



Visual Evoked Potentials in Joubert Syndrome: A Suggested Useful Method for Evaluating Future Approaches Targeted to Improve Visual Pathways' Function

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ABSTRACT

Introduction: Joubert syndrome (JS) is a recessive disorder characterized by a congenital malformation of the mid-hindbrain and a large spectrum of clinical features including optic nerve morphologic abnormalities. The function of the visual pathways, including the optic nerve, can be objectively evaluated by visual evoked potential (VEP) recordings. Our work aims to employ VEP to evaluate the neural

conduction along the visual pathways in JS patients with or without optic nerve morphologic abnormalities (ONMA).

Methods: In this observational and prospective study, 18 children with genetic diagnosis of JS (mean age 8.78 ± 5.87 years) and 17 healthy age-similar control subjects (control group, 9.05 ± 6.02 years) were enrolled. Based on presence/absence of ONMA at fundus examination, JS patients were divided into two groups: the JS-A group (eight patients with ONMA) and JS-N group (ten patients without ONMA). Following the ISCEV standards, pattern VEPs were recorded in patients and controls in response to 60' and 15' checks to obtain a prevalent activation of large or small axons, respectively.

Results: Compared to controls, both the JS-A and JS-N groups showed significant abnormalities in 60' and 15' VEP implicit time and amplitude. Only in the JS-N group were values of 15' VEP implicit significantly correlated with the corresponding values of visual acuity.

Conclusions: Our results suggest that a visual pathways dysfunction (of both large and small axons) detectable by VEP may occur in JS patients regardless of the presence of ONMA. Since clinical trials are envisaged in the near future to address JS-related ocular problems, our results might provide information about the potential usefulness of VEP recordings to assess the efficacy of treatments targeted to improve the visual pathways' function.

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Keywords: Joubert syndrome; Ophthalmology; Optic nerve; Visual evoked potentials; Visual pathways

Key Summary Points

Why carry out this study?

Joubert syndrome (JS) is an autosomal recessive disorder characterized by congenital malformation of the mid-hindbrain and a large spectrum of clinical features including optic nerve morphologic abnormalities (ONMA).

The function of all of the visual pathways, including the optic nerve, can be objectively evaluated by visual evoked potential (VEP) recordings, and, by using appropriate characteristics of visual stimuli (60' and 15' checks), it is possible to obtain an objective evaluation of the neural conduction along “large” and “small” axons.

This observational and prospective study was carried out to evaluate the functional condition of different components (large and small axons) of visual pathways in JS patients with (JS-A) or without (JS-N) ONMA. Our results should provide information about the possible usefulness of VEP recordings in the efficacy assessment of treatments targeted to improve the visual pathway condition.

What was learned from the study?

Compared to controls, in both the JS-A and JS-N groups, significant abnormalities of the values of 60' and 15' VEP implicit time and amplitude were found. Only in the JS-N group were the values of 15' VEP implicit time significantly correlated with the corresponding values of visual acuity.

In JS patients with or without ONMA, a visual pathway dysfunction (in both large and small axons) occurs and is detectable by VEP recordings. VEP methods should be proposed to evaluate the effectiveness of adequate treatment aimed to improve the visual pathway impairment of JS patients.

DIGITAL FEATURES

This article is published with digital features, including a summary slide, to facilitate understanding of the article. To view digital features for this article go to <https://doi.org/10.6084/m9.figshare.13084130>.

INTRODUCTION

In 1969, Dr. Marie Joubert first described four siblings presenting with cognitive impairment, ataxia, episodic tachypnea, eye movement abnormalities and cerebellar vermis agenesis. Subsequently, several patients with Joubert syndrome (JS) were described, presenting a peculiar congenital malformation of the mid-hindbrain associated with neurologic signs and variable multiorgan involvement [1]. The diagnosis of JS is currently based on the presence of the typical “molar tooth sign” on brain imaging, characterized by cerebellar vermian hypoplasia, thickened and horizontalized superior cerebellar peduncles and a deepened interpeduncular fossa [2]. JS can occur as an isolated neurologic disorder or be associated with variable involvement of the retina and optic nerve, kidneys, liver and skeleton [2, 3]. The prevalence of JS has been estimated at approximately 1:100,000 in the US [3] and 0.47:100,000 in Italy [4].

Over 40 genes are known to cause JS with recessive inheritance [5, 6], all encoding for proteins of the primary cilium, a subcellular organelle found on the surface of most cellular types, making JS part of the expanding spectrum of “ciliopathies” [7].

An involvement of the visual system is described in about 30% of JS patients [8], including congenital retinal dystrophy [9–11],

jerky eye movements, nystagmus and strabismus [1], oculomotor apraxia [12, 13], ptosis and extraocular muscle limitation [14]. Morphologic changes of the optic nerve, such as pallor or coloboma [15, 16], can also be detected, albeit more rarely.

Interestingly, a single study on JS [17] suggested mitochondrial dysfunction similar to that seen in Leber's hereditary optic neuropathy (LHON), a distinct neurodegenerative optic neuropathy in which mitochondrial failure is responsible for optic nerve impairment with consequent severe visual loss [18]. In LHON, idebenone administration was found to improve psychophysical measurements, such as the visual acuity, visual field and color vision [19, 20]. However, these subjective tests do not allow an objective evaluation of the effects of this treatment on optic nerve function.

The function of the visual pathways, including the optic nerve, can be objectively evaluated by visual evoked potential (VEP) recordings, well standardized by ISCEV protocols [21].

By using appropriate characteristics of visual stimuli, it is possible to obtain an objective evaluation of the neural conduction along "large" and "small" axons forming visual pathways [22, 23]. For instance, in LHON, an optic nerve dysfunction, prevalent in the component of the small axons, but also involving the large axons, was detected by using VEP recordings with appropriate visual stimuli [24, 25].

In rare diseases such as JS, the monitoring of visual pathway function over time and/or after specific treatments is a debated topic, because it is not easy to find appropriate tests that can evaluate both visual function and visual pathway function at the same time. Indeed, despite being a sensitive and objective tool to measure visual pathway function, VEPs are generally neglected.

The aim of this study was to evaluate the functional condition of different components (large and small axons) of the visual pathways in JS patients with or without morphologic optic nerve involvement. In addition, since clinical trials aimed at restoring visual function in JS patients are envisaged in the near future, our results might suggest new breakthroughs

related to the possible usefulness of VEP recordings to evaluate the efficacy of treatments targeted to improve the visual pathways condition.

METHODS

Patients

In this observational and prospective study, 18 Italian children (mean age 8.78 ± 5.87 years) with a neuroradiologically proven "molar tooth sign" were selected (see inclusion criteria below) from a larger cohort of JS patients recruited within the frame of a larger clinical-genetic project on cerebellar and brainstem congenital defects (CBCD).

Seventeen healthy age- and gender-matched (mean age 9.05 ± 6.02 years) control subjects were also enrolled. JS patients and healthy controls were enrolled at the Department of Child Neurology and Child Psychiatry IRCCS C. Mondino, Pavia, and submitted to complete ophthalmologic evaluation (see below) at the Section of Ophthalmology, University of Pavia, IRCCS Fondazione Policlinico San Matteo, Pavia. All subjects underwent a complete diagnostic work-up including a detailed assessment of kidney, liver and heart function.

All patients receiving a neuroradiologic diagnosis of JS underwent an NGS-based molecular analysis of a large panel of ciliary genes, including known JS-related genes [5, 6] and genes responsible for other primary ciliopathies (e.g., skeletal ciliopathies, renal ciliopathies, Bardet-Biedl syndrome, Meckel syndrome, etc.) as well as candidate genes that emerged from whole-exome sequencing studies. Identified pathogenic variants were confirmed by Sanger sequencing, and segregation with the disease in the family was assessed by sequencing family members, when available. Genes responsible for LHON or other non-ciliopathy retinal dystrophies were not included in the panel, as this was mainly aimed at testing patients with Joubert syndrome and other primary ciliopathies.

JS patients and controls had a complete ophthalmologic examination including best

corrected visual acuity (BCVA) assessments. Anterior segment evaluation with a slit lamp was performed depending on the patients' cooperation. Cycloplegic refraction (cyclopentolate 1%) and dilated fundus examination with indirect ophthalmoscopy were performed for each patient.

Since VEP recordings were performed by using a binocular stimulation (see below, VEP assessment), patients were divided in two groups based on the presence (JS-A, $n = 8$) or absence (JS-N, $n = 10$) of binocular characteristics of the optic nerve morphologic involvement (i.e., coloboma, pale optic disc head, optic disc head cupping). Since a retinal dysfunctional condition may influence VEP responses [26, 27], the main inclusion criterion was the absence of retinal dystrophies at fundus examination. For JS patients and controls, exclusion criteria were optic media opacities, previous history of optic neuropathy or glaucoma, refractive error $< \pm 3$ equivalent spherical diopters, and concomitant general (i.e., diabetes) or neurologic diseases.

Demographic and genetic data, visual acuity and type of morphologic optic nerve involvement in the control, JS-A and JS-N groups are reported in Table 1.

The research followed the tenets of the Declaration of Helsinki, and the study was approved by local Institutional Review Board (Scientific Committee of Section of Ophthalmology, University of Pavia, IRCCS Fondazione Policlinico San Matteo, Pavia, Italy). Informed consent was obtained from the parents of each child.

Visual Acuity Evaluation

In verbal children, BCVA was assessed by the modified Early Treatment Diabetic Retinopathy Study (ETDRS) Charts (Lighthouse, Low Vision Products, Long Island City, NY, USA) and expressed in logMAR values obtained at the distance of 4, 2, 1 and 0.5 m. In preverbal and nonverbal children, BCVA was measured with Teller acuity cards (TACs), and the values were converted to logMAR.

VEP Assessment

VEP recordings were performed according to ISCEV standard [21] protocols.

Briefly, subjects were seated and adapted to room light in a semi-dark, acoustically isolated room for 10 min in front of the display and surrounded by a uniform field of luminance of five candelas per m^2 . Pupil diameter was approximately 5 mm. No mydriatic or miotic drugs were used. Visual stimuli were checkerboard patterns (contrast, 80%; mean luminance, $110 \text{ cd}/m^2$) generated on a TV monitor and reversed in contrast at the rate of two reversals per second. At the viewing distance of 114 cm, the check edges subtended 60 min (60') and 15 min (15') of the visual angle. As suggested by the ISCEV standards, VEPs were recorded in response to 60' (60' VEP) and 15' (15' VEP) checks to obtain a prevalent activation of large (with 60' checks) or small (with 15' checks) axons [22, 25]. The monitor screen subtended 23° . A small fixation target, subtending a visual angle of approximately 0.5° (estimated after considering spectacle-corrected individual refractive errors), was placed at the center of the pattern stimulus. For every VEP acquisition, each patient positively reported that he/she could clearly perceive the fixation target.

In JS patients and controls, VEPs were recorded by using a binocular stimulation. About this, the ISCEV standards recommend a monocular stimulation for separating the bio-electrical signals of each eye, but it also reports the following: "Monocular stimulation is standard. This may not be practical in infants or other special populations; in such cases binocular stimulation may be used to assess visual pathway function from both eyes" [21]. Since our JS patients can be considered a "special population," we believe that the binocular stimulation was appropriate with respect to the aim of the study. The monocular stimulation is preferred in patients with different optic nerve conditions between the eyes (i.e., absence of nerve abnormalities in the right eye and coloboma in left eye); in fact, in this case, if a binocular stimulation is performed, the VEP responses are highly related to the normal eye

Table 1 (A) Demographic, identified genetic variant, visual acuity and (B) type of morphologic optic nerve involvement (with relative identified genetic variant) observed in control subjects and in patients with Joubert syndrome with (JS-A group) or without (JS-N group) optic nerve morphologic abnormalities

A	Controls	JS-A group	JS-N group
Number	17	8	10
Age (years, mean \pm 1 SD)	9.05 \pm 6.02	8.79 \pm 7.38	9.00 \pm 4.76
Sex (male/female)	10/7	5/3	4/6
Mutated gene			
C5orf42	0	1	3
CC2D2A	0	0	3
KIF7	0	1	0
INPP5E	0	1	0
TMEM67	0	2	0
AHI1	0	2	2
RPGRIP1L	0	1	1
NPHP1	0	0	1
Visual acuity (logMAR, mean \pm 1 SD)	0.005 \pm 0.01	0.63 \pm 0.18	0.54 \pm 0.21
B	Controls	JS-A group	JS-N group
		Mutated gene	
Optic nerve morphologic abnormalities			
Binocular coloboma	0	3	TMEM67, RPGRIP1L
Binocular pale optic disc head	0	4	KIF7, INPP5E, C5orf42, KIF7
Binocular optic disc head cupping	0	1	AHI1
Binocular absence of abnormalities	17	0	0

with a negligible contribution of the abnormal eye. To contrast this source of bias, we enrolled selected JS patients based on the presence of the same optic nerve morphologic abnormalities in both eyes or with binocular absence of optic nerve morphologic abnormalities (see inclusion criteria and Table 1). The transient VEP response is characterized by several waves with three subsequent peaks of negative, positive and negative polarity, respectively. In visually normal subjects, these peaks have the following implicit times: 75, 100 and 145 ms (N75, P100 and N145). VEP P100 implicit time (IT) and

N75-P100 peak-to-peak amplitude (A) were measured, in milliseconds (ms) and microvolts (μ V), respectively, directly on the displayed records by means of a pair of cursors (see Fig. 1).

During a recording session, VEPs were recorded at least twice (between 2 to 5 times), and the resulting waveforms were superimposed to check the consistency of results. Based on previous studies [24, 25], we know that intra-individual variability (evaluated by test-retest) is approximately \pm 2 ms for VEP P100 IT and approximately \pm 0.18 μ V for VEP N75-P100 A. During the recording session we considered two

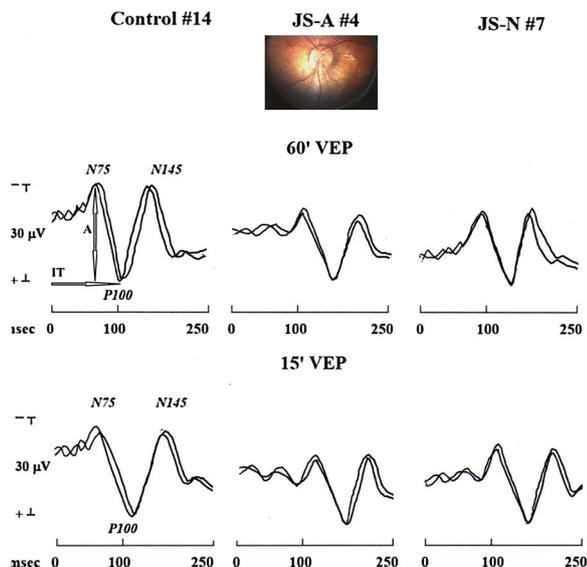


Fig. 1 Examples of visual evoked potential (VEP) responses assessed in one control subject (control #14), in one patient with Joubert syndrome with optic nerve morphologic abnormalities (JS-A #4) and in one patient with Joubert syndrome without optic nerve morphologic abnormalities (JS-N #7); 60' and 15' refer to visual stimuli in which each check subtended 60 and 15 min (') of the visual arc, respectively. With respect to the control eye, both JS-N and JS-N patients showed VEP responses with delayed P100 implicit times (IT, →) and with a reduction in N75-P100 peak-to-peak amplitude (A, ↑). Relative to JS-A #4, the fundus camera image of the optic nerve morphologic abnormality (coloboma) is also presented

successive waveforms “superimposable,” and therefore repeatable, with a difference in ms (for VEP P100 IT) and in μV (for VEP N75-P100 A) less than the above-reported values of intra-individual variability. At times, the first two recordings were sufficient to obtain repeatable waveforms, while other times, further recordings were required (albeit never more than 5 in the cohort of JS patients). For statistical analyses (see below), we considered the VEP P00 IT and N75-P100 A values measured in the recording with the shorter VEP P100 IT.

Statistical Analysis

The Anderson-Darling and Kolmogorov-Smirnov tests were applied to verify that data were normally distributed.

Differences in values of BCVA and VEP parameters (P100 IT and N75-P100 A) between the control and JS groups were evaluated by the one-way analysis of variance (ANOVA). Pearson's test was used to assess the relationship between the values of BCVA and those of VEP parameters. All statistical analyses were performed using SPSS V.26 (Statistical Package for Social Science IBM), and $p < 0.01$ was considered as statistically significant.

RESULTS

Visual Acuity Data

On average, a significant ($p < 0.01$) reduction in BCVA values was observed when data from both the JS-A and JS-N groups were compared to those of controls.

VEP Data

Figure 1 reports examples of VEP responses assessed in one control subject (C #14), in one representative patient with JS and ONMA (JS-A #4) and in one representative patient with JS without ONMA (JS-N #7).

Table 2 presents the mean data of VEP P100 ITs and N75-P100 As (in response to visual stimuli in which each check subtended 60' and 15' of the visual arc, respectively) detected in controls, JS, JS-A and JS-N groups and relative statistical analyses among groups.

The VEP results are reported separately as follows:

60' VEP

In the JS group, P100 ITs and N75-P100 As values were significantly ($p < 0.01$) increased and reduced, respectively, compared to controls.

Considering individual JS-A patients, the P100 ITs and 75-P100 As were not significantly ($p > 0.01$) linearly correlated with the corresponding values of BCVA.

On average, compared to controls, in the JS-A group a significant ($p < 0.01$) increase in P100 ITs values and a significant ($p < 0.01$) reduction in N75-P100 As values were found.

Table 2 Mean values and standard deviation of visual evoked potential (VEP) parameters detected in control subjects (C), patients with Joubert syndrome with (JS-A) or without (JS-N) ophthalmoscopic signs of optic nerve morphologic abnormalities

	VEP 60'		VEP 15'	
	P100 IT (ms)	N75-P100 A (μ V)	P100 IT (ms)	N75-P100 A (μ V)
Controls ($N = 17$)				
Mean	98.12	31.82	101.65	30.47
SD	4.23	10.19	4.89	8.77
JS ($N = 18$)				
Mean	108.39	17.40	114.06	12.65
SD	11.74	12.64	12.25	7.60
ANOVA vs. C				
$f(1.34)$	45.08	13.70	15.15	41.41
$p =$	< 0.001	< 0.001	< 0.001	< 0.001
JS-A ($N = 8$)				
Mean	111.00	13.23	111.50	10.31
SD	12.31	9.21	9.24	7.16
ANOVA vs. C				
$f(1.24)$	15.41	19.27	12.38	31.99
$p =$	< 0.001	< 0.001	0.002	< 0.001
JS-N ($N = 10$)				
Mean	106.30	20.74	116.10	14.52
SD	10.48	9.42	14.38	7.76
ANOVA vs. C				
$f(1.26)$	8.26	7.86	14.65	22.59
$p =$	0.008	0.010	< 0.001	< 0.001
ANOVA vs. JS-A				
$f(1.17)$	0.77	2.88	0.61	1.40
$p =$	0.344	0.109	0.445	0.254

SD 1 standard deviation, 60' and 15' visual stimuli in which each check subtended 60 and 15 min of the visual arc, respectively, IT implicit time, ms milliseconds, A amplitude, μ V microvolt, N number of subjects or patients, ANOVA one-way analysis of variance between groups

On average, in the JS-N group a significant ($p < 0.01$) increase in P100 ITs values and a significant ($p < 0.01$) reduction in N75-P100 A values were observed with respect to controls.

No significant ($p > 0.01$) differences in mean values of P00 Its and N75-P100 As with respect to those of the JS-N group were found.

In the JS-N group, no significant ($p > 0.01$) linear correlations between the individual values of P00 ITs and N75-P100 As and the corresponding values of BCVA were observed.

15' VEP

In the JS group, the mean values of P100 ITs and N75-P100 As were significantly ($p < 0.01$) increased and reduced, respectively, compared to those of the control group.

Considering JS-A patients, no significant ($p > 0.01$) linear correlation between individual P100 ITs and 75-P100 As values with the corresponding BCVA values were found.

On average, in the JS-A group, a significant ($p < 0.01$) increase in P100 ITs values and a significant ($p < 0.01$) reduction in N75-P100 As values were detected with respect to controls.

On average, with respect to the control group, in the JS-N group a significant ($p < 0.01$) increase in P100 ITs values and a significant ($p < 0.01$) reduction in N75-P100 As values were detected. No significant ($p > 0.01$) differences in the mean values of all VEP parameters with respect to those of JS-N were found.

In the JS-N group, a significant ($r = 0.0827$, $p = 0.003$) linear correlation between the individual values of P00 ITs and corresponding values of BCVA was found. No significant correlation between the individual values of N75-P100 A and corresponding values of BCVA was observed.

In the JS group or JS-A and JS-N groups, considering each VEP parameter (P100 IT and N75-P100 A), no significant ($p > 0.01$) differences were observed between the values obtained with 60' checks with respect to 15' checks.

DISCUSSION

The aim of our work was to evaluate the neural conduction along the visual pathways (by VEP recordings) in JS patients with or without optic nerve morphologic abnormalities, with the aim of evaluating this technique as a potential valid and objective method to evaluate the effectiveness of future treatments.

In our study, both JS groups showed VEP P100 ITs significantly delayed and N75-P100 reduced compared to controls, in keeping with the few published studies demonstrating abnormal VEP responses in JS [28–30].

It is very interesting that in both the JS-A and JS-N groups, considering each VEP parameter (VEP 100 Its and N75-P100 As), there were no significant differences between the bioelectrical responses obtained with 60' of visual stimulation and 15' checks ones. This led us to believe that in JS patients with or without optic nerve morphologic abnormalities, there is a concomitant dysfunction of small and large axons [22, 25].

To explain our VEP findings detected in JS patients, several hypotheses must be considered.

First, there could be a relationship between pathogenic variants in specific JS-related genes and VEP abnormalities. In previous studies in JS, attempts at genotype-phenotype correlations have not been based on VEP recordings [27–29], but only on morphologic findings [30, 31]. We acknowledge that the patients' cohort ($n = 18$) is too small to draw any meaningful correlations.

A second hypothesis to explain the observed VEP findings is an underlying mitochondrial dysfunction possibly causing optic nerve impairment and consequent visual loss, similar to that seen in LHON [18]. Indeed, this hypothesis has been proposed once about a JS patient [17]. In LHON, several studies reporting VEP abnormalities [24, 25, 32–35] suggest that a prevalent impairment of small axons (forming the macular-papillo bundle of the optic nerve) may happen. However, different from LHON, in our JS patients we did not detect significant differences between the impaired neural conduction along large axons (delay in 60' VEP P100IT) and along small axons (delay in 15' VEP P100IT). This indicates that in JS both large and small axons can be affected, the latter correlating with a reduction of visual acuity exclusively in patients without optic nerve morphologic abnormalities (JS-N group).

A third possibility is demyelination or structural abnormalities of the visual pathways. Maria et al. [36], reporting neuroradiologic data

of a cohort of 46 JS patients, suggested an involvement of the myelination process in 33% of cases. This process is crucial for normal neural conduction along visual pathways, and it is well known that in demyelinating neurodegenerative diseases (i.e., multiple sclerosis), there is a delay in neural conduction along the visual pathways detectable by abnormal VEP responses (see Carcelén-Gadea et al. for a review [37]). Thus, it is conceivable that also in JS patients there could be an abnormal myelination of nerve fibers forming the visual pathways, leading to the observed VEP abnormalities.

Regarding the possible selective morphologic abnormalities of visual pathways, postmortem neuropathologic and MRI studies have reported several malformation of multiple brainstem structures, but not of the optic pathways (see Yachnis and Rorke for a review [38]).

Finally, when considering VEP abnormalities in our JS cohort (which reflect a dysfunction of the whole visual pathways, from photoreceptors to the visual cortex [21, 27]), a contribution of possible retinal impairment must be taken in consideration. Indeed, we recently reported retinal dysfunction in JS patients with or without retinal dystrophies, which could be detectable by abnormal electroretinographic responses [39]. Thus, although we enrolled JS patients without retinal dystrophy at fundus examination (see inclusion criteria), a possible contribution of an underlying retinal dysfunction cannot be entirely excluded. Nevertheless, the JS patients enrolled in this study were distinct from those presenting electroretinographic abnormalities [39], and therefore this study does not provide information about a possible relationship between VEP abnormalities and potential retinal dysfunction in the absence of retinal dystrophies at fundus examination.

VEP findings detected in the present cohort of JS patients were overall similar to those observed in patients with other diseases inducing a visual pathway dysfunction (see Creel for a review [27]). Recently, to improve the visual pathway function, several clinical trials have been started in patients with different types of optic neuropathies (LHON, ischemic optic

neuropathy, glaucoma) by using treatments with idebenone [19, 20], citicoline [40–43], coenzyme Q10 [44] or nerve growth factor [45]. In many of these studies [40–45], the efficacy of the supplementation treatment was evaluated by measuring the changes of the VEP responses, and it can be foreseen that VEP can be adopted as a useful methodology to evaluate the outcome of future trials aimed at improving visual function in JS patients. A limitation of this study was the small number of JS patients tested ($n = 18$), which did not allow assessing potential meaningful relationships between VEP findings and variants in specific genes.

CONCLUSION

In conclusion, our results suggest that in JS patients (with or without optic nerve morphologic abnormalities) there is an impaired neural conduction along the visual pathways, involving both large and small axons. This impairment is detectable by VEP recordings, and this method, associated with psychophysical visual function measures (visual acuity and visual field), may be proposed as a valuable tool for evaluating the effectiveness of adequate treatment targeted to improve the visual pathway function in JS patients.

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Compliance with Ethics Guidelines. All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the local Institutional Review Board (Scientific Committee of Section of Ophthalmology, University of Pavia, IRCCS Fondazione Policlinico San Matteo, Pavia, Italy). Informed consent was obtained from all individual participants included in the study.

Data Availability. All authors had full access to all of the data in this study and take complete responsibility for the integrity of the data and accuracy of the data analysis. The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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