Epidemiology of central nervous system malformations in South-Eastern Hungary

Ph.D. Thesis

Nóra Zsuzsanna Szabó, M.D.

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Epidemiology of central nervous system malformations in South-Eastern Hungary

by

Nóra Zsuzsanna Szabó, M.D.

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Department of Paediatrics, Faculty of Medicine, University of Szeged

Consultant: László Sztriha, M.D., Ph.D., D.Sc.

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Full papers

I. Szabó N, Szabó H, Hortobágyi T, Túri S, Sztriha L. Pontocerebellar hypoplasia type 1. *Pediatr Neurol* 2008;39:286-8. *IF: 1.505*

II. Szabó N, Hegyi A, Boda M, Páncsics M, Pap C, Zágonyi K, Romhányi E, Túri S, Sztriha L. Bilateral operculum syndrome in childhood. *J Child Neurol* 2009;24:544-50. *IF: 1.592*

III. Szabó N, Pap C, Kóbor J, Svékus A, Túri S, Sztriha L. Primary microcephaly in Hungary: epidemiology and clinical features. *Acta Paediatr* 2010;99:690-3. *IF: 1.955*

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VI. 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;44:420-6. *IF: 1.513*

Other publications

VII. Sztriha L, Panzeri C, Kálmánchey R, Szabó N, Endreffy E, Túri S, Baschirotto C, Bresolin N, Vekerdy Z, Bassi MT. First case of compound heterozygosity in ALS2 gene in infantile-onset ascending spastic paralysis with bulbar involvement. *Clin Genet* 2008;73:591-3. *IF: 3.206*

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ACRONYMS AND ABBREVIATIONS

aCGH	array comparative genomic hybridisation
ASD	atrial septal defect
CC	corpus callosum
CNS	central nervous system
CSP	cavum septum pellucidum
CT	computer tomography
CVH	cerebellar vermis hypoplasia
DD	developmental delay
DHCARS	Department of Hungarian Congenital Abnormality Registry and Surveillance
DWM	Dandy-Walker malformation
EUROCAT	European Surveillance of Congenital Anomalies
EURORDIS	European Organisation for Rare Diseases
F	female
FCD	focal cortical dysplasia
FISH	fluorescent in situ hybridisation
HPE	holoprosencephaly
ID	intellectual disability
L	left
LB	live births
LBPR	live birth prevalence rate
M	male
MRI	magnetic resonance imaging
MTS	molar tooth sign
No	number
NR	not relevant
NTD	neural tube defects
PCH	pontocerebellar hypoplasia
PDA	patent ductus arteriosus
PFO	patent foramen ovale
PMG	polymicrogyria
R	right

Rh	rhombomere
SB	stillbirths
SBH	subcortical band heterotopia
SOD	septo-optic dysplasia
TOP	termination of pregnancy
TP	total prevalence
TBPR	total birth prevalence rate
VSD	ventricular septal defect
95% CI	95% confidence interval

SUMMARY

Progress in brain imaging revealed that dysgenesis of the central nervous system is a common finding in patients with developmental disabilities and epilepsy. Despite the important aetiological role of brain malformations in mental and physical handicap, and epilepsy only a few reliable data are available on the birth prevalence of these abnormalities in Hungary.

Objectives

The aim of this study was to survey the birth prevalence of CNS malformations in South-Eastern Hungary and establish a registry for these malformations at the Department of Paediatrics, University of Szeged. Further objectives were to recognize environmental and genetic factors responsible for CNS dysgenesis.

Patients and methods

A population-based retrospective survey of patients born with malformations of the CNS in South-Eastern Hungary between July 1, 1992 and June 30, 2006 was performed. The diagnosis of CNS malformations was always confirmed by cranial ultrasound, CT and/or MRI. A detailed analysis of the patients with CNS malformations was carried out. Chromosomal analysis was requested for children with dysmorphic features or multiple anomalies. Molecular cytogenetic studies or mutation analysis of genes responsible for CNS malformations were also performed for several patients. Total and live birth prevalences per 10 000 births for NTD, and live birth prevalences per 10 000 live births for the other types of malformations were calculated.

Results

There were 185 486 live births (95 241 male and 90 245 female) in the area between July 1, 1992 and June 30, 2006. The total number of patients born alive with CNS malformation totalled 214 (120 boys and 94 girls). The overall LBPR of CNS malformations was 11.54 per 10 000 live births, 12.60 per 10 000 among males and 10.42 per 10 000 among girls, which means that one newborn out of 870 live births was affected by some kind of CNS malformation. The total birth prevalence of myelomeningocele and anencephaly increased during the study period, while the live birth prevalence of myelomeningocele decreased with a parallel increase in the number of terminations for this defect.

Four syndromes (otopalatodigital syndrome spectrum disorder, bilateral operculum syndrome, mega corpus callosum, polymicrogyria, epilepsy, psychomotor retardation syndrome and PHACE syndrome) were recognized by us and three mutations in genes (*LIS1, DCX*, *CEP290*) were identified as a result of our efforts to reveal the molecular basis of the malformations.

Conclusions

The birth prevalence of some major CNS malformations (e.g. HPE, CC anomalies, MTS, DWM) in South-Eastern Hungary was similar to those ones published from elsewhere. However, our survey revealed novel population-based prevalence data as well, not available in the literature, for several malformations (e.g. primary microcephaly, various types of cortical dysgenesis). An increase in the total birth prevalence of myelomeningocele and anencephaly in the region requires public health measures in order to replace termination of pregnancy with primary prevention. Based on our data the burden imposed by CNS malformations on the health service in Hungary can be predicted. Our malformation registry provides a database for further environmental and molecular genetic studies as new information on the aetiology of various malformations become known, facilitating prenatal counselling.

ÖSSZEFOGLALÁS

A képalkotó eljárások fejlődésével kiderült, hogy a megkésett fejlődés és az epilepszia hátterében gyakran található központi idegrendszeri fejlődési rendellenesség. A fejlődési rendellenességek gyakorisága ellenére Magyarországon kevés adat áll rendelkezésünkre azok epidemiológiájáról.

Célkitűzések

A központi idegrendszer fejlődési rendellenességeinek születési prevalenciáját kívántuk tanulmányozni Dél-Kelet Magyarországon 1992. július 1. és 2006. június 30. között. A központi idegrendszer fejlődési rendellenességeiben szenvedő gyermekek regiszterét kívántuk létrehozni a SZTE Gyermekgyógyászati Klinikáján. További célkitűzésünk volt azoknak a környezeti és genetikai tényezőknek a tanulmányozása, amelyek felelősek lehetnek a gerincvelő és az agy kóros fejlődéséért.

Betegek és módszerek

A központi idegrendszer fejlődési rendellenességeinek születési prevalenciáját egy retrospektív, népesség alapú felméréssel vizsgáltuk az 1992. július 1. és 2006. június 30. között Dél-Kelet Magyarországon született gyermekek körében. A fejlődési rendellenesség pontos diagnózisa érdekében koponya CT és MRI vizsgálat történt az ultrahanggal végzett szűrést követően. Az általános gyermekgyógyászati és neurológiai vizsgálaton túlmenően kromoszóma vizsgálatot kértünk azoknak a betegeknek, akiken többszörös minor anomáliát, vagy multiplex fejődési rendellenességet diagnosztizáltunk. Célzott molekuláris genetikai vizsgálatot igyekeztünk megszervezni az olyan fejlődési rendellenességben szenvedő gyermekek részére, akiknél gén mutáció volt feltételezhető a dysgenesis hátterében. Teljes és élveszületési prevalenciát számítottunk a velőcső záródási defektusokra, míg csupán élveszületési prevalenciát tudtunk számolni a többi fejlődési rendellenességre 10 000 újszülöttre vonatkoztatva.

Eredmények

Az élveszületések száma 185 486 (95 241 fiú és 90 245 leány) volt a régióban 1992. július 1. és 2006. június 30. között. Az élveszülöttek között 214 (120 fiú és 94 leány) újszülöttnek volt központi idegrendszeri fejlődési rendellenessége, tehát a teljes élveszületési prevalencia 11,54/10 000 volt, 12,60/10 000 a fiúk és 10,42/10 000 a leányok között. A számok azt jelentik, hogy 870 élveszületésre jutott egy központi idegrendszeri fejlődési rendellenesség. A myelomeningocele és az anencephalia teljes

születési prevalenciája növekedett a vizsgált időszak alatt, míg a myelomeningocele élveszületési prevalenciája csökkent a terminálások számának egyidejű emelkedése mellett.

Négy szindrómát (otopalatodigitalis szindróma spektrum rendellenesség, bilateralis operculum szindróma, mega corpus callosum, polymicrogyria, epilepszia, psychomotoros retardáció szindróma és PHACE szindróma) diagnosztizáltunk, továbbá három új esetben sikerült gén mutációt (*LIS1, DCX, CEP290*) azonosítani az anyagunkban.

Következtetések

Több fejlődési rendellenesség (pl. holoprosencephalia, corpus callosum rendellenességek, moláris fog és Dandy-Walker malformáció) élveszületési prevalenciája hasonló volt a vizsgált régióban a más országokból közölt adatokkal. Vizsgálataink azonban kiderítettek új, eddig nem közölt születési prevalencia adatokat is több fejlődési rendellenességre vonatkozóan (pl. primér microcephalia, corticalis dysgenesis különböző típusai). A myelomeningocele és az anencephalia teljes prevalenciájának növekedése felhívja a figyelmet a primér prevenció terén folyó felvilágosító tevékenység javítására. Adataink alapján felbecsülhető az a terhelés, amit a központi idegrendszeri fejlődési rendellenességek rónak az egészségügyi ellátó rendszerre. Munkánkkal egy adatbázist hoztunk létre, amely alapjául szolgál további molekuláris genetikai vizsgálatoknak, a központi idegrendszeri dysgenesisek genetikai okainak feltárása pedig megkönnyíti a fogamzás előtti és a prenatalis genetikai tanácsadást.

INTRODUCTION

Congenital anomalies (birth defects) can be defined as structural, functional, and/or biochemical-molecular defects developing during foetal life caused by genetic and/or environmental factors and discovered either prenatally or after birth at any age (1). As congenital anomalies impose a great burden not only on health professionals and government officials but also on the society, it is necessary for the government and health care providers to be aware of the exact data on congenital anomalies in their region (2).

Prevalence is a descriptive epidemiological term that is used for estimating the frequency of a disease in a certain population. Prevalence is defined as the total number

of affected persons present in the population at a specific time divided by the number of persons in the population at that time (3). The incidence of a disease is defined as the number of new cases of a disease that occur during a specified period of time in a population at risk for developing the disease (3). Conventionally, the frequency of occurrence of congenital anomalies was referred to as "prevalence" rather than an "incidence", because the number of early spontaneous abortions of the affected foetuses is generally not available in the databases (2). Different methods can be used for measuring the prevalence rate of congenital anomalies, such as total prevalence (TP) and live birth prevalence rates (LBPRs). Total prevalence includes all the affected live births (LB), foetal deaths, and terminations of pregnancies (TOPs) for foetal anomaly as a proportion of all live births and stillbirths (SB), while the LBPR is based on liveborn cases as a proportion of all livebirths in the population. Calculation of LBPR is particularly important for health service providers because it shows the proportion of the affected newborns in the population (2).

Wide variability in prevalence data in the regions of the world was reported in the World Atlas of Births Defects published by World Health Organization (4). In Europe, the total birth prevalence of the major congenital anomalies registered by European Surveillance of Congenital Anomalies (EUROCAT), the European network of population-based registries for the epidemiologic surveillance of congenital anomalies was 239.5 per 10 000 births between 2003–2007 (2). The total birth prevalence of congenital anomalies reported to EUROCAT was 270.15 per 10 000 births in Hungary in the same period of time (5).

Some difficulties may arise at the estimation of the proportion of central nervous system (CNS) malformations among congenital anomalies as a whole. The registers, like EUROCAT usually provide data on neural tube defects (NTD), microcephaly, and arhinencephaly/holoprosencephaly (HPE), however figures on the various forms of cortical malformations or posterior fossa abnormalities are not available (5). In addition, "hydrocephaly", which can have heterogeneous aetiology is also regarded as a group of congenital nervous system anomalies by EUROCAT (5). By all means it appears that 10.61% of all anomalies affected the nervous system by the EUROCAT data between 2003 and 2007 (2). In Hungary 5.97% of all malformations, reported to the EUROCAT, involved the nervous system over 2003-2007 (5).

Special types of congenital anomalies of the central nervous system [e.g. HPE or molar tooth sign (MTS)] are rare diseases (orphan diseases). Any disease affecting less

than 1 in 2000, i.e. 5 people in 10 000 is considered rare (6). Living with a rare disease raises several special difficulties. Studies by the European Organisation 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 (6). Rare diseases impose heavy social consequences on the patients, who often lack appropriate quality healthcare (6).

The burden of CNS malformations is enormous. They account for a high percentage of foetal and infant deaths (as high as 40%) (7). A pregnancy with a dysmorphic baby has a higher risk to result in premature birth. CNS malformations cause severe mental disabilities and they are very common in children with cerebral palsy (8). Unfortunately, the aetiology of CNS defects remains unknown in more than 60 % of the affected children (9).

The surveillance of malformations was initiated in the 1960's and the establishment of birth defect registries has been among the highlighted programmes of the World Health Organization since then. The largest European network of population-based registries is the EUROCAT (5). Hungary became a member of EUROCAT in 2003. The following objectives have been targeted by the EUROCAT (5):

- To provide essential epidemiologic information on congenital anomalies in Europe
- To facilitate the early warning of new teratogenic exposures
- To evaluate the effectiveness of primary prevention
- To assess the impact of developments in prenatal screening
- To act as an information and resource centre for the population, health professionals and managers regarding clusters or exposures or risk factors of concern
- To provide a ready collaborative network and infrastructure for research related to the causes and prevention of congenital anomalies and the treatment and care of affected children
- To act as a catalyst for the setting up of registries throughout Europe collecting comparable, standardised data

As mentioned earlier, EUROCAT and several birth defect registries collect data only on NTD, hydrocephaly, microcephaly and arhinencephaly/HPE (5). Progress in brain imaging, however, revealed large groups of CNS malformations, like corpus callosum (CC) anomalies, cortical abnormalities, or posterior fossa malformations that

should be included in an epidemiological surveillance on CNS dysgenesis. This was the reason why a decision has been made by our research group to compile a registry as complete as possible on all types of CNS malformations in South-Eastern Hungary.

Objectives

The following objectives were targeted by our study:

1. To perform a population-based retrospective comprehensive survey of the epidemiology and clinical spectrum of all types of CNS malformations in South-Eastern Hungary, which represents a region in the European Union (Dél-Alföld)
   - to calculate the birth prevalence of CNS malformations
   - to describe the spectrum of associated CNS and extra-CNS malformations
   - to give an account of the main clinical features [developmental delay (DD), intellectual disability (ID) and neurological defects (ND)] associated with the various groups of malformations
   - to estimate the occurrence of epilepsy in patients with CNS dysgenesis
   - to make an attempt to reveal the aetiology of CNS malformations
   - to compare our results with other published data
2. To establish and manage a regional register on patients living with CNS malformations which can serve as a database for future studies
   - to make environmental and genetic studies feasible in the future as progress in this field occurs
   - to be able to compile homogeneous groups of patients with CNS malformations for genetic studies
   - to help health care providers with their work
   - to draw the attention of decision-makers to special problems of patients living with CNS or multiplex malformations

PATIENTS AND METHODS

A population-based retrospective survey of patients born with CNS malformations in South-Eastern Hungary between July 1, 1992 and June 30, 2006 was performed. A primary structural defect of the CNS occurring during the morphogenesis was regarded as a malformation (9). Patients with CNS abnormalities secondary to obvious destructive events (e.g. hypoxic-ischemic encephalopathy, injuries, intracranial haemorrhage, etc.) were excluded from the study.

Data collection

All children in Hungary are assigned to a paediatrician and his/her clinic, therefore children born with CNS malformations in the South-Eastern region in Hungary between July 1, 1992 and June 30, 2006 were ascertained by searching the databases of the paediatric clinics. All paediatricians in the region received questionnaires and were requested to report on patients with CNS malformations. In addition, they were encouraged also by telephone interviews and field researchers to provide information on these patients in order to compile a register as complete as possible. As severe cases with CNS malformations accompanied by striking dysmorphic features were treated in the Neonatal Intensive Units, or Neonatal Wards in the hospitals, the survey was extended to these departments in the region as well. Demographic data were collected from the Hungarian Central Statistical Office.

The diagnosis of CNS malformations was always confirmed by cranial ultrasound, computer tomography (CT) and/or magnetic resonance imaging (MRI), performed by conventional protocols. The images were reviewed visually by two examiners (Nóra Szabó and the consultant László Sztriha).

A detailed analysis of the patients with CNS malformations was carried out. Clinical records were retrospectively reviewed for family history, parental consanguinity, maternal and birth history, possible environmental factors, neonatal course, developmental milestones, and epileptic seizures. Detailed clinical and neurological examinations were performed in cases when it was possible. Dysmorphic features and extra-CNS malformations received special attention. Electroencephalograpy was performed on epileptic patients, according to the international 10-20 system of electrode placement.

Chromosomal analysis with G-band technique was carried out for patients with dysmorphic features or multiple anomalies.

Molecular cytogenetic studies or mutation analysis of genes responsible for CNS malformations were performed for a few patients in collaboration with the Clinical Genetics Centre, Department of Paediatrics, Medical and Health Science Centre, University of Debrecen, Debrecen, Hungary (for *LIS1* and *DCX* gene), Atlas Biolabs GmBH, Berlin, Germany [for array comparative genomic hybridisation (aCGH)], Diagnostics Lab PreventionGenetics, Molecular Diagnostics and Biobanking, Marshfield, Wisconsin, USA (for *AHI1* and *CEP290*), Molecular Genetics Laboratory, Medical Genetics Institute, Faculty of Medicine, University of Pécs, Pécs, Hungary (for

MECP2, *CDKL5*, *FOXG1*, *FMR1)* Department of Genome Analysis, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands (for *TSEN54)*. Financial coverage for most of the molecular genetic studies was provided by the Hungarian National Healthcare Fund.

Calculation of birth prevalence

As the number of terminations for and foetal deaths with NTD were available from the Department of Hungarian Congenital Abnormality Registry and Surveillance (DHCARS), the calculation of the total and live birth prevalences was possible for these malformations. Data on terminations and foetal deaths were not available for the other CNS malformations therefore only live birth prevalences were calculated for these anomalies.

Calculation of the total prevalence in neural tube defects (Groups 1-3): The numerator included all affected live births, foetal deaths from 20 weeks gestation and terminations of pregnancy for NTD after prenatal diagnosis, at any gestational age. The denominator was the number of LB and SB (after 24 weeks of gestation) in the population (2). The gender of the terminated foetuses remained unknown.

Calculation of the live birth prevalence in all groups: The numerator included all affected LB and the denominator was the number of LB in the region (2).

All prevalence data were calculated for 10 000 births. 95% confidence intervals (95% CI) were calculated on the basis of approximation to the binomial distribution. Statistical analysis was performed by using the SPSS 15.0 program (SPSS Inc., an IBM Company, Chicago, Illinois, USA).

This study was approved by the Ethics Committee of the Faculty of Medicine, University of Szeged (Szeged, Hungary). Informed consent was requested from the parents of patients before participation in the study.

Classification of malformations

The CNS malformations were classified according to the schemes published by Volpe (10), Barkovich et al. (11) and Parisi and Dobyns (12). The malformation regarded as fundamental from developmental point of view was the basis of classification in cases with complex CNS dysgenesis.

NEURAL TUBE DEFECTS

Group 1. Myelomeningocele

Group 2. Anencephaly

Group 3. Encephalocele

Group 4. Closed (occult) spinal dysraphism

MALFORMATIONS OF THE PROSENCEPHALON

Group 5. Holoprosencephaly

5a HPE only with craniofacial abnormalities

5b HPE with craniofacial and non-craniofacial abnormalities

5c HPE in association with chromosomal anomalies

Disorders of midline prosencephalic development

Group 6. Corpus callosum anomalies

6a Isolated agenesis/hypoplasia of the CC

6b Agenesis/hypoplasia of the CC in association with other CNS abnormalities

6c Agenesis/hypoplasia of the CC associated with both CNS and extra-CNS abnormalities

6d Agenesis/hypoplasia of the CC associated only with extra-CNS abnormalities

Miscellaneous groups of prosencephalic malformations

Group 7. Septo-optic dysplasia (SOD)

Group 8. Cavum septum pellucidum (CSP)

Only patients with large CSP (10 mm, or wider) were included in this study.

NEURONAL AND GLIAL PROLIFERATION DEFECTS

Group 9. Microcephaly

9a Primary microcephaly

9b Congenital microcephaly associated with chromosomal abnormalities or as part of a genetic syndrome

9c Congenital microcephaly with other CNS and/or extra-CNS abnormalities

Group 10. Microlissencephaly

Microlissencephaly refers to congenital microcephaly in association with extremely poor gyral formation.

NEURONAL MIGRATION DEFECTS

Group 11. Agyria/pachygyria - subcortical band heterotopia (SBH) spectrum

Group 12. Neuronal heterotopia

MALFORMATIONS OF CORTICAL ORGANIZATION

Group 13. Polymicrogyria (PMG)

Group 14. Schizencephaly

Group 15. Focal cortical dysplasia (FCD)

MIDBRAIN AND HINDBRAIN MALFORMATIONS

Malformations of both midbrain and hindbrain

Group 16. Molar tooth sign

Malformations affecting predominantly the cerebellum and its derivates [Rhombomere (Rh)1]

Group 17. Dandy–Walker malformation (DWM)

Group 18. Cerebellar vermis hypoplasia (CVH) without molar tooth sign

Group 19. Unilateral cerebellar hypoplasia

Group 20. Cerebellar aplasia/hypoplasia

Malformations affecting predominantly the lower hindbrain (Rh 2-8)

Group 21. Chiari I malformation

Malformations associated with degeneration of prenatal onset

Group 22. Pontocerebellar hypoplasia (PCH)

OTHER CNS MALFORMATIONS

Group 23. Arachnoid cysts

RESULTS

The live birth prevalence rate of the various CNS malformations in South-Eastern Hungary is summarised in Table 1. There were 185 486 live births (95 241 male and 90 245 female) in the area between July 1, 1992 and June 30, 2006. The total number of patients born alive with CNS malformation totalled 214 (120 boys and 94 girls). The overall LBPR of CNS malformations was 11.54 per 10 000 live births (95% CI: 9.99-13.08), 12.60 per 10 000 among males (95% CI: 10.35-14.85) and 10.42 per 10 000 among girls (95% CI: 8.31-12.52). According to our data one newborn out of 870 live births was affected by some kind of CNS malformation between July 1, 1992 and June 30, 2006.

Table 1. Live birth prevalence of CNS malformations in South–Eastern Hungary (1 July 1992–30 June 2006)

Malformations	Number of cases (M/F)	Prevalence per 10 000 live births (1:No of live births)	95% CI	Prevalence of males per 10 000 live births (1: No of live births)	95% CI	Prevalence of females per 10 000 live births (1: No of live births)	95% CI
Myelomeningocele	31 (14/17)	**1.67 (1:6000)**	1.08-2.26	**1.47 (1:6800)**	0.70-2.24	**1.88 (1:5300)**	0.99-2.78
Anencephaly	0	**NR**	NR	**NR**	NR	**NR**	NR
Encephalocele	6 (1/5)	**0.32 (1:31 000)**	0.06–0.58	**0.10 (1:100 000)**	-0.10-0.31	**0.55 (1:18 000)**	0.07-1.04
Closed (occult) spinal dysraphism	4 (2/2)	**0.22 (1:46 000)**	0.02-0.43	**0.21 (1:47 600)**	-0.08-0.50	**0.22 (1:45 000)**	-0.09-0.53
Holoprosencephaly	9 (5/4)	**0.49 (1:20 000)**	0.17-0.58	**0.52 (1:19 000)**	0.06-0.99	**0.44 (1:23 000)**	0.01-0.88
Corpus callosum anomalies	38 (26/12)	**2.05 (1:4900)**	1.40-2.70	**2.73 (1:3700)**	1.68-3.78	**1.33 (1:7500)**	0.58-2.08
Septo-optic dysplasia	2 (1/1)	**0.11 (1:91 000)**	-0.04-0.26	**0.10 (1:100 000)**	-0.10-0.31	**0.11 (1:91 000)**	-0.11-0.33
Cavum septum pellucidum	11(7/4)	**0.59 (1:17 000)**	0.24-0.94	**0.73 (1:14 000)**	0.19-1.28	**0.44 (1:23 000)**	0.01-0.88
Microcephaly	30 (11/19)	**1.62 (1:6200)**	1.04-2.20	**1.15 (1:8700)**	0.47-1.84	**2.11 (1:4700)**	1.16-3.05
Microlissencephaly	2 (1/1)	**0.11 (1:91 000)**	-0.04-0.26	**0.10 (1:100 000)**	-0.10-0.31	**0.11 (1:91 000)**	-0.11-0.33
Agyria/pachygria-subcortical band heterotopia spectrum	5 (3/2)	**0.27 (1:37 000)**	0.03-0.51	**0.31 (1:32 000)**	-0.04-0.67	**0.22 (1:45 000)**	-0.09-0.53
Neuronal heterotopia	3 (1/2)	**0.16 (1:62 000)**	-0.02-0.34	**0.10 (1:100 000)**	-0.10-0.31	**0.22 (1:45 000)**	-0.09-0.53
Polymicrogyria	8 (7/1)	**0.43 (1:23 000)**	0.13-0.73	**0.73 (1:14 000)**	0.19-1.28	**0.11 (1:91 000)**	-0.11-0.33
Schizencephaly	10(6/4)	**0.54 (1:19 000)**	0.20-0.87	**0.63 (1:16 000)**	0.13-1.13	**0.44 (1:23 000)**	0.01-0.88
Focal cortical dysplasia	1 (1/0)	**0.05 (1:200 000)**	-0.05-0.16	**0.10 (1:100 000)**	-0.10-0.31	**NR**	NR
Molar tooth sign	2 (1/1)	**0.11 (1:91 000)**	-0.04-0.26	**0.10 (1:100 000)**	-0.10-0.31	**0.11 (1:91 000)**	-0.11-0.33
Dandy-Walker malformation	6 (1/5)	**0.32 (1:31 000)**	0.06-0.58	**0.10 (1:100 000)**	-0.10-0.31	**0.55 (1:18 000)**	0.07-1.04
Cerebellar vermis hypoplasia without molar tooth sign	9 (3/6)	**0.49 (1:20 000)**	0.17-0.80	**0.31 (1:32 000)**	-0.04-0.67	**0.66 (1:15 000)**	0.13-1.20
Unilateral cerebellar hypoplasia	2 (1/1)	**0.11 (1:91 000)**	-0.04-0.26	**0.10 (1:100 000)**	-0.10-0.31	**0.11 (1:91 000)**	-0.11-0.33
Cerebellar aplasia/hypoplasia	7 (5/2)	**0.38 (1:26 000)**	0.10-0.66	**0.52 (1:19 000)**	0.06-0.99	**0.22 (1:45 000)**	-0.09-0.53
Chiari I malformation	2 (2/0)	**0.11 (1:91 000)**	-0.04-0.26	**0.21 (1:48 000)**	-0.08-0.50	**NR**	NR
Pontocerebellar hypoplasia	2 (0/2)	**0.11 (1:91 000)**	-0.04-0.26	**NR**	NR	**0.22 (1:45 000)**	-0.09-0.53
Arachnoid cysts	24 (21/3)	**1.29 (1:7700)**	0.78-1.81	**2.20 (1:4500)**	1.26-3.15	**0.33 (1:30 000)**	-0.04-0.71
Total	**214 (120/94)**	**11.54 (1:870)**	**9.99-13.08**	**12.60 (1:790)**	**10.35-14.85**	**10.42 (1:960)**	**8.31-12.52**

Epidemiology of neural tube defects

As mentioned in the PATIENTS and METHODS section, the TBPR was also calculated for NTD in addition to the LBPR, therefore the number of SB (874 in the study period) was added to the number of LB in order to perform the calculation of the total prevalence data correctly. Eventually the total number of births was 186 360, NTD occurred in 110 cases corresponding to a TBPR of 5.90 per 10 000 births (95% CI: 4.80-7.01, Table 2).

Table 2. Total and live birth prevalence of neural tube defects in South–Eastern Hungary (1 July 1992–30 June 2006)

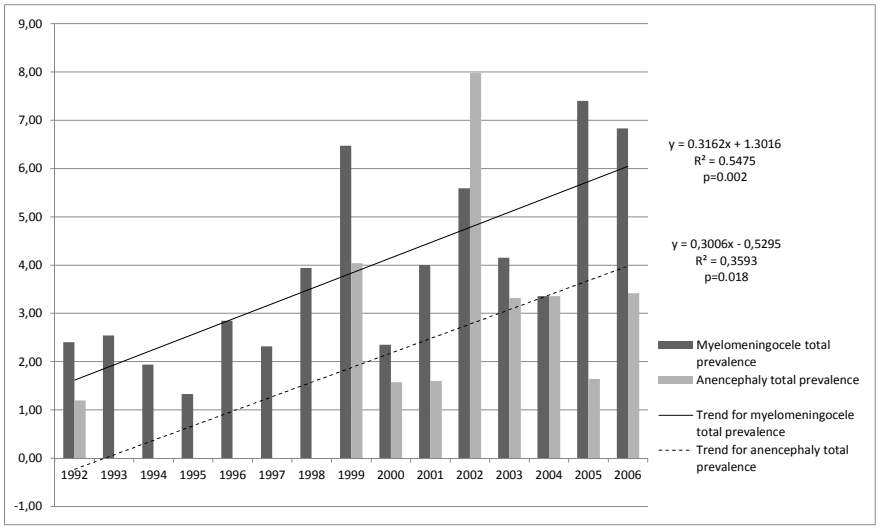
Malformations	Number of cases (total)	Total prevalence per 10 000 births	95% CI	Number of cases (live birth)	Live birth prevalence per 10 000 births	95% CI
Myelomeningocele	68	**3.65**	2.78–4.52	31	**1.67**	1.08–2.26
Anencephaly	32	**1.72**	1.12–2.31	0	**NR**	NR
Encephalocele	10	**0.54**	0.20–0.87	6	**0.32**	0.06–0.58
Total	110	**5.90**	4.80–7.01	37	**1.99**	1.35–2.64

Table 3. Detailed yearly epidemiologic data of neural tube defects in South–Eastern Hungary (1 July 1992–30 June 2006)

Year	Myelomeningocele					Anencephaly					Encephalocele				
	LB	TOP	SB	Total	**TP per 10 000 births**	LB	TOP	SB	Total	**TP per 10 000 births**	LB	TOP	SB	Total	**TP per 10 000 births**
1992	2	0	0	2	**2.40**	0	0	1	1	**1.20**	0	0	0	0	**NR**
1993	2	0	2	4	**2.54**	0	0	0	0	**NR**	0	0	0	0	**NR**
1994	3	0	0	3	**1.94**	0	0	0	0	**NR**	0	0	0	0	**NR**
1995	2	0	0	2	**1.33**	0	0	0	0	**NR**	1	0	0	1	**0.66**
1996	3	0	1	4	**2.85**	0	0	0	0	**NR**	0	0	0	0	**NR**
1997	2	0	1	3	**2.32**	0	0	0	0	**NR**	0	0	0	0	**NR**
1998	5	0	0	5	**3.94**	0	0	0	0	**NR**	2	0	0	2	**1.57**
1999	3	4	1	8	**6.47**	0	5	0	5	**4.04**	1	0	0	1	**0.81**
2000	2	1	0	3	**2.35**	0	2	0	2	**1.57**	1	0	0	1	**0.78**
2001	1	3	1	5	**4.00**	0	2	0	2	**1.60**	0	0	0	0	**NR**
2002	2	5	0	7	**5.59**	0	9	1	10	**7.98**	0	1	1	2	**1.60**
2003	0	5	0	5	**4.15**	0	4	0	4	**3.32**	1	0	0	1	**0.83**
2004	0	4	0	4	**3.36**	0	4	0	4	**3.36**	0	1	0	1	**0.84**
2005	4	5	0	9	**7.40**	0	2	0	2	**1.64**	0	0	0	0	**NR**
2006	0	4	0	4	**6.83**	0	2	0	2	**3.42**	0	1	0	1	**1.71**
Total	**31**	**31**	**6**	**68**	**3.65**	**0**	**30**	**2**	**32**	**1.72**	**6**	**3**	**1**	**10**	**0.54**

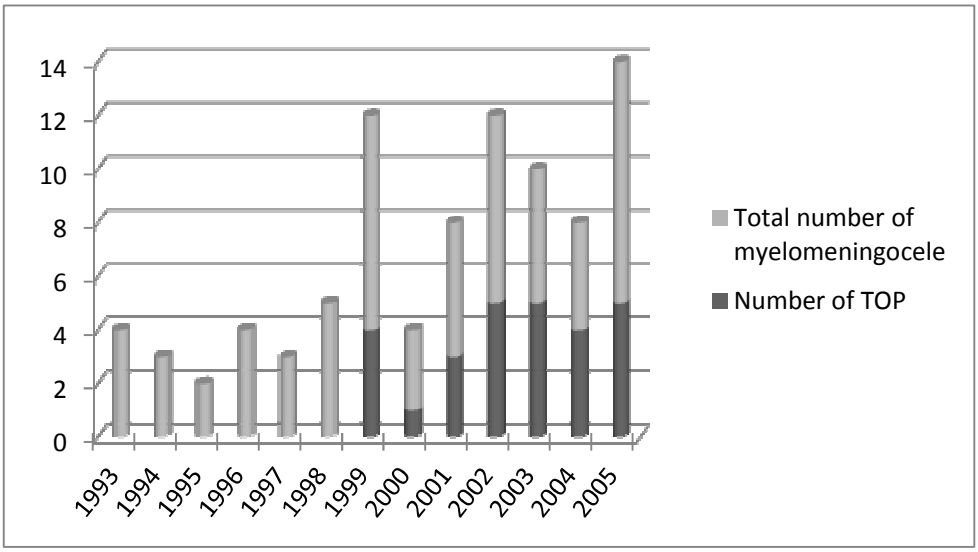
Group 1. Myelomeningocele (Tables 1–3, Figures 1, 2) was found in 68 cases (61.8% of all NTD) which corresponded to a TBPR of 3.65 per 10 000 births (95% CI: 2.78-4.52, Table 2). There was a trend of increase in the TBPR after 1999 (Table 3, Figure 1). The pregnancy was terminated after prenatal diagnosis of the malformation in 31 cases, however, the same number of newborns (14 males and 17 females) was born alive (LBPR: 1.67 per 10 000, 95% CI: 1.08-2.26, Table 2, 3). It is noteworthy that the majority of live births occurred before 1999 while the number of terminations increased after 1999 parallel with a trend of increase in the total birth prevalence (Table 3, Figure 1, 2).

Figure 1. Total birth prevalence of myelomeningocele and anencephaly in South-Eastern Hungary (1 July 1992-30 June 2006)



Myelomeningocele was associated with other CNS and extra-CNS abnormalities in 21 cases (31%, 10 livebirths, 8 terminated foetuses, 3 stillbirths). It co-occurred with trisomy 13 in a case and trisomy 18 in another terminated pregnancy. Myelomeningocele was accompanied by encephalocele in 2 cases. Anomalies of CC (4 cases), subependymal nodular heterotopia and cerebellar aplasia were among the CNS malformations in association with myelomeningocele. Congenital heart disease occurred in 5, renal and urinary tract abnormalities in 3 cases, intestinal abnormalities in 2 and cleft lip and palate in one case out of the total of 68 cases. These figures, however, can not be accurate, because accompanying malformations might have been missed in terminated foetuses and stillbirths.

Figure 2. Total number of myelomeningocele and number of terminations for myelomeningocele per year in South–Eastern Hungary (1993-2005)



Group 2. Anencephaly (Tables 1–3, Figure 1) occurred in 32 cases (29.1% of all NTD, all terminations, except 2 stillborns), which means a TBPR of 1.72 per 10 000 births (95% CI: 1.12–2.31, Tables 2, 3). There was a trend of increase in the total prevalence of anencephaly after 1999 (Table 3, Figure 1).

Extra-CNS abnormalities, such as renal, gastrointestinal and musculoskeletal defects were revealed in 6 out of 32 cases, although several associated anomalies might have been missed in terminated foetuses and stillbirths.

Group 3. Encephalocele (Tables 1–3) was diagnosed in 10 cases (9.1% of all NTD, 6 livebirths, 3 terminated foetuses, 1 stillbirth), which corresponded to a TBPR of 0.54 per 10 000 births (95% CI: 0.20–0.87) and LBPR of 0.32 per 10 000 (95% CI: 0.06–0.58, Tables 1-3). A female predominance (male/female ratio: 0.2) was observed among the livebirths.

The encephalocele was occipital in 9 cases. It was part of Meckel-Gruber syndrome in one and associated with multiple anomalies (cleft lip, cleft palate, club foot and eye defects) in another newborn. Nasofrontal encephalocele was found in a case with trisomy 21. Patients who were born alive showed developmental delay.

Group 4. Closed (occult) spinal dysraphism (Table 1) occurred in 4 patients (2 males and 2 females), which means a live birth prevalence of 0.22 per 10 000 (95% CI: 0.02–0.43). Lipomyelomeningocele was found in 3 children and dermal sinus in one patient

without any other associated defect. Neurological deficits (lower limb weakness and incontinence) led to the diagnosis in each case.

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Group 5. Holoprosencephaly (Tables 4–6) was diagnosed in 9 patients (5 males and 4 females), which means that the LBPR was 0.49 per 10 000 (95% CI: 0.17–0.58). The male/female ratio was 1.25. All types (lobar, semilobar and alobar) of holoprosencephaly were included and the patients were classified into 3 groups based on the associated abnormalities. Molecular cytogenetic studies (aCGH), or mutation analysis of genes responsible for holoprosencephaly were not performed. The main clinical features and outcome can be seen in Tables 4-6.

5a. HPE with craniofacial (eye, nose, ear, mouth and jaw) abnormalities only was found in 4 patients (Table 4).

Table 4. Holoprosencephaly only with craniofacial abnormalities

No	Year of birth	Sex	Associated abnormalities	Outcome	Epilepsy	Comments
1	1994	F	Cebocephaly	DD, ID, ND	-	Spastic quadriplegia Death at the age of 5 years, no autopsy
2	2001	M	Hypotelorism Midfacial dysmorphic features	DD, ID, ND	+	Spastic quadriplegia
3	1995	M	Hypotelorism Midfacial dysmorphic features	DD, ID, ND	+	Spastic quadriplegia
4	1993	M	Bilateral microphthalmos Bilateral optic nerve coloboma Cleft lip and palate	DD, ID, ND	+	Spastic quadriplegia

5b. Holoprosencephaly in association with non-craniofacial abnormalities in addition to craniofacial abnormalities was found in another 4 patients (Table 5).

Table 5. Holoprosencephaly with craniofacial and non-craniofacial abnormalities

No	Year of birth	Sex	Associated abnormalities	Outcome	Epilepsy	Comments
1	1997	F	Bilateral microphthalmos Polydactyly Thyroid hypoplasia Congenital heart disease: ASD Imperforate anus Rectovaginal fistula	NR	NR	Neonatal death Autopsy, no histology
2	1994	M	Cyclopia Proboscis Cryptorchism	NR	NR	Neonatal death Autopsy, no histology
3	1999	M	Microphthalmos (left) Micropenis	DD, ID, ND	+	Generalized hypotonia Paucity of spontaneous movements
4	1999	F	Cleft lip and palate Pyloric stenosis	DD, ID, ND	+	Maternal diabetes Spastic quadriplegia

5c. HPE was associated with chromosomal anomaly (trisomy 13) in a newborn (Table 6).

Table 6. Holoprosencephaly in association with chromosomal anomaly

No	Year of birth	Sex	Associated abnormalities	Outcome	Epilepsy	Comments
1	1998	F	Cleft lip and palate Postaxial polydactyly (hands, bilateral), Polysyndactyly (right foot) Hypoplastic left heart	NR	NR	Patau syndrome (trisomy 13) Neonatal death Autopsy, no histology

Group 6. Corpus callosum anomalies (total/partial agenesis and hypoplasia, Tables 7–11) were found in 38 patients (26 males and 12 females), which means an overall live birth prevalence of 2.05 per 10 000 live births (95% CI: 1.40–2.70) for all types of CC anomalies, 2.73 per 10 000 (95% CI: 1.68–3.78) among males and 1.33 per 10 000 (95% CI: 0.58–2.08) among females (Table 7). The LBPR was 1.02 per 10 000 (95% CI: 0.56–1.48) for both total/partial agenesis and hypoplasia, 1.36 per 10 000 (95% CI: 0.62–2.11) among males and 0.66 per 10 000 (95% CI: 0.13–1.20) among females (Table 7). The male/female ratio was 2.2 for both total/partial agenesis and hypoplasia of the CC (Table 7), as the same number of patients (13 males, 6 females) occurred in both groups. Parental consanguinity or familial occurrences were not found. CC anomalies were associated with other CNS malformation in 32% of patients while extra-CNS abnormalities occurred in 40% of the cases. Patients after an obvious destructive cerebral event, particularly preterm infants with a thin corpus callosum accompanied by periventricular leukomalacia were excluded from the study.

Table 7. Birth prevalence of corpus callosum anomalies

Corpus callosum anomaly	Overall prevalence per 10 000 live births	Prevalence for males per 10 000 live births	Prevalence for females per 10 000 live births	Sex ratio (M/F)
Agenesis (total and partial) and hypoplasia	2.05 (95% CI:1.4–2.7)	2.73 (95% CI: 1.68–3.78)	1.33 (95% CI:0.58–2.08)	2.2
Agenesis (total and partial)	1.02 (95% CI:0.56–1.48)	1.36 (95% CI:0.62–2.11)	0.66 (95% CI:0.13–1.20)	2.2
Hypoplasia	1.02 (95% CI:0.56–1.48)	1.36 (95% CI:0.62–2.11)	0.66 (95% CI:0.13–1.20)	2.2

The patients with CC anomalies were classified into 4 groups based on their associated abnormalities.

6a. Isolated agenesis/hypoplasia of CC was evident in 18 patients (14 males and 4 females, Table 8). DD and ID occurred in several patients in this group but abnormal

neurological findings were found in only 2 children. Epilepsy was diagnosed in half of the cases.

Table 8. Isolated agenesis/ hypoplasia of the corpus callosum

No	Year of birth	Sex	CC malformation	Outcome	Epilepsy	Comments
	1992	M	Agenesis	DD, ID	+	
2	2000	M	Agenesis	DD	+	
3	2000	M	Agenesis	DD, ND	–	Generalized hypotonia
4	2002	M	Agenesis	DD	+	
5	1995	M	Agenesis Interhemispheric cyst	–	–	Prenatal diagnosis
6	1999	M	Agenesis Interhemispheric cyst	–	–	Prenatal diagnosis
7	1993	M	Hypoplasia	DD, ID	+	
8	1996	M	Hypoplasia	–	+	
9	1996	M	Hypoplasia	DD, ID, ND	+	Spastic quadriplegia
10	1999	M	Hypoplasia	DD, ID	–	
11	2000	F	Hypoplasia	DD, ID	–	
12	2000	F	Hypoplasia	DD, ID	+	
13	2001	M	Hypoplasia	DD, ID	+	
14	2004	F	Hypoplasia	DD	–	
15	2005	F	Hypoplasia	–	+	
16	2005	M	Hypoplasia	–	–	Prenatal diagnosis
17	2003	M	Hypoplasia Interhemispheric cyst	–	–	Prenatal diagnosis
18	2005	M	Hypoplasia Interhemispheric cyst	–	–	Prenatal diagnosis

6b. Agenesis/hypoplasia of the CC was associated with another CNS malformation without any extra CNS anomaly in 5 children (3 males and 2 females). The callosal anomaly was accompanied by microcephaly, optic nerve hypoplasia, CVH or wide CSP (Table 9). DD and ID were observed in 4 patients, abnormal neurological findings were evident in 3 cases, and epilepsy was diagnosed in 2 children of this group.

Table 9. Agenesis/hypoplasia of the corpus callosum in association with other CNS abnormalities

No	Year of birth	Sex	CC malformation	Associated CNS abnormalities	Outcome	Epilepsy	Comments
1	1994	F	Agenesis	Microcephaly	DD, ID, ND	–	Axial hypotonia Nystagmus
2	1996	M	Agenesis	Bilateral optic nerve hypoplasia	DD, ID, ND	+	Spastic quadriplegia
3	2003	F	Partial agenesis (rostrum is absent)	Cerebellar vermis hypoplasia (no molar tooth malformation)	DD, ID	+	
4	1996	M	Hypoplasia	Wide CSP	–	–	MRI because of headache
5	2002	M	Hypoplasia	Microcephaly Bilateral optic nerve hypoplasia	DD, ID, ND	–	Spastic quadriplegia

6c. Both CNS and extra-CNS abnormalities accompanied agenesis/hypoplasia of the corpus callosum in 7 patients (4 males and 3 females, Table 10). Callosal anomalies were associated with encephalocele, hemimegalencephaly, PMG, FCD or wide CSP in addition to extra-CNS abnormalities, such as dysmorphic features, congenital heart disease, limb anomalies or hip dysplasia (Table 10). DD, ID and abnormal neurological findings were evident in all patients, and epilepsy was diagnosed in 3 children of this group. One patient died in childhood.

Table 10. Agenesis/hypoplasia of the corpus callosum associated with both CNS and extra-CNS abnormalities

No	Year of birth	Sex	CC malformation	Associated CNS and extra-CNS abnormalities	Outcome	Epilepsy	Comments
1	1993	M	Agenesis	Occipital cephalocele (small) Dysmorphic features	DD, ID, ND	–	Spastic quadriplegia
2	1994	F	Agenesis	Occipital cephalocele (small) Dysmorphic features Syndactyly	DD, ID, ND	–	Spastic quadriplegia
3	1999	F	Agenesis	Right hemimegalencephaly with agyria-pachygyria Congenital heart disease: ASD	DD, ID, ND	+	Spastic diplegia Amaurosis
4	2003	M	Agenesis	Polymicrogyria Dysmorphic features	DD, ID, ND	+	Dystonia Death in childhood
5	1997	F	Partial agenesis (splenium is absent)	Bi-occipital cortical dysplasia Polydactyly	DD, ID, ND	–	Generalized hypotonia
6	2006	M	Hypoplasia	Wide CSP Developmental dysplasia of the hip	DD, ID, ND	–	Generalized hypotonia Nystagmus
7	1993	M	Hypoplasia Interhemispheric cyst	Polymicrogyria Supernumerary nipples	DD, ID, ND	+	Spastic quadriplegia

6d. Agenesis/hypoplasia of CC was associated only with extra-CNS abnormalities in 8 cases (5 males and 3 females, Table 11). Various somatic abnormalities were recognised in this group. Two syndromes, such as short rib-polydactyly syndrome type II (Majewski) and Edwards syndrome (trisomy 18) were also identified. Half of the patients died during the neonatal period, and autopsies confirmed the callosal and extra-CNS abnormalities. Developmental delay and intellectual disability were observed in patients surviving the neonatal period and 3 out of these 4 children showed abnormal neurological findings and/or epilepsy.

Table 11. Agenesis/hypoplasia of the corpus callosum associated with extra-CNS abnormalities

No	Year of birth	Sex	CC malformation	Associated extra-CNS abnormalities	Outcome	Epilepsy	Comments
1	1992	M	Agenesis	Dysmorphic features Congenital heart disease: ASD, ventricular septal defect (VSD), coarctation of the aorta, patent ductus arteriosus (PDA) Hypospadias	NR	NR	Neonatal death
2	1998	F	Agenesis	Short rib-polydactyly syndrome type II (Majewski) Cleft palate	NR	NR	Neonatal death
3	1999	M	Agenesis	Dysmorphic features	DD, ID	+	
4	2001	M	Agenesis	Dysmorphic features Congenital heart disease: PDA double-outlet right ventricle Bilateral inguinal hernia (Edwards syndrome)	NR	NR	Trisomy 18 Neonatal death
5	2005	M	Agenesis	Dysmorphic features Blepharophimosis Corneal opacities of unknown aetiology	DD, ID, ND	–	Generalized hypotonia
6	1997	F	Hypoplasia	Congenital heart disease: VSD, coarctation of the aorta, PDA	NR	NR	Neonatal death
7	1997	M	Hypoplasia	Dyscrania	DD, ID, ND	+	Spastic quadriplegia
8	2005	F	Hypoplasia	Dysmorphic features, Congenital heart disease: ASD Pyelectasis Umbilical hernia	DD, ID, ND	+	Sensorineural hearing loss

Group 7. Septo-optic dysplasia (Table 12) was ascertained in 2 patients (1 male and 1 female), which means a live birth prevalence of 0.11 per 10 000 (95% CI: -0.04–0.26). A patient who was available for follow up had congenital heart disease (atrial septal defect), epilepsy, and poor neurodevelopmental outcome (Table 12).

Table 12. Septo-optic dysplasia

No	Year of birth	Sex	Associated abnormalities	Outcome	Epilepsy	Comments
1	2003	M	Unknown	Unknown	Unknown	Lost for our follow-up
2	2003	F	Dilated lateral ventricles Congenital heart disease: ASD	DD, ID, ND	+	Spastic diplegia with contractures

Group 8. Cavum septum pellucidum (Table 13) (10 mm, or wider) occurred in 11 patients (7 males and 4 females), which corresponded to a live birth prevalence of 0.59 per 10 000 (95% CI: 0.24–0.94, Table 13). The male/female ratio was 1.75. The CSP was isolated in all cases, except one patient (Patient 7, Table 13) who had multiple

extra-CNS anomalies. Genetic testing was carried out in a girl (Patient 1, Table 13) with delayed development, autistic features and epilepsy, however the routine chromosomal study and mutation analysis of the *MECP2, CDKL5, FOXG1* and *FMR1* genes failed to show any abnormalities.

Developmental delay and intellectual disability were found in a few cases, while behavioural problems dominated the clinical features in others (Table 13). Febrile convulsions and epilepsy occurred in more than half of the patients.

Table 13. Cavum septum pellucidum

No	Year of birth	Sex	Associated abnormalities	Outcome	Epilepsy	Comments
1	2005	F	-	DD, ID	+	Autistic features Normal karyotype No mutation in *MECP2, CDKL5, FOXG1* and *FMR1* genes
2	1999	F	-	DD, ID, ND	-	Generalized hypotonia
3	2002	M	-	-	+	
4	2002	F	-	-	+	Behavioural problems
5	1998	M	-	-	+	
6	1994	M	-	-	-	Headache
7	1992	M	Congenital heart disease: truncus arteriosus Inguinal hernia (right side) Undescendent testis (left side)	-	-	Death in infancy
8	1997	F	-	DD, ID	-	Psychiatric problems Speech disorder Febrile convulsion
9	2005	M	-	-	-	Perinatal asphyxia Neonatal seizures Febrile convulsion
10	1998	M	-	DD	-	Psychiatric problems
11	1998	M	-	-	-	Psychiatric problems

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Group 9. Microcephaly (Tables 14–16). Congenital microcephaly was diagnosed in 30 patients (11 males and 19 females), which means a live birth prevalence of 1.62 per 10 000 (95% CI: 1.04–2.20). Patients with congenital microcephaly were classified into 3 groups, such as (a) primary microcephaly, (b) microcephaly associated with chromosomal abnormalities and as part of genetic syndromes, and (c) microcephaly with other CNS and/or extra-CNS abnormalities.

9a. Primary microcephaly (Table 14) was found in 10 patients (2 males and 8 females), which means a live birth prevalence of 0.54 per 10 000 (95% CI: 0.20–0.87). The male/female ratio was 0.25. Chromosomal analysis was normal in all children. Each of them had DD and ID, however, other neurological signs were not observed,

except for dyskinesia in one patient (Patient 4, Table 14). Two children suffered from epilepsy.

Table 14. Primary microcephaly

No	Year of birth	Sex	Outcome	Epilepsy	Comments
1	2005	F	DD, ID	-	
2	2004	F	DD, ID	-	
3	1992	F	DD, ID	-	
4	2005	F	DD,ID,ND	+	Dyskinesia
5	2004	F	DD, ID	-	Patients 5 and 6 are twins
6	2004	F	DD, ID	-	Patients 5 and 6 are twins
7	1997	F	DD, ID	-	
8	2004	M	DD, ID	-	
9	1992	F	DD, ID	+	
10	2005	M	DD, ID	-	

Table 15. Congenital microcephaly associated with chromosomal abnormalities and as part of a recognisable syndrome

No	Year of birth	Sex	Chromosomal abnormalities/ syndromes	Extra-CNS abnormalities	Outcome	Epil epsy	Comments
1	2004	F	XXXXX syndrome (Penta X syndrome)	Facial dysmorphism Short stature Renal dysplasia Hip dysplasia	DD, ID	_	
2	1993	F	Trisomy 13 (Patau syndrome)	Simian creases Cleft lip, cleft palate Congenital heart disease: cor triloculare Imperforate anus Absent coccygeal bone	NR	NR	Neonatal death
3	1996	M	Otopalalatodigital syndrome spectrum disorder (Xq deletion)	Cleft palate Micrognathia Low set and dysmorphic ears Syndactyly of fourth and fifth toes on both sides Congenital heart disease: ASD, PDA	DD, ID, ND	-	Spastic quadriplegia
4	2003	M	Cornelia de Lange syndrome	Syndrome specific features	DD, ID, ND	-	Generalized hypotonia Febrile convulsion
5	2006	M	Cornelia de Lange syndrome	Syndrome specific features	DD, ID, ND	-	Generalized hypotonia
6	1993	F	Nijmegen breakage syndrome (Published by Erdős M et al., ref. 13)	Syndrome specific features	DD, ID	-	Patients 6 and 7 are siblings Primary cutaneous tuberculosis Mutation in *NBS1* gene (c.657_661del5bp, p.K219fs*234)
7	2002	F	Nijmegen breakage syndrome (Published by Erdős M et al., ref. 13)	Syndrome specific features	DD, ID	-	Patients 6 and 7 are siblings Primary cutaneous tuberculosis Mutation in *NBS1* gene (c.657_661del5bp, p.K219fs*234)

9b. Congenital microcephaly was associated with chromosomal abnormalities or was ascertained as part of a recognisable syndrome in 7 patients (3 males and 4 females, Table 15). The live birth prevalence was 0.38 per 10 000 (95% CI: 0.10-0.66) of this heterogeneous group of microcephaly. The male/female ratio was 0.75. Five distinct syndromes, such as Penta X, Patau, Cornelia de Lange (2 cases) and Nijmegen breakage (2 siblings) syndromes, and otopalatodigital syndrome spectrum disorder were identified. A deletion (c.657_661del5bp, p.K219fs*234) in the *NBS1* gene identified in Debrecen confirmed the diagnosis of Nijmegen breakage syndrome (13).

Table 16. Congenital microcephaly with other CNS and/or extra-CNS anomalies

No	Year of birth	Sex	Associated abnormalities	Outcome	Epilepsy	Comments
1	2005	F	Hypertelorism, epicanthus Clinodactyly, syndactyly of second, third and fourth toes on both sides	DD,ID	-	
2	2000	M	Hypotelorism, microphthalmos Congenital heart disease: ASD	DD,ID,ND	+	Spastic quadriplegia
3	1996	M	Micrognathia, cleft palate Deep set ears Oesophageal atresia and tracheo-oesophageal fistula Congenital heart disease: tetralogy of Fallot	NR	NR	Neonatal death
4	2003	M	Facial dysmorphism Choanal atresia Pterygium colli Abnormal toes Congenital heart disease: ASD, pulmonary stenosis, PDA	NR	NR	Death in infancy (2004)
5	1995	F	Craniofacial dysmorphism Cleft lip, cleft palate	DD,ID	-	
6	2004	F	Craniofacial dysmorphism Corpus callosum dysgenesis	DD,ID,ND	+	Spastic quadriplegia
7	2004	M	Facial dysmorphism Hypospadias	DD,ID	-	
8	1994	M	Optic nerve hypoplasia	DD,ID,ND	+	Spastic quadriplegia Death in childhood (2003)
9	1998	F	Facial dysmorphism	DD,ID,ND	+	Spastic quadriplegia
10	2003	F	Facial dysmorphism	ND	-	
11	2003	F	Facial dysmorphism	DD,ID	-	Foetal alcohol syndrome
12	2002	F	Facial dysmorphism	ID	-	Maternal drug use (ACE inhibitor) during pregnancy
13	1998	M	Facial dysmorphism	DD,ID	-	Familial mental retardation of unknown aetiology

9c. Congenital microcephaly with other CNS and/or extra-CNS abnormalities was found in 13 patients (6 males and 7 females, Table 16) with a birth prevalence rate of 0.70 per 10 000 live births (95% CI: 0.32-1.08). A syndrome was not recognisable in these patients. The male/female ratio was 0.86. The microcephaly was accompanied by CC dysgenesis in a girl and optic nerve hypoplasia in a boy (Table 16). Craniofacial

dysmorphic features were the most common extra-CNS abnormalities. Almost all of these patients had DD and ID. Epilepsy occurred in 4 out of these 13 children. Three patients with multiple abnormalities died.

Group 10. Microlissencephaly (Table 17) was found in 2 patients (1 male and 1 female), which means that the live birth prevalence was 0.11 per 10 000 (95% CI: -0.04–0.26, Table 17). Both children had extreme microcephaly with shallow sulci on brain imaging. The abnormalities proved to be more severe than those ones of simplified gyral pattern usually seen in congenital microcephaly. The clinical features were dominated by severe DD, ID and ND. One of the patients (Patient 2, Table 17) with multiple abnormalities died in infancy.

Table 17. Microlissencephaly

No	Year of birth	Sex	Associated abnormalities	Outcome	Epilepsy	Comments
1	2002	M	-	DD, ID, ND	+	Spastic quadriplegia
2	2002	F	Oesophageal atresia and tracheo-oesophageal fistula Congenital heart disease: PDA	DD, ID, ND	-	Spastic quadriplegia Death in infancy (2003)

Epidemiology of neuronal migration defects

Migration defects (including agyria/pachygyria-SBH spectrum and heterotopias) were diagnosed in 8 patients (4 males and 4 females), which means that the live birth prevalence was 0.43 per 10 000 (95% CI: 0.13–0.73).

Group 11. Agyria/pachygyria - SBH spectrum (Table 18) was ascertained in 5 patients (3 males and 2 females, Table 18), which corresponded to a live birth prevalence of 0.27 per 10 000 (95% CI: 0.03–0.51). The male/female ratio was 1.5. The routine karyotype was negative in all cases. Mutation analysis identified a novel mutation in the *LIS1* gene (c.83_84 delAT, p.Tyr28Phefs*31) in a boy (Patient 1, Table 18) with typical classical lissencephaly with postero-anterior gradient and in the *DCX* gene (c.200delG, p.Ile68Leufs*87) in a girl with SBH (Patient 5, Table 18). DD and ID were seen in nearly all cases and epilepsy occurred in 3 patients out of 5.

Table 18. Agyria/pachygyria-subcortical band heterotopia spectrum

No	Year of birth	Sex	Malformation	Associated abnormalities	Outcome	Epilepsy	Comments
1	2001	M	Lissencephaly (posterior >anterior gradient)	Dysmorphic features	DD, ID, ND	+	Novel mutation in the *LIS1* gene (c.83_84delAT, p.Tyr28Phefs*31)
2	2005	M	Pachygyria (mainly bilateral frontal)	-	ND	+	Spastic hemiplegia
3	1999	F	Pachygyria (mainly in the bilateral central area)	Dysmorphic features Microcephaly	DD, ID, ND	-	Spastic quadriplegia
4	1998	M	Agyria/pachygyria (mainly bilateral parietal)	-	DD, ID	-	Febrile convulsions
5	2000	F	Subcortical band heterotopia	-	DD, ID	+	Novel mutation in the *DCX* gene (c.200delG, p.Ile68Leufs*87)

Group 12. Neuronal heterotopia (subependymal nodular heterotopia, Table 19) occurred in 3 patients (1 male and 2 females) with a live birth prevalence of 0.16 per 10 000 (95% CI: -0.02–0.34, Table 19). All cases had subependymal nodular heterotopia. Congenital heart disease and facial dysmorphism were reported as associated abnormalities. The neurodevelopmental outcome was poor and ID was evident in all patients. Febrile convulsions or epilepsy were diagnosed in 2 out of 3 patients.

Table 19. Neuronal heterotopia

No	Year of birth	Sex	Malformation	Associated abnormalities	Outcome	Epilepsy	Comments
1	1999	F	Subependymal nodular heterotopia	Dilated lateral ventricles Congenital heart disease: patent foramen ovale (PFO)	DD, ID, ND	-	Spastic hemiplegia Strabismus Febrile seizures
2	1999	M	Subependymal nodular heterotopia	Dysmorphic features	ID	-	
3	2002	F	Subependymal nodular heterotopia (frontal)	-	DD, ID, ND	+	Generalized hypotonia

Epidemiology of abnormal cortical organization

Group 13. Polymicrogyria (Table 20) was found in 8 patients (7 males and 1 female), which means that the live birth prevalence was 0.43 per 10 000 (95% CI: 0.13–0.73). Significant male predominance was reported, the male/female ratio was 7.0 in the sample. Table 20 shows the details of MRI findings and clinical features in this heterogeneous group of patients. Patient 7 represented a unique combination of bilateral

PMG and mega corpus callosum. DD and ND were observed in each case in association with ID and epilepsy in the majority of them.

Table 20. Polymicrogyria

No	Year of birth	Sex	Malformation	Associated abnormalities	Outcome	Epilepsy	Comments
1	1999	M	Unilateral (right) polymicrogyria	_	DD, ND	-	Spastic hemiplegia
2	1994	M	Unilateral (right) polymicrogyria	_	DD, ID, ND	+	Spastic hemiplegia
3	2003	M	Unilateral (left parietal) polymicrogyria	Dilated lateral ventricles Dysmorphic features: epicanthus, deep set ears Möbius sequence	DD, ND	-	Abnormal facial expression Abnormal eye movements
4	2001	M	Unilateral (right temporo-parietal) polymicrogyria	Microcephaly Micrognathia Congenital heart disease: ASD	DD, ID, ND	+	Spastic hemiplegia
5	1992	M	Bilateral generalized polymicrogyria	Microcephaly Dilated lateral ventricles Enlarged extraaxial cerebrospinal fluid spaces	DD, ID, ND	+	Spastic quadriplegia
6	1999	M	Bilateral fronto-parietal and perisylvian polymicrogyria	Dysmorphic features, dyscrania White matter T2 signal hyperintensity Septum pellucidum cyst Abnormal corpus callosum Hypoplasia of medulla oblongata Polydactyly (both hands and feet) Congenital heart disease: VSD	DD, ID, ND	+	Gestational diabetes Bilateral perisylvian syndrome Spastic quadriplegia
7	2000	M	Bilateral parietal polymicrogyria	Dysmorphic features Mega corpus callosum	DD, ID, ND	+	Syndrome with mega corpus callosum, PMG, epilepsy and psychomotor retardation (aCGH was negative)
8	2002	F	Polymicrogyria (Details are unknown)	Dyscrania Dysmyelination	DD, ID, ND	+	Spastic quadriplegia Death in childhood

Group 14. Schizencephaly (Table 21) was diagnosed in 10 patients (6 males and 4 females), which means that the prevalence at birth was 0.54 per 10 000 live births (95% CI: 0.20–0.87, Table 21), and the male/female ratio was 1.5. The schizencephaly was unilateral in 7 cases (with closed lips in 5 and open lips in 2 patients) and bilateral in 3 children (with closed lips in 2 and open lips in 1). In most cases, the unilateral cleft was localized to the perisylvian region, whereas the bilateral clefts were localized in various areas. The vast majority of patients had DD, ID and ND. Signs of bilateral operculum

syndrome were observed in Patient 5 (Table 21). Seizures appeared and epilepsy was diagnosed in 3 patients.

Table 21. Schizencephaly

No	Year of birth	Sex	Malformation	Associated abnormalities	Outcome	Epilepsy	Comments
1	1993	M	Unilateral (left) open lip, perisylvian	Dysmorphic features Absence of septum pellucidum Right perisylvian polymicrogyria Right cerebellar hypoplasia Intracerebral calcification Pigmentary retinopathy Congenital heart disease	DD, ID, ND	+	Spastic quadriplegia, more severe on the right side Normal karyotype
2	2004	M	Unilateral (left) closed lip, perisylvian	Dysmorphic features Absence of septum pellucidum	DD, ID, ND	-	Spastic hemiplegia (R) Normal karyotype
3	2001	F	Unilateral (right) closed lip, perisylvian	-	DD, ID, ND	-	Spastic hemiplegia (L)
4	2004	F	Unilateral (left) closed lip, perisylvian	Absence of septum pellucidum	ND	-	Spastic hemiplegia (R)
5	1998	M	Unilateral (left) closed lip, perisylvian	Absence of septum pellucidum Right perisylvian polymicrogyria	DD, ID, ND	+	Spastic hemiplegia (R) Bilateral operculum syndrome Normal karyotype
6	1995	F	Unilateral (right) closed lip, parietal	-	ND	-	Spastic hemiplegia (L)
7	1996	M	Unilateral (right) open lip, parietal	-	DD, ID, ND	-	Spastic hemiplegia (L)
8	2005	M	Bilateral open lip, perisylvian	Absence of septum pellucidum Agenesis of corpus callosum	DD, ID, ND	+	Spastic quadriplegia
9	1993	F	Bilateral closed lip, parietal	-	DD, ID, ND	-	Generalized hypotonia
10	2001	M	Bilateral, closed lip, right parietal and left frontal	Dysgenesis of corpus callosum	DD, ID, ND	-	Spastic quadriplegia

Group 15. Focal cortical dysplasia (Table 22) was diagnosed in 1 patient (male), which means that the prevalence was 0.05 per 10 000 live births (95% CI: -0.05–0.16). The MRI revealed isolated, frontal, focal cortical dysplasia. The child had ID and epilepsy.

Table 22. Focal cortical dysplasia

No	Year of birth	Sex	Localization	Associated abnormalities	Outcome	Epilepsy	Comments
1	1995	M	Frontal	-	ID	+	

Epidemiology of midbrain and hindbrain malformations

Hindbrain malformations were diagnosed in 30 patients (13 males and 17 females), which means that the overall live birth prevalence was 1.62 per 10 000 (95% CI: 1.04–2.20), 1.36 per 10 000 (95% CI: 0.62–2.11) among boys and 1.88 per 10 000 (95% CI: 0.99–2.78) among girls. The male/female ratio was 0.76.

Malformations affecting both the midbrain and the hindbrain

Group 16. Molar tooth sign (Table 23) was ascertained in 2 cases (1 male and 1 female), which corresponded to a live birth prevalence of 0.11 per 10 000 (95% CI:-0.04–0.26, Table 23).

Joubert syndrome was ascertained in a boy (Patient 1,Table 23), which suggested a birth prevalence of 1: 200 000 live births. Mutation analysis revealed compound heterozygosity in the *CEP290* gene: frameshift mutation was found in exon 46 (c.6277delG, p.Val2093SerfsStop4) and nonsense mutation in exon 38 (c.5182>T, p.Glu1728Stop) resulting in truncated proteins.

MTS accompanied by multiple brain malformations was also noticed in a girl with short rib-polydactyly syndrome (Patient 2, Table 23). Chromosomal analysis showed normal karyotype and aCGH did not reveal genome imbalance.

Table 23. Molar tooth sign

No	Year of birth	Sex	Associated abnormalities	Outcome	Epilepsy	Comments
1	2005	M	Ptosis (R) Bilateral optic nerve atrophy	DD, ID, ND	-	Joubert syndrome: hypotonia, ataxia, jerky eye movements, oculomotor apraxia *CEP290* mutation: Allele 1: exon 38 (c.5182G>T, p.Glu1728Stop) – from the father Allele 2: exon 46 (c.6277delG, p.Val2093SerfsStop4) – from the mother (compound heterozygosity)
2	2005	F	Micrencephaly (cerebral hemispheric hypoplasia) Hypoplastic pons and cerebellum Short ribs and limbs Polydactyly	DD ID, ND	-	Ellis-van Creveld syndrome (atypical form?) Ventilator dependence Generalized hypotonia and abnormal innervation by several cranial nerves Death in infancy aCGH was negative

Malformations affecting predominantly the cerebellum and its derivates (Rh1)

Group 17. Dandy-Walker malformation (Table 24) was found in 6 cases (1 male and 5 females), which means a live birth prevalence of 0.32 per 10 000 (95% CI: 0.06–0.58). The male/female ratio was 0.2. In 5 out of 6 patients the DWM was associated with several CNS and extra-CNS malformations. A girl (Patient 2, Table 24) had

PHACE syndrome. Three patients died in the neonatal period and the outcome was very poor in the other patients as well.

Table 24. Dandy–Walker malformation

No	Year of birth	Sex	Associated abnormalities	Outcome	Epilepsy	Comments
1	2005	F	-	Unknown	-	
2	2003	F	Segmental haemangioma of the face and scalp Myelomeningocele Microcephaly Anophthalmia Cleft lip, cleft palate Ear hypoplasia Vertebral abnormalities Reduction defects of the fingers Congenital heart disease: ASD, VSD Omphalocele Bilateral renal hypoplasia	DD, ID, ND	+	PHACE syndrome Sensorineural hearing loss Normal karyotype
3	2000	F	Microcephaly Optic nerve hypoplasia Congenital heart disease: ASD Renal hypoplasia	DD, ID, ND	+	Spastic quadriplegia (Sibling had microcephaly and died in late infancy)
4	1992	F	Hydrocephalus Polydactyly, syndactyly Congenital heart disease: hypoplastic left heart syndrome, truncus arteriosus communis, PDA	NR	NR	Neonatal death (1992)
5	1993	F	Hydrocephalus	NR	NR	Prematurity and neonatal death (1994)
6	2004	M	Hydrocephalus Cleft lip, cleft palate Supernumerary nipples (right side) Congenital heart disease: tetralogy of Fallot Hypoplasia of penis	NR	NR	Neonatal death (2004)

Group 18. Cerebellar vermis hypoplasia without molar tooth sign (Table 25) was ascertained in 9 cases (3 males and 6 females) with a live birth prevalence of 0.49 per 10 000 (95% CI: 0.17–0.80). The male/female ratio was 0.5. Nearly half of the children with CVH did not have any associated malformations. A seemingly balanced chromosomal translocation: XX,t(3q;16q)(2.6;2.4) was recognized by G-band technique and FISH in a girl with CVH, blepharophimosis and atypical autism (Patient 9, Table 25). The outcome was rather poor in the majority of cases, and epilepsy occurred in 4 patients.

Table 25. Cerebellar vermis hypoplasia without molar tooth sign

No	Year of birth	Sex	Associated abnormalities	Outcome	Epilepsy	Comments
1	1993	M	Unknown	Unknown	-	
2	1992	M	_	Normal development	-	Headache
3	2002	F	-	DD, ID, ND	-	Generalized hypotonia
4	1996	F	_	DD, ID, ND	+	Generalized hypotonia Behavioural problems
5	1998	F	Hyperpigmented spot on the skin (left abdominal wall)	DD, ID	-	
6	1998	M	Cavum septum pellucidum	DD	+	
7	2003	F	Corpus callosum dysplasia Optic nerve hypoplasia (no septo-optic dysplasia) Suspected peripheral neuropathy (?)	DD, ID, ND	+	Hypotonia Ataxia
8	1993	F	Dilated lateral ventricles Cavum septum pellucidum Optic nerve hypoplasia (no septo-optic dysplasia) Congenital heart disease: ASD Renal hypoplasia	DD, ID, ND	+	
9	2004	F	Dysmorphic features Blepharophimosis	DD, ID, ND	-	Hypothyroidism Autism spectrum disorder Chromosomal abnormality: 46, XX,t(3q;16q)(2.6;2.4) balanced translocation (G-band and fluorescence in situ hybridization (FISH))

Group 19. Unilateral cerebellar hypoplasia (Table 26) occurred in 2 cases (1 male and 1 female), which corresponded to a live birth prevalence of 0.11 per 10 000 (95% CI: -0.04–0.26). One of the patients (Patient 1, Table 26) had trisomy 21 (Down syndrome) while the other did not show any recognizable syndrome. Both patients had epilepsy.

Table 26. Unilateral cerebellar hypoplasia

No	Year of birth	Sex	Associated abnormalities	Outcome	Epilepsy	Comments
1	1997	F	Down syndrome (trisomy 21)	DD, ID, ND	+	
2	1997	M	Congenital heart disease: mitral insufficiency	Unknown	+	Abducens nerve paresis

Group 20. Cerebellar aplasia/hypoplasia, affecting both the vermis and hemispheres (Table 27) was found in 7 cases (5 males and 2 females), which means that the live birth prevalence was 0.38 per 10 000 (95% CI: 0.10–0.66). The male/female ratio was 2.5. **Cerebellar aplasia** was ascertained in one case (Patient 1, Table 27) accompanied by multiple anomalies. This patient died in the neonatal period.

Cerebellar hypoplasia was diagnosed in 6 patients in association with other abnormalities in 4 of them. The neurodevelopmental outcome was poor in most of the patients, epilepsy occurred in 4 of them and one patient (Patient 5, Table 27) died in infancy.

Table 27. Cerebellar aplasia/hypoplasia

No	Year of birth	Sex	Malformation	Associated abnormalities	Outcome	Epilepsy	Comments
1	1997	M	Cerebellar aplasia	Diaphragm agenesis Hypoplasia of the ribs (R) Cervical rib (right side) Absence of forearm bones (right side) Syndactyly	NR	NR	Prematurity Neonatal death
2	2000	M	Cerebellar hypoplasia	Inguinal hernia	DD	+	Strabismus Behavioural problems (attention deficit hyperactivity disorder)
3	1997	M	Cerebellar hypoplasia	–	ND	-	Ataxia
4	1992	F	Cerebellar hypoplasia	–	DD, ID, ND	+	Dyskinesia Spastic hemiplegia (left)
5	2002	M	Cerebellar hypoplasia	Hydrocephalus	DD, ID, ND	-	Prematurity Death in infancy
6	1995	F	Cerebellar hypoplasia	Microcephaly, Arachnodactyly	DD, ID, ND	+	Generalized hypotonia
7	1993	M	Cerebellar hypoplasia	Facial dysmorphism Optic nerve atrophy, Congenital heart disease: mitral insufficiency	DD, ID, ND	+	Strabismus Amblyopia (left eye) Limited tongue movements

Malformations affecting predominantly the lower hindbrain (Rh 2-8)

Group 21. Chiari type I malformation (Table 28) was diagnosed in 2 patients (2 males), which corresponded to a live birth prevalence of 0.11 per 10 000 (95% CI:-0.04–0.26). One of them was diagnosed accidentally, while the other child showed neurological signs and autistic features.

Table 28. Chiari I. malformation

No	Year of birth	Sex	Associated abnormalities	Outcome	Epilepsy	Comments
1	1995	M	-	-	-	Migraine
2	1993	M	-	DD, ID, ND	-	Twin (A) Generalized hypotonia Uncoordinated movements Autistic features

Malformations associated with prenatal onset degeneration

Group 22. Pontocerebellar hypoplasia, type 2 (Table 29) was confirmed in 2 patients (2 females), which means that the live birth prevalence was 0.11 per 10 000 (95% CI:-0.04–0.26). One of the patients (Patient 1, Table 29) had mental retardation and movement disorder (dyskinesia and dystonia), and the other (Patient 2, Table 29) was presented with severe mental retardation, spastic quadriplegia, sensorineural hearing loss and moderate facial dysmorphism. Chromosomal abnormalities and inborn errors of metabolism were excluded. Mutation analysis of the *TSEN54* gene was negative in both patients. The other *TSEN* genes have not been tested yet. Epilepsy was diagnosed in both of them.

Table 29. Pontocerebellar hypoplasia

No	Year of birth	Sex	Malformation	Associated abnormalities	Outcome	Epilepsy	Comments
1	1993	F	Pontocerebellar hypoplasia, type 2	Microcephaly	DD, ID, ND	+	Strabismus Generalized hypotonia Dyskinesia, dystonia No mutation in *TSEN54* gene
2	2005	F	Pontocerebellar hypoplasia, type 2	Progressive microcephaly (simplified gyral pattern) Facial dysmorphism	DD, ID, ND	+	Spastic quadriplegia Sensorineural hearing loss Strabismus No mutation in *TSEN54* gene

Other CNS malformations

Group 23. Arachnoid cysts (Table 30) were found in 24 patients (21 males and 3 females), which means that the prevalence was 1.29 per 10 000 live births (95% CI: 0.78–1.81). The male predominance was significant; the male/female ratio was 7.0. Most of the cysts were found in the middle cranial fossa. Other CNS and/or extra-CNS abnormalities were rarely associated with the arachnoid cysts. Epilepsy occurred in 29% of the cases.

Table 30. Arachnoid cysts

No	Year of birth	Sex	Localization	Associated abnormalities	Outcome	Epilepsy	Comments
1	2002	M	Temporal	–	Favourable	-	Headache
2	1997	M	Temporal	-	Favourable	-	Headache
3	1994	F	Temporal	-	Favourable	-	Recurrent vomiting
4	1995	M	Temporal	-	Favourable	+	
5	1993	M	Temporal	-	Favourable	+	
6	1995	F	Temporal	-	Favourable	-	Febrile seizure
7	1993	M	Sylvian, left	-	Not known	+	
8	2004	M	Temporal, left	Congenital heart disease: valvular aortic stenosis	DD	-	Headache
9	2001	M	Temporal, right	Dilated Virchow-Robin spaces	DD, ND	-	Generalized hypotonia
10	2004	M	Temporal, right	-	DD, ID	+	Autism spectrum disorder
11	2000	M	Temporal, left	-	DD, ID, ND	+	Generalized hypotonia
12	1996	M	Frontal, right	–	DD, ND	-	Facial weakness Problems with coordination Speech delay
13	1993	M	Frontal, left	Dysmorphic features (broad philtrum) Conjunctival telangiectasis Polydactyly, syndactyly,	DD, ID	+	Familial mental retardation
14	1995	M	Suprasellar	Congenital heart disease: ASD, PDA	DD, ID	-	Autism spectrum disorder
15	2000	F	Quadrigeminal area	–	ND	-	Parinaud syndrome
16	1995	M	Quadrigeminal area	–	Not known	-	
17	1993	M	Quadrigeminal area	–	Not known	-	
18	1994	M	Quadrigeminal area	–	DD, ID, ND	-	Sensorineural deafness Ataxia Problems with coordination Behavioural problems
19	2004	M	Quadrigeminal area	Dysmorphic features Syndactyly Cerebral atrophy Sacrococcygeal dermal sinus	DD, ID, ND	+	Inborn error of metabolism: urea cycle defect Generalized hypotonia
20	1992	M	Posterior fossa	-	Not known	-	
21	1995	M	Posterior fossa	-	Favourable	-	Temporary visual disturbance
22	1996	M	Posterior fossa	-	Favourable	-	Headache
23	2003	M	Posterior fossa	–	ND	-	Facial nerve palsy (lower motor neurone type)
24	1993	M	Posterior fossa	–	ND	-	Neck pain and numbness in the limbs

DISCUSSION

Epidemiology of neural tube defects

Groups 1-3. Myelomeningocele, anencephaly and encephalocele.

The total birth prevalence rate of neural tube defects was relatively low in South-Eastern Hungary for 1992-2006 with a figure of 5.90 per 10 000 births in contrast to 8.55 per 10 000 averaged across EUROCAT registers for the same period of time (5). The TBPRs of myelomeningocele (3.65 per 10 000), anencephaly (1.72 per 10 000) and encephalocele (0.54 per 10 000 births) were also lower in South-Eastern Hungary than the average (4.55, 2.92, and 1.08 per 10 000 births, respectively) across EUROCAT registers (5). Similar, or lower prevalence rates of NTD were reported from several Italian regions, South Portugal, Spain, and Zagreb, whereas the prevalence was higher in the other EUROCAT registers (5).

According to our previous survey (details not described in this study) the total birth prevalence of NTD was 11.06 per 10 000 in South-Eastern Hungary in the eighties (1980-1991), therefore a very significant decline to 5.90 per 10 000 births occurred between 1992 and 2006, parallel with the international trends. At the beginning of the nineties it became clear from the Medical Research Council study (14) and the Hungarian study by Czeizel and Dudás (15) that folic acid had a preventive effect on neural tube defects. A health education campaign (Hungarian Periconceptional Service) to promote periconceptional folic acid supplementation was launched in Hungary in the eighties, and the sharp decline in the total prevalence rate of neural tube defects after 1992 might have been the result of these recommendations (16). Food fortification with folic acid has not been implemented in Hungary (17).

In spite of the success with folic acid prevention in the late eighties-early nineties an increase in the TBPR of myelomeningocele and anencephaly was observed in South-Eastern Hungary after 1999. These data suggest that the primary prevention of NTD requires further public health measures. Indeed, a study revealed that despite an official recommendation, formulated in 1996 and 1998, large number of the Hungarian women did not take folic acid in the periconceptional period (18). On the contrary, a decline in the total prevalence rate of NTD has been observed in countries where food fortification with folic acid has been introduced (19), although not all cases of NTD are preventable by increasing folate intake (20). A trend of increase in terminations for

myelomeningocele in South-Eastern Hungary suggests an improved prenatal recognition of this malformation.

As the several tables in this study show, CNS malformations very often occur in combination with other CNS, or extra-CNS anomalies. This was the case with NTD, 27% of them was associated with other abnormalities, similarly to findings in the literature (21).

Group 4. Closed (occult) spinal dysraphism refers to spinal anomalies covered with skin (22). Population-based epidemiological data were not found in the literature. The live birth prevalence of 0.22 per 10 000 in this study probably underestimates the real prevalence because of the difficulties with diagnosis.

Epidemiology of prosencephalon malformations

Group 5. Holoprosencephaly. Diverse data are available on the epidemiology of HPE. In a review, based on several studies from various parts of the world it has been suggested that the birth prevalence rate of HPE was lower than 1 per 10 000 if LB and SB were only included, while the rate was above 1 per 10 000 if terminated pregnancies were also included (23). LBPR surveyed in this study and the figure of 0.49 per 10 000 was similar to figures found in New York State (24), a region in the UK, and three Italian regions (5).

The range of craniofacial abnormalities in the patients in this study extended from severe defects with cyclopia to milder forms of midfacial dysmorphic features described in the literature (25). Non-craniofacial abnormalities can occur in more than half of the cases without chromosomal abnormalities (25). The ratio was similar in our study, 4 patients out of the 8 cases without chromosomal abnormalities had non-craniofacial defects as well. Maternal diabetes appears to be a risk factor for holoprosencephaly, 5 cases were found in a series of 63 patients in the literature (25). The only case associated with maternal diabetes in our series represents a similar ratio despite the small number of cases. Trisomy 13 is frequently associated with HPE (26), the only case in this study represents approximately the same ratio as described in the literature. Mutations, responsible for holoprosencephaly have been found in several genes (*SIX3, SHH, TGIF, ZIC2, PTCH1, GLI2, DISP1, NODAL, FOXH1*), however molecular genetic testing has not been performed for our cases (27).

Group 6. Corpus callosum anomalies (partial/total agenesis and hypoplasia) were the most common CNS malformations in this survey with a prevalence of 2.05 per 10 000

live births. This result is very similar to the birth prevalence of 1.8 per 10 000 ascertained in California by a population-based survey that included infants aged less than 1 year (28).

Both agenesis (partial or total) and hypoplasia of CC were included in our study because these two conditions may be linked by a common genetic background (29). Partial/total agenesis was evident in exactly half of the patients, whereas hypoplasia occurred in the other 50% of cases. The agenesis/hypoplasia ratio was 1.25 in another retrospective study by Schell-Apacik et al. (30), but almost reached 3 in the California-based study (28). We found a male predominance among patients with callosal anomalies in Hungary. The sex distribution was almost equal in the California study (28), however a male/female sex ratio of 2.3 was described by Chadie et al. (31), a figure very close to the ratio of 2.2 in the present study.

In a number of cases (31.6%) CC anomalies were accompanied by other CNS malformations, such as optic nerve hypoplasia, wide cavum septum pellucidum, microcephaly, polymicrogyria, cortical dysplasia, or cerebellar vermis hypoplasia. According to our classification principles the CC malformation was the most striking dysgenesis in these cases. Interhemispheric cyst occurred in one seventh of patients, which was a similar ratio found by others (32). Extra-CNS abnormalities were observed in 39.5% of patients with CC anomalies, however recognisable syndromes were ascertained only in 2 patients (Edwards syndrome and short rib–polydactyly syndrome type II).

Group 7. Septo-optic dysplasia (de Morsier syndrome) is a rare, highly heterogeneous condition comprising variable phenotypes including midline forebrain abnormalities (absence of septum pellucidum), optic nerve and pituitary hypoplasia (33). A survey in Sweden showed that optic nerve hypoplasia occurred at a rate of 0.69 per 10 000 births, the rate of SOD was about 0.06 per 10 000 births, and the rate increased to 0.08 per 10 000 births including all midline brain defects associated with optic nerve hypoplasia (34). (Our own calculation based on the data available in the article). The prevalence rate of SOD (0.11 per 10 000 live births) in our study was similar to the Swedish figure, both fell in the same range of magnitude. However, all studies, including ours are likely to underestimate the true birth prevalence of SOD because of the diagnostic challenges. The aetiology (environmental or genetic) was not revealed in our cases.

Group 8. Cavum septum pellucidum. Patients with isolated, large CSP (separation of the leaves 10 mm or greater) were included in this group. All patients were older than one year, and the prevalence was 0.59 per 10 000 births. Whereas small (<5 mm) CSP is quite common, wide cavum seems to be rare and it may suggest impaired brain development with frequent occurrence of seizures, cognitive dysfunction, behavioural and psychiatric problems (35). A population-based epidemiological study on the prevalence of wide CSP has not been available in the literature. Our survey very likely underestimated the prevalence of CSP.

Epidemiology of neuronal and glial proliferation defects

Group 9. Microcephaly was defined as occipitofrontal circumference 2 standard deviation or more below the mean for the patient's age and gender at birth (9). It was the third most frequent form of cerebral dysgenesis with a birth prevalence of 1.62 per 10 000 live birth. For reasons of the clinical and aetiological heterogeneity and complexity of methodology in epidemiological studies, there are controversial data on the birth prevalence of microcephaly. Including all cases with microcephaly of various aetiologies 7 cases per 10 000 infants were reported from Missouri (USA) (36) and 0.77 per 1000 (7.7 per 10 000) from Metropolitan Atlanta (USA) (9). The lower prevalence rate in this study can be explained by our strict inclusion criteria: only cases with congenital microcephaly presumably of genetic aetiology without any exogenous and/or destructive aetiological factor, and without any other major brain malformation were included in this study. It appeared to be justified to classify the patients with microcephaly in three groups, such as (a) primary microcephaly, (b) microcephaly associated with chromosomal abnormalities or as part of a recognizable syndrome, and (c) microcephaly accompanied by other CNS and/or extra-CNS abnormalities.

9a. Congenital microcephaly without any other abnormalities or environmental aetiological factors can be defined as primary microcephaly or microcephalia vera. Its prevalence was 0.54 per 10 000 live births in our survey. A population-based epidemiological study for primary microcephaly was not available in the literature. Böök et al. (37) found 1.6 microcephalic individuals per 10 000 births and they estimated that the frequency of the syndrome of "genetic microcephaly" was of the magnitude of 1:25 000-1:50 000 in the total population between 5 and 55 years of age. According to Qazi and Reed (38) the incidence of 'genetic microcephaly' was approximately 1 per 40 000. Patients with "genetic microcephaly" in these publications

appear to be comparable to patients classified as having primary microcephaly in this study with birth prevalence of approximately 0.54 per 10 000 (2.16 per 40 000) live births. There is no clear explanation for the higher figures found in our study at the moment. The earlier studies probably were biased towards adults, or cases with familial occurrence, while all cases with primary congenital microcephaly were included in our survey. Primary microcephaly is probably the sequel of a genetic defect. Several genes (*MCPH1*, *CDK5RAP2*, *ASPM, CENPJ, STIL)* were indentified in association with primary microcephaly (39).

9b. Microcephaly is frequently associated with well known chromosomal abnormalities and it can be part of several syndromes. A search on OMIM database revealed several hundred syndromes with microcephaly. Seven patients with 5 distinct syndromes were ascertained in this subgroup. Microcephaly is a major feature of Cornelia de Lange and Nijmegen breakage syndromes (13, 40). The 2 cases with Cornelia de Lange syndrome corresponded to a prevalence of 0.11 per 10 000 live births, similar to the data of 0.12 per 10 000 found by Barisic et al. in Europe (40). Epidemiological data for Nijmegen breakage syndrome were not found in the literature, its prevalence appeared to be 0.11 per 10 000 live births in our study. The diagnosis of Nijmegen breakage syndrome was confirmed by mutation analysis of the *NBS1* gene (c.657_661del5bp, p.K219fs*234) at the University of Debrecen (13).

9c. This group comprises heterogeneous patients with congenital microcephaly. The aetiology of the malformations is probably genetic in these cases. Chromosomal analysis showed normal karyotype in all patients and further studies (aCGH, whole-genome sequencing) are warranted in order to reveal the genetic background. Since the routine use of aCGH has been implemented large number of complex malformations with microcephaly due to genome imbalances have been described (41). In spite of the strict inclusion criteria the aetiological role of exogenous substances can not be ruled out completely in a few cases (Patients 11 and 12, Table 16) in this group. The clinical manifestations were more severe and the outcome was worse in children with multiple anomalies than in cases with isolated microcephaly. The prevalence of epilepsy is relatively high among patients with microcephaly accompanied by other abnormalities.

In summary it can be stated that the identification of the genetic aetiology of microcephaly in patients of groups (a) and (b) can be expected and tests in this direction have been initiated. In group (c) congenital microcephaly is the only common feature

among the diverse abnormalities and aCGH might be the first step to approach the aetiology of these complex malformations.

Group 10. Microlissencephaly. Data on the prevalence of microlissencephaly is unknown, the Orphanet database lists it merely as a 'rare disease' (42). We found only two patients (1 male and 1 female) with a birth prevalence to 0.11 per 10 000 live births in the region. Based on the presence of severe mental retardation, seizures, neurological signs and poor life expectancy as seen in our patients, microlissencephaly can be distinguished from primary microcephaly. Associated malformations are common, and the aetiology is still unclear (11).

Epidemiology of malformations of cortical development (neuronal migration and cortical organization defects)

Population-based data on the epidemiology of the neuronal migration and organization defects were not available in the international registries (e.g. EUROCAT) for congenital anomalies. We were able to retrieve only a few population-based study on lissencephaly type I (43) and schizencephaly (44), most reports were published on cohorts (45, 46), and were unsuitable for the estimation of the epidemiology of these abnormalities in a given population. Our efforts, therefore, to provide population-based data on the epidemiology of these malformations appear to be a new challenging endeavour.

Cortical malformations (agyria/pachygyria-SBH spectrum, neuronal heterotopia, PMG, schizencephaly and FCD) occurred in 27 cases in our survey (Table 1), which corresponded to a prevalence of 1.46 per 10 000 live births (95% CI: 0.91-2.00). Five patients (18.5%) were diagnosed with agyria/pachygyria - SBH spectrum, 3 patients (11.0%) with neuronal heterotopia, 8 children (29.6%) had PMG, 10 patients (37.0%) showed schizencephaly and one child (3.7%) had FCD. For comparison the following distribution of malformations of cortical development were published by Leventer et al. (45) and Güngör et al. (46) in cohorts of children referred to tertiary paediatric centres: agyria/pachygyria 15% and 23%, heterotopia 19% and 12%, polymicrogyria 16% and 54%, schizencephaly 5% and 12%, respectively, focal cortical dysplasia 16% (by Leventer et al.(45), not reported by Güngör et al (46). Our data for agyria/pachygyria - SBH, neuronal heterotopia and PMG were between or close to figures reported by these authors, however schizencephaly was commoner and FCD less frequent in our series.

Epidemiology of neuronal migration defects

Group 11. Agyria/pachygyria - subcortical band heterotopia spectrum. The prevalence of the agyria/pachygyria - SBH spectrum was 0.27 per 10 000 live births, whereas the live birth prevalence of the agyria/pachygyria spectrum without SBH proved to be 0.22 per 10 000. A lower prevalence of 11.7 per million (0.12 per 10 000) for lissencephaly type I was reported from The Netherlands in the eighties before the routine use of MRI (43). DD, ID and epilepsy were common clinical findings in these patients.

Mutations in several genes (*LIS1*, *DCX, TUBA1A, RELN, VLDLR, ARX)* can be responsible for the malformation in the agyria/pachygyria and SBH spectrum disorders (47). Efforts have been made to reveal the molecular genetic causes in our patients as well. Patient 1 (Table 18) had a novel frameshift mutation in the third exon of the *LIS1* gene (c.83_84 delAT) resulting in a truncated protein (p.Tyr28Phefs*31), and Patient 5 (Table 18) with SBH had an already known mutation, a base pair deletion in exon 4 of the *DCX* gene (c.200delG) also leading to a truncated protein (p.Ile68Leufs*87). Mutations in the *LIS1* and *DCX* gene account for 85% of classical lissencephaly. The *LIS1* and *DCX* gene products are microtubule associated proteins, which play a significant role in neuroblast division, migration and maturation (47).

Group 12. Neuronal heterotopia. The prevalence of neuronal heterotopia (subependymal/periventricular type) was 0.16 per 10 000 live births. The population-based epidemiology of this malformation is unknown. Developmental delay, intellectual disability, epilepsy and extra-CNS abnormalities were found in our patients, similarly to cases studied by others (48). The underlying aetiology might be heterogeneous (48), it was not revealed in our cases.

Epidemiology of cortical organization defects

Group 13. Polymicrogyria. The prevalence of PMG corresponded to 0.43 per 10 000 live births in South-Eastern Hungary. Population-based epidemiological data were not found in the literature. Significant male predominance was found in our series, similarly to other studies (49). PMG appears to be a highly heterogeneous disorder in term of its pathogenesis, topographic distribution, pathological appearance, and clinical and imaging features (49). This variability in localization, MRI features, and accompanied CNS and extra-CNS abnormalities was observed in our patients as well (Table 20). The outcome was rather poor, and all children, except two suffered from epilepsy. The

aetiology of polymicrogyria is unclear in the majority of cases, evidence for both environmental and genetic causes has been found. Consistent chromosome abnormalities identified PMG loci according to Dobyns et al. (50), and mutations in several genes (*GPR56, WDR62, TUBA8, TUBB2B, TUBB3, etc.*) were found in association with PMG (51,52,53,54). As the causes of PMG remained unknown in our cases, genetic tests would be planned in the future as new and cheaper methods become available.

Mega corpus callosum was associated with bilateral symmetrical polymicrogyria, epilepsy and psychomotor retardation in Patient 7 (Table 20). Mega corpus callosum has not been described in large series of polymicrogyria (49). Based on several case reports this combination of malformations may represent a new cerebral dysgenesis syndrome awaiting confirmation by molecular genetic techniques (55).

Group 14. Schizencephaly. The prevalence of schizencephaly was 0.54 per 10 000 live births in the region (Table 1). This was a higher figure than reported from California, where an overall prevalence of 1.54 per 100 000 (0.15 per 10 000) was found (44). However the California Birth Defects Monitoring Program conducted surveillance only up to age 1 year, while our survey included all children born between July 1, 1992 and June 30, 2006 in South-Eastern Hungary, and diagnosed with schizencephaly at any age. The diagnosis was established only in 5 out of 10 patients up to 1 year in our study. Similar results were reported by Denis and co-workers, who found that only half of 30 patients presented with neurological symptoms prior to age one (56). We can conclude that an underascertainment of mild schizencephalic defects likely occurred in the California survey and the marked difference between the figures might be the result of differing methodologies.

The patterns of accompanying CNS abnormalities and the outcome of the patients were similar as reported previously (44, 56). Epilepsy with partial, occasionally generalised seizures is a common complication of schizencephaly, however it occurred only in 3 out of 10 patients in this survey. The aetiology of schizencephaly might be diverse (44,56); it was not identified in any of our cases.

Group 15. Focal cortical dysplasia was diagnosed only in a single case that corresponded to a prevalence of 0.05 per 10 000 live births. Population-based epidemiological data have not been found in the literature, although FCD proved to be a frequent cause of intractable epilepsy in children (57). In many cases only special

imaging techniques and histopathology of surgically removed tissue could provide evidence of FCD (57), hence our data very likely underestimated its prevalence.

Epidemiology of midbrain and hindbrain malformations

Midbrain and hindbrain malformations occurred in 30 cases, 14% of all malformations in our survey. This figure corresponded to a prevalence of 1.62 per 10 000 (95% CI: 1.04 - 2.20) live births, close to the data on the incidence of posterior fossa malformations estimated to be 1 out of every 5000 live births by the Metropolitan Atlanta Congenital Defects Program (58).

Malformations of both midbrain and hindbrain

Group 16. Molar tooth sign. The prevalence of anomalies with MTS was 0.11 per 10 000 live births. A single case with typical features of Joubert syndrome and another patient with a unique combination of skeletal dysplasia and complex brain malformation, including molar tooth malformation were classified in this group. Therefore the prevalence of Joubert syndrome corresponded to 0.05 per 10 000 live births (i.e. 0.5 in 100 000), which rate was in the range of 1 in 100 000-300 000 births estimated by others (59). Joubert syndrome and Joubert syndrome-related disorders are genetically heterogeneous and causative mutations in several genes (*INPP5E, TMEM216, AHI1, NPHP1, CEP290, TMEM67, RPGRIP1L, ARL13B, CC2D2A and OFD1)* have been identified (60). In our patient, a compound heterozygous mutation was found in the *CEP290* gene: in allele 1, exon 38 (c.5182G>T, p.Glu1728Stop) from the father and in allele 2, exon 46 (c.6277delG, p.Val2093SerfsStop4) from the mother. Mutations in the *CEP290* gene can be responsible for at least 10% of the cases with Joubert syndrome (59).

Several genes responsible for various forms of short rib-polydactyly syndrome have been identified recently (61) and further molecular genetic tests will be carried out for our patient presented with this syndrome in combination with a complex brain dysgenesis.

Malformations affecting predominantly the cerebellum and derivates (Rh1)

Group 17. Dandy-Walker malformation. The birth prevalence of DWM was 0.32 per 10 000 live births. Similar data, prevalences between 1 in 25 000 and 1 in 100 000 live births were reported earlier (62, 63). A considerable female preponderance (M/F: 0.2) was observed among our patients, not found by others (63). DWM is very often associated with other CNS and extra-CNS anomalies (64) as it occurred in our patients

as well. Hydrocephalus was a common complication, as described in the literature (65). Cleft lip and palate, cardiac defects, genitourinary malformations were the most significant extra-CNS abnormalities. The outcome depends on the associated malformations and it was very poor in our series. The aetiology of DWM is heterogeneous, it was found in association with various chromosomal abnormalities and mutations in *ZIC2* and *ZIC4* genes (66, 67). However, most of the cases are sporadic and the aetiology remains unknown.

One of our cases (Patient 2, Table 24) fulfilled the criteria of PHACE (**P**osterior fossa malformation, **H**aemangioma, **A**rterial anomalies, **C**oarctation of the aorta or other cardiac defects, and **E**ye abnormalities) syndrome (68). This syndrome was probably underdiagnosed in the past because the facial haemangioma was mistakenly regarded as part of an atypical Sturge-Weber syndrome.

Group 18. Cerebellar vermis hypoplasia without molar tooth sign. The prevalence of CVH was 0.49 per 10 000 live births. Twice as many females as males were identified in this series. CVH is likely to be underdiagnosed and often misdiagnosed as Dandy-Walker variant or mega cisterna magna (12). Population-based epidemiological data were not available for CVH. It can be associated with other CNS and extra-CNS abnormalities, like in this survey, or CVH can be part of several genetic syndromes (69). A balanced chromosomal translocation: XX, t(3q;16q)(2.6;2.4) was revealed in a patient with CVH, blepharophimosis and atypical autism (Patient 9, Table 26); involvement of genes at the breakpoints might be responsible for the symptoms. DD and ID were common clinical findings in our patients emphasising the role of cerebellum in cognitive development (70).

Group 19. Unilateral cerebellar hypoplasia. The prevalence of unilateral cerebellar hypoplasia was 0.11 per 10 000 live births. Population-based epidemiological data were not available, only a few cohort studies have been published so far (71,72). The malformation was associated with Down syndrome in one of the 2 cases (Patient 1, Table 26) and congenital heart disease also occurred in the other child (Patient 2, Table 26). The underlying pathomechanism of unilateral cerebellar hypoplasia is not fully understood. Acquired disruption (pre/perinatal haemorrhage) can be responsible for the majority of cases, therefore this condition cannot be regarded as a true malformation (71,72).

Group 20. Cerebellar aplasia/hypoplasia. The prevalence of this entity was 0.38 per 10 000 live births. Moderate male predominance was found. The prevalence of

cerebellar aplasia/hypoplasia is unknown. It has been described in context of several genetic and metabolic syndromes (69,73), therefore its population-based survey would be a great challenge. The clinical spectrum of our cases was similar to those ones reported by Wassmer et al. (74). These features showed a significant overlap with the clinical symptoms of isolated CVH. Epilepsy was found in more than half of the patients, similarly to the published data (74). The aetiology was not revealed in our cases.

Malformations affecting predominantly the lower hindbrain (Rh 2-8)

Group 21. Chiari type I malformation. The prevalence of Chiari type I malformation was 0.11 per 10 000 live births in our survey. A frequency of Chiari I malformation in another paediatric population was found to be 0.7 per 10 000 (75). All the head and spine MRI studies performed for patients under the age of 20 years in a certain population were reviewed from an electronic database in the latter survey (75). The symptoms of Chiari type I malformation often appear during early adulthood, or this malformation can remain asymptomatic (76), therefore the prevalence data in our survey for symptomatic cases below the age of 14 years underestimated the real live birth prevalence of this abnormality.

Malformations associated with prenatal onset of degeneration

Group 22. Pontocerebellar hypoplasia. The prevalence of PCH was 0.11 per 10 000 live births in our study. PCH is a group of very rare, inherited progressive neurodegenerative disorders with prenatal onset (77,78). Up to now seven different subtypes have been reported (PCH1-7). The prevalence of each subtype is unknown. Two cases were found in our survey, one of them (Patient 2, Table 29) showed typical features of PCH2, while the other patient (Patient 1, Table 29) presumably represented a less severe form of PCH2. Both children had severe cognitive and motor handicaps and seizures. Mutations in three tRNA splicing endonuclease subunit genes (*TSEN54, TSEN2, TSEN34)* were found to be responsible for PCH2, PCH4 and PCH5 (77). Mutations in the nuclear encoded mitochondrial arginyl-tRNA synthetase gene (*RARS2*) underlie PCH6 (77,78). The tRNA splicing endonuclease (*TSEN54*), the mitochondrial arginyl-tRNA synthetase (*RARS2*) and the vaccinia related kinase 1 (*VRK1*) genes were found to be mutated in the minority of PCH1 cases (77,78). There were no mutations in the *TSEN54* gene in our cases.

Other CNS malformations

Group 23. Arachnoid cysts. The prevalence of arachnoid cysts was 1.29 per 10 000 live births. Arachnoid cysts made up 11.2% of all CNS malformations in our survey. Population-based live birth prevalence data on arachnoid cysts are not available, an estimated prevalence of about 1: 5 000 has been published by Orphanet (42). A male predominance was observed in our survey, similarly to other studies (79). The vast majority of the cysts occurred in the middle fossa, as observed previously (79,80). Arachnoid cysts have often been diagnosed incidentally by brain imaging requested for rather heterogeneous clinical symptoms (79,80); seizures and headache were relatively common indications for performing CT or MRI in our series.

Limitations

Some inaccuracy probably cannot be avoided in a retrospective epidemiological study; hence this survey also bears some limitations. Several patients had multiple malformations and they were classified on the basis of the abnormality believed to be the major one. As the molecular background of more and more malformations and malformation syndromes become known obviously more appropriate classifications will be available. The prevalence rates in this study might be an underestimate of the real prevalence, since affected children might have been missed from surveillance. The TBPR was calculated only for the NTD because data on stillbirths with other CNS malformations or terminations for CNS dysgenesis other than NTD were not available. Aetiology has not been found yet in the majority of cases with malformations. An adequate search for environmental risk factors, which might interfere with CNS development, was not possible in this retrospective survey. The launch of a prospective study, which includes cases diagnosed intrauterine and provides more data on the patients with CNS malformations will be considered in the future.

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REFERENCES

1. Czeizel AE. Birth defects are preventable. *Int J Med Sci* 2005;2:91-2.
2. Dolk H, Loane M, Garne E. The prevalence of congenital anomalies in Europe. *Adv Exp Med Biol* 2010;686:349-64.
3. Bonita R, Beaglehole R, Kjellström T. Measuring health and disease. In: Bonita R, Beaglehole R, Kjellström T. Basic epidemiology. 2nd ed. Geneve: WHO Press, 2006:15-38.
4. World Atlas of Birth Defects. World Health Organization. 2nd ed. Geneva: WHO Press, 2003.
5. EUROCAT. http://www.eurocat.ulster.ac.uk
6. EURORDIS. http://www.eurordis.org/
7. Aicardi J. Malformations of the central nervous system. In: Aicardi J, editor. Diseases of the nervous system in childhood. 3rd ed. London: Mac Keith Press, 2009:41-102.
8. Croen LA, Grether JK, Curry CJ, Nelson KB. Congenital abnormalities among children with cerebral palsy: more evidence for prenatal antecedents. *J Pediatr* 2001;138:804-10.
9. Gleeson JG, Dobyns WB, Plawner L, Ashwal S. Congenital structural defects. In: Swaiman KF, Ashwal S, Ferriero DM, editors. Pediatric neurology. Principles and practice. 4th ed. Philadelphia: Mosby, Elsevier, 2006:363-490.
10. Volpe JJ. Human brain development. In: Volpe JJ, editor. Neurology of the newborn. 5th ed. Philadelphia: WB Saunders, Elsevier, 2008:3-118.
11. Barkovich AJ, Kuzniecky RI, Jackson GD, Guerrini R, Dobyns WB. A developmental and genetic classification for malformations of cortical development. *Neurology* 2005;65:1873-87.
12. Parisi MA, Dobyns WB. Human malformations of the midbrain and hindbrain: review and proposed classification scheme. *Mol Genet Metab* 2003;80:36-53.
13. Erdös M, Tóth B, Veres I, Kiss M, Remenyik E, Maródi L. Nijmegen breakage syndrome complicated with primary cutaneous tuberculosis. *Pediatr Infect Dis J* 2011;30:359-60.
14. MRC Vitamin Study Research Group. Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. *Lancet* 1991;338:131-7.

15. Czeizel AE, Dudás I. Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. *N Engl J Med* 1992;327:1832-5.

16. Czeizel AE. Primary prevention of neural-tube defects and some other major congenital abnormalities. Recommendations for the appropriate use of folic acid during pregnancy. *Paediatr Drugs* 2000;2:437-49.

17. Sandor J. Report on periconceptional folic acid supplementation for Hungary. In: EUROCAT special report: Prevention of neural tube defects by periconceptional folic acid supplementation in Europe. htpp://www.eurocat-network.eu/content/NTD-Dec2007-Hungary.pdf

18. Bognár M, Ponyi A, Hauser P, et al. Improper supplementation habits of folic acid intake by Hungarian pregnant women: improper recommendations. *J Am Coll Nutr* 2008;27:499-504.

19. Boulet SL, Yang Q, Mai C, et al. Trends in the postfortification prevalence of spina bifida and anencephaly in the United States. *Birth Defects Res A Clin Mol Teratol* 2008;82:527-32.

20. Heseker HB, Mason JB, Selhub J, Rosenberg IH, Jacques PF. Not all cases of neural-tube defect can be prevented by increasing the intake of folic acid. *Br J Nutr* 2009;102:173-80.

21. Stoll C, Alembik Y, Dott B. Associated malformations in cases with neural tube defects. *Genet Couns* 2007;18:209-15.

22. Pacheco-Jacome E, Ballesteros MC, Jayakar P, Morrison G, Ragheb J, Medina LS. Occult spinal dysraphism: evidence-based diagnosis and treatment. *Neuroimag Clin N Am* 2003;13:327-34.

23. Orioli IM, Castilla EE. Epidemiology of holoprosencephaly: prevalence and risk factors. *Am J Med Genet Part C Semin Med Genet* 2010;154C:13-21.

24. Olsen CL, Hughes JP, Youngblood LG, Sharpe-Stimac M. Epidemiology of holoprosencephaly and phenotypic characteristics of affected children: New York State, 1984-1989. *Am J Med Genet* 1997;73:217-26.

25. Rasmussen SA, Moore CA, Khoury MJ, Cordero JF. Descriptive epidemiology of holoprosencephaly and arhinencephaly in Metropolitan Atlanta, 1968-1992. *Am J Med Genet* 1996;66:320-33.

26. Bendavid C, Dupé V, Rochard L, Gicquel I, Dubourg C, David V. Holoprosencephaly: an update on cytogenetic abnormalities. *Am J Med Genet Part C Semin Med Genet* 2010;154C:86-92.
27. Roessler E, Muenke M. The molecular genetics of holoprosencephaly. *Am J Med Genet Part C Semin Med Genet* 2010;154C:52-61.
28. Glass HC, Shaw GM, Ma C, Sherr EH. Agenesis of the corpus callosum in California 1983-2003: a population-based study. *Am J Med Genet Part A* 2008;146A:2495-500.
29. van Bon BWM, Koolen DA, Borgatti R, et al. Clinical and molecular characteristics of 1qter microdeletion syndrome: delineating a critical region for corpus callosum agenesis/hypogenesis. *J Med Genet* 2008;45:346-54.
30. Schell-Apacik CC, Wagner K, Bihler M, et al. Agenesis and dysgenesis of the corpus callosum: clinical, genetic and neuroimaging findings in a series of 41 patients. *Am J Med Genet Part A* 2008;146A:2501-11.
31. Chadie A, Radi S, Trestard L, et al. Neurodevelopmental outcome in prenatally diagnosed isolated agenesis of the corpus callosum. *Acta Paediatr* 2008;97:420-4.
32. Hetts SW, Sherr EH, Chao S, Gobuty S, Barkovich AJ. Anomalies of the corpus callosum: an MR analysis of the phenotypic spectrum of associated malformations. *AJR Am J Roentgenol* 2006;187:1343-8.
33. McCabe MJ, Alatzoglou KS, Dattani MT. Septo-optic dysplasia and other midline defects: the role of transcription factors: *HESX1* and beyond. *Best Pract Res Clin Endocrinol Metab* 2011;25:115-24.
34. Tornqvist K, Ericsson A, Källén B. Optic nerve hypoplasia: risk factors and epidemiology. *Acta Ophtalmol Scand* 2002;80:300-4.
35. Bodensteiner JB, Schaefer GB. Wide cavum septum pellucidum: a marker of disturbed brain development. *Pediatr Neurol* 1990;6:391-4.
36. Krauss MJ, Morrissey AE, Winn HN, Amon E, Leet TL. Microcephaly: an epidemiologic analysis. *Am J Obstet Gynecol* 2003;188:1484-90.
37. Böök JA, Schut JW, Reed SC. A clinical and genetical study of microcephaly. *Am J Ment Defic* 1953;57:637-60.
38. Qazi QH, Reed TE. A possible major contribution to mental retardation in the general population by the gene for microcephaly. *Clin Genet* 1975;7:85-90.

39. Thornton GK, Woods CG. Primary microcephaly: do all roads lead to Rome? *Trends Genet* 2009;25:501-10.

40. Barisic I, Tokic V, Loane M, et al. Descriptive epidemiology of Cornelia de Lange syndrome in Europe. *Am J Med Genet A* 2008;146A:51-9.

41. Firth HV, Richards SM, Bevan AP, et al. DECIPHER: Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources. *Am J Hum Genet* 2009;84:524-33.

42. ORPHANET. http://www.orpha.net/consor/cgi-bin/index.php

43. de Rijk-van Andel JF, Arts WF, Hofman A, Staal A, Niermeijer MF. Epidemiology of lissencephaly type I. *Neuroepidemiology* 1991;10:200-4.

44. Curry CJ, Lammer EJ, Nelson V, Shaw GM. Schizencephaly: heterogeneous etiologies in a population of 4 million California births. *Am J Med Genet* 2005;137A:181-9.

45. Leventer RJ, Phelan EM, Coleman LT, Kean MJ, Jackson GD, Harvey AS. Clinical and imaging features of cortical malformations in childhood. *Neurology* 1999;53:715-22.

46. Güngör S, Yalnizoğlu D, Turanli G, Saatçi I, Erdoğan-Bakar E, Topçu M. Malformations of cortical development: clinical spectrum in a series of 101 patients and review of the literature (Part I). *Turk J Pediatr* 2007,49:120-30.

47. Guerrini R, Parini E. Neuronal migration disorders. *Neurobiol Dis* 2010;38:154-66.

48. Srour M, Rioux MF, Varga C, et al. The clinical spectrum of nodular heterotopias in children: report of 31 patients. *Epilepsia* 2011;52:728-37.

49. Leventer RJ, Jansen A, Pilz DT, et al. Clinical and imaging heterogeneity of polymicrogyria: a study of 328 patients. *Brain* 2010;133:1415-27.

50. Dobyns WB, Mirzaa G, Christian SL, et al. Consistent chromosome abnormalities identify novel polymicrogyria loci in 1p36.3, 2p16.1-p23.1, 4q21.21-q22.1, 6q26-q27, and 21q2. *Am J Med Genet A* 2008;146A:1637-54.

51. Jansen A, Andermann E. Genetics of the polymicrogyria syndromes. *J Med Genet* 2005;42:369-78.

52. Piao X, Chang BS, Bodell A, et al. Genotype-phenotype analysis of human frontoparietal polymicrogyria syndromes. *Ann Neurol* 2005;58:680-7.

53. Bilgüvar K, Oztürk AK, Louvi A, et al. Whole-exome sequencing identifies recessive *WDR62* mutations in severe brain malformations. *Nature* 2010,467:207-10.

54. Tischfield MA, Cederquist GY, Gupta ML Jr, Engle EC. Phenotypic spectrum of the tubulin-related disorders and functional implications of disease-causing mutations. *Curr Opin Genet Dev* 2011;21:1-9.

55. Hengst M, Tücke J, Zerres K, Blaum M, Häusler M. Megalencephaly, mega corpus callosum, and complete lack of motor development: delineation of a rare syndrome. *Am J Med Genet A* 2010;152A:2360-4.

56. Denis D, Chateil JF, Brun M, et al. Schizencephaly: clinical and imaging features in 30 infantile cases. *Brain Dev* 2000;22:475-83.

57. Krsek P, Maton B, Korman B, et al. Different features of histopathological subtypes of pediatric focal cortical dysplasia. *Ann Neurol* 2008;63:758-69.

58. Bolduc ME, Limperopoulos C. Neurodevelopmental outcomes in children with cerebellar malformations: a systematic review. *Dev Med Child Neurol* 2009;51:256-67.

59. Doherty D. Joubert syndrome: insights into brain development, cilium biology, and complex disease. *Semin Pediatr Neurol* 2009,16:143-54.

60. Lee JE, Gleeson JG. Cilia in the nervous system: linking cilia function and neurodevelopmental disorders. *Curr Opin Neurol* 2011;24:98-105.

61. Mill P, Lockhart PJ, Fitzpatrick E, et al. Human and mouse mutations in *WDR35* cause short-rib polydactyly syndromes due to abnormal ciliogenesis. *Am J Hum Genet* 2011;88:508-15.

62. Lavanya T, Cohen M, Gandhi SV, Farrell T, Whitby EH. A case of a Dandy-Walker variant: the importance of a multidisciplinary team approach using complementary techniques to obtain accurate diagnostic information. *Br J Radiol* 2008;81:e242-5.

63. Ohaegbulam SC, Afifi H. Dandy-Walker syndrome: incidence in a defined population of Tabuk, Saudi Arabia. *Neuroepidemiology* 2001;20:150-2.

64. Salihu HM, Kornosky JL, Druschel CM. Dandy-Walker syndrome, associated anomalies and survival through infancy: a population-based study. *Fetal Diagn Ther* 2008;24:155-60.

65. Spennato P, Mirone G, Nastro A, et al. Hydrocephalus in Dandy-Walker malformation. *Childs Nerv Syst* 2011;27:1665-81.

66. Imataka G, Yamanouchi H, Arisaka O. Dandy-Walker syndrome and chromosomal abnormalities. *Congenit Anom (Kyoto)* 2007;47:113-8.

67. Blank MC, Grinberg I, Aryee E, et al. Multiple developmental programs are altered by loss of *Zic1* and *Zic4* to cause Dandy-Walker malformation cerebellar pathogenesis. *Development* 2011;138:1207-16.

68. Metry D, Heyer G, Hess C, et al. Consensus statement on diagnostic criteria for PHACE syndrome. *Pediatrics* 2009;124:1447-56.

69. Ramaekers VT, Heimann G, Reul J, Thron A, Jaeken J. Genetic disorders and cerebellar structural abnormalities in childhood. *Brain* 1997;120:1739-51.

70. Steinlin M. Cerebellar disorders in childhood: cognitive problems. *Cerebellum* 2008;7:607-10.

71. Poretti A, Limperopoulos C, Roulet-Perez E, et al. Outcome of severe unilateral cerebellar hypoplasia. *Dev Med Child Neurol* 2010;52:718-24.

72. Benbir G, Kara S, Yalcinkaya BC, et al. Unilateral cerebellar hypoplasia with different clinical features. *Cerebellum* 2011;10:49-60.

73. Boltshauser E. Cerebellum-small brain but large confusion: a review of selected cerebellar malformations and disruptions. *Am J Med Genet A* 2004;126A:376-85.

74. Wassmer E, Davies P, Whitehouse WP, Green SH. Clinical spectrum associated with cerebellar hypoplasia. *Pediatr Neurol* 2003,28:347-51.

75. Aitken LA, Lindan CE, Sidney S, et al. Chiari type I malformation in a pediatric population. *Pediatr Neurol* 2009;40:449-54.

76. Tubbs RS, Lyerly MJ, Loukas M, Shoja MM, Oakes WJ. The pediatric Chiari I malformation: a review. *Childs Nerv Syst* 2007;23:1239-50.

77. Namavar Y, Barth PG, Kasher PR, et al. Clinical, neuroradiological and genetic findings in pontocerebellar hypoplasia. *Brain* 2011;134:143-56.

78. Namavar Y, Barth PG, Poll-The BT, Baas F. Classification, diagnosis and potential mechanisms in pontocerebellar hypoplasia. *Orphanet J Rare Dis* 2011;6:50 (http:/www.ojrd.com/content/6/1/50).

79. Al-Holou WN, Yew AY, Boomsaad ZE, Garton HJL, Muraszko KM, Maher CO. Prevalence and natural history of arachnoid cysts in children. *J Neurosurg Pediatr* 2010;5:578-85.

80. Gosalakkal JA. Intracranial arachnoid cysts in children: a review of pathogenesis, clinical features, and management. *Pediatr Neurol* 2002,26:93-8.