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Is the prolongation latency of visual evoked potentials a pathological sign in children with Down's syndrome without ocular abnormalities? Case–control study of children with Down's syndrome

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ABSTRACT

Objective To evaluate retino-cortical function in children with Down's syndrome (DS) and no evident ocular abnormalities beyond mild refractive error, by recording visual evoked potentials (VEP) in response to pattern-reversal stimuli and comparing to those of age-matched healthy controls.

Methods and analysis All the children with DS registered at Split-Dalmatia County who met inclusion criteria of no ocular abnormalities and with refraction error between −0.5 and +2.0 D, and their age-matched healthy controls were included in the study (n=36 children, N=72 eyes, for both groups, respectively, with the same age of 9±2 years). Transient VEP was recorded and the waves with a positive peak as a response to a pattern-reversal stimulus, were analysed. The peak P100 latency, defined as the time from the stimulus onset to the main positive peak, and peak to peak amplitudes were measured.

Results While P100 wave amplitudes were comparable between two groups (p=0.804), P100 latencies were from 4.3 to 28.5 ms longer in children with DS (p<0.001). Interocular latency difference between a VEP dominant and an inferior eye was pronounced in healthy (1.2 ms (0.2–4.0), but was almost diminished in children with DS (0.3 ms (0.1–0.5), p<0.001).

Conclusion Our study has demonstrated that VEP response is divergent in children with DS compared with their age-matched healthy controls, indicating possible structural or functional abnormalities of the visual cortex. As VEP results are helpful in the diagnosis and treatment planning of vision-related disorders, we should reconsider the use of common VEP diagnostic criteria in subpopulation of children with DS.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Ocular abnormalities are more prevalent in children with Down's syndrome (DS) than in healthy population, putting them at particularly high risk of developing amblyopia. The visual evoked potentials (VEP) responses provide information on both ocular abnormalities and structural or functional integrity of the visual cortex. VEP response in people with DS was shown to be atypical with somewhat conflicting results on prolongation of P100 latency and amplitude with no clear distinction between individuals with or without ocular abnormalities/normal vision.

WHAT THIS STUDY ADDS

⇒ Our findings of the bilateral prolongation of peak P100 latencies and the reduction in interocular differences in peak P100 latencies accompanied with opposed patterns of its associations to age and refraction error demonstrate that even in population of children with DS and no ocular abnormalities/normal vision, VEP response is quite atypical. Moreover, in children with DS, unlike in healthy individuals, interocular difference in peak P100 latencies between a VEP inferior and a dominant eye is almost diminished.

HOW THE STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The clinical significance of our study is illustrated by the fact that 70% of the children with DS would have been misdiagnosed with pathological VEP, if the common criteria of VEP assessment had been applied. We propose that VEP diagnostic criteria for DS children should be reconsidered with special attention on latency duration criteria. Namely, P100 latency prolongation is least affected by technical factors and degree of patient cooperation and is considered the most reliable indicator of clinically significant abnormality.

INTRODUCTION

Down's syndrome (DS) is caused by trisomy 21, the most common genetic cause of developmental delay and intellectual disability. Ocular abnormalities such as refractive errors of the eye and strabismus,[1–4] as well as inaccurate accommodation,[5 6] are more prevalent in people with DS than in healthy population. These abnormalities affect visual function by blurring or distorting the retinal image. In children with DS such degradation of the retinal image occurs during a sensitive period

of visual development, making them at particularly high risk of developing amblyopia.[4] To avoid the onset of the disease and to ensure its early diagnosis and treatment, it is important that ophthalmologists monitor carefully visual development of these children by measuring spatial vision, such as acuity. The assessment by standard acuity tests depends on the cognitive ability of the patient and the experience of the examiner. Due to mental impairment in these children, visual acuity in most of them could not be accurately measured, resulting in some cases of undiagnosed amblyopia.[7]

Visual evoked potential (VEP) testing is an alternative method used to assess visual performance without relying on patient's response or observer accuracy. The VEP responses depend on functional integrity of central vision at all levels of visual pathway including eye, retina, optic nerve, optic radiations and the occipital cortex[8]; and thus, provide not only information on ocular abnormalities but also on structural or functional integrity of the visual cortex.[9] So far, several studies have examined transient pattern-reversal VEPs in people with DS. Kakigi *et al* studied adult patients with DS and noticed that their P100 response latency was longer, while the amplitude was lower than in age-matched healthy controls.[10] However, the authors did not find a significant difference in response latency between the patients without any ophthalmological abnormalities and the healthy controls, suggesting that ophthalmological abnormalities in the people with DS are one of the main factors affecting abnormal VEP response. Contrary, Suttle and Lloyd who studied a small sample of adult DS patients with no significant ocular abnormality showed that, compared with healthy controls, P100 component of their response was significantly delayed, and its amplitude was significantly lower,[11] thus demonstrating that an abnormal function of neural visual pathway in this population caused abnormal VEP response. The same group of authors compared VEP responses in children aged 4–15-years old with DS, who were free of strabismus but had some refraction error, with their age-matched healthy controls.[12] Although the authors observed delayed P100 response latencies and lower P100 amplitudes at various spatial frequencies of the stimulus, the proposed differences in P100 parameters were not significant, neither in the overall sample of patients nor in the subset of patients with low refractive error. Moreover, within the same study, these non-significant differences disappeared when children with DS were matched to healthy controls by both age and refractive status. Several other authors investigated VEP responses using different stimuli and/or recording conditions. Del Viva *et al* analysed steady state-pattern VEP responses in adult patients with mild retardation and concluded that VEP response of primary visual areas is preserved and comparable to age-matching controls,[13] whereas Fenton *et al* reported delayed latency of the flash P2 component in adults with DS, but also found normal P100 pattern VEP latencies.[14]

Although the above-mentioned findings suggest that the VEP response in people with DS is atypical, it is still not clear if and to what extent this atypicality reflects ocular or neural abnormalities. This is especially relevant for children for which VEP based diagnostic criteria are of considerable importance, but findings on atypical VEP response are scarce and ambiguous. Given that even high functioning children with DS, who are free from any ophthalmic anomaly beyond mild refractive error, also experience sensory visual impairment that cannot be fully corrected with corrective lenses,[15 16] structural and/or functional changes in the neural visual pathway of these patients seems likely phenomenon.[17]

The aim of this study was to evaluate retino-cortical function in children with DS who had no evident ocular abnormality beyond mild refractive error by recording electrophysiological activity of primary visual areas. For that purpose, we used standard VEP stimuli and recording setting performed by an experienced ophthalmologist who was in charge of long-term care for ocular health of the target population, and a representative sample of children with DS registered in the County, resulting in a large sample size of VEP recordings from this population and analysed P100 amplitudes, latencies, interocular differences in amplitude/latency and their relationship with age, refractive error, health status or sex.

METHODS AND ANALYSIS

All the children diagnosed with DS in the Split-Dalmatia County are referred by health professionals to the Croatian Association for Down Syndrome Split (CADSS), whose register is currently the most comprehensive resource for listing these patients. Parents of all the children from the CADSS's list, who were between 6 and 12 years of age with medically confirmed full trisomy 21 by cytogenetic testing, were contacted by phone (N=67). As children with DS are regularly checked by an ophthalmologist at the Department of Ophthalmology, University Hospital Center Split since 2016, contacted children were already familiar with the environment, procedure and an examiner, resulting in the response rate of 90%. We have also recruited age-matched healthy controls for systematic checkups, during the daily routine work with patients in the clinic (N=167).

Ocular status of participants was assessed at the Children Outpatient Clinic of the Department of Ophthalmology, University Hospital Center Split. Accommodation was estimated by dynamic retinoscopy and by measuring monocular and binocular distance and near vision, whereas refractive error was determined by retinoscopy. The pupils were isocoric and of normal size in all subjects. The retinoscopy was performed using the sphero-spherical method at a distance of 1 m, 60 min after local instillation of 1% tropicamide drops in order to achieve cycloplegia. Visual acuity was measured first binocularly, then monocularly at near (35 cm) and at the distance using (6 m) Pflüger charts and was expressed as a decimal value. Pflüger chart is similar to Tumbling E

chart. It works on the same principle as Snellen chart and could be used to estimate visual acuity in small children, younger than 4 or of illiterate. Optotypes on the tables are ordered from the largest, whose size corresponds to visual acuity of 6/60 (0.1) to the line with the smallest, which corresponds to visual acuity of 6/6 (1.0).

Final inclusion criteria were:

1. monocular visual acuity ≥0.8.
2. near vision equal or better than distance 1.
3. refraction between −0.50 D and +2.00 D in spherical equivalent.

Participants were excluded if they met any of the following criteria:

1. Strabismus.
2. Hypermetropies and myopes.
3. Astigmatism greater than physiological (any astigmatism higher than 1 D axis 90°, every oblique and astigmatism against the rule).
4. Nystagmus.
5. Congenital cataract.
6. Congenital glaucoma.
7. Keratoconus.
8. Anisometropia (difference between two eyes higher than 1.5 D).

Final number of participants was N=36, for both DS and healthy children with age of 9±2 years for both groups. The groups had good gender (50% and 44% of boys, respectively) and refraction error balance.

Flow diagram showing the recruitment processes is presented in online supplemental figure 1.

VEP procedure

Visually evoked potential (VEP) on monocular and binocular pattern stimulation were recorded in accordance with the International Society for Clinical Electrophysiology of Vision (ISCEV guidelines[18]). All refractive deficits were corrected with appropriate lenses during all the tests.

The recording of transient pattern-reversal VEPs was done on a Tomey EP-1000 device (TOMEY Am Weichselgarten Erlangen, Germany). VEPs were elicited using checkerboard pattern stimuli with large, 1° checks and a presentation rate of 2 reversals/s. The distance between the patient and the screen (stimulus field) was 50 cm. Testing of each eye was performed separately and independently; first, we tested the right and then the left eye.

The subjects' attention during the recording was monitored with a camera installed in the device, as well as by analysing the number of recorded errors. To obtain the most accurate results, we repeated each test at least three times. We also used standard recording conditions, as recommended by ISCEV. VEP was detected by placing silver skin electrodes on the cortical projection of the visual sphere. Electrode placement was in accordance with the international 10–20 system, with the active electrode placed at Oz position and the common reference electrode at Fz position. The ground electrode was positioned at the ear lobe. Electrodes were placed on the skin previously cleaned with the abrasive paste (Nuprep Skin Prep gel, Weaver and company, Aurora, Colorado, USA). The attachment of electrodes on the clean skin was done by filling the electrode disk with conductive paste (Ten 20 conduction, Weaver and company, Aurora, Colorado, USA). The average number of sweeps was 64 and at least two sweep series were performed. The values of the amplitude and latency of P100 wave, the repeatability of the waves and their morphology were recorded. The average with the smallest P100 latency and preferably the largest P100 amplitude was taken for analysis. VEP responses in all 72 participants were recorded successfully.

Data analysis

Quantitative data were described with mean and SD, or median and IQR, depending on the distribution of data. To describe qualitative data, we used absolute frequencies and percentages.

As we used the information on VEP responses from both eyes, repeated measures of the general linear models (RMGLM) were used to explore differences in amplitude or latency of P100 response between healthy children and children with DS. Dependent variables in RMGLM models were either: (A) amplitude, or (B) latency values; recorded on both eyes. The health status (healthy/DS) and sex were included in models as between-subject factors, whereas refractive error and age were used as covariates. The rationale behind building of RMGLM models was the reduction of type I error and the increase in sensitivity of a model due to the usage of multiple dependent and independent variables in a single model. It is important to note that RMGLM models were built using two sets of repeated measures: one included P100 data of a left and a right eye, and another set which contained P100 data of a VEP dominant and a VEP inferior eye. We defined VEP dominant eye as an eye which exhibited higher P100 amplitude, and whose P100 latency was shorter or equal to the value of the other eye.

Multiple regression model as well as Kendall's correlation analysis were used to further examine the relationship between an interocular difference in amplitude/latency and age, refractive error, heath status or sex. Distributions of interocular differences between two study groups were compared by Mann-Whitney U test and Moses test of extreme reaction, whereas independent t-test and χ^2 test were used to assess the differences in age and sex distributions between the respective study groups. Data analysis was carried out in SPSS v.19.0 (IBM). The level of statistical significance was set to 0.05.

RESULTS

Sociodemographic and clinical characteristics of children by study group are presented in table 1. The groups were comparable in terms of age (t-test, p=0.957), sex (χ^2, p=0.637) and refractive error (t-test, p=0.575) distributions.

VEP features of representative patients are presented in online supplemental figures 2,3. The peaks used to measure latency and amplitude are marked by a circle.

Table 1 Sociodemographic and clinical characteristics of children by study group

Characteristics		Down's syndrome mean±SD or N (%)	Healthy
Age (years)		9±2	9±2
Sex	Males	18 (50%)	16 (44%)
	Females	18 (50%)	20 (56%)
Refractive error (D)		1.26±0.72	1.17±0.64

The distributions of P100 amplitude and latency which were observed in both study groups are shown in online supplemental table 1.

When we analysed the differences in P100 parameters between the study groups, while considering measurements from both eyes of a participant and with an additional control of sex, age and refractive error, we established that P100 latencies were significantly longer in the group of children with DS (online supplemental tables 1,2).

In fact, all the latencies of children with DS were from 4.3 to 28.5 ms longer than observed latencies of healthy controls. No other variable (including sex, refractive error, age or interaction of health status and sex) affected latency differences between the subjects (table 2). Summary of the RMGLM examining latency values between subjects is shown in table 2.

Another interesting result emerged for the within-subject effects of the same RMGLM model shown in table 2 (P100 latencies model). When we used data on the left, and the right eye as repeated measures in the model, none of the independent variables listed in table 2 had a significant impact on the interocular latency difference in a person (RMGLM multivariate test, p≥0.122 for all). However, when data on P100 latency of a VEP dominant and an inferior eye of a person were used, we identified a significant effect of the health status on interocular (VEP inferior-dominant) latency difference (RMGLM multivariate test with p<0.001 for health status, p≥0.277 for other variables). The scatter plot of latencies measured at VEP-dominant and VEP-inferior eye explains this finding in more detail (figure 1, upper panel). In the majority of healthy children (92% (n=33)), a VEP-inferior eye exhibits a maximum latency of 106.1 ms whereas a VEP-dominant eye shows the latency which is on average shorter by 1.2 ms (median interocular difference of 1.2 ms, IQR from 0.2 to 4.0). Contrary, in children with DS both eyes exhibit comparable latencies ranging from 114.2 to 116.5 ms. Furthermore, there is no clear cut-off value for the maximum latency, and interocular latency difference is almost completely diminished (median of 0.3 ms, IQR from 0.1 to 0.5). In other words, interocular latency differences between VEP inferior and dominant eye were on average larger (Mann Whitney test, p=0.006; figure 1, lower panel) and much more dispersed (Moses test of extreme reaction, p<0.001) in healthy children than in children with DS.

Table 2 Variables affecting differences in p100 amplitude and latency in healthy children and children with Down's syndrome

RMGLM model	Variable	F (1,65)	P value
P100 latencies	Refractive error	0.95	0.334
	Age	1.20	0.277
	Health status (Down/healthy)	449.82	<0.001
	Sex	0.15	0.705
	Interaction health status × sex	0.01	0.942
P100 amplitudes	Refractive error	0.51	0.822
	Age	0.17	0.685
	Health status (Down/healthy)	0.06	0.804
	Sex	2.10	0.153
	Interaction health status × sex	1.59	0.212

RMGLM, repeated measures general linear models.

Correlation analysis performed separately in healthy and in children with DS corroborated the effect of health status on interocular VEP inferior/dominant latency difference as the analysis showed different patterns of association between this difference and the refractive error, or age in the study groups (table 3).

Specifically, whereas in healthy children an interocular latency difference increased moderately with refractive error, in children with DS no such association was identified. Also, while interocular latency difference moderately decreased with age in the healthy individuals, in children with DS there was a moderate increase with age.

Regarding the P100 amplitude, its RMGLM model revealed that none of the factors or covariates affected significantly the amplitude differences between the subjects (table 2). In other words, P100 response amplitudes in children with DS were comparable to that of healthy controls. However, within-subject effects of this model showed that the amplitude measured on a VEP dominant eye of a person was significantly higher than the one measured on his/her inferior eye (RMGLM multivariate test, p<0.001; online supplemental table 1 and figure 2). This interocular VEP dominant-inferior amplitude difference was 1.5±0.6 µV and was not affected by health status or sex of a participant (RMGLM, p≥0.376). Nevertheless, it interacted significantly with the refractive error (p=0.004), and age (p<0.001). As it was delineated by the multiple regression model of this amplitude difference, the older the subject (τ=−0.15, p<0.001) or the higher his/her refractive error (τ=−0.30, p=0.004), the smaller was the interocular difference between VEP dominant and inferior P100 amplitudes.

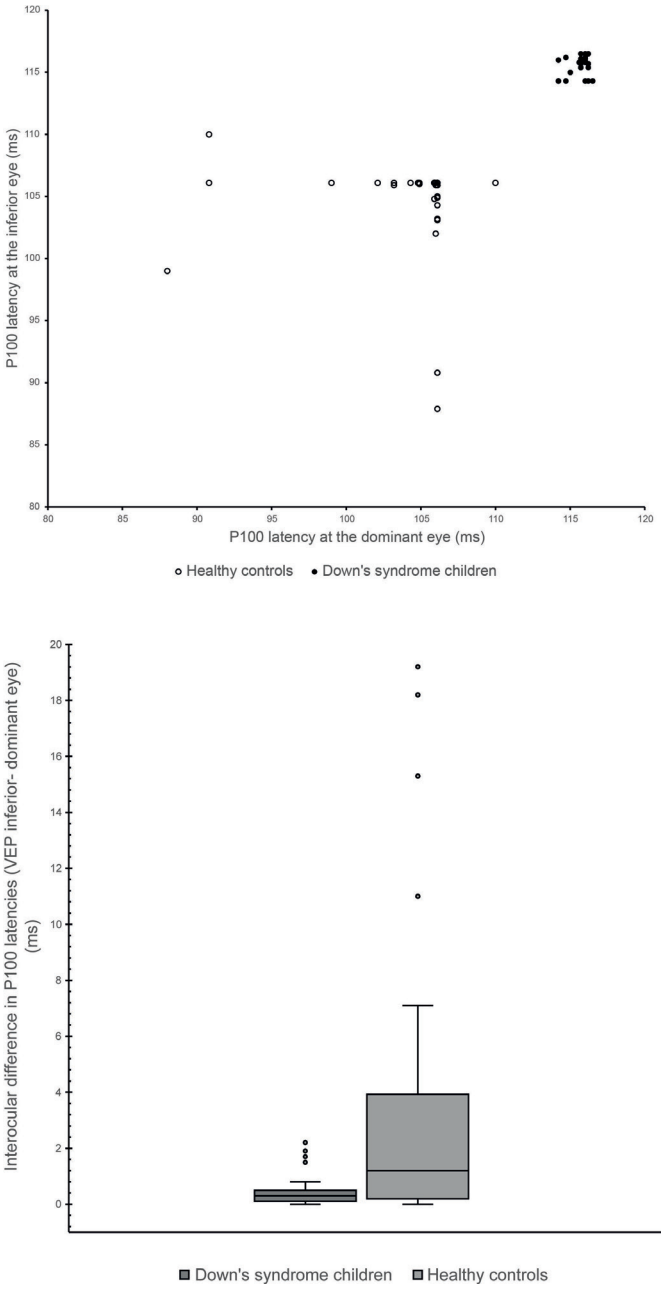

Figure 1 P100 latencies and differences in latencies in healthy children and children with Down's syndrome. (Upper panel) The scatter plot of latencies measured at VEP dominant and inferior eye in healthy children (empty grey circles) and children with Down's syndrome (full black circles). (Lower panel) Distributions of within-subject differences in latencies between VEP dominant and inferior eye, shown by the study group. VEP, visual evoked potentials.

DISCUSSION

Our study clearly showed that peak P100 latencies were significantly longer in children with DS who did not have any evident ocular abnormalities beyond mild refractive error, whereas P100 amplitudes were comparable to those of healthy children. The prolongation of latencies was bilateral with similar delays on both eyes and was so marked that the shortest latency in children with DS was still 4 ms longer than the longest latency in age-matching controls. The study results have demonstrated that children with DS do have atypical VEP response which, at least in part, reflects atypical structure/function of their neural visual pathway. Since all the children in our study had normal vision (visual acuity of ≥0.8, no ocular abnormalities), such atypicality evidently does not affect their visual acuity to an extent which is clinically relevant. The clinical significance of our finding is illustrated by the fact that 25% or 70% of the children with DS in our study would have been misdiagnosed with pathological VEP, if the common criteria of VEP assessment were applied (mean latency +2.5 SD of the control group to consider a VEP pathological).[18] Since P100 latency prolongation is least affected by technical factors and the degree of patient cooperation, it is considered the most reliable indicator of clinically significant abnormality,[18] so a special caution should be taken when assessing VEP in this particular population based on the latency duration criteria.

Other authors who recorded transient-pattern VEP responses in people with DS also observed an increase in peak P100 latencies compared with healthy group, but conclusions of these studies were inconsistent. Whereas

Table 3 Associations of interocular latency difference with refractive error, age and sex—shown by study groups

	Down's syndrome	Healthy
Refractive error	p=0.777	τ=0.39, p=0.002*
Age	τ=0.38, p=0.009*	τ=−0.45, p<0.001*
Sex	p=0.363	p=0.949

-; no significant associations, τ; Kendall's τ correlation coefficient, *; correlation coefficient is shown only for significant associations.

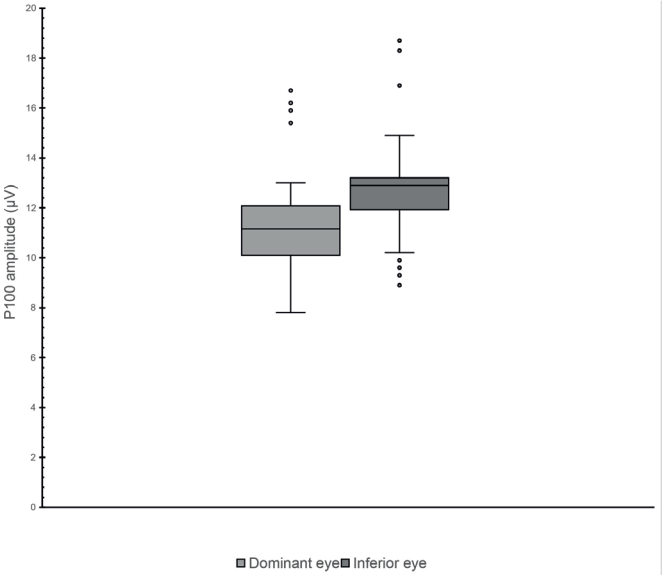

Figure 2 Distributions of the p100 amplitudes by the VEP dominancy of an eye. VEP, visual evoked potentials.

Suttle and Lloyd reported significant peak P100 prolongation in a sample of just seven adults with DS who did not have any ocular abnormalities[11]; Kakigi *et al* initially demonstrated prolongation in the overall adult sample but failed to do so when comparing results of normal subjects to nine patients who were similar to those recorded in the Suttle's sample.[10] In nine children with DS and without ocular abnormalities, however, Suttle and Turner did not find significant differences in latencies between these children and their age-matched controls.[12] It is likely that in these studies small sample sizes and the usage of group averages instead of patient-level data on P100 wave parameters masked the effect of DS on peak P100 latencies which was observed in our study. The above-mentioned studies have also reported inconsistent results for P100 amplitudes, with Kakigi and Suttle supporting our finding on no significant difference in amplitudes between people with DS and healthy controls[10 11]; and Suttle reporting significantly lower amplitudes in adults with DS.[12]

The bilateral delay of the peak P100 with similar delays on testing of each eye is often found in demyelination and in other disorders in which the reduction of conduction velocity is widely disseminated.[19] A typical VEP finding in patients with multiple sclerosis, the most common chronic inflammatory demyelinating disease; is the prolongation of P100 wave latencies with normal amplitude and relatively preserved wave shape which is similar to our findings in children with DS.[20] Bilateral delays, but accompanied with reduction of P100 amplitude, have also been reported in patients with obstructive sleep apnea,[21] or following alcohol intake[22]; both conditions with widely spread change in the conduction velocity. In DS, defects in white matter development and function are well known. Postmortem studies reported reduced myelin content[23] and fewer oligodendrocytes in striatum of these patients.[24] Most recently, the analysis of the transcriptomes from DS brains and a trisomic mouse model revealed that hypomyelination in mouse model is in part due to cell-autonomous effects of trisomy on oligodendrocyte differentiation and the production of neocortical myelin, and that it results in slower neocortical action potential transmission between cerebral hemispheres.[25] Thus, the prolongation of latencies that we observed in children with DS could likely be, at least in part, due to hypomyelination.

An interesting finding of our study is the phenomenon related to interocular differences in peak P100 latencies. While none of the children with DS in our study had pathological VEP according to this latency criteria,[26] we detected a distinct effect of DS condition on this value. Specifically, asymmetries in peak P100 latency (and amplitude) which we observed in healthy children are commonly assigned to differences between dominant and non-dominant eye, and interpreted as electrophysiological evidence of lateralisation in the nervous system.[27 28] Once we defined VEP dominant eye in our participants, we clearly demonstrated that in children with DS, unlike in healthy controls, interocular difference in peak P100 latencies between a VEP inferior and a dominant eye is insignificant. When the difference was calculated between the left and the right eye the effect was masked by averaging, which is probably the reason why the phenomenon had not been reported previously. It should be noted that we did not perform any eye dominance test so the discussion on the link between the electrophysiological VEP dominance and the actual sighting eye dominance is out of scope. Studies which analysed the association between transient-pattern VEP responses and eye dominance tests showed that on average, peak P100 latency of a dominant eye in healthy subjects is decreased and P100 amplitude is increased,[29] but the congruence was not absolute. Nevertheless, the lack of asymmetry in visual information processing which we observed in children with DS is in line with atypical cerebral lateralisation in this syndrome that has been reported previously.

The study on hand, foot, ear and eye laterality, which were used as nonintrusive measures of cerebral lateralisation, demonstrated that laterality in persons with DS was distinct from that of normally developed persons, or patients with other types of mental retardation.[30] In addition, neuropsychological studies performed on adults with DS have shown a pattern of cognitive deficits comparable to the one seen in patients with left-hemisphere brain damage.[31] Finally, the studies of perceptual asymmetries which applied dichotic listening to explore lateral dominance of brain function in healthy, persons with DS and persons with some other form of mental retardation, have found that right-handed individuals with DS exhibit a unique, syndrome-specific pattern of ear dominance which cannot be attributed solely to mental retardation.[32] Since volumetric evidence for an asymmetry of the brain in persons with DS was inconsistent, it has been suggested that the basis for lateralised dysfunction in this syndrome might be functional and not structural.[33 34] Functional MRI of cognitive processes in young adults with DS compared with those of age-matched normally developing controls, revealed atypical patterns of brain activation for the individuals with DS.[35] Building on this finding, Anderson *et al* have found evidence on immature development of connectivity in DS with increased, shorter-range inter-regional synchrony and impaired ability to integrate information from distant brain regions into coherent distributed networks.[36]

Regardless of the reason for abnormal asymmetry of peak P100 latency, it is important to note that interocular latency difference as a marker of this asymmetry also showed opposed association patterns with age and refraction error in two study groups. The finding suggests that in children with DS this marker indicates an aspect of visual information processing which is completely altered from that in healthy children, or it points towards a different aspect of such processing assumedly generated by atypical patterns of brain activation in DS.

The limitation of this study is that children with DS were not tested for IQ. Severity of mental retardation

could influence children's state of arousal and degree of attention to the test stimulus, which is of critical importance for VEP testing. However, severe mental retardation would normally result in the inability to record a VEP, whereas the variation in attention/arousal would introduce larger differences in measures between left and right eyes. By enrolling children with no ocular abnormalities in this study, we filtered out severe cases of mental retardation ensuring successful VEP recording in all participants. Also, peak P100 latencies between left and right eye in children with DS were more similar than in healthy individual, excluding the possibility that the variability of attention/arousal introduced differences in this parameter. Finally, to assure an adequate level of arousal we enrolled children between 6 and 12 years old who, according to our experience, have the highest attention during the recording; children were already familiar with the environment, procedure and examiner. Moreover, we recruited the examiner who was experienced in VEP recording in this population. Additionally, their attention was monitored through a camera installed inside the device's dome during the VEP recording procedures, and in children with decreased attention level, the testing was paused to let them rest. Also, as the alteration of the spatial frequency changes the sampling frequency and significantly affects the attention of the examinees and the obtained test results, we used checkerboard pattern stimuli with large, 1° checks, instead of small 0.25° checks. However, we did not perform any sophisticated test to measure participant's attention to stimulus. Since attention affects the VEP, including response latency, amplitude and waveform, lack of precise control over participant's attention to stimulus might be limitation of the study, despite of all above-mentioned procedures.

In conclusion, the bilateral prolongation of peak P100 latencies and the reduction in interocular differences in peak P100 latencies accompanied with opposed patterns of its associations to age and refraction error have demonstrated that even in the population of children with DS and no ocular abnormalities/normal vision, VEP response is quite atypical—probably reflecting global structural and functional changes in the DS brain. We propose that VEP diagnostic criteria for these children is reconsidered, possibly with the inclusion of this target population (DS and no ocular abnormalities) as a norm. In addition, it would be interesting to know if a level of mental retardation affects the magnitude of latency prolongation or its interocular differences.

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Contributors Full access to all the data in the study and responsibility for the integrity of the data, the data analysis accuracy and responsible for the overall content: DKU. Concept and design, analysis or interpretation of data, critical revision of the manuscript for important intellectual content and approval of the final manuscript: all authors. Acquisition of data, obtained funding and administrative, technical or material support: DKU and IM. Drafting of the manuscript: AJ, IB, HK and IM. Statistical analyses: AJ, HK, IB. Guarantor: DKU.

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Patient consent for publication Consent obtained from parent(s)/guardian(s).

Ethics approval The study was conducted adhering to the tenets of the Declaration of Helsinki and approved by the Ethical Committee of the University Hospital Center Split, Croatia (No. 500-03/20-01/10). Parents of all included children gave written informed consent to the measurement procedures, analysis and the use of the data for publication.

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Data availability statement Data are available on reasonable request. The datasets used and analysed during the current study are available from the corresponding author on request.

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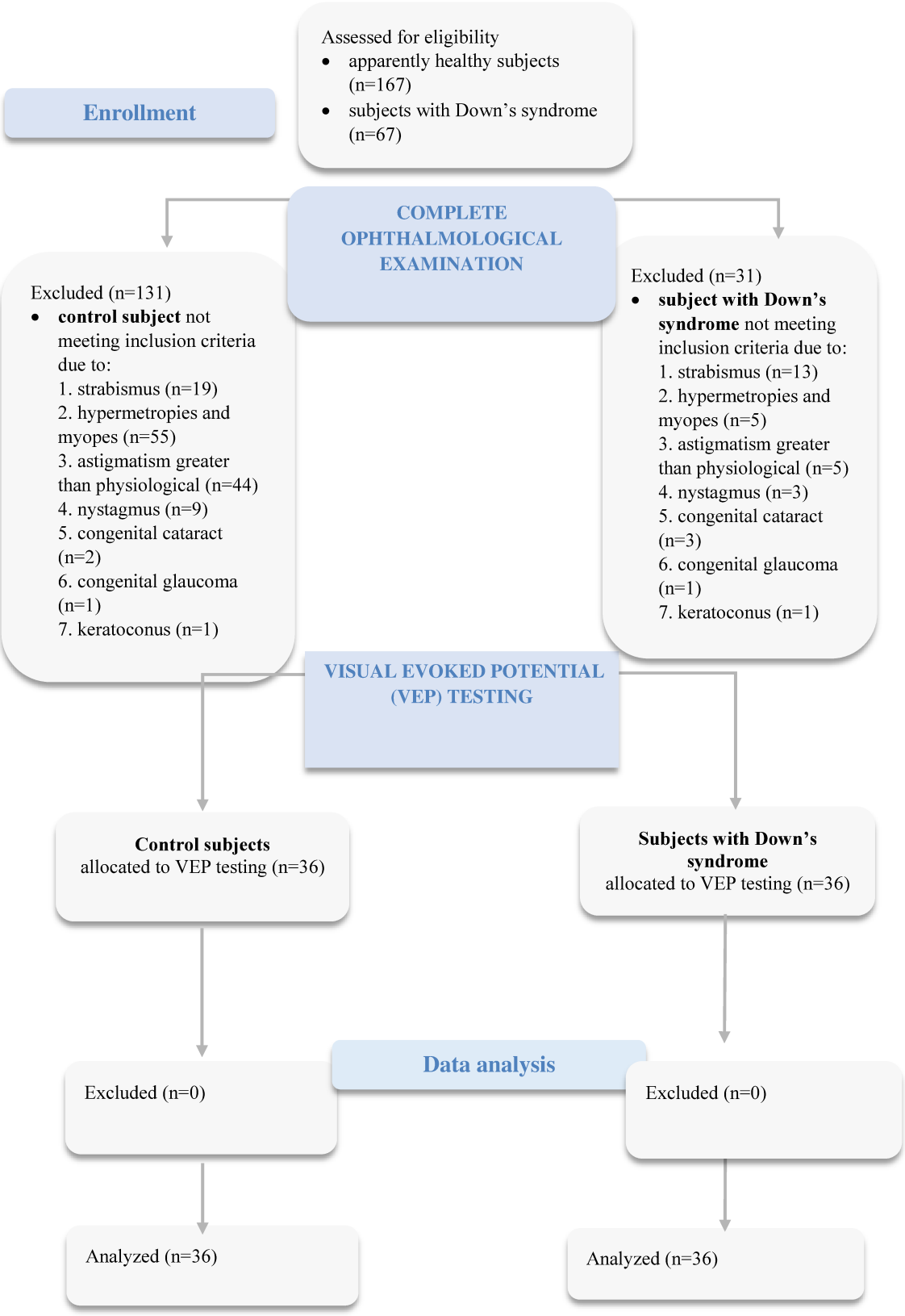
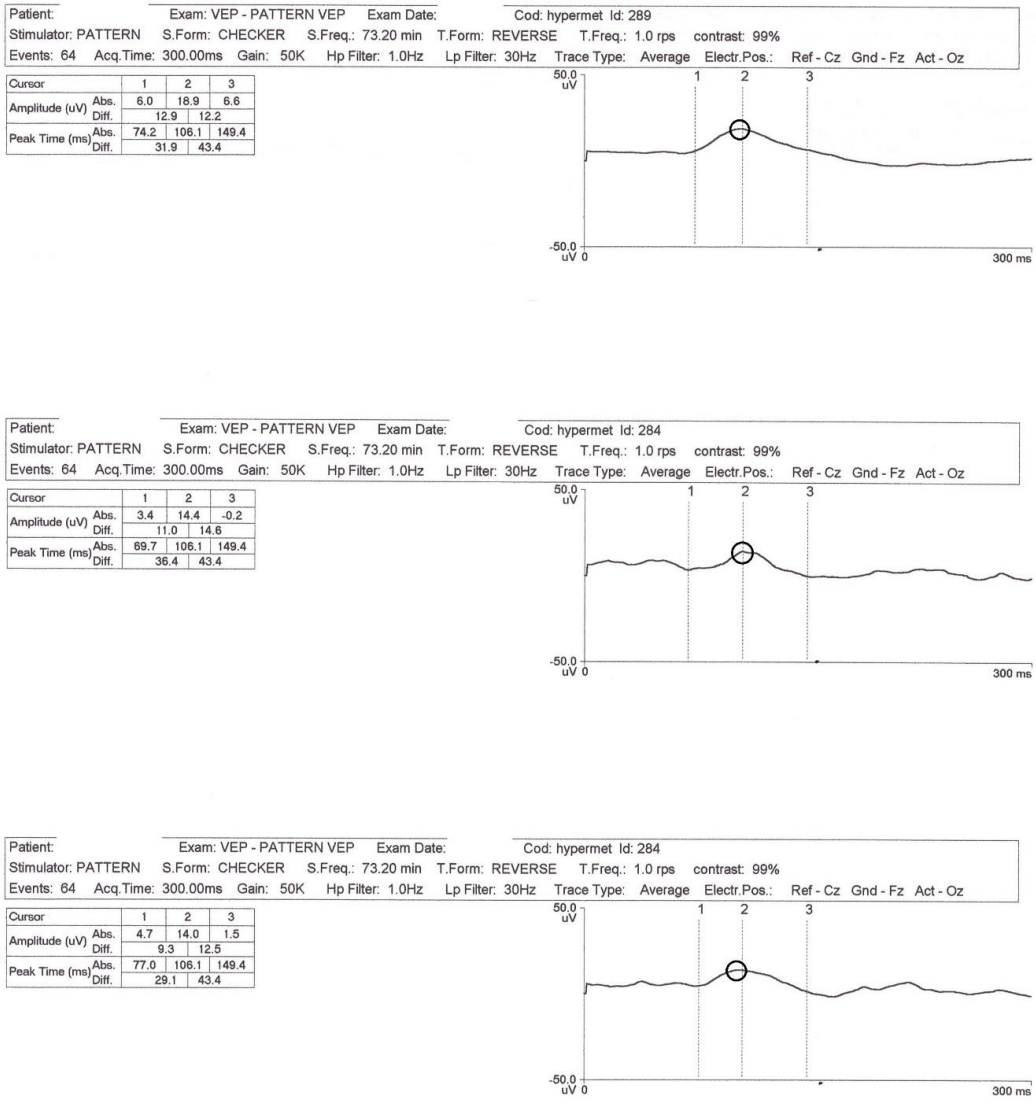


Supplemental Figure 1. Flow diagram showing the recruitment processes and study protocol

Supplemental Table 1. Average P100 parameters in children with Down syndrome and in healthy children

		Down syndrome	**Healthy**
		mean±SD	
Latencies (ms)	Left eye	115.6±0.7	104.2±4.7
	Right eye	115.7±0.6	104.2±4.7
Amplitude (μV)	Left eye	11.4±1.7	12.0±2.0
	Right eye	11.9±1.8	12.0±1.9
Latencies (ms)	VEP dominant	115.4±0.7	102.3±5.9
	VEP inferior	115.9±0.6	106.1±1.5
Amplitude (μV)	VEP dominant	12.7±1.8	12.7±1.9
	VEP inferior	11.2±1.7	11.1±1.8

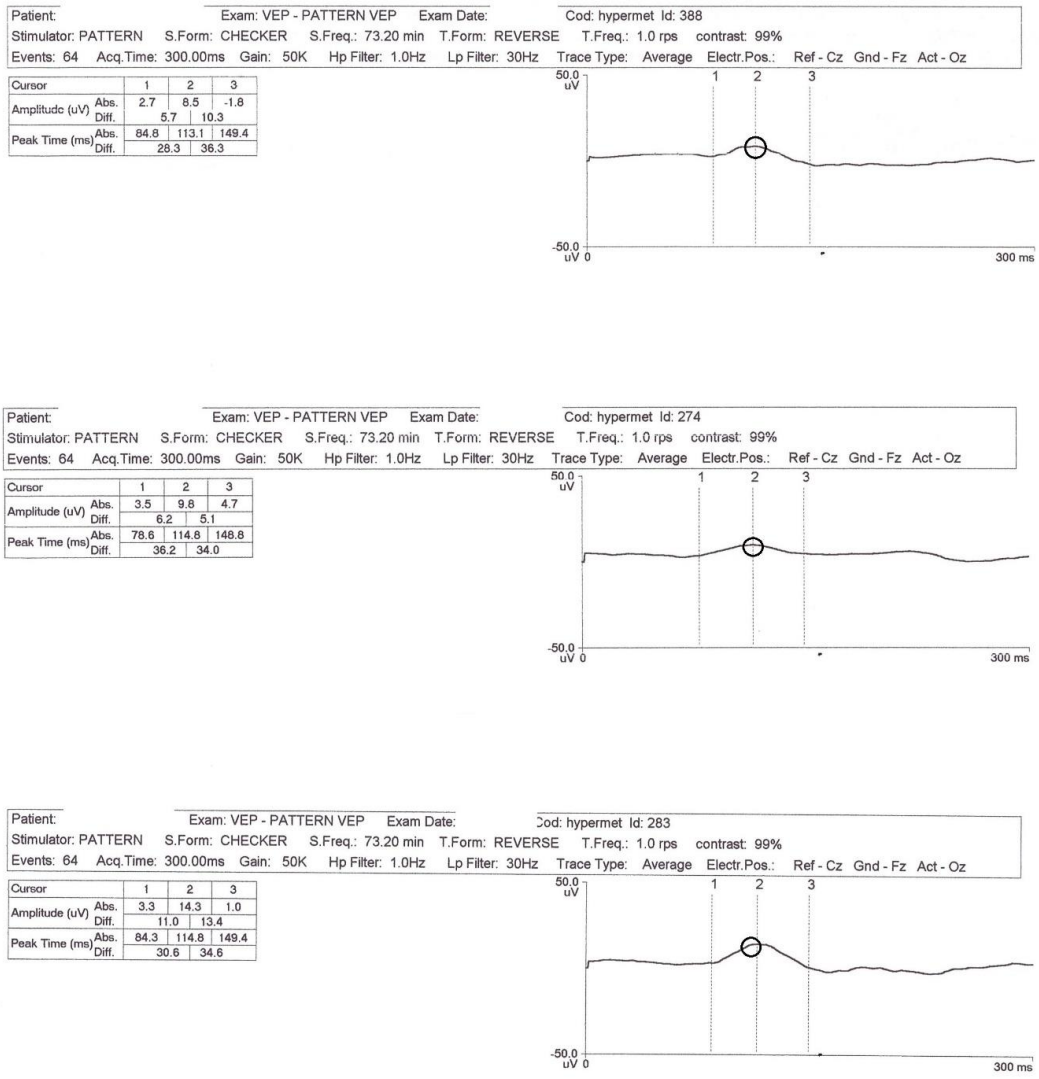
VEP dominant eye was defined as an eye which exhibited: higher P100 amplitude, and P100 latency that was either shorter or equal to the value of the other eye.



Supplemental Figure 2 VEP recordings of healthy children

The peaks used to measure latency and amplitude are marked by a circle.

Three VEP recordings represent three distinct participants



Supplemental Figure 3. VEP recordings of Down syndrome

The peaks used to measure latency and amplitude are marked by a circle.

Three VEP recordings represent three distinct participants