SPECTRUM OF NEURODEVELOPMENTAL DISABILITIES IN A COHORT OF CHILDREN IN HUNGARY

Ph.D. Theses

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THIS THESIS IS BASED ON THE FOLLOWING PUBLICATIONS

1. Gergev G, Máté A, Zimmermann A, Rárosi F, Sztriha L. Spectrum of neurodevelopmental disabilities: a cohort study in Hungary. *J Child Neurol. Accepted for publication.*
2. Gajda A, Horváth E, Hortobágyi T, Gergev G, Szabó H, Farkas K, Nagy N, Széll M, Sztriha L. Nemaline myopathy type 2 (NEM2): two novel mutations in the nebulin (*NEB*) gene. *J Child Neurol. 2013 Sep 20. [Epub ahead of print]*.
3. Horváth E, Horváth Z, Isaszegi D, Gergev G, Nagy N, Szabó J, Sztriha L, Széll M, Endreffy E. Early detection of Angelman syndrome resulting from de novo paternal isodisomic 15q UPD and review of comparable cases. *Mol Cytogenet. 2013 Sep 8;6(1):35. Doi: 10.1186/1755-8166-6-35.*
4. Gajda A, Szabó H, Gergev G, Karcagi V, Szabó N, Endreffy E, Túri S, Sztriha L. Congenital myasthenic syndromes and transient myasthenia gravis. *Clin Neurosci/Ideggyógyászati Szemle.* 2013;66:200-203.
5. Mokánszki A, Körhegyi I, Szabó N, Bereg E, Gergev G, Balogh E, Bessenyei B, Sümegi A, Morris-Rosendahl DJ, Sztriha L, Oláh É. Lissencephaly and band heterotopia: *LIS1, TUBA1A,* and *DCX* mutations in Hungary. *J Child Neurol.* 2012;27:1534-1540.
6. Szabó N, Gergev G, Kóbor J, Bereg E, Túri S, Sztriha L. Corpus callosum anomalies: birth prevalence and clinical spectrum in Hungary. *Pediatr Neurol.* 2011;44:420-426.
7. Szabó N, Gergev G, Kóbor J, Szűcs P, Túri S, Sztriha L. Holoprosencephaly in Hungary: birth prevalence and clinical spectrum. *J Child Neurol.* 2011;26:1029-1032.
8. Szabó N, Gyurgyinka G(ergev), Kóbor J, Bereg E, Túri S, Sztriha L. Epidemiology and clinical spectrum of schizencephaly in South-Eastern Hungary. *J Child Neurol.* 2010;25:1335-1339.

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SUMMARY

Neurodevelopmental disabilities are a group of chronic heterogeneous disorders, which include clinically distinct, chronic disorders whose essential and unifying feature is a disturbance in developmental progress in one or more developmental domains.

Objectives

The aims of this study were to estimate the share of neurodevelopmental disabilities among patients referred to a Paediatric Neurology Service in a region in Hungary and study the spectrum of disability subtypes in this cohort. Our further objective was to search for the aetiological factors leading to neurodevelopmental disability, estimate the degree of intellectual disability (mental retardation) in the various subgroups and compare our results with figures in the literature. Setting up a manageable database for further research and public health services was also intended.

Patients and methods

A retrospective survey of patients with neurodevelopmental disabilities referred to the Paediatric Neurology Service at the Department of Paediatrics, Division B, University of Szeged, Hungary between 1 January 2006 and 31 December 2011 was performed. Detailed history was taken followed by physical and neurologic examinations and neuropsychological testing. Further investigations (imaging, metabolic screening, histopathology, cytogenetic, molecular cytogenetic tests, or mutation analysis of candidate genes) were directed by the history and examinations.

Results

This study included 241 (131 boys, 110 girls) out of 1764 children (13.7%) referred to the Paediatric Neurology Service. Neurodevelopmental disability occurred without any known prenatal, perinatal, and/or neonatal averse events in 167 patients (69.3%), while known prenatal, perinatal, and/or neonatal adverse events were found in 74 children (30.7%).

Patients without adverse events were classified into the following subgroups: genetic syndromes with recognized aetiology (12.0%), global developmental delay/intellectual disability (mental retardation) in association with dysmorphic features not recognized as specific syndromes (9.5%), global developmental delay/intellectual disability (mental retardation) without recognized aetiology and dysmorphic features (18.7%), brain malformations (14.5%), inborn errors of metabolism (2.5%), leukoencephalopathies (1.7%), epileptic syndromes (1.7%), developmental (specific) language impairment (3.7%), and neuromuscular disorders 5.0%). Patients with adverse

events comprised the following subgroups: cerebral palsy after preterm delivery (14.9%), after delivery at term (8.3%), and disability without cerebral palsy (7.5%).

Overall the aetiology of the neurodevelopmental disabilities was identified in 66.4% of the patients and well documented genetic diagnosis was found in 19.5%.

Conclusions

Recognition of causes of neurodevelopmental disabilities helps starting adequate treatment, when feasible, and establishing a health maintenance plan and rehabilitation. It provides prediction of the outcome, helps in avoiding unnecessary diagnostic tests and contributes to the prevention of the disorder. Our database can be used for further studies on the genetic and environmental causes of neurodevelopmental disorders and it provides useful information for the health authorities on the special needs of children living with neurodevelopmental disabilities.

ÖSSZEFOGLALÁS

Az idegrendszeri funkciók fejlődése késlekedhet egy vagy több részterületen különböző súlyosságú fogyatékosságot okozva. A fogyatékosság hátterében különböző klinikailag diagnosztizálható kórképek állnak, amelyek közös jellemzője, hogy a beteg motoros és/vagy szellemi fejlődése, beszédkészsége változó mértékben elmarad az életkori átlagtól.

Célkitűzések

Az egy vagy több részterületen fogyatékos gyermekek arányát kívántuk tanulmányozni Magyarország egyik régiójának gyermekneurológiai szakrendelésére beutalt betegek között, továbbá meg kívántuk állapítani a fogyatékosság fő típusait. Célul tűztük ki a fogyatékosság okának kiderítését és szellemi fogyatékosság esetén vizsgálni kívántuk annak súlyosságát a különböző diagnosztikus csoportokban. Célunk volt, hogy eredményeinket összehasonlítsuk a nemzetközi szakirodalomban talált adatokkal. Egy adatbázist kívántunk létesíteni, amely alkalmazható további kutatási célra és hasznos lehet az egészségügyi szolgálat számára is.

Betegek és módszerek

A Szegedi Tudományegyetem Gyermekgyógyászati Klinika B Részlegének neurológiai szolgálatán 2006. január 1. és 2011. december 31. között megjelent gyermekek körében végeztük a vizsgálatainkat. Az anamnézis részletes felvételére, fizikális, neurológiai és neuropszichológiai vizsgálatra került sor a gondozás során. További vizsgálatok (képalkotó módszerek alkalmazása, anyagcsere vizsgálat, szövettan, citogenetikai, molekuláris citogenetikai tesztek, mutáció analízis) az anamnézis és a klinikai vizsgálatok eredményétől függően történtek.

Eredmények

Hat év alatt 1764 beteget vizsgáltunk, ebből 241 gyermeket (13.7%, 131 fiú, 110 leány) vettünk be a tanulmányunkba. Fogyatékosságot találtunk valamilyen praenatalis, perinatalis és/vagy újszülöttkori ismert rizikótényező nélkül 167 betegnél (69.3%), míg a fogyatékosság hátterében rizikó faktornak tartható praenatalis, perinatalis és/vagy újszülöttkori esemény állt 74 betegnél (30.7%).

Az azonosítható rizikófaktor nélkül kialakult fogyatékossággal élő gyermekeket a következő alcsoportokba soroltuk be: ismert etiológiájú genetikai szindrómák (12.0%), dysmorphismussal társuló, de szindromológiailag be nem sorolható globálisan késlekedő fejlődés/szellemi fogyatékosság (9.5%), ismeretlen etiológiájú globálisan késlekedő fejlődés/szellemi fogyatékosság dysmorphismus nélkül (18.7%), agyi

fejlődési rendellenességek (14.5%), veleszületett anyagcsere betegségek (2.5%), a fehérállomány betegségei (1.7%), epilepsziás szindrómák (1.7%), megkésett beszédfejlődés mint részfunkció-zavar (3.7%) és neuromuscularis betegségek (5.0%). Az ismert rizikótényező következtében kialakult fogyatékossággal élő betegek körében 3 alcsoportot különítettünk el: cerebralis paresis koraszülöttekben (14.9%), cerebralis paresis érett újszülöttekben (8.3%) és különböző típusú fogyatékosság cerebralis paresis nélkül (7.5%).

Az összes beteget tekintve a fogyatékosság oka a gyermekek 66.4%-ában derült ki, genetikai okot pedig 19.5%-ban találtunk.

Következtetések

Az idegrendszeri fejődés egy vagy több részterületét érintő fogyatékosság oka az esetek több mint felében diagnosztizálható. Az etiológia kiderítése alapvetően fontos annak érdekében, hogy az újabb kutatások által feltárt terápiás lehetőségeket alkalmazhassuk. A megfelelő gondozás és az egyénre szabott rehabilitációs program kidolgozása is megkívánja az etiológia ismeretét. A specifikus kórokok kiderítése révén elkerülhetőek a beteget terhelő szükségtelen vizsgálatok, prognosztizálható a kimenetel és számos esetben megelőzhető a kórkép ismétlődése egy családban.

Adatbázisunk hozzájárul ahhoz, hogy az új tudományos eredményeket megfelelő időben alkalmazni tudjuk betegeinknél és segíti az egészségügyi ellátórendszert a fogyatékosok speciális igényeinek felmérésében.

INTRODUCTION

Neurodevelopmental disabilities are a group of chronic heterogeneous disorders, which include clinically distinct, chronic disorders whose essential and unifying feature is a documented disturbance in developmental progress, either quantitative or qualitative, or both, compared with established norms in one or more recognized developmental domains.[1] These domains traditionally include: motor (gross or fine), speech/language, cognition, personal/social, and activities of daily living.[1-3]

Neurodevelopmental disabilities have been divided into subtypes.[1,2] These subtypes are essentially "symptom complexes" rather than specific disorders or diseases and they share marked heterogeneity within each subtype.[1,2] Based on previous conceptualization global developmental delay, mental retardation/intellectual disability, cerebral palsy, gross motor delay including mostly the neuromuscular disorders, developmental language impairment as a single domain developmental delay and autistic spectrum disorders can be regarded as the main categories of neurodevelopmental disabilities.[1-3]

Global developmental delay applies to children less than 5 years and it is defined as a significant delay in two or more developmental domains.[4,5] Global developmental delay may be an early marker of what is termed mental retardation, which is typically diagnosable in the child older than 5 years of age.[5] The present consensus definition of "mental retardation," put forward by the American Association on Mental Retardation (now the American Association on Intellectual and Developmental Disabilities) in 2002, describes this entity as "a disability characterized by significant limitation both in intellectual functioning and adaptive behaviour as expressed in conceptual, social, and practical adaptive skills".[5] Intellectual disability is the currently preferred term for mental retardation.[6,7]

Cerebral palsy describes a group of permanent disorders of the development of movement and posture, causing activity limitation, that are attributed to non-progressive disturbances that occurred in the developing foetal or infant brain.[8] Neurodegenerative, neurometabolic, and neoplastic processes do not underlie cerebral palsy; however some controversy exists regarding the inclusion of potentially progressive vascular (i.e. moyamoya) or traumatic (i.e. shaken baby syndrome) aetiologies under this term.[8]

Single domain neurodevelopmental delay in the motor or language area has been suggested by Shevell and coworkers.[1,9] Indeed acquisitions of motor skills is the main area of delay in diseases of the motor unit, however the cognition can also be affected in

several muscle disorders suggesting an overlap between different categories of neurodevelopmental disabilities. Developmental (specific) language impairment refers to a significant delay in speech and language skills with normal performance in other developmental domains or activities of daily living.[1,9,10]

In addition to the main categories of neurodevelopmental disabilities other subtypes, such as isolated hearing loss, isolated visual impairment, autism, specific learning disability, attention-deficit-hyperactivity disorder, developmental co-ordination disorder ("clumsy" children) etc. also occur in the setting of the neurodevelopmental disabilities,[1,3] however these conditions will not be dealt with in this study .

The role of the paediatric neurologist/neuropsychologist when assessing these patients is several-fold: to verify that neurodevelopmental disability does indeed exist, carefully search for an underlying aetiology, provide access and referral to adequate therapeutic and rehabilitation resources, and offer prognostication.[11-14]

Several syndromes and disorders with neurodevelopmental disability (e.g. genetic syndromes, inborn errors of metabolism, or certain types of muscle diseases) are rare diseases (orphan diseases).[15,16] Any disease affecting less than 1 in 2000, i.e. 5 people in 10 000 is considered rare in the European Union.[16] Living with a rare disease raises several special difficulties.[16] Studies by the European Organization for Rare Diseases (EURORDIS) revealed that there is a lack of quality information on and scientific knowledge of these diseases, therefore the correct diagnosis is delayed and the patients lack appropriate quality healthcare .[16]

Several population- and cohort-based studies and reviews on the various features of global developmental delay and intellectual disability/mental retardation have been published earlier,[17-25] and efforts have been made for the characterization of single domain disorders as well.[9] Based on the progress in paediatric neurosciences, acknowledging the merits of previous classification schemes, a more refined classification of neurodevelopmental disabilities, including more distinct subtypes of disorders, seems to be required. Our aim was to establish a comprehensive classification scheme, rather dissimilar to the previous ones, in order to characterize the spectrum of neurodevelopmental disabilities and demonstrate the clinical utility of this classification scheme as an organizing framework for clinical investigations. Aetiology of the disabilities has been searched for and the degree of intellectual disability in each diagnostic subtype has also been assessed.

OBJECTIVES

The following objectives were targeted by our study:

1./ To perform a retrospective survey of children with neurodevelopmental disabilities in a cohort of patients referred to a paediatric neurology service at a university hospital in South-Eastern Hungary in order

- to estimate the share of neurodevelopmental disabilities among all patients referred to the service
- to establish the spectrum of neurodevelopmental disability subtypes in this cohort of patients
- to search for aetiological factors leading to neurodevelopmental disability
- to estimate the incidence of different severity degrees of intellectual disability (mental retardation) among patients with neurodevelopmental disabilities
- to compare our results with data published by other cohort studies

2./ To establish and manage a regional database for the following purposes:

- to search for environmental factors beyond obstetrical complications responsible for neurodevelopmental disabilities
- to encourage genetic studies to promote preimplantation and prenatal diagnosis in subsequent pregnancies
- to draw the attention of decision-makers to the special needs of children living with neurodevelopmental disabilities

PATIENTS AND METHODS

A retrospective survey of patients with neurodevelopmental disabilities referred to the Paediatric Neurology Service (outpatient and inpatient) at the Department of Paediatrics, Division B, University of Szeged, Hungary between 1 January 2006 and 31 December 2011 was carried out.

An algorithm was designed for the evaluation of patients with neurodevelopmental disabilities. This scheme for diagnostic work-up followed the recommendations by the Quality Standards Subcommittee of the American Academy of Neurology and The Practice Committee of the Child Neurology Society[4], van Karnebeek and co-workers[20] and Wilska and Kaski[26] (Table 1). Standard assessment included clinical history of the antenatal, perinatal, neonatal, and postnatal period. Developmental milestones and behavioural phenotype were also assessed. Physical and neurological examinations were performed with attention even to soft neurological signs. Hearing was always tested and neuro-ophthalmologic consultation was also an integral part of the physical examination (Table 1).

Special attention has been paid to the presence of malformations and/or dysmorphic features. Data on malformations of any organ system(s) were retrieved from the previous medical records. Physical anomalies detectable by surface examination were described as recommended in the literature.[27-35]

Developmental and cognitive evaluations were performed by the Hungarian adaptations of the revised Brunet-Lézine[36] (below 3 years of age), Stanford-Binet[37] (Budapest Binet, between the ages of 3 and 6 years) tests, WISC-IV (Wechsler Intelligence Scale for Children – Fourth Edition, between the ages of 6 and 16 years),[38] and Woodcock Johnson III Tests of Achievement (above 16 years of age).[39] In cases where cognitive functioning was extremely reduced and/or behaviour was substantially disturbed, not allowing the administration of formal test, DQ/IQ scale was assessed indirectly based upon clinical descriptions of the child's functioning.

Developmental delay and intellectual disability (mental retardation) was classified according to the DSM-IV-TR[40] by measure of DQ or IQ as severe to profound (DQ/IQ level below 35-40), moderate (DQ/IQ level 35-40 to 50-55), mild (DQ/IQ level 50-55 to approximately 70), and borderline (DQ/IQ 70-85). Children with severe and profound developmental delay and intellectual disability (mental retardation) were grouped together in a single category.

Neuropsychological assessment was carried out partly by our own clinical testing, partly by personal or telephone interviews. There is a national network of Committees for the Assessment of Learning Abilities and Rehabilitation in Hungary. Infants or children with suspected global developmental delay, or intellectual disability (mental retardation) are entitled to be tested and advised by these committees. Special institutions for children with visual (National Committee for the Assessment of Vision Abilities and Rehabilitation), or hearing impairments (National Committee for the Assessment of Hearing Abilities and Rehabilitation), speech (National Committee for Speech Assessment and Rehabilitation), or movement disorders (National Committee for the Assessment of Movement and Rehabilitation) also provide assessment in Hungary. DQ/IQ scores were retrieved by personal or telephone interviews from the reports issued by these committees for those patients with neurodevelopmental disabilities whose parents were reluctant to expose their children to another test at our department.

Cerebral palsy and its complications were diagnosed by neurologic examination and classified as spastic diplegia, quadriplegia, hemiplegia, extrapyramidal, and hypotonic cerebral palsy.[41]

Brain imaging (ultrasound, CT, and MRI), EEG, tests for intrauterine infection and screening for inborn errors of metabolism were carried out by conventional protocols. Muscle biopsy specimen was taken from patients with suspected congenital myopathies. Chromosomal analysis with G-band technique was carried out for patients with intellectual disability (mental retardation), dysmorphic features, or multiple anomalies. *FMR1* gene test was performed for children with intellectual disability (mental retardation) if no other etiology was evident. Fluorescence in situ hybridization (FISH) with specific probes or mutation analysis of putative genes were requested for the confirmation of particular diagnoses suspected on the basis of clinical, imaging, laboratory, biochemical, or histopathological features. For a few patients with intellectual disability (mental retardation) and dysmorphic features subtelomeric FISH, or array comparative genome hybridization (aCGH) were also carried out.

Table 1. Algorithm for the aetiological diagnosis of neurodevelopmental disabilities

(Modified from Shevell et al.[4], van Karnebeek et al.[20] and Wilska and Kaski[26])

DETAILED HISTORY

Antenatal, perinatal, neonatal, medical, developmental, psycho-social, environmental, and family history

EXAMINATIONS

Physical and neurologic examination
(Special attention to dysmorphic features /major/minor malformations)
Neuropsychological testing
Consultations: audiologist /ophthalmologist

ARE THERE FEATURES SUGGESTING A SPECIFIC DIAGNOSIS?

A. Are there historical or physical findings (e.g. dysmorphic features) to suggest specific syndromes (e.g. Down, Fragile X, Rett syndrome, etc.), or any other genetic disorders?
B. Are there historical data (e.g. intrauterine infection, intrapartum asphyxia, very low birth weight, etc.), physical findings (e.g. cerebral palsy, focal findings, microcephaly, etc.), or seizures to suggest CNS injury/malformation?
C. Are there historical data (e.g. weak foetal movements, etc.), or signs (e.g. hypotonia, weakness, areflexia) to suggest neuromuscular disorders?
D. Is there a known metabolic disorder in the family, history of episodic decompensation, loss or regression of developmental milestones, history of parental consanguinity, prior unexplained loss of a child, or multiple miscarriages?

YES				**NO**
A	**B**	**C**	**D**	MRI Bone x-ray Cytogenetic and molecular cytogenetic (subtelomeric FISH, microarray-based cytogenetic technology) tests *FMR1* and/or *MeCP2* gene tests Metabolic testing*
Specific tests for that disorder Cytogenetic and molecular cytogenetic (subtelomeric FISH, microarray-based cytogenetic technology) tests Specific gene tests Bone X-ray Metabolic testing*	MRI (CT) Tests for infections if appropriate	Serum CK EMG, ENG Specific gene tests Muscle biopsy	MRI EEG Bone X-ray Metabolic testing* Histopathology (lymphocytes, skin, conjunctiva, liver, bone marrow, peripheral nerve) Cytogenetic and molecular cytogenetic (subtelomeric FISH, microarray-based cytogenetic technology) tests Specific gene tests	

*Based on historical features, clinical, physical and/or imaging findings metabolic tests were requested if appropriate: plasma amino acids, carnitine esters, carnitine, uric acid, lactate, pyruvate, ammonia, very long chain fatty acids, phytanic acid, copper, ceruloplasmin, transferrin isoelectric focusing (for congenital defects of glycosylation), leukocyte lysosomal enzymes, urinary organic acids, mucopolysaccharides, sulphites, blood/urine screening for disorders of creatine metabolism, cerebrospinal fluid (CSF/blood ratio) glucose, lactate, pyruvate and amino acids

Patients with traumatic brain injury and CNS infections beyond the neonatal period and children with CNS tumours were not included in this study because special services provide care for these patients in Hungary.

Chi-squared goodness-of-fit test for uniform distribution was used to determine whether any gender predominance existed within the diagnostic groups or any degree of intellectual disability prevailed within a diagnostic subtype. $p \leq 0.05$ was used for establishing statistical significance. Pearson residuals were used as a measure of deviance from the hypothesized uniform distribution.

An informed consent to participate in this study was requested from the parents. This study was approved by the Ethics Committee of the Faculty of Medicine, University of Szeged (Szeged, Hungary).

The classification of neurodevelopmental disabilities in this study can be seen in Table 4.

RESULTS

Total number of 1764 patients was referred to the Paediatric Neurology Service at the Department of Paediatrics, Division B, University of Szeged between 1 January 2006 and 31 December 2011. Neurodevelopmental disability was ascertained in 316 patients (17.9%) and eventually 241 patients (13.7%) were included in this study. The mean age of these children was 7.2 ± 4.6 years (age range: 0.5-22 years) at the last follow up. There were 131 boys and 110 girls and the male/female ratio was 1.19, however the male preponderance was statistically not significant. The gender distribution in the subgroups of patients with different categories of neurodevelopmental disabilities is shown in Table 2. There was a trend of male predominance in the moderate, mild and borderline categories, while more girls were observed among patients with severe/profound global developmental delay/intellectual disability (Table 2).

The neuropsychological assessment was carried out in our institution for 74 children (Brunet-Lézine test: 23, Budapest Binet: 33 and WISC-IV: 18) and the scores for cognitive abilities were obtained by interviews for 167 patients. The assessment methods applied in different categories of disabilities can be seen in Table 3.

Neurodevelopmental disability occurred **without known prenatal, perinatal, and/or neonatal adverse events** in 167 patients (69.3%), while **known prenatal, perinatal, and/or neonatal adverse events** were responsible for the neurodevelopmental disability in 74 children (30.7%, Table 4).

Table 2. Gender distribution in different subgroups of patients with neurodevelopmental disabilities

Patient subgroups	Gender distribution and ratios in different categories of global developmental delay/intellectual disability (mental retardation)[+]																	
	SP[+]			MO[+]			MI[+]			BL[+]			NO[#]			Total		
	M	F	M/F ratio	M	F	M/F ratio	M	F	M/F ratio	M	F	M/F ratio	M	F	M/F ratio	**M**	**F**	**M/F ratio**
No adverse events																		
Global developmental delay/intellectual disability (mental retardation)																		
Genetic syndromes with recognized aetiology	2	3	0.67	5	5	1.00	5	3	1.67	3	3	1.00	0	0	NR	**15**	**14**	**1.07**
Children with dysmorphic features not recognized as specific syndromes	8	7	1.14	3	0	NR	2	3	0.67	0	0	NR	0	0	NR	**13**	**10**	**1.30**
Children without recognized aetiology and dysmorphic features	8	13	0.62	6	1	6.00	8	5	1.60	1	3	0.33	0	0	NR	**23**	**22**	**1.05**
Brain malformations	11	10	1.10	2	2	1.00	2	3	0.67	3	1	3.0	0	1	NR	**18**	**17**	**1.06**
Inborn errors of metabolism	2	0	2.00	2	1	2.00	1	0	NR	0	0	NR	0	0	NR	**5**	**1**	**5.00**
Leukoencephalopathies	1	1	1.00	0	1	NR	1	0	NR	0	0	NR	0	0	NR	**2**	**2**	**1.00**
Epileptic syndromes	0	2	NR	0	0	NR	2	0	NR	0	0	NR	0	0	NR	**2**	**2**	**1.00**
Developmental (specific) language impairment	0	0	NR	0	0	NR	0	0	NR	4	0	NR	5	0	NR	**9***	**0**	**NR**
Neuromuscular disorders	1	0	NR	1	0	NR	3	0	NR	0	0	NR	2	5	0.40	**7**	**5**	**1.40**
Adverse events (prenatal, perinatal, neonatal)																		
Cerebral palsy after preterm delivery	8	7	1.14	1	1	1.00	3	1	3.00	6	2	3.00	2	5	0.40	**20**	**16**	**1.25**
Cerebral palsy after delivery at term	4	4	1.00	2	1	2.00	0	1	NR	1	1	1.00	3	3	1.00	**10**	**10**	**1.00**
Neurodevelopment disabilities without cerebral palsy	2	6	0.33	1	0	NR	3	5	0.60	1	0	NR	0	0	NR	**7**	**11**	**0.64**
Total	**47**	**53**	**0.89**	**23**	**12**	**1.92**	**30**	**21**	**1.43**	**19**	**10**	**1.90**	**12**	**14**	**0.86**	**131**	**110**	**1.19**

M = male, F = female, [+]Degree of global developmental delay/intellectual disability (mental retardation): SP = severe to profound, MO = moderate, MI = mild, BL = borderline, [#]NO = no global developmental delay/intellectual disability (mental retardation), only defect in the motor or speech development, NR = not relevant, * = < 0.05

Table 3. Evaluation methods used in patients with neurodevelopmental disabilities

	Distribution of evaluation methods in patients with different degrees of global developmental delay/intellectual disability (mental retardation)[+]																							
	SP[+]				MO[+]				MI[+]				BL[+]				NO[#]				Total			
	B-L	B	W	I	B-L	B	W	I	B-L	B	W	I	B-L	B	W	I	B-L	B	W	I	B-L	B	W	I
No of patients	**5**	**7**	**0**	**87**	**4**	**6**	**3**	**23**	**12**	**9**	**8**	**22**	**2**	**6**	**5**	**16**	**0**	**5**	**2**	**19**	**23**	**33**	**18**	**167**

No = number of patients, [+]Degree of global developmental delay/intellectual disability (mental retardation): SP = severe to profound, MO = moderate, MI = mild, BL = borderline, [#]NO = no global developmental delay/intellectual disability (mental retardation), only defect in the motor or speech development, B-L = Brunet-Lézine test, B = Budapest Binet, W = Wechsler Intelligence Scale for Children – Fourth Edition, I = personal or telephone interview

Table 4. Classification of 241 patients with neurodevelopmental disability

Subgroups	*No (%)*
Neurodevelopmental disabilities without known prenatal, perinatal, and/or neonatal adverse events	
Global developmental delay/intellectual disability (mental retardation)	
Genetic syndromes with recognized aetiology	29 (12.0)
Children with dysmorphic features not recognized as specific syndromes	23 (9.5)
Children without recognized aetiology and dysmorphic features	45 (18.7)
Brain malformations	35 (14.5)
Inborn errors of metabolism	6 (2.5)
Leukoencephalopathies	4 (1.7)
Epileptic syndromes	4 (1.7)
Developmental (specific) language impairment	9 (3.7)
Neuromuscular disorders	12 (5.0)
Neurodevelopmental disabilities subsequent known prenatal, perinatal, and/or neonatal adverse events	
Cerebral palsy after preterm delivery	36 (14.9)
Cerebral palsy after delivery at term	20 (8.3)
Neurodevelopmental disabilities without cerebral palsy	18 (7.5)
Total	241 (100.0)

Subgroups without known prenatal, perinatal, and/or neonatal adverse events

Genetic syndromes with recognized aetiology (Table 5) comprised 29 patients, 15 boys and 14 girls (male/female ratio: 1.07) with mean age of 7.3 ± 4.7 years (age range: 1-18 years) at the last follow up. Syndromes with numerical/structural chromosomal abnormalities and single gene defects were included in this group. Trisomy 21 was found in 5 patients (Patients 1-5) and numerical anomalies of the sex chromosomes in 2 casas (Patients 16 and 17). Chromosomal structural anomalies were found in 4 boys and 6 girls (male/female ratio: 0.67, Patients 6-15) either by banding technique or FISH. FISH studies identified Prader-Willi (Patients 6-8), Williams (Patient 10) and DiGeorge (Patient 11) syndromes. Angelman syndrome occurred due to a rare de novo balanced translocation involving chromosome 15 (Patient 9). Molecular study with polymorphic short tandem repeat markers of the fibrillin-1 gene located in the region of 15q21.1 revealed that both chromosomes 15 were inherited from the father, therefore paternal uniparental disomy was proven as responsible for the Angelman syndrome. Patient 15 had a seemingly balanced translocation by G-band technique. Molecular testing was not available for cases with tuberous sclerosis (Patients 21, 22), neurofibromatosis 1 (Patient 23), VACTERL association (Patient 27) and one of the patients with Rett syndrome (Patient 19); however the diagnosis was evident on clinical grounds. Molecular genetic testing failed to reveal the aetiology of Cornelia de Lange (Patient 26) and blepharophimosis-mental retardation syndromes (Patients 28 and 29) until the time of writing. Visual impairment in 2 cases (Patients 12 and 13) and hearing loss in 2 children (Patients 13 and 24) were part of the syndromes, and epilepsy occurred in 6 patients (Table 5). Moderate intellectual disability was the most frequent (34.5%) among these patient, followed by mild (27.6%) borderline (20.7%) and severe/profound (17.2%) disability (Tables 5, 17).

Global developmental delay/intellectual disability (mental retardation) were associated **with dysmorphic features without** being **recognized as specific syndromes (Table 6)** in 23 children, 13 boys and 10 girls (male/female ratio: 1.30), and their mean age was 7.4 ± 4.0 years (age range: 2-16 years) at the last follow up. The phenotypic anomalies observed on these patients are listed in Table 6. The parents of 3 siblings (Patients 6, 7 and 8) and another child (Patient 9) were consanguineous (first cousins).

Table 5. Genetic syndromes with recognized aetiology

Patient No	Age* (years)	Sex	Diagnosis	Intellectual disability[+]	Comments
1	1	M	M. Down	MO	Trisomy 21
2	5	M	M. Down	MO	Trisomy 21
3	5	M	M. Down	MO	Trisomy 21
4	3	M	M. Down	MI	Trisomy 21
5	1	F	M. Down	BL	Trisomy 21
6	1	F	Prader-Willi syndrome	MI	15q11-13 deletion (FISH)
7	13	F	Prader-Willi syndrome	MI	15q11-13 deletion (FISH)
8	16	M	Prader-Willi syndrome	MO	15q11-13 deletion (FISH)
9	5	M	Angelman syndrome	MI	Epilepsy 45,XY,der(15;15)(q10;q10) (Balanced 15q;15q translocation)
10	8	M	Williams syndrome	BL	7q11.23 deletion (FISH)
11	7	F	DiGeorge syndrome	MI	22q11.2 deletion (FISH)
12	5	F	WAGR syndrome	SP	Visual impairment 11p13 deletion
13	6	F	HDR (hypoparathyroidism, sensorineural deafness and renal dysplasia) syndrome	SP	Visual impairment Hearing impairment 46,XX, del 10p(12.1):10qterm+
14	18	M	Ring chromosome 22	SP	Epilepsy Cerebellar vermis hypoplasia 46,XY,r(22)
15	8	F	Chromosomal abnormality	SP	Atypical autism Blepharophimosis Cerebellar vermis hypoplasia 46,XX,t(3q;16q)(2.6;2.4)
16	6	M	XXXXY syndrome	BL	
17	13	F	Triple X	BL	47, XXX, trisomy X
18	3	M	Fragile X syndrome	MI	*FMR1*gene: CGG repeat expansion (288-293 repeats)
19	15	F	Rett syndrome	MO	Epilepsy
20	6	F	Rett syndrome	MO	Epilepsy *MECP2* gene: heterozygous deletion in exon 4 (44 nucleotides)
21	9	F	Tuberous sclerosis	MO	Epilepsy
22	14	M	Tuberous sclerosis	MI	Epilepsy
23	6	M	Neurofibromatosis 1	MO	
24	13	M	Waardenburg syndrome (WS1)	BL	Hearing impairment *PAX3* gene: heterozygous deletion (c.751-757delTTCAGCT p.Phe129Glyfs*21) in exon 3
25	6	M	Myotonic dystrophy	SP	*DMPK* gene: CTG repeat expansion
26	3	M	Cornelia de Lange syndrome	MI	No mutations in *NIPBL* and *SMC1A* genes
27	5	F	VACTERL association	MO	
28	6	F	Blepharophimosis-mental retardation syndrome	BL	Patients 28 and 29 are sisters Cerebellar vermis hypoplasia
29	4	F	Blepharophimosis-mental retardation syndrome	MO	Patients 28 and 29 are sisters aCGH negative No mutation in *FOXL2* gene

No = number of patients, *Age at last follow up, M = male, F = female, [+]Degree of global developmental delay/intellectual disability (mental retardation): SP = severe to profound, MO = moderate, MI = mild, BL = borderline, aCGH = array comparative genome hybridization

Table 6. Global developmental delay/intellectual disability (mental retardation) with dysmorphic features not recognized as specific syndromes

Patient No	Age* (years)	Sex	Phenotypic anomalies	Intellectual disability[+]	Comments
1	3	F	Microcephaly, deeply-set eyes, large, long nose, short philtrum, everted lower lip, congenital heart disease: pulmonary stenosis, atrial septal defect,	SP	aCGH normal
2	3	F	Strabismus, broad nose, macrostomia, full upper lip	SP	Intrauterine growth retardation
3	4	F	Short stature, hypertelorism, epicanthi, small nose, anteversion of nares, long philtrum, high palate, low-set, abnormally modelled ears, muscle hypotonia and weakness	SP	Hearing impairment aCGH normal
4	4	M	Microcephaly, arched eyebrows, epicanthi, flat and wide nasal bridge, long philtrum, low-set, dysplastic ears	SP	
5	5	F	Hypertelorism, cleft palate, low-set, abnormally modelled ears, talipes equinovarus	SP	
6	7	M	Deeply-set eyes, abnormally modelled ears, macrostomia, thick upper lip	SP	Consanguineous parents (first cousins) Patients 6, 7 and 8 are brothers Patients 6 and 7 are twins Subtelomeric FISH: normal aCGH normal
7	7	M	Microcephaly, deeply-set eyes, dysplastic ears, macrostomia, thick upper lip	SP	Consanguineous parents (first cousins) Patients 7, 6 and 8 are brothers Patients 7 and 6 are twins
8	16	M	Deeply-set eyes, prominent, dysplastic ears, macrostomia, prominent lower jaw, truncal obesity	SP	Consanguineous parents (first cousins) Patients 8, 6 and 7 are brothers
9	7	F	Microcephaly, wide nasal bridge, epicanthi, clinodactyly, syndactyly of the toes II-III and III-IV	SP	Parental consanguinity
10	8	F	Prominent forehead, macrostomia, low-set, abnormally modelled ears, overriding toes	SP	
11	8	M	Microcephaly, strabismus, muscle hypotonia, pectus excavatum	SP	Epilepsy Visual impairment No mutations in *ARX*, *MECP2*, *FOXG1* and *CDKL5* genes
12	10	F	Microcephaly, ptosis, choanal stenosis, large nose, long fingers, partial syndactyly of toes II-III	SP	
13	11	M	Prominent forehead, flat, wide nasal bridge, epicanthi, thick upper lip, drooping lower lip, low-set ears, clinodactyly, hypermobility of small and large joints	SP	Intrauterine growth retardation
14	12	M	Deeply-set eyes, flat nasal bridge, short philtrum, macrostomia, low-set, prominent ears, short dig V, clinodactyly, hypotonia	SP	
15	16	M	Macrocephaly, narrow, elongated face, wide nasal bridge, hypertelorism, high palate	SP	Epilepsy
16	4	M	Microcephaly, frontal bossing, epicanthi, strabismus, narrow, elongated face, long philtrum, thick upper lip, abnormally shaped teeth, high palate, large, abnormally modelled ears	MO	MRI: occipital hypomyelination
17	6	M	Macrocephaly, micrognathia, anteversion of nares, pectus excavatum, hypotonia	MO	No mutation in *NSD1* gene
18	11	M	Prominent forehead, wide, flat nasal bridge, epicanthi, thick upper lip, drooping lower lip, low-set ears, clinodactyly, wide-spaced nipples, joint hypermobility	MO	
19	2	F	Microcephaly, epicanthi, short philtrum, low-set, abnormally modelled ears	MI	Epilepsy
20	2	M	Macrocephaly, large, abnormally modelled ears, decreased flexion palmar creases	MI	
21	6	M	Short palpebral fissures, dental crowding, malocclusion, polydactyly (post-axial) on the left hand, short fingers, polydactyly and syndactyly on both feet, small penis and testes	MI	
22	7	F	Microcephaly, alopecia totalis, microstomia, short philtrum, pinched nose, arachnodactyly, skeletal muscle hypoplasia	MI	
23	11	F	Short stature, hypotelorism, kyphoscoliosis, increased lumbar lordosis, bowed femur and tibia, multiple naevi, hirsutism	MI	

No = number of patients, *Age at last follow up, M = male, F = female, [+]Degree of global developmental delay/intellectual disability (mental retardation): SP = severe to profound, MO = moderate, MI = mild, aCGH = array comparative genome hybridization

Intrauterine growth retardation occurred in two patients (Patients 2 and 13). Routine chromosomal studies, *FMR1* gene testing and metabolic screening were normal for all cases and cranial MRI did not reveal any abnormalities except Patient 16, who had delayed occipital myelination. Subtelomeric FISH in one case (Patient 6) and array comparative genome hybridization in 3 children (Patients 1, 3 and 6) did not detect any abnormalities. Search for the aetiology by mutation analysis was not successful in finding any mutations in 2 cases (Patients 11 and 17). Visual loss was found in one patient (Patient 11) and hearing impairment occurred in another child (Patient 3) and 3 patients (Patients 11, 15 and 19) were epileptic. The majority of patients (65.2%, $p<0.05$) suffered from severe/profound developmental delay/intellectual disability (mental retardation) in this group (Tables 6, 17).

Global developmental delay/intellectual disability (mental retardation) occurred **without any recognized aetiology and dysmorphic features (Table 7)** in 45 patients, 23 boys and 22 girls (male/female ratio: 1.05) with a mean age of 7.7 ± 4.2 years (age range: 2-18 years) at the last follow up. Two patients were siblings (Patients 16 and 17). Routine chromosomal studies, *FMR1* gene testing and metabolic screening were normal for all cases and cranial MRI did not reveal any abnormalities. Array comparative genome hybridization was available only for a single child (Patient 16) and it was normal. Mutation analysis for 3 patients (Patients 27, 29 and 40) did not identify any abnormalities in the genes tested. Epilepsy was diagnosed in 13 out of these 45 children (28.9%). Almost half of these patients (46.7%, $p<0.05$) showed severe/profound defects in their cognitive functions, the cognitive impairment was mild in 28.9%, moderate in 15.5% and borderline in 8.9% (Tables 7, 17).

Brain malformations (Table 8) were revealed by MRI in 35 children, 18 boys and 17 girls (male/female ratio: 1.06). Their mean age was 7.2 ± 3.5 years (age range: 0.54-18 years) at the last follow up. Two patients (Patients 31 and 32) who were born from consanguineous parents and had pontocerebellar hypoplasia type 1 died in infancy, and another patient (Patient 27) with bilateral schizencephaly died at the age of 6 years.

Table 7. Global developmental delay/intellectual disability (mental retardation) without recognized aetiology and dysmorphic features

Patient No	Age* (years)	Sex	Intellectual disability[+]	Comments
1	3	F	SP	
2	3	F	SP	
3	3	M	SP	
4	4	F	SP	
5	4	F	SP	Epilepsy
6	4	F	SP	
7	5	M	SP	
8	5	F	SP	
9	5	F	SP	Epilepsy
10	5	F	SP	
11	6	M	SP	
12	8	M	SP	
13	8	M	SP	Epilepsy
14	8	F	SP	
15	8	M	SP	
16	8	M	SP	Patients 16 and 17 are siblings aCGH normal
17	18	F	SP	Patients 17 and 16 are siblings
18	9	F	SP	
19	10	F	SP	
20	17	F	SP	
21	18	M	SP	Epilepsy
22	4	M	MO	
23	7	M	MO	
24	8	M	MO	
25	9	M	MO	
26	9	F	MO	Epilepsy
27	9	M	MO	Epilepsy No mutation in *SCN1A* gene
28	10	M	MO	
29	2	M	MI	Epilepsy No mutation in *SLC2A1* gene
30	3	M	MI	
31	5	M	MI	
32	5	F	MI	
33	5	M	MI	
34	6	M	MI	
35	7	M	MI	
36	8	M	MI	
37	11	M	MI	Epilepsy
38	11	F	MI	
39	13	F	MI	Epilepsy
40	13	F	MI	Epilepsy No mutation in *PCDH19* gene
41	14	F	MI	Epilepsy
42	2	F	BL	
43	5	F	BL	Epilepsy
44	7	M	BL	
45	16	F	BL	Epilepsy

No = number of patients, *Age at last follow up, M = male, F = female, [+]Degree of global developmental delay/intellectual disability (mental retardation): SP = severe to profound, MO = moderate, MI = mild, BL = borderline, aCGH = array comparative genome hybridization

Table 8. Brain malformations

Patient No	Age* (years)	Sex	Malformation	Intellectual disability+	Comments
1	14	F	Holoprosencephaly	SP	Visual impairment
2	13	M	Agenesis of the corpus callosum	SP	Epilepsy
3	2	M	Corpus callosum and vermis hypoplasia	MI	
4	11	M	Corpus callosum and vermis hypoplasia	SP	
5	3	F	Corpus callosum, vermis and optic nerve hypoplasia	BL	Visual impairment
6	9	F	Corpus callosum, vermis and optic nerve hypoplasia	MI	Epilepsy Visual impairment
7	4	F	Corpus callosum, vermis and white matter hypoplasia	SP	Epilepsy
8	1	M	Agenesis of the corpus callosum Greig cephalosyndactily syndrome	BL	*GLI3* gene: heterozygous deletion of exon 2
9	4	F	Acrocallosal syndrome	MO	Visual impairment Hearing impairment aCGH normal No mutation in *KIF7* and *GLI3* genes
10	7	F	Wide cavum septi pellucidi	MO	
11	13	F	Wide cavum septi pellucidi	SP	
12	7	F	Microcephaly	SP	
13	7	F	Microcephaly	SP	
14	1	M	Micrencephaly with simplified gyral pattern, hypoplasia of the corpus callosum and cerebellum	SP	Epilepsy Visual impairment Hearing impairment
15	2	M	Megalencephaly	SP	
16	5	M	Megalencephaly	BL	
17	13	F	Bilateral pachygyria	SP	
18	11	M	Lissencephaly (agyria-pachygyria)	SP	Cerebral palsy: spastic quadriplegia Epilepsy Visual impairment Hearing impairment *LIS1*gene : heterozygous deletion (c.83-84delAT, p.Tyr28Phefs*31)
19	12	F	Subcortical band heterotopia	MI	Epilepsy *DCX* gene: heterozygous deletion (c.200delG, p.Ile68Leufs*87)
20	11	M	Bilateral polymicrogyria	SP	Cerebral palsy: spastic quadriplegia
21	12	M	Bilateral polymicrogyria and mega corpus callosum	MO	Cerebral palsy: spastic hemiplegia on the left side Epilepsy aCGH normal No mutation in *VPS13B* gene
22	14	M	Schizencephaly on the left side and polymicrogyria on the right side	MI	Cerebral palsy: spastic hemiplegia on the right side Epilepsy
23	8	F	Schizencephaly on the left side	NO	Cerebral palsy: spastic hemiplegia on the right side
24	1	M	Bilateral schizencephaly	SP	Cerebral palsy: spastic quadriplegia Epilepsy
25	2	F	Bilateral schizencephaly	SP	Hypotonic cerebral palsy
26	5	M	Bilateral schizencephaly	SP	Cerebral palsy: spastic quadriplegia Epilepsy Visual impairment
27	6	M	Bilateral schizencephaly	SP	Cerebral palsy: spastic quadriplegia Epilepsy Visual impairment Died at the age of 6 years
28	11	F	Bilateral opercular hypoplasia	MI	Pseudobulbar paresis
29	18	M	Vermis and optic nerve hypoplasia	MO	
30	5	M	Molar tooth malformation (Joubert syndrome)	SP	Visual impairment *CEP290* gene: Allele 1: mutation in exon 38 (c.5182G>T, p.Glu1728Stop) from the father Allele 2: mutation in exon 46 (c.6277delG, p.Val2093SerfsStop4) from the mother

Table 8. Brain malformations (continued)

31	0,4 (4,5m)	M	Pontocerebellar hypoplasia type 1	SP	Consanguineous parents Visual impairment Hearing impairment Generalized hypotonia No development *EXOSC3* gene: homozygous mutation (c.92G>C p.Gly31Ala) in exon 1 Died at the age of 4,5 months
32	0,6 (7m)	F	Pontocerebellar hypoplasia type 1	SP	Consanguineous parents Visual impairment Hearing impairment Generalized hypotonia No development *EXOSC3* gene: homozygous mutation (c.92G>C p.Gly31Ala) in exon 1 Died at the age of 7 months
33	3	F	Pontocerebellar hypoplasia	SP	Generalized hypotonia *CASK* gene: heterozygous mutation (c.1034delG p.Arg345Lysfs*24) in exon 12
34	7	F	Pontocerebellar hypoplasia	SP	Cerebral palsy: spastic quadriplegia Epilepsy Visual impairment Hearing impairment No mutation in *CASK* and *TSEN54* genes
35	10	M	Vena Galeni aneurysm	BL	

No = number of patients, *Age at last follow up, M = male, F = female, [+]Degree of global developmental delay/intellectual disability (mental retardation): SP = severe to profound, MO = moderate, MI = mild, BL = borderline, NO = no global developmental delay/intellectual disability (mental retardation), only defect in the motor development, aCGH = array comparative genome hybridization

The various types of malformations are listed in Table 8. Anomalies of the corpus callosum (Patients 2-9) and schizencephaly (Patients 22-27) were the most frequent malformations in this series of patients. Chromosomal abnormalities or inborn errors of metabolism were not found by routine testing. Array comparative genome hybridization failed to reveal any abnormalities in 2 cases (Patients 9 and 21). Molecular genetic studies identified mutations in Patients 8, 18, 19, 30, 31/32, and 33 in the *GLI3*, *LIS1*, *DCX*, *CEP290*, *EXOSC3* and *CASK* genes, respectively, which means that in 20% of brain malformations the molecular background was detected (Table 8). Mutation analysis in another 3 patients (Patients 9, 21 and 34) did not identify any mutations in the genes tested (Table 8). Cerebral palsy occurred in 10 cases (28.6%) visual impairment in 12 (34.3%), and hearing loss in 6 patients (17.1%). Epileptic seizures appeared in 12 children (34.3%). As shown in Tables 8 and 17, the vast majority (60.0%, $p<0.05$) of patients with brain malformations had severe/profound global developmental delay/intellectual disability (mental retardation).

Inborn errors of metabolism (Table 9) were diagnosed in 6 cases, 5 boys and one girl (male/female ratio: 5:1), their mean age was 8.5 ± 7.8 years (age range: 2-22 years) at the last follow up. Mutations in the *HPRT* gene were identified in three patients (Patients 1-3) with the X-linked Lesch-Nyhan syndrome, two of them were

relatives. Mutations in the mitochondrial DNA occurred in another three patients, one with Leigh syndrome (Patient 4) and 2 with Kearns-Sayre syndrome (Patients 5 and 6). Visual impairment was observed in all 3 children with mitochondrial disease and hearing impairment appeared in 2 patients (Patients 5 and 6) with Kearns-Sayre syndrome. The child with Leigh syndrome (Patient 4) died at the age of 4 years and one of the Kearns-Sayre cases (Patient 6) at the age of 22 years. Two patients in this group (Patients 3 and 5) had severe/profound intellectual disability; the cognitive defect was moderate in 3 children (Patients 1, 2 and 4) and mild in one (Patient 6) child (Tables 9, 17).

Table 9. Inborn errors of metabolism

Patient No	Age* (years)	Sex	Diagnosis	Intellectual disability^{+}	Comments
1	2	M	Lesch-Nyhan syndrome	MO	*HPRT* gene: hemizygous mutation (c.609+1G>A) with change in the first base after exon 8, probably leading to defective splicing
2	4	M	Lesch-Nyhan syndrome	MO	*HPRT* gene: hemizygous mutation (c.151C>T p.Arg51*) in exon 3 The mothers of Patients 2 and 3 are sisters
3	5	M	Lesch-Nyhan syndrome	SP	*HPRT* gene: hemizygous mutation (c.151C>T p.Arg51*) in exon 3 The mothers of Patients 3 and 2 are sisters
4	4	F	Leigh syndrome	MO	Visual impairment Mitochondrial *ND5* gene mutation (m.13513G>A), 60-70% heteroplasmy rate Died at the age of 4 years
5	14	M	Kearns-Sayre syndrome	SP	Epilepsy Visual impairment Hearing impairment Mitochondrial DNA (m.8646_15647del) deletion Died at the age of 14 years
6	22	M	Kearns-Sayre syndrome	MI	Visual impairment (pigmentary degeneration of the retina) Hearing impairment Mitochondrial DNA deletion Died at the age of 22 years

No = number of patients, *Age at last follow up, M = male, F = female, $^{+}$Degree of global developmental delay/intellectual disability (mental retardation): SP = severe to profound, MO = moderate, MI = mild

Leukoencephalopathies (Table 10) were diagnosed in 4 cases, 2 boys and 2 girls (male/female ratio: 1.00). Their mean age was 4.4 ± 4.0 years (age range: 0.5-8 years) at the last follow up. Clinical features and abnormal brain MRI were the clues to the diagnosis in Alexander disease (Patient 1), X-linked adrenoleukodystrophy (Patient 2) and Krabbe disease (Patient 3). Metabolic tests (Patients 2 and 3) and mutation analysis of the *GFAP*, *ABCD1* and *GALC* genes (Patients 1, 2 and 3, respectively) confirmed the diagnosis in 3 cases. In spite of an extended molecular genetic search (mutation testing of the *PLP1*, *GJA12/GJC, FAM126A2* and *POLR3A/POLR3B* genes) the aetiology remained unknown in Patient 4. Visual impairment was obvious in Patient 2 with X-linked adrenoleukodystrophy at the last follow up and epilepsy occurred in

Patients 2 and 3. Patient 1 with infantile form of Alexander disease died at the age of one year. The global developmental delay/intellectual disability was severe/profound in patients with Alexander disease and X-linked adrenoleukodystrophy, moderate in the infant with Krabbe disease at 6 months of age and mild in Patient 4, who suffered from leukoencephalopathy of unknown aetiology (Tables 10, 17).

Table 10. Leukoencephalopathies

Patient No	Age* (years)	Sex	Diagnosis	Intellectual disability^{+}	Comments
1	1	F	Alexander disease	SP	*GFAP* gene: heterozygous mutation (c.1175C>T p.Thr392Ile) in exon 8 Died at the age of 1 year
2	8	M	X-linked adrenoleukodystrophy	SP	Epilepsy Visual impairment Accumulation of very long chain fatty acids *ABCD1* gene: mutation (c.631C>T p.Leu211Phe) in exon 1
3	0.5 (6m)	F	Krabbe disease	MO	Epilepsy Low activity of galactocerebroside-beta galactosidase *GALC* gene: Allele 1: heterozygous mutation (c.1586C>T p.Thr529Met) in exon 14 Allele 2: heterozygous deletion of exons 11-17
4	8	M	Leukoencephalopathy with unknown aetiology	MI	No mutation in *PLP1, GJA12/GJC, FAM126A2, POLR3A* and *POLR3B* genes

No = number of patients, *Age at last follow up, M = male, F = female, $^{+}$Degree of global developmental delay/intellectual disability (mental retardation): SP = severe to profound, MO = moderate, MI = mild

Epileptic syndromes (Table 11) were responsible for the neurodevelopmental disability in 4 patients, 2 girls and 2 boys (male/female ratio: 1.00) with mean age of 4.8 ± 4.5 years (age range 1-11 years). Devastating infantile migrating partial seizures in association with visual, hearing impairment and severe/profound disability occurred in 2 girls (Patients 1 and 2) and West syndrome started in infancy in 2 boys (Patients 3 and 4) who were left with mild cognitive disability later (Tables 11, 17). The aetiology of the epileptic syndromes was not identified.

Table 11. Epileptic syndromes

Patient No	Age* (years)	Sex	Diagnosis	Intellectual disability^{+}	Comments
1	1	F	Malignant migrating partial seizures in infancy (unknown aetiology)	SP	Visual impairment Hearing impairment
2	11	F	Malignant migrating partial seizures in infancy (unknown aetiology)	SP	Visual impairment Hearing impairment
3	2	M	West syndrome (unknown aetiology)	MI	
4	5	M	West syndrome (unknown aetiology)	MI	

No = number of patients, *Age at last follow up, M = male, F = female, $^{+}$Degree of global developmental delay/intellectual disability (mental retardation): SP = severe to profound, MI = mild

Developmental (specific) language impairment (Table 12) was found in 9 boys. Their mean age was 7.7 ± 2.8 years (age range: 4-12 years) at the last follow up. Four boys (Patients 1-4) had borderline cognitive impairment, while intellectual

disability was not revealed by neuropsychological testing in 5 boys (Patients 5-9, Tables 12, 17). No aetiology was found in any of these patients (Table 12).

Table 12. Developmental (specific) language impairment

Patient No	Age* (years)	Sex	Intellectual disability[+]
1	5	M	BL
2	8	M	BL
3	10	M	BL
4	12	M	BL
5	4	M	NO
6	5	M	NO
7	6	M	NO
8	9	M	NO
9	10	M	NO

No = number of patients, *Age at last follow up, M = male [+]Degree of global developmental delay/intellectual disability (mental retardation): BL = borderline, NO = no global developmental delay/intellectual disability (mental retardation), only delay in the speech development

Neuromuscular disorders (Table 13) comprised a group of 12 children, 7 boys and 5 girls (male/female ratio: 1.40). The mean age was 9.8 ± 5.8 years (age range 4-21 years) at the last follow up. Patient 1 with spinal muscular atrophy type 1 was supported by mechanical ventilation and died at the age of 4 years. Patients 2-4 suffered from spinal muscular atrophy type 2 and had serious defect in their motor development. Delay in the motor development was also observed in patients with congenital myasthenia (Patient 5), nemaline (Patients 6 and 7), myotubular (Patient 8) and centronuclear (Patient 9) myopathies. Two boys (Patients 10 and 11) had Duchenne muscular dystrophy and a young man (Patient 12) was diagnosed with facioscapulohumeral dystrophy. Molecular genetic studies of the *SMN1, CHRNE, NEB, MTM1* and *DMD* genes confirmed the diagnoses as shown in Table 13. Contraction of the D4Z4 repeat on chromosome 4q35 was identified in the patient with facioscapulohumeral dystrophy. Molecular testing was not successful so far only for 2 children (Patients 7 and 9). There was no intellectual disability in 58.4% ($p<0.05$) of the patients; it was mild in 25.0%, moderate in 8.3% and severe/profound also in 8.3% (Tables 13, 17).

Table 13. Neuromuscular disorders

Patient No	Age* (years)	Sex	Diagnosis	Intellectual disability[+]	Comments
1	4	F	Spinal muscular atrophy type 1	NO	*SMN1* gene: homozygous deletion exons 7 and 8 Died at the age of 4 years
2	6	F	Spinal muscular atrophy type 2	NO	*SMN1* gene: homozygous deletion exons 7 and 8
3	7	F	Spinal muscular atrophy type 2	NO	*SMN1* gene: homozygous deletion exons 7 and 8
4	12	F	Spinal muscular atrophy type 2	NO	*SMN1* gene: homozygous deletion exons 7 and 8
5	8	F	Congenital myasthenia	NO	*CHRNE* gene*:* homozygous mutation (c.1267delG p.422fs*) in exon 12
6	6	M	Nemaline myopathy	MI	*NEB* gene: Allele 1: mutation in exon 174 (c.24527_24528delCT p.P8176fs) from the father Allele 2: mutation in exon 171 (c.24250_24253dupGTCA p.T8085fs) from the mother
7	6	M	Nemaline myopathy	NO	No mutation in *ACTA1, TPM2, TPM3 and NEB* genes
8	7	M	Myotubular myopathy (X-linked)	SP	*MTM1* gene: heterozygous mutation (c.1315_1316insT p.439fs*) in exon 12
9	5	M	Centronuclear myopathy	NO	No mutation in *BIN1* gene
10	19	M	Duchenne muscular dystrophy	MI	*DMD* gene: deletion exons 45 to 52 Patients 10 and 11 are brothers
11	21	M	Duchenne muscular dystrophy	MO	*DMD* gene: deletion exons 45 to 52 Patients 11 and 10 are brothers
12	16	M	Facioscapulohumeral dystrophy	MI	Contraction of the D4Z4 repeat on chromosome 4q35

No = number of patients, *Age at last follow up, M = male, F = female, [+]Degree of global developmental delay/intellectual disability (mental retardation): SP = severe to profound, MO = moderate, MI = mild, NO = no global developmental delay/intellectual disability (mental retardation), only defect in the motor development

Subgroups with known prenatal, perinatal, and/or neonatal adverse events

Cerebral palsy after preterm delivery (Table 14) was diagnosed in 36 children, 20 boys and 16 girls (male/female ratio: 1.25). Their mean age was 5.8 ± 3.7 years (age range 1-20 years) at the time of the last follow up. Eleven patients (30.6%, 6 boys and 5 girls) were born from twin pregnancy, one of them (Patient 4) after in vitro fertilization. Intrauterine growth retardation occurred in 2 cases (Patients 5 and 6). Spastic quadriplegia was observed in 19 patients (52.8%), spastic diplegia in 9 (25%) and spastic hemiplegia in 7 children (19.4%). Mixed form of cerebral palsy was found in one patient. Some details of perinatal data, adverse events and findings by neuroimaging can be found in Table 14. Visual loss occurred in 7 patients (19.4%), hearing impairment in 4 (11.1%) and epilepsy in 10 (27.8%) children (Table 14). The global developmental delay/intellectual disability (mental retardation) was severe/profound in 41.7% ($p<0.05$), moderate, mild and borderline in 5.6%, 11.1% and 22.2%, respectively, while 19.4% of the patients showed normal intellectual abilities (Tables 14, 17).

Table 14. Cerebral palsy after preterm delivery

Patient No	Age* (years)	Sex	Gestational age (weeks)	Birth weight (g)	Adverse events Abnormalities found by brain imaging	Type of cerebral palsy	Intellectual disability+	Comments
1	1	M	34	2420	Twin pregnancy, twin-twin transfusion syndrome Hydranencephaly	Spastic quadriplegia	SP	Epilepsy Visual impairment Hearing impairment
2	2	F	37	2250	Twin pregnancy Intrapartum asphyxia	Spastic quadriplegia	SP	Visual impairment Hearing impairment Died at the age of 2 years
3	2	M	33	1490	Intrauterine CMV infection	Spastic quadriplegia	SP	Epilepsy Visual impairment Hearing impairment
4	2	M	31	1700	IVF, twin pregnancy PWMI	Spastic quadriplegia	SP	Epilepsy West syndrome
5	3	M	31	990	Intrauterine growth retardation GMH-IVH, PWMI	Spastic quadriplegia	SP	Epilepsy
6	4	F	31	860	Intrauterine growth retardation PWMI	Spastic quadriplegia	SP	
7	4	F	30	1340	GMH-IVH, PWMI Ventriculitis	Spastic quadriplegia	SP	Epilepsy Visual impairment Hearing impairment
8	6	M	30	1510	Twin pregnancy PWMI	Spastic quadriplegia	SP	
9	7	M	35	2180	GMH-IVH, PWMI Hydrocephalus	Spastic quadriplegia	SP	Epilepsy
10	8	F	28	1190	GMH-IVH, PWMI	Spastic quadriplegia	SP	
11	8	M	30	1690	PWMI	Spastic quadriplegia	SP	
12	9	M	33	1320	PWMI	Spastic quadriplegia	SP	
13	10	F	27	1380	PWMI	Spastic quadriplegia	SP	Epilepsy
14	11	F	33	1290	PWMI	Spastic quadriplegia	SP	Visual impairment
15	2	F	30	1490	PWMI	Spastic quadriplegia	MO	
16	2	M	31	1900	PWMI	Spastic quadriplegia	MI	
17	3	M	32	1650	PWMI	Spastic quadriplegia	MI	
18	6	M	32	1910	Twin pregnancy PWMI	Spastic quadriplegia	MI	Epilepsy
19	6	M	35	2460	PWMI	Spastic quadriplegia	BL	
20	2	M	32	1800	PWMI	Spastic diplegia	BL	
21	4	M	31	1400	PWMI	Spastic diplegia	BL	
22	5	F	26	960	PWMI	Spastic diplegia	BL	
23	6	F	29	1460	Twin pregnancy PWMI	Spastic diplegia	BL	
24	8	M	28	990	PWMI	Spastic diplegia	BL	Visual impairment
25	10	M	30	1200	Twin pregnancy PWMI	Spastic diplegia	BL	

Table 14. Cerebral palsy after preterm delivery (continuation)

26	5	F	31	1900	PWMI	Spastic diplegia	NO	
27	7	F	30	1290	Twin pregnancy PWMI	Spastic diplegia	NO	
28	6	M	31	2380	Diabetic foetopathy PWMI	Spastic diplegia	NO	
29	3	M	29	1430	GMH-IVH Periventricular haemorrhagic infarction on the right side Hydrocephalus	Spastic hemiplegia on the left side	MO	
30	10	F	30	1300	Asymmetrical PWMI more severe on the right side	Spastic hemiplegia on the left side	MI	
31	8	M	27	990	Asymmetrical PWMI more severe on the left side	Spastic hemiplegia on the right side	BL	Epilepsy
32	4	F	30	1780	Twin pregnancy PWMI	Spastic hemiplegia on the right side	NO	
33	5	F	26	840	GMH-IVH Periventricular haemorrhagic infarction on the right side	Spastic hemiplegia on the left side	NO	
34	6	M	32	1770	Twin pregnancy Intrapartum asphyxia Asymmetrical PWMI more severe on the right side	Spastic hemiplegia on the left side	NO	
35	20	F	32	1720	Placental abruption Asymmetric PWMI more severe on the right side	Spastic hemiplegia on the left side	NO	Epilepsy
36	3	F	23	630	Twin pregnancy GMH-IVH	Mixed form (spastic and extrapyramidal) of cerebral palsy	SP	Visual impairment

No = number of patients, *Age at last follow up, M = male, F = female, [+]Degree of global developmental delay/intellectual disability (mental retardation): SP = severe to profound, MO = moderate, MI = mild, BL = borderline, NO = no global developmental delay/intellectual disability (mental retardation), only defect in the motor development, CMV = cytomegalovirus, IVF = in vitro fertilisation, PWMI = periventricular white matter injury, GMH-IVH = germinal matrix-intraventricular haemorrhage

Cerebral palsy after delivery at term (Table 15) developed in 20 children, 10 boys and 10 girls (male/female ratio: 1.00), one girl (Patient 12) was born from twin pregnancy. The mean age was 6.4 ± 3.6 years (age range: 2-16 years) in this group at the last follow up. Intrauterine growth retardation occurred in 3 cases (15.0%, Patients 11, 13 and 19). Spastic quadriplegia was diagnosed in 10 (50.0%), spastic hemiplegia in 9 (45.0%) patients and extrapyramidal type of cerebral palsy was found in one case. The details of perinatal data, suspected aetiology and findings by neuroimaging can be found in Table 15. Visual loss occurred in 3 patients (15.0%) hearing impairment in 2 (10.0%) and epilepsy in 4 (20.0%) children (Table 15). The global developmental delay/intellectual disability (mental retardation) was severe/profound in 40.0%, moderate, mild and borderline in 15.0%, 5.0% and 10.0%, respectively, while 30.0% of the patients showed normal intellectual abilities (Tables 15, 17).

Table 15. Cerebral palsy after delivery at term

Patient No	Age* (years)	Sex	Gestational age (weeks)	Birth weight (g)	Adverse events Abnormalities found by brain imaging	Type of cerebral palsy	Intellectual disability[+]	Comments
1	16	M	39	2760	Intrapartum asphyxia	Spastic quadriplegia	SP	Epilepsy
2	11	F	41	3700	Intrapartum asphyxia	Spastic quadriplegia	SP	
3	2	M	38	3000	Intrapartum asphyxia	Spastic quadriplegia	SP	
4	5	F	41	2600	Intrapartum asphyxia	Spastic quadriplegia	SP	Epilepsy
5	4	M	39	4000	Intrapartum asphyxia	Spastic quadriplegia	SP	
6	9	M	37	3200	Intrapartum asphyxia	Spastic quadriplegia	SP	Epilepsy Visual impairment Hearing impairment
7	2	F	40	3700	Intrapartum asphyxia	Spastic quadriplegia	SP	Visual impairment Hearing impairment
8	7	F	37	3040	Neonatal bacterial meningitis	Spastic quadriplegia	SP	
9	3	M	41	3970	Intrapartum asphyxia	Spastic quadriplegia	MO	Visual impairment
10	8	M	39	2870	Neonatal bacterial meningitis	Spastic quadriplegia	NO	Epilepsy
11	11	F	39	1930	Intrauterine growth retardation	Spastic hemiplegia on the left side	MO	
12	3	F	37	2750	Twin pregnancy Asymmetrical PWMI more severe on the right side	Spastic hemiplegia on the left side	MI	
13	9	F	40	2400	Intrauterine growth retardation	Spastic hemiplegia on the right side	BL	
14	3	M	40	3310	Intrapartum asphyxia	Spastic hemiplegia on the right side	BL	
15	7	F	39	3200	Neonatal stroke on the right side	Spastic hemiplegia on the left side	NO	
16	4	F	40	3350	Neonatal stroke on the right side	Spastic hemiplegia on the left side	NO	
17	8	M	40	3140	Neonatal infection	Spastic hemiplegia on the left side	NO	
18	6	M	38	3430	Intrapartum asphyxia Neonatal stroke on the left side	Spastic hemiplegia on the right side	NO	
19	3	F	38	2390	Intrauterine growth retardation	Spastic hemiplegia on the right side	NO	
20	7	M	38	3200	Intrapartum asphyxia	Extrapyramidal cerebral palsy	MO	

No = number of patients, *Age at last follow up, M = male, F = female, [+]Degree of global developmental delay/intellectual disability (mental retardation): SP = severe to profound, MO = moderate, MI = mild, BL = borderline, NO = no global developmental delay/intellectual disability (mental retardation), only defect in the motor development, PWMI = periventricular white matter injury

Neurodevelopmental disabilities subsequent known prenatal, perinatal, and/or neonatal adverse events but **without cerebral palsy (Table 16)** comprised 18 patients, 7 boys and 11 girls (male/female ratio: 0.64) with a mean age of 8.2 ± 5.3

years (age range: 2-20 years). Intrauterine growth retardation occurred in 3 cases (16.67%, Patients 9, 14 and 15). The details of perinatal data, suspected aetiology and findings by neuroimaging can be found in Table 16. Visual impairment occurred in 3 patients (16.7%) hearing loss in 2 (11.1%) and epilepsy in 2 (11.1%) children (Table 16). The global developmental delay/intellectual disability (mental retardation) was severe/profound, or mild in 44.4% ($p<0.05$), while the classification criteria excluded patients with normal intellectual abilities from this subgroup (Tables 16, 17).

Table 16. Neurodevelopmental disabilities subsequent known prenatal, perinatal, and/or neonatal adverse events without cerebral palsy

Patient No	Age* (years)	Sex	Gestational age	Birth weight (g)	Adverse events Abnormalities found by brain imaging	Intellectual disability[+]	Comments
1	7	F	36	2300	Preeclampsia, placental insufficiency	SP	Epilepsy
2	5	M	27	740	Prematurity GMH-IVH, PWMI	SP	Visual impairment
3	9	F	30	1400	Prematurity PWMI	SP	Visual impairment
4	9	F	?	2500	Foetal alcohol syndrome	SP	
5	3	F	23	630	Prematurity GMH-IVH, PWMI	SP	Visual impairment
6	18	M	36	2380	Prematurity	SP	
7	20	F	41	3900	Neonatal bacterial meningitis	SP	
8	3	F	40	3420	Intrauterine CMV infection	SP	Hearing impairment
9	17	M	40	2520	Intrauterine growth retardation	MO	Epilepsy
10	10	F	38	3440	Neonatal asphyxia	MI	
11	6	F	39	4750	Intrapartum asphyxia	MI	
12	5	F	37	2220	Twin-twin transfusion syndrome	MI	
13	6	M	36	2280	Intrapartum asphyxia	MI	
14	9	F	38	1980	Intrauterine growth retardation	MI	
15	2	M	34	1800	Intrauterine growth retardation Prematurity Intrapartum asphyxia	MI	
16	5	M	38	3370	Threatened miscarriage	MI	
17	3	F	39	2800	Intrauterine CMV infection	MI	Hearing impairment
18	10	M	35	2270	Placental abruption Prematurity Intrapartum asphyxia	BL	

No = number of patients, *Age at last follow up, M = male, F = female, [+]Degree of global developmental delay/intellectual disability (mental retardation): SP = severe to profound, MO = moderate, MI = mild, BL = borderline, CMV = cytomegalovirus, GMH-IVH = germinal matrix-intraventricular haemorrhage, PWMI = periventricular white matter injury

Distribution of different severity categories of global developmental delay/intellectual disability (mental retardation) in the various subgroups of neurodevelopmental disabilities in our series of patients is demonstrated on Table 17. The cognitive disability was severe/profound in 41.5% ($p<0.05$) of the total of 241 patients; it was moderate, mild or borderline in 14.5%, 21.2% and 12.0%, respectively. Defect only in the motor or speech development without global developmental delay/intellectual disability (mental retardation) was found in 10.8% of patients, the majority of them occurred in the groups with developmental language impairment, neuromuscular disorders and cerebral palsy (Table 17).

The occurrence of cerebral palsy (27.4% of the total 241 patients), epilepsy (23.6%), visual (14.0%) and hearing (8.7%) impairment, the combination of these complications and their distribution in various categories of global developmental delay/intellectual disability (mental retardation) are demonstrated on Table 18. It can be seen that cerebral palsy, epilepsy, sensory losses and their combination were most common in patients with severe/profound cognitive defect (Table 18).

Since the classification of patients into various subgroups rested mainly on aetiological bases, the aetiological yield was very high in certain subgroups (i.e. genetic syndromes with recognized aetiology, or neurodevelopmental disabilities subsequent known prenatal, perinatal, and/or neonatal adverse events, etc.), while the aetiology remained unknown in all patients in other subgroups (i.e. children with dysmorphic features not recognized as specific syndromes, or developmental language impairment, etc.). Overall the aetiology of neurodevelopmental disabilities was identified in 66.4% of the 241 children (Table 19). Well documented genetic diagnosis (chromosomal numerical/structural abnormalities, or single gene defects) was established in 19.5% of the entire study population. This figure grew up to 28.1% if the number of positive test results was related only to the first major group of patients without known prenatal, perinatal, and/or neonatal adverse events.

Table 17. Distribution of severity of global developmental delay/intellectual disability (mental retardation) in different subgroups of patients with neurodevelopmental disability

Patient subgroups	No. of patients in different categories of global developmental delay/intellectual disability (mental retardation)[+]					
	SP[+]	**MO[+]**	**MI[+]**	**BL[+]**	**NO[#]**	**Total**
	No (%)	No (%)	No (%)	No (%)	No (%)	**No (%)**
No adverse events						
Global developmental delay/intellectual disability						
Genetic syndromes with recognized aetiology	5 (17.2)	10 (34.5)	8 (27.6)	6 (20.7)	0	**29 (100)**
Children with dysmorphic features not recognized as specific syndromes*	15 (65.2)[a]	3 (13.1)	5 (21.7)	0	0	**23 (100)**
Children without recognized aetiology or dysmorphic features*	21 (46.7)[a]	7 (15.5)	13 (28.9)	4 (8.9)	0	**45 (100)**
Brain malformations*	21 (60.0)[a]	4 (11.4)	5 (14.3)	4 (11.4)	1 (2.9)	**35 (100)**
Inborn errors of metabolism	2	3	1	0	0	**6**
Leukoencephalopathies	2	1	1	0	0	**4**
Epileptic syndromes	2	0	2	0	0	**4**
Developmental (specific) language impairment	0	0	0	4	5	**9**
Neuromuscular disorders*	1 (8.3)	1 (8.3)	3 (25.0)	0	7 (58.4)[a]	**12 (100)**
Adverse events (prenatal, perinatal, neonatal)						
Cerebral palsy after preterm delivery*	15 (41.7)[a]	2 (5.6)	4 (11.1)	8 (22.2)	7 (19.4)	**36 (100)**
Cerebral palsy after delivery at term	8 (40.0)	3 (15.0)	1 (5.0)	2 (10.0)	6 (30.0)	**20 (100)**
Neurodevelopmental disabilities without cerebral palsy*	8 (44.4)[a]	1 (5.6)	8 (44.4)[a]	1 (5.6)	0	**18 (100)**
Total*	**100 (41.5)[a]**	**35 (14.5)**	**51 (21.2)**	**29 (12.0)**	**26 (10.8)**	**241 (100)**

No = number of patients, % = percentage of the total number of patients in each subgroup, [+]Degree of global developmental delay/intellectual disability: SP = severe to profound, MO = moderate, MI = mild, BL = borderline, [#]NO = no global developmental delay/intellectual disability, only defect in the motor or speech development,
* = The distribution of the degrees of global developmental delay/intellectual disability significantly differs from uniform distribution ($p<0.05$),
[a] = Largest deviation from the uniform distribution (maximal Pearson residual)

Table 18. Distribution of cerebral palsy, epilepsy, visual and hearing impairments in different categories of global developmental delay/intellectual disability

	No. of patients with various impairments in different categories of global developmental delay/intellectual disability[+]					
	SP[+]	**MO[+]**	**MI[+]**	**BL[+]**	**NO[#]**	**Total**
	No (%)	No (%)	No (%)	No (%)	No (%)	**No (%)**
Cerebral palsy	30 (12.4)	6 (2.5)	6 (2.5)	10 (4.2)	14 (5.8)	**66 (27.4)**
Epilepsy	30 (12.4)	8 (3.3)	14 (5.8)	3 (1.2)	2 (0.8)	**57 (23.6)**
Visual impairment	27 (11.2)	3 (1.2)	2 (0.8)	2 (0.8)	0	**34 (14.0)**
Hearing impairment	17 (7.1)	1 (0.4)	2 (0.8)	1 (0.4)	0	**21 (8.7)**
Two of these impairments	14 (5.8)	3 (1.2)	4 (1.6)	2 (0.8)	2 (0.8)	**25 (10.2)**
Three of these impairments	8 (3.3)	0	0	0	0	**8 (3.3)**
Four of these impairments	6 (2.5)	0	0	0	0	**6 (2.5)**

No = number of patients, % = percentage of the total 241 patients, [*]Degree of global developmental delay/intellectual disability (mental retardation): SP = severe to profound, MO = moderate, MI = mild, BL = borderline. [#]NO = no global developmental delay/intellectual disability (mental retardation), only defect in the motor or speech development

Table 19. Association between categories of global developmental delay/intellectual disability and recognized aetiology

Degree of disability[+]	**Aetiology detected** No	**Total** No	%
SP[+]	64	100	64.0
MO[+]	25	35	71.4
MI[+]	30	51	58.8
BL[+]	21	29	72.4
NO[#]	20	26	76.9
Total	160	241	**66.4**

No = number of patients, % = percentage of patients in each category,
[+]Degree of global developmental delay/intellectual disability (mental retardation):
SP = severe to profound, MO = moderate, MI = mild, BL = borderline,
[#]NO = no global developmental delay/intellectual disability (mental retardation),
only defect in the motor or speech development

DISCUSSION

This retrospective study presents the results of an extensive investigation into the profile of neurodevelopmental disabilities in a cohort of children. We followed the recent concept of Shevell and patients with single domain disabilities, such as isolated motor defect, or developmental language disorder without intellectual disability were also included in the study.[1,2,9] Classification of patients into two major groups based on the absence or presence of definite proof of prenatal, perinatal, and/or neonatal adverse events seems to be justified, because detailed analysis of the prenatal/perinatal/neonatal history can shape the direction of evaluation and has a significant role in determining the aetiology of neurodevelopmental disabilities.[4,21,26] Applying this classification scheme aetiology was found in 66.4% of patients. Comparison with other data reported in the literature appears to be difficult as referral pattern, inclusion criteria and methodology are different in almost each study. The aetiological yield figures tended to be about 50-54.1%[20,42] in studies, which enrolled patients with intellectual disability only. Analysis of a group of patients with global developmental delay revealed the aetiology in 53% of children without autistic features.[21] A retrospective chart review of 60 patients with global developmental delay in the same institution found an aetiology in 63% of cases if autistic spectrum disorder was excluded.[21] A retrospective cohort study on global developmental delay in Turkey identified aetiology in 64% of patients.[19] Although the latter cohort study data apply to global developmental delay only, they are very similar to our findings. Due to significant advances in laboratory testing substantial improvement in the diagnostic yield is expected in the future.

Boys were overrepresented among children with neurodevelopmental disabilities in this study. Similar trend was reported in the literature.[3,20,21]

Subgroups without known prenatal, perinatal, and/or neonatal adverse events

Almost seventy per cent of our patients with neurodevelopmental disability in this study did not have any known adverse events in the prenatal, perinatal, and/or neonatal period.

Genetic syndromes with recognized aetiology. Patients in this subgroup had chromosomal abnormalities, or syndromes due to single gene defects. In agreement with the literature[25] the dysmorphologic examination was a key element of the diagnostic evaluation and it provided essential guidance which additional investigations were indicated in these children. A systematic review of the usefulness of various diagnostic investigations in

individuals with mental retardation revealed that the frequency of detected chromosomal aberrations was around one in every 10 investigated patients.[24] We found numerical or structural abnormalities (including those ones, recognized only by FISH) in 7.0 per cent of the total patient population in this study. This figure of positive findings however grew up to 12.9 per cent if related to the number of patients in those 4 groups in which chromosomal analysis was mandatory (genetic syndromes with recognized aetiology, global developmental delay/intellectual disability with or without dysmorphic features, and brain malformations, Tables 5-8). Chromosomal structural anomalies reported in this study showed female predominance similarly to other observations.[24]

Clinical symptoms provided the lead to FISH studies in Prader-Willi, Williams and DiGeorge syndromes (Table 5, Patients 6-11).[43] Prader-Willi and Angelman syndromes are imprinting disorders. In this study Angelman syndrome was the result of a rare form of balanced translocation and paternal uniparental disomy involving chromosome 15 in association with the loss of the maternally inherited *UBE3A* gene.[44]

Williams syndrome was caused by deletion of the WS critical region on chromosome 7q11.2 including the elastin (*ELN*) gene as well as numerous genes believed to contribute to the phenotype.[43,45] WAGR syndrome (Table 5, Patient 12) is a rare genetic disorder characterized by a de novo deletion of 11p13. *PAX6* and *WT1* genes reside in the 11p13 region, the former is involved in ocular and central nervous system development, whereas the latter (Wilms tumour gene) is a tumour suppressor gene.[46] The 10p deletion in HDR syndrome (Table 5, Patient 13) probably was associated with *GATA3* haploinsufficiency, which proved to be the cause of the syndrome.[47] Patient 14 (Table 5) with ring chromosome 22 accompanied by severe intellectual disability and epilepsy presumably was hemizygous for the *SHANK3* gene.[48] The translocation in Patient 15 (Table 5) was seemingly balanced, however involvement of genes located near the breaking point on chromosome 3 and responsible for atypical autism, blepharophimosis and vermis hypoplasia might explain the clinical symptoms.[49,50]

Fragile X syndrome (Table 5, Patient 18) arose from an expansion of triplet repeats in the *FMR1* gene with the result of hypermethylation of the CpG islands in the promoter region of the gene preventing gene expression.[51] Mutations in the gene encoding methyl-CpG-binding protein 2 (*MECP2*), as shown in Patient 20 (Table 5), are the most common causes of classical Rett syndrome.[52] Heterozygous mutations in *TSC1* or *TSC2* and *NF1*

genes cause tuberous sclerosis and neurofibromatosis type 1 (Table 5, Patients 21-23), respectively.[53,54] Loss of *TSC1* or *TSC2* encoded proteins (hamartin, or tuberin) leads to overactivation of mammalian target of rapamycin (mTOR) and disinhibition of cell growth and proliferation.[45] The *NF1* product neurofibromin-1 is a negative regulator of Ras-kinase and its loss also leads to overactivation of mTOR through a sophisticated signalling process leading to excessive mRNA translation in both neuronal and glial cells.[45] Waardenburg syndrome (WS1) due to *PAX3* mutation (Table 5, Patient 24) is a neurocristopathy usually with preserved cognition;[55] our patient however showed borderline intellectual disability. RNA toxicity leading to severe clinical symptoms is the result of CTG repeat expansion in the *DMPK* gene in congenital myotonic dystrophy (Table 5, Patient 25).[56]

A few genes have been implicated in Cornelia de Lange syndrome,[57] VACTERL association[58] and blepharophimosis–mental retardation syndrome,[59] however our cases are still awaiting their molecular diagnoses.

The cognitive and behavioural phenotype of several neurodevelopmental syndromes with well documented genetic bases have been reviewed by Siegel and Smith.[60] Intellectual disability with a wide range in severity can be associated with certain syndromes (i.e. Down, or Fragile X syndromes), while the cognitive defect remains usually mild/moderate in other syndromes (i.e. Prader-Willi, Williams, DiGeorge syndromes) and in numerical anomalies of the sex chromosomes.[24,60-62] Our results presented in Tables 5 and 17 are essentially in agreement with the findings described in the literature.

Global developmental delay/intellectual disability (mental retardation) with dysmorphic features not recognized as specific syndromes. Unfortunately dysmorphologic evaluation of children with neurodevelopmental disabilities remained unsuccessful in terms of syndrome recognition in almost 10% of our patients with male preponderance (Table 6). Routine chromosomal analysis and *FMR1* gene testing failed to show any abnormalities. The availability of subtelomeric FISH (1 patient only) and chromosomal microarray (3 cases only) was highly limited so far.

The co-occurrence of mental retardation and minor anomalies has been suggested long time ago.[34,63] The application of the FISH technique to examine the subtelomere region of each chromosome led to the awareness that submicroscopic subtelomeric chromosome rearrangements can be a significant cause of malformation and mental retardation.[64] Submicroscopic subtelomeric chromosome defects have been found in 7.4% of children

with moderate to severe mental retardation and in 0.5% of children with mild retardation of unknown aetiology, both groups of patients with normal G banded chromosomes.[25,65] Meanwhile subtelomeric FISH has been replaced by a new technology, chromosomal microarray, which permits genome-wide detection of copy number variants (deletions and duplications) at a significantly higher resolution than G-banded karyotyping.[66,67] A 2010 consensus statement by the International Standard Cytogenetic Array Consortium indicated that chromosomal microarray should be the first-line diagnostic test for individuals with global developmental delay or intellectual disability, autism spectrum disorders, or multiple congenital anomalies.[66] As a next step in the diagnostic work up whole-exome sequencing should be considered for the array negative cases.[67,68] According to these guidelines all patients in this group are candidates for chromosomal microarray followed by whole-exome sequencing depending on the results gained by the array.[67]

Global developmental delay/intellectual disability without recognized aetiology and dysmorphic features. Patients with non-syndromic forms of intellectual disability belong into this group (Table 7). The availability of chromosomal array (in 2 cases only) and mutation analysis (for 3 children) was limited for these patients as well and these tests failed to show any abnormalities. Extensive genetic heterogeneity can be experienced in this type of intellectual disability. Large number of autosomal and X-linked causative genes have been recognized by various approaches.[68] Application of new technologies, i.e. whole exome sequencing has made it theoretically possible to identify causal mutations in most individuals with intellectual impairment regardless of frequency, heterogeneity, and inheritance.[68,69] The extensive research in the last decade provided evidence that molecular networks involving intellectual disability genes can be found in presynaptic pathways, postsynaptic protein complexes, cytoskeleton dynamics, intracellular signal transduction pathways, transcription regulation, epigenetic modulation of the chromatin structure and post-transcriptional mechanisms for controlling gene expression.[45,70] Hence our patients with non-syndromic intellectual disability, similarly to patients with dysmorphic features in the previous group are candidates for being tested by new techniques.

Brain malformations. Brain abnormalities can often be found by brain imaging in patients with neurodevelopmental disabilities.[24,25] With special attention only to malformations, 16.3% and 18% of patients with global developmental delay had cerebral dysgenesis as reported by Özmen et al.[19] and Srour et al.[21] These figures are close to the frequency of brain malformations in 14.5% of children with neurodevelopmental disabilities in this study.

The presence of cerebral dysgenesis explains neurodevelopmental delay,[71-79] which proved to be severe/profound in the majority of patients in this survey. Brain malformations are a major cause of cerebral palsy,[80] and indeed, almost one third of our cases with brain malformations had cerebral palsy. On the other hand 15% of all cerebral palsy cases in this cohort were caused by brain malformations. Brain malformations were responsible for cerebral palsy only in 8.6% and 11.3% of patients according to Garne et al.[80] and Self et al.,[81] respectively. The differing data probably reflect some bias in the referral system.

Although brain malformation was regarded as aetiology in our calculations, the malformations themselves are the sequels of genetic defects or environmental factors.[25] Gene defects causing brain malformations and revealed in 20% of our patients, can be considered as real aetiologies, while the majority of cases with cerebral dysgenesis await recognition of their real causes. Rare copy number variants has been found recently by array comparative genomic hybridization in cerebral dysgenesis[82] and whole-exome sequencing has also been successful in identifying mutations responsible for brain malformations.[83] Application of these new technologies in our cases with unknown aetiology is under way.

Schizencephaly was found as the second most common malformation in this survey after corpus callosum anomalies. It has been suggested by a recent study that important non-genetic factors, such as young maternal age, lack of prenatal care and alcohol use can be significantly associated with risk of schizencephaly.[84] Indeed; the aetiological role of environmental factors should be considered, however difficult it is in a retrospective study.

Inborn errors of metabolism. Neonatal screening programme for metabolic disorders identify children with several treatable inborn errors of metabolism in Hungary. There are however metabolic disorders, like Lesch-Nyhan syndrome or mitochondrial diseases, found in this study, which escape the diagnosis by routine neonatal screening.

Overall the yield of metabolic studies is low in patients with neurodevelopmental disabilities. Van Karnebeek et al. reported in their review that the positive results varied

from 0.2 to 8.4% (median 1.0%) among patients with mental retardation; the higher figures were from countries where specific entities were more common, or the population was highly inbred.[24] Unselected metabolic screening provides extremely low yield hence the metabolic tests should be targeted at the suspected category of disorders.[25] The family history in Lesch-Nyhan syndrome and the clinical features/MRI abnormalities in mitochondrial diseases provided the clue to the correct diagnosis in our cases. The frequency of metabolic disorders among children with global developmental delay was reported by Özmen et al. and Srour et al. as 4% and 2%, respectively,[19,21] which figures are in a good agreement with the result of 2.5% in this survey.

The gender distribution was strongly shifted towards the male sex in this group. The X-linked Lesch-Nyhan syndrome was found in half of the patients due to mutation in the *HPRT* gene, which encodes the purine salvage enzyme hypoxanthine-guanine phosphoribosyltransferase. Mental retardation and other clinical symptoms in these patients were similar to the findings described in the literature.[85]

Leigh syndrome is a devastating neurodegenerative disorder[86] and it was caused by a mutation in the mitochondrial DNA in our patient. Mitochondrial DNA deletions were responsible for Kearns-Sayre syndrome in two patients. Cognitive dysfunction was observed in addition to ataxia, visual and hearing impairment in these patients similarly to features described in the literature.[87] Characteristic patterns of MRI abnormalities were suggestive of mitochondrial disease in all 3 cases.

Leukoencephalopathies. White matter disorders or leukoencephalopathies comprise all disorders that exclusively or predominantly affect the white matter of the brain.[88] Many leukoencephalopathies are the result of inborn error of metabolism, however the major involvement of the white matter justifies classifying these disorders separately in spite of the obvious overlap with the previous group of patients with neurodevelopmental disorders.[88] Distinct patterns of MRI abnormalities provided the clue to the diagnosis in all cases and molecular genetic tests confirmed the specific aetiology in 3 patients (Alexander disease, X-linked adrenoleukodystrophy and Krabbe disease). All patients in this series had cognitive disability associated with a progressive clinical course. Srour et al reported on the occurrence of leukodystrophy in 2% of patients in a cohort with global developmental delay,[21] which figure is rather close to the figure of 1.6% in this study.

The role of MRI in the diagnosis of neurodevelopmental disabilities is highlighted by our findings. It proved to be essential in the diagnosis of brain malformations and leukoencephalopathies and guided the investigations in certain types of inborn errors of metabolism. Altogether it had a fundamental diagnostic value in 42 cases, which equals 17.4% of all patients and 25.1% of children without known adverse events or risk factors.

Epileptic syndromes. Early onset epileptic encephalopathies such as malignant migrating partial seizures and West syndrome in this study, are characterized by frequent severe seizures, neurodevelopmental disability and usually poor outcome.[89,90] Risk factors or adverse events were not found in the history of our patients and the MRI was normal at the beginning of the seizures. Causative mutations in several genes have been identified recently in both syndromes,[89,90] however molecular genetic tests were not available for our patients.

Early onset epileptic encephalopathies with unknown aetiology as causes of global developmental delay have not been mentioned in earlier cohort studies,[19,21] hence comparison was not feasible.

Developmental (specific) language impairment. In developmental (specific) language impairment predominantly a single developmental domain is affected, although longitudinal studies provided evidence that the delay may not be solely restricted to the language domain over time.[1,91] This disorder is supposed to be the result of an interplay between genetic, environmental, neurobiological and cognitive (perception, speed of processing, working memory and phonological short-term memory) factors.[91,92] Developmental language impairment is a heterogeneous disorder with various subgroups and a changing profile for each individual across development.[91]

The prevalence of specific language impairment was estimated as 7.4% among kindergarten children in the USA.[93] Only 0.5% of the total 1764 patients referred to our Paediatric Neurology Service had developmental language impairment and it occurred only in 3.7% of the children with neurodevelopmental disabilities. Obviously most of the children with this impairment were referred to speech therapist and escaped registration by us. All patients in our cohort were boys and the aetiology of the disorder remained unknown. Almost half of them had borderline intellectual abilities. These findings were in agreement with data in the literature.[91]

Neuromuscular disorders. Gross motor delay and/or decline with or without cognitive impairment are the main clinical features of neuromuscular disorders and these genetic diseases constitute an important group of neurodevelopmental disabilities. Data on the prevalence of neuromuscular disorders among children with global developmental delay were not available for comparison.[19,21] All patients in our study showed motor disability as described in the literature[94] and the diagnosis was confirmed by molecular testing in all cases, except a patient with nemaline and another one with centronuclear myopathy (Table 13). A review has been published on the cognition in neuromuscular disorders by D'Angelo[95] and our experiences were in good agreement with the findings summarized by this publication. There was no cognitive impairment in spinal muscular atrophy, congenital myasthenia,[96] centronuclear myopathy and one of the patients with nemaline myopathy. Mild or moderate intellectual disability was found in another case with nemaline myopathy,[97] Duchenne muscular dystrophy and facioscapulohumeral dystrophy. The patient with congenital myotubular myopathy had very severe intellectual disability in addition to ventilator dependency, as described in the literature.[98] A high correlation has been found between cognitive features and cerebral protein expression in Duchenne muscular dystrophy.[95] It has been proven recently that intelligence quotient is related to the D4Z4 repeat number in facioscapulohumeral dystrophy.[99] Correlation between tissue-specific protein expression and cognitive deficits, however, is still elusive in congenital myopathies.[95]

Subgroups with known prenatal, perinatal, and/or neonatal adverse events

Cerebral palsy due to known adverse events and including patients after preterm, or term delivery was the most common form of neurodevelopmental disability in this study, affecting 23.2 % of the patients altogether. Cerebral palsy occurred in association with brain malformations as well in 4.2 % of the total population of the cohort, since the total rate of cerebral palsy was 27.4%. These figures are in agreement with literature data, which claim that cerebral palsy is the commonest cause of physical disability in childhood.[100] Based on the differing risk factors and pathophysiology it was justified to classify patients with cerebral palsy into two groups depending on the gestational age at birth.

Cerebral palsy after preterm delivery. Cerebral palsy prevalence increases with lower birth weight and higher immaturity.[100] Typical periventricular lesions were found in

our patients, which were „complex amalgam of destructive and developmental disturbances" as reviewed by Khwaja and Volpe.[101,102] According to the population based Surveillance of Cerebral Palsy in Europe around half of the children with cerebral palsy were born at term, 20% had a gestational age of 32-36 weeks and 25% were below 32 weeks of gestation between 1977-1996.[100,103] In contrast to these European data recorded earlier, our cohort study showed that 45.5% of the total 66 cerebral palsy cases (preterm, term after adverse events plus patients with brain malformations, all term) was born at term, 54.5% was preterm, only 9% had a gestational age of 32-36 weeks and 43.9% of them were born at or below 32 weeks of gestation. These data emphasize the risk that the high preterm delivery rate in Hungary means, although some bias in the referrals might have influenced the data in this cohort study.

A male excess of cerebral palsy among preterm infants has been reported[104] and sex differences in cell death pathways in the foetal or neonatal period have been suggested.[105] There were more boys in our cohort as well, however the male preponderance was statistically not significant.

There are controversial data on the prevalence of cerebral palsy among preterm twins.[106] In our cohort almost one third of the preterm infants developing cerebral palsy later, were products of twin pregnancy suggesting that twinning was a risk factor in this population. Assisted conception occurred only in a single case (Table 14, Patient 4) among these twins. Neurodevelopmental disability, including cerebral palsy is more common in infants with intrauterine growth retardation.[107] Both patients in this study (Table 14, Patients 5 and 6) had spastic quadriplegia and severe intellectual disability. Intrauterine CMV infection is a well-known aetiology of cerebral palsy,[108] the preterm infant (Table 14, Patient 3) in this study also had very severe spastic quadriplegia in addition to visual and hearing impairment, epilepsy and intellectual disability. Although the majority of preterm infants with cerebral palsy had severe intellectual disability as well, almost one fifth of them showed borderline and another fifth normal intellect.

Cerebral palsy after delivery at term. Ten risk factors have been identified recently for cerebral palsy in children born at term: placental abnormalities, major and minor birth defects, low birth weight, meconium aspiration, instrumental/emergency Caesarean delivery, birth asphyxia, neonatal seizures, respiratory distress syndrome, hypoglycaemia, and neonatal infections.[109] The underlying pathology generally seems to be

different in patients with cerebral palsy born at term than in children born preterm. Malformations, cortical and deep grey matter lesions and infarct (stroke) are more common in term born cerebral palsy cases.[100]

The spectrum of risk factors and pathologies in our patients with cerebral palsy after delivery at term is in agreement with literature data. Intrapartum asphyxia was the most common aetiology followed by intrauterine growth retardation due to placental abnormalities and infections. Neonatal stroke was diagnosed following intrapartum asphyxia in a case (Table 15, Patient 18), while the aetiology of stroke remained unknown in 2 children (Table 15, Patients 15, 16). As discussed earlier 10 patients with brain malformations, all born at term, had cerebral palsy (Table 8). A genetic cause was identified in the patient with lissencephaly (Table 8, Patient 18), while a genetic cause, not identified yet, is very likely in another patient with pontocerebellar hypoplasia (Table 8, Patient 34). In the other 8 cases with polymicrogyria and/or schizencephaly the aetiology might be environmental or genetic.

The intellectual disability was severe in the majority of patients with cerebral palsy born at term, however the intellectual performance remained normal based on our testing in almost one third of the patients.

Neurodevelopmental disabilities subsequent known prenatal, perinatal, and/or neonatal adverse events but **without cerebral palsy.** There were patients in our cohort, born preterm or at term, who were exposed to some adverse events however did not have cerebral palsy. This was a heterogeneous group of children, all of them with intellectual disability without major motor defect. The risk factors and pathophysiology of cerebral injury very likely showed the same pattern as in patients with cerebral palsy discussed above.

Limitations

This was a retrospective study; therefore, some of the limitations of retrospective studies, such as incomplete data assessment, apply.

Our sample represents a cohort of children with neurodevelopmental disabilities referred to subspecialty evaluation to a Paediatric Neurology Service, therefore the results may not generalize to other populations where medical practice, expertise, referral patterns, accessibility to medical care may be different.

Although age specific developmental and intelligence tests were performed for large number of children at our Department, the majority of patients were tested by the Committees for the Assessment of Learning Abilities and Rehabilitation responsible for children's evaluation in Hungary.

There are also limitations as to the intensity of diagnostic workup. For example, only few patients underwent subtelomeric chromosomal rearrangement, or aCGH screening because of financial limitations. It applies also to gene tests and next generation sequencing. If these methods were employed more commonly during the routine workup, we would anticipate a higher etiologic yield than that obtained.

Conclusions

This study provides data on the distribution of the various diagnostic categories of neurodevelopmental disabilities in a cohort of patients referred to a Paediatric Neurology Service in Hungary. The degree of global developmental delay/intellectual disability has been assessed in each group. Aetiology was found in 66.4% of patients, which compares with data in the literature. Recognition of the causes of neurodevelopmental disabilities helps starting adequate treatment, when feasible and establishing a health maintenance plan and rehabilitation. It provides prediction of the outcome, helps in avoiding unnecessary diagnostic tests and contributes to the prevention of the recurrence of the disorder.

A manageable database has been set up, which can be used for further studies on the genetic and environmental causes of neurodevelopmental disorders. This database provides useful information for the public health authorities as well on the special needs of children living with neurodevelopmental disabilities.

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