = 0.000$, $Z = -2.546$, $p = 0.011$), with the USH group scoring lower results in all three. The USH group score the highest in “Block Design” (Mdn = 11.5) and the lowest in “Picture Arrangement” (Mdn = 1.5). However, it is worth noting that only 50.0% (two children) had measurable scores in this subtest. The GJB2 group, on the other hand, had the highest score in “Picture Completion” (Mdn = 12.50) and the lowest in the “Information” subtest (Mdn = 4.00). For specific comparisons and details on exact *p* values please refer **Table 3.**

TABLE 3 WISC-III STANDARD SCORES AND IQS (USH VS. GJB2)

	USH		GJB2		Differences between groups		
	n	Median (IQR; min-max)	n	Median (IQR; min-max)	*U*	*Z*	*p*
Evaluation Age	4	135 (37.75; 119 – 160)	11	119 (24.00; 84 – 151)	10.500	-1.507	0.132
FSIQ	4	82.00 (31.5; 58 – 94)	10	76.00 (14.75; 72 – 118)	16.500	-0.496	0.620
VIQ	4	81.00 (34.75; 49 –92)	10	70.00 (9.75; 66 – 117)	15.000	-0.709	0.478
PIQ	4	85.50 (39.00; 70 – 115)	11	103.00 (28.00; 86 – 132)	9.500	-1.641	0.101
VERBAL SCALE							
Information	4	7.00 (6.50; 1 – 9)	9	4.00 (3.50; 2 – 17)	14.000	-0.622	0.534
Similarities	4	8.00 (6.25; 4 – 11)	8	9.00 (2.75; 7 – 14)	12.000	-0.684	0.494
Arithmetic	3	9.00 (2.00; 8 – 10)	7	7.00 (4.00; 3 – 11)	6.000	-1.035	0.301
Vocabulary	5	8.50 (5.50; 2 – 9)	9	5.00 (4.50; 4 – 11)	15.000	-0.471	0.638
Comprehension	4	4.00 (5.00; 1 – 7)	8	6.50 (4.50; 2 – 11)	8.000	-1.364	0.173
Digit Span	4	5.00 (7.25; 1 – 10)	8	6.00 (4.50; 1 – 9)	15.000	-0.172	0.863
PERFORMANCE SCALE							
Picture Completion	4	11.00 (8.25; 7 – 16)	10	12.50 (5.25; 7 – 19)	16.500	-0.498	0.618
Coding	4	7.50 (6.22; 2 – 10)	10	10.50 (2.25; 8 – 13)	5.000	-2.145	0.032*
Picture Arrangement	2	1.50 (4.25; 0 – 3)	7	12.00 (7.00; 9 – 19)	0.000	-2.067	0.039*
Block Design	4	11.50 (5.50; 8 – 15)	10	11.50 (2.72; 7 – 13)	19.000	-0.144	0.885
Object Assembly	4	8.50 (7.50; 3 – 12)	9	9.00 (4.00; 7 – 16)	14.000	-0.623	0.533
Symbol Search	3	5.00 (6.00; 0 – 6)	10	10.00 (7.50; 7 – 16)	0.000	-2.546	0.011*

WISC-III, Wechsler Intelligence Scale for Children—third edition; FSIQ, Full Scale Intelligence Quotient; VIQ, Verbal Intelligence Quotient; PIQ, Performance Intelligence Quotient; USH, Usher Syndrome; GJB2, Gap junction beta 2 protein; IQR, Interquartile range; min, minimum; max, maximum.
Comparisons signaled with * represent statistically significant *p* values (Mann–Whitney *U, p* < 0.05).
All age data is presented in months.

Although both groups exhibited a tendency to score higher in the Performance Scale subtests, the USH group displayed a more heterogeneous profile. Specifically, some subtests within the Performance Scale scored lower that any of the others, as illustrated in **Fig. 2**.

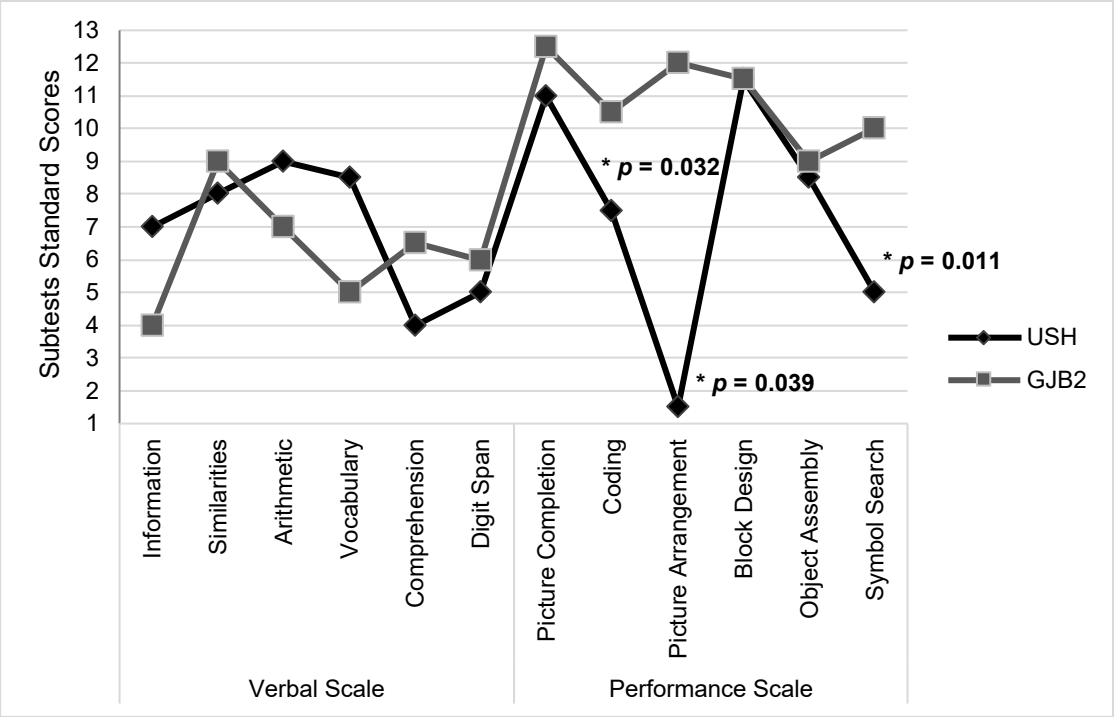

FIG. 2 WISC-III SUBTESTS STANDARD SCORES PROFILE FOR USH AND GJB2 GROUPS.
Comparisons signaled with * represent statistically significant p values (Mann–Whitney *U*, $p < 0.05$).

3.4. Molecular and clinical characterization of the USH group

Molecular and clinical characterization included all children and adolescents diagnosed with USH or with high suspicion of this diagnosis, as clinically and genetically determined by the specialized neurodevelopmental and genetics multidisciplinary team of CDC HP-CHUC. This also included one subject who was previously excluded from the analysis due to severe intellectual disability**.** A comprehensive molecular characterization and a summary of the clinical findings reported until the last medical visit can be found in **Table 4.**

The cases examined in this study were all diagnosed as USH type 1. This may be attributed to the fact that this type of the disease typically presents with earlier ophthalmological findings, making it easier to detect during childhood compared to USH type 2 or type 3. Of the ten cases analyzed, five (50.0%) presented homozygous variants and five (50.0%) had composed heterozygous variants. *MYOA7* was the most frequently affected gene, accounting for six cases (60.0%), followed by *CDH23* and *PCHD15* with two cases (20.0%) each. The variant *MYOA7* (NM_000260.4): c.999T>G (p.Tyr333*) was the most frequently observed, identified

TABLE 4 MOLECULAR AND CLINICAL CHARACTERIZATION OF THE USH GROUP

ID	Last Medical Visit Age (years)	USH type	Type of Variation	Gene	Variant	Variant Classification [a]	Reference	Associated Phenotypes	General Clinical Features					
									SNHL	RP	VD	AH	MD	Other
1	16	USH1B	HMZ	*MYO7A*	c.999T>G (p.Tyr333*)	Pathogenic	Weston *et al.* [27]	USH1B; DFNB2	+	+	+	+	+	OCD; motor stereotypies; restricted interests
2	7	USH1B	HMZ	*MYO7A*	c.999T>G (p.Tyr333*)	Pathogenic	Weston *et al.* [27]	USH1B; DFNB2	+	+	-	+	+	
3	4	USH1B*	CHET	*MYO7A*	c.3508G>A (p.Glu1170Lys)	Likely Pathogenic	Cuevas *et al.* [28]	USH1B; DFNB2; DFNA11	+	-	-	+	+	Motor stereotypies
					c.4489G>C (p.Gly1497Arg)	Likely Pathogenic	Bonnet *et al.* [29]	USH1B; DFNB2						
4	2	USH1F	HMZ	*PCDH15*	c.3661C>T (p.Gln1221*)	Likely Pathogenic	Santana *et al.* [30]	USH1F	+	-	-	+	+	
5	15	USH1B	CHET	*MYO7A*	c.999T>G (p.Tyr333*)	Pathogenic	Weston *et al.* [27]	USH1B; DFNB2	+	+	+	+	+	Severe intellectual disability of unknown cause; solitary kidney with hydronephrosis
					c.3508G>A (p. Glu1170Lys)	Likely Pathogenic	Cuevas *et al.* [28]	USH1B; DFNB2; DFNA11						

(Continue on next page)

ID	Last Medical Visit Age (years)	USH type	Type of Variation	Gene	Variant	Variant Classification [a]	Reference	Associated Phenotypes	General Clinical Features					
									SNHL	RP	VD	AH	MD	Other
6	18	USH1F	CHET	*PCDH15*	c.401G>A (p.Arg134Gln)	Likely Pathogenic	Aller *et al.* [31]	USH1F; DFNB23	+	+	+	+	+	Mild intellectual disability
					c.3661C>T (p.Gln1221*)	Likely Pathogenic	Santana *et al.* [30]	USH1F						
7	11	USH1B	CHET	*MYO7A*	c.3764delA (p.Lys1255Argfs*8)	Pathogenic	Weston *et al.* [27]	USH1B; DFNB2; DFNA11	+	+	+	-	-	Depression
					c.6070C>T (p.Arg2024*)	Pathogenic	Weston *et al.* [27]	USH1B; DFNB2; DFNA11						
8	17	USH1	*No detailed information available.*						+	+	-	+	+	Motor stereotypies
9	18	USH1D	CHET	*CDH23*	c.6319C>T (p.Arg2107*)	Pathogenic	Bork *et al.* [32]	USH1D	+	+	+	-	-	ADHD
					c.6049+1G>A	Pathogenic	Wayne *et al.* [33]	USH1D; DFNB						
10	11	USH1D	HMZ	*CDH23*	c.753+2T>A	Likely Pathogenic	Novel		+	+	+	+	+	
11	17	USH1B	HMZ	*MYO7A*	c.4489G>C (p.Gly1497Arg)	Likely Pathogenic	Bonnet *et al.* [29]	USH1B; DFNB2	+	+	+	+	+	Mild intellectual disability

USH, Usher Syndrome; HMZ, Homozygous; CHET, Compound Heterozygous; DFNB2, Deafness Autosomal Recessive 2; DFNB23, Deafness Autosomal Recessive 23; DFNA11, Deafness, Autosomal Dominant 11; SNHL, Sensorineural Hearing Loss; RP, Retinitis Pigmentosa; VD, Vestibular Dysfunction; AH, Axial Hypotonia as neonate; MD, Motor Delay; OCD, Obsessive-compulsive Disorder; ADHD, Attention-deficit / hyperactivity disorder.

Reference sequences: *MYO7A* (NM_000260.4); *PCDH15* (NM_001384140.1); *CDH23* (NM_022124.6).
[a] American College of Medical Genetics and Genomics (ACMG) Classification [34]

* Long-term follow-up is needed to address new clinical findings, particularly the presence of RP, which will be crucial for distinguish USH from DFNB2.

Note In the “Reference” column only the first report of the variant was mentioned.

in five pathogenic alleles from three different children. Details about the molecular study for Case 8 were not available as it was conducted at another hospital.

It is noteworthy that Case 3 did not received a definitive diagnosis of USH, as there are not yet retinal abnormalities and the variants c.3508G>A (p.Glu1170Lys) and c.4489G>C (p.Gly1497Arg) in *MYOA7* (NM_000260.4) could also be associated with DFNB2. Long-term follow-up and clinical findings will be crucial in distinguish between these two conditions, particularly the presence of RP in USH, which may appear late in life but is absent in DFNB2. Case 11 was a similar case, with USH diagnosis being confirmed only at the age of 17 when ophthalmologic findings became apparent. Additionally, in Case 4, the absence of RP could likely be attributed to the subject's age, as it was prior to the typical onset of this symptom. The previous identification of variant *PCDH15* (NM_001384140.1): c.3661C>T (p.Gln1221*) in patients with USH type 1F suggests a high likelihood that this is the definitive diagnosis. In Case 10, the variant c.753+2T>A in intron 7 of *CDH23* (NM_022124.6) has not been previously described in the literature. As it leads to altered splicing with potential formation of aberrant splicing products, it is most likely pathogenic and allows molecular confirmation of USH1 type D, which is further supported by the clinical features.

In Case 5, despite undergoing whole exome sequencing and array-CGH studies, the results of these investigations do not provide a clear explanation for the intellectual disability observed.

4. Discussion

USH is a clinically and genetically heterogenous disease making its diagnosis very challenging. Hearing loss appears in an early stage, prior to ophthalmological symptoms and is usually misdiagnosed as non-syndromic deafness. There are certain early indicators, however, that could help highlight the need for a more meticulous investigation. One such early marker, which has been widely reported and confirmed in this study, is the presence of motor delay in children with USH. Most of the children in our USH group (9 out of 11) exhibited axial hypotonia as neonates, which could serve as an important indicator of possible delayed motor development.

Our findings regarding the age of acquisition of early developmental milestones are also consistent with the literature with USH children showing delayed age of sitting without support (> 9 months) and delay in age of walking (> 18 months). [15,35,36] In the non-syndromic deafness group, no delay seems to occur. Additionally, both GJB2 and USH children show a delay in their age of first words, which is typically over 12 months. The onset of first words is even later in the USH group, although no evidence in literature reports this finding.

When considering the GMDS scores, children with USH and GJB2 have a similar cognitive profile, except for in the “Practical Reasoning” subscale where those with USH tend to score lower. This is expected because the GMDS administration is typically conducted at a younger age before ophthalmologic involvement is expected to appear. However, the “Practical Reasoning” subscale cannot be administered to children aged bellow 24 months. This means that those who are able take this subscale are older and thus may have a more advanced progression of the disease. The “Practical Reasoning” subscale evaluates the child’s ability to apply their acquired knowledge from the environment to solve real-world challenges, organize sequences, grasp mathematical concepts, and solve moral issues. [24,25] Children who are visually impaired may encounter difficulties in ordering events, making accurate inferences about case and effect, and conceptualizing ideas. Based on limited visual input, children with low vision must mentally process fragmented visual information, making it harder for them to perceive and learn from their environment. [37,38] Consistently, a study in the Portuguese population had shown that low vision children tend to score lower on this subscale. [38]

In WISC-III assessment, as expected, both groups scored higher in PIQ and lower in VIQ. This is a well-documented finding in deaf children, with the Performance Scale being widely used to measure intelligence and distinguish between the effects of deafness and cognitive delays. [39] The low scores of USH children on certain PIQ subtests may account for the smaller difference between PIQ and VIQ in this group. Specifically, USH children scored significantly lower in “Coding”, “Picture Arrangement” and “Symbol Search”. These subtests are heavily reliant on visual perception, visuo-spatial memory, and visual discrimination. However, performance on “Picture Completion”, “Block Design” and “Object Assembly” – that also rely on visual perception of stimuli – appears to remain unaffected. This could be explained by the fact that “Coding” and “Symbol Search” are also measures of processing speed, that could be easily affected by visual impairments. On the other hand, low scores in “Picture Arrangement” can be justified by difficulties on social adjustment, which can be compromised in these children. To validate this finding, it must be concordant with the score registered in the “Comprehension” subtest, which in this case is lower than the GJB2 group as well. [26,40]

The clinical and molecular characterization of our USH sample highlighted the challenges in confirming a genetic diagnosis. USH group exhibit heterogenous clinical features, including mental and behavioral disorders, which are prevalent within these children. [41] Variants in the USH causative genes are also associated with non-syndromic hearing loss, which is consistent with previous studies in the literature. [42–44] This empathizes the need for early and regular ophthalmologic examinations for children with congenital SNHL. Since routine ophthalmology may not always detect ocular signs in young children, electroretinography is recommended. However, this test may not always be available and often RP still non-detectable at

presentation. [45] These factors underscore the need for vigilance of other early signs of the disease to prompt target investigation from the outset.

Given the limited number of participants in this study, more research is needed to validate and replicate the findings. To improve the understanding of the neurodevelopment and cognitive profile of USH, larger population studies and multicenter collaborative efforts are necessary. Nonetheless, our study included a significant number of USH and GBJ2 children from the surrounding area of a tertiary hospital. As a reference center for cochlear implants, CDC HP-CHUC receives patients from across the country making it a small but representative sample. Moreover, the USH subjects have undergone thorough molecular characterization, rendering them a reliable sample for the disease. Another limitation of our study could be the fact that the neuropsychological tests used may not be the most accurate measures for assessing children with deafblindness, potentially leading to missing values that are more likely related to sensory rather than cognitive difficulties. Adequate assessment tools for this population are necessary, but the low incidence makes it challenging to obtain large sample sizes for the development of normative data. Therefore, clinicians and researchers should be aware of the limitations of norm-referenced tests when assessing the cognitive abilities of a deafblind child. [46] Additionally, three children did not have cochlear implants, which could potentially confound their test results. However, their individual test scores did not fall below the group's median values on most subtests. Nonetheless, further research is needed to determine if the absence of cochlear implants had any impact on their scores.

Despite the limitations, we consider our findings valuable and useful for inspiring future studies and raising awareness about the distinctive features of USH in its early stages. For instance, future studies could explore the phenotype-genotype correlation and the possibility of using a deaf child's cognitive profile to predict their likelihood of developing RP and be classified as USH. It would also be interesting to address the hypothesis raised by Jacobson et al. [47], that *MYO7A* alleles with stop mutations within the motor domain coding region [e.g., c.999T>C p.(Tyr333*)], may cause milder visual dysfunction than missense variants.

Identifying the distinctive characteristics of USH will be critical for timely implementation of strategies like cochlear implants that can enhance communication skills before the onset of visual loss. This is essential for maintaining social interaction and to minimize isolation, psychological co-morbidities, and social exclusion. Furthermore, an early diagnosis offers the opportunity to participate in ongoing clinical trials, including gene replacement therapy targeting the retina, cochlea, and vestibular system.

5. Conclusion

Confirming the USH diagnosis can be challenging due to the significant clinical and genetical variability, which can sometimes result in misidentification as non-syndromic hearing loss. Therefore, a USH diagnosis should be suspected in children with SNHL loss and a history of axial hypotonia as neonates or delayed motor milestones, regardless of the normal ophthalmology initial screen. Additionally, USH children may face difficulties with tasks involving processing speed and adequate social/environmental adjustment. Conducting a detailed evaluation with appropriate instruments will also be crucial to address cognitive difficulties caused by progressive hearing and vision loss. This will aid in the implementation of early and more tailored intervention and rehabilitation strategies that focus not only on vision and hearing but also on the psychosocial consequences of the disease.

Conflict of interest statement

The authors declare no conflict of interest.

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