Clinical Characteristics and Cognitive Functioning of Hungarian Children Born with Orofacial Clefts

Doctoral (PhD) Thesis

Dr. Kinga Amália Sándor-Bajusz

University of Pécs, OGYDHT Pécs

Doctoral School of Clinical Neurosciences

Head of Doctoral School: Prof. Dr. József Janszky

Psychiatry Program **(B-1/2012)**

Thesis Supervisor: Prof. Dr. Györgyi Csábi

Program Director: Prof. Dr. Tamás Tényi

Pécs, 2023

Table of Contents

Abstract

Orofocial clefts are the most common congenital abnormality of the craniofacial structures. They are defined as syndromic or non-syndromic clefts based on the underlying etiology. The optimal clinical care of these patients is ensured by a multidisciplinary team, and a long-term treatment plan in which well-timed cleft repair surgeries are of priority. In both syndromic and non-syndromic cases, the defect is associated with additional medical conditions and/or a higher risk for mental disorders that further complicate the overall care of these patients. The aim of the current thesis work was to analyze the clinical and mental health outcomes of children born with orofacial clefts, which was achieved in three levels by: (1) evaluating the impact of genetic syndromes on the algorithm of cleft repair surgeries, (2) identifying a subpopulation of children of non-syndromic orofacial clefts at risk for abnormal neurodevelopment, and (3) by summarizing the available evidence on brain structural differences in individuals with orofacial clefts and their controls.

Abbreviations

ADHD: Attention deficit hyperactivity disorder

CBCL: Child Behavior Checklist

CL: Cleft lip

CLP: Cleft lip and palate

CP: Cleft palate

CPT: Continuous Performance Task

FS-IQ: Full-scale IQ.

HCAR: Hungarian Congenital Abnormality Registry

ID: Intellectual disorder

IQ: Intelligence Quotient

MD: Mean difference

MRI: Magnetic resonance imaging

OFC: Orofacial cleft

OMIM: Online Mendelian Inheritance in Man database

PRI: Perceptual Reasoning Index

PRS: Pierre Robin syndrome

PSI: Processing Speed Index

SD: Standard deviation(s)

SES: Socio-economic status

TOL: Tower of London

VCI: Verbal Comprehension Index

WISC-IV: Wechsler Intelligence Scale for Children, Fourth Edition

WMI: Working Memory Index

Introduction

Orofacial clefts (OFCs) are the most common congenital abnormality that affect the development of the craniofacial structures. The anomaly is characterized by the presence of a cleft on the lip and/or palate. OFCs are defined as syndromic or non-syndromic clefts and can be further classified as cleft lip (CL), cleft palate (CP), and combined cleft lip and palate (CLP) (Figure 1). The optimal clinical care of children with OFCs is carried out by a multidisciplinary team that ensures an individualized long-term treatment plan. Specialists including pediatric surgeons, oral and maxillofacial surgeons, plastic surgeons, dentists, orthodontics, otolaryngologists, speech, and language pathologists work together on a case-by-case basis to provide carefully coordinated and well-timed interventions for these children. The multidisciplinary Cleft Team of the University of Pécs (further mentioned as the Pécs Cleft Team) has over 25 years of experience in treating this population and is an important center for cleft patient care in Hungary.

Cleft research and clinical experience both underline that syndromic and non-syndromic OFCs represent two distinct groups of patients that clinically differ in etiology, severity, timing of treatment, and prognosis. Children with syndromic OFCs often present with additional complications that affect the timing of their cleft repair including failure to thrive, feeding and respiratory difficulties. As a result, the cleft repair protocol used for non-syndromic OFCs is often altered for syndromic patients. Children with non-syndromic OFCs have a higher risk for later neuropsychiatric disabilities compared to the general population. This observed risk was explained by the presence of multiple chronic stressors present in the life of children with OFCs and their families, including repetitive cleft repair surgeries, aesthetics, and functional consequences such as speech difficulties. However, these underlying mechanisms have not been able to further explain the atypical neurodevelopment and the higher risk for mental difficulties observed in some of these children. Delays in developmental milestones, learning difficulties in preschool, and brain structural differences identified with MRI indicate a primary dysfunction of early developmental processes involving both facial and brain structures.

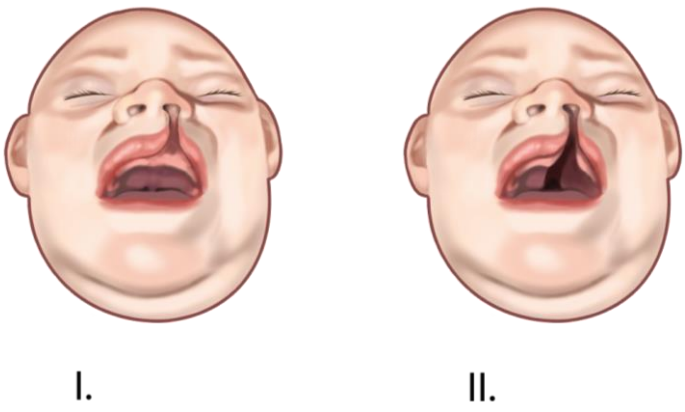

Figure 1. Types of orofacial clefts. I: unilateral cleft lip, II: unilateral combined cleft lip and palate.

List of original publications

Study I

Sándor-Bajusz KA, Maros TB, Olasz L, Sándor GK, Hadzsiev K, Vástyán AM. The Influence of Genetic Syndromes on the Algorithm of Cleft Lip and Palate Repair - A Retrospective Study. Ann Maxillofac Surg. 2021 Jul-Dec;11(2):270-273. doi: 10.4103/ams.ams_77_21.

Study II

Sándor-Bajusz KA, Dergez T, Molnár E, Hadzsiev K, Till Á, Zsigmond A, Vástyán A, Csábi G. Cognitive functioning and clinical characteristics of children with non-syndromic orofacial clefts: A case-control study. Frontiers in Psychology. 2023 Feb 28;14:1115304. doi: 10.3389/fpsyg.2023.1115304.

Study III

Sándor-Bajusz KA, Sadi A, Varga E, Csábi G, Antonoglou GN, Lohner S. The Brain in Oral Clefting: A Systematic Review with Meta-Analyses. Frontiers in Neuroanatomy. 2022 Jun 10;16:863900. doi: 10.3389/fnana.2022.863900.

Aims

The aim of the current thesis work was to analyze the clinical and mental outcomes of children born with orofacial clefts (OFCs).

The first study aimed to identify Hungarian syndromic OFC patients and evaluate how their genetic syndrome influenced the timing of the algorithm of cleft repair surgeries.

The second study aimed to identify a subpopulation of Hungarian children with non-syndromic OFCs that are at risk for abnormal neurodevelopment by assessing their developmental history and present cognitive functioning.

The final study aimed to summarize the available evidence on potential brain structural differences in individuals with non-syndromic OFCs and their matched controls.

Study I

Materials and methods

Participants

The records of syndromic and non-syndromic CLP patients managed by the Pécs Cleft Team between January 1999 and December 2015 were analyzed retrospectively. Detailed clinical documentation of all patients, including genetic and epidemiological data, was required for inclusion in the study.

Data collection and statistical analyses

The data were collected retrospectively without personal identifying details. Special permission was obtained and granted for data collection from the Hungarian Congenital Abnormality Registry (HCAR). The Ethics Committee of the University of Pécs waived the need for ethical approval due to the retrospective nature of the study. All procedures performed in the study were conducted in accordance with the ethics standards given in the 1964 Declaration of Helsinki, as revised in 2013.

The Online Mendelian Inheritance in Man database (OMIM) was used to identify the genetic syndromes. Epidemiological data were obtained from the HCAR. Special emphasis was placed on the syndromic features of the patients and their associated anomalies. The timing of the CL and/or CLP repair was recorded and was compared with the algorithm used for non-syndromic cleft patients. The type and timing of the surgeries or interventions unrelated to the clefts were listed and categorized. The study used descriptive statistics consisting of percentages and frequencies of the surgical interventions, presenting syndromes and participants of the study.

Results

Syndromes and cleft types

A total of 607 patients were managed by the cleft team during the study between 1999 and 2015. Among the patients, 25 children (4.1%) had associated anomalies and sixteen patients (2.6%) were noted to be afflicted with a particular identifiable syndrome. Ten patients (60%) were boys and six (40%) were girls of the syndromic CLP group. The majority of the syndromic CLP patients had CP only (n = 13, 81%). Seven different genetic syndromes and one sequence were present in the study. The Pierre Robin sequence occurred most often, comprising 50% of the cohort. The other syndromes observed in the cohort included: Smith-Lemli Opitz syndrome, Dandy–Walker syndrome, DiGeorge syndrome, Ectrodactyly-ectodermal dysplasia-clefting syndrome, Treacher Collins syndrome, Turner syndrome, and Weissenbacher–Zweymüller syndrome (Figure 2).

Modified treatment algorithm

The treatment algorithm used by the PCT in managing non-syndromic clefts required modification in 13 of the 16 syndromic patients (81%). The timing of the cleft repair procedure in the syndromic cohort is illustrated in Figure 2. There were notable delays in the timing of the palate repair in syndromic patients. In two syndromic patients, the palatoplasty procedure was completed much later, at four years of age. In addition, 15 patients underwent additional surgeries due to the presence of the syndromes and associated medical conditions, including

heart and urogenital tract diseases. These operations had of necessity priority over cleft repair. Tracheostomies were needed in three patients with PRS. Secondary operations for CLP were required in six patients (37.5%). Speech improvement operations or pharyngoplasty and tympanostomy tube placements were the most common secondary operations and were mainly required by PRS patients.

Study II

Materials and Methods

Participants

A case-controlled study was carried out at the Department of Pediatrics of the University of Pécs between July 2020 and March 2022. The study was approved by the Regional Ethics Committee of the University of Pécs (approval number: 7967-PTE 2020) and was performed according to the principles of the Declaration of Helsinki. All participating children with non-syndromic OFCs were patients of the Pécs Cleft Team. Medical geneticists examined all participants of the cleft group to rule out the presence of additional congenital malformations and/or underlying syndromes. Controls were recruited from the community of Baranya County, specifically from public elementary, high schools, and post advertisements on social media. The inclusion criteria for the OFC group consisted of the following: children with non-syndromic forms of OFC, 6–16 years old and an IQ ≥ 70. An OFC was considered non-syndromic when the cleft was the only single malformation without additional physical or developmental anomalies. The inclusion criteria of the controls included the following: healthy children born without OFCs, 6–16 years old and IQ ≥ 70.

Materials

The study consisted of three phases including questionnaires to collect retrospective clinical data and psychometric tools to assess executive functioning and Intelligence Quotient (IQ). Initially all psychometric tests were completed on site. The study was converted into an online platform due to restrictions related to the ongoing COVID-19 pandemic at the time. Measurements that required in-person completion (IQ test) were postponed onto a later period once the pandemic situation improved.

The Hungarian version of the Child Behavior Checklist (CBCL) was used to screen for behavioral and emotional problems in children and adolescents for the previous six months. A parental questionnaire was developed for the study to collect demographic data. This included prenatal and postnatal history, birth, motor and language development, education, previous psychiatric treatment, and history of somatic and neuropsychiatric disorders. Parental socio-economic data were additionally collected, including parental age, education, and employment status. Parents were also asked about a possible family history of neuropsychiatric disorders and/or any previous psychiatric treatment.

Four computer-based tests were used to assess the main domains of executive functioning. All tests were provided by the Psyway Hungarian psychometric website and all tests are standardized and norm-referenced (PsyWay, 2020). The official Hungarian version of the

WISC-IV (Nagyné Réz et al., 2007) was used to measure full-scale IQ, which was important for the assessment of executive functioning.

Statistical analysis

Statistical analysis was carried out using IBM SPSS Statistics 28 Software. A descriptive statistical analysis was performed. The primary aim of the analysis was to compare the differences in the results of cognitive test for executive function assessment (London Tower, Stroop, Corsi, and Continuous Performance Test), IQ (WISC-IV), the CBCL questionnaire (Child Behavior Checklist), and the demographic parameters between the two study groups.

Occupational statuses of the parents were classified as follows: employed, not employed, or retired. Academic levels of the parents were initially grouped into basic (elementary, lower secondary education), intermediate (upper secondary) and advanced (college or university). We later grouped these levels as either higher education (upper secondary education, college, or university) or lower education (elementary, lower secondary education) to increase statistical power.

The raw score is an untransformed score from a measurement of the above listed cognitive tests and the CBCL questionnaire. The raw scores were converted into a scale called T-score scale, which assumes a normal distribution with the mean = 50 and the standard deviation = 10. The T-scores of all psychometric tests were expressed as means ± standard deviations. The categorical data of the cleft and control groups were analyzed using contingency tables and the chi-squared or Fischer's test, as appropriate. For quantitative variables, two-sided independent samples Student's t-test were used. The Welch test was applied in cases when the variance was not homogenous. Analysis of variance (ANOVA) was used to test the difference among more than two groups (e.g., in case of analysis based on the type of cleft). These variables follow a normal distribution. Statistical significance was established as a value of $p < 0.05$. Effect sizes were defined as Cohen's d value in case of two independent groups, $\eta 2$ in case of ANOVA test, and ϕ value in case of Chi-square test (Coe, 2002).

Results

We recruited 43 children with non-syndromic OFCs and 44 controls for the study. Past medical history revealed two syndromic OFCs and these participants were excluded from the study. One participant of the cleft group was lost to follow up. The data of 84 study participants were analyzed (Figure 2).

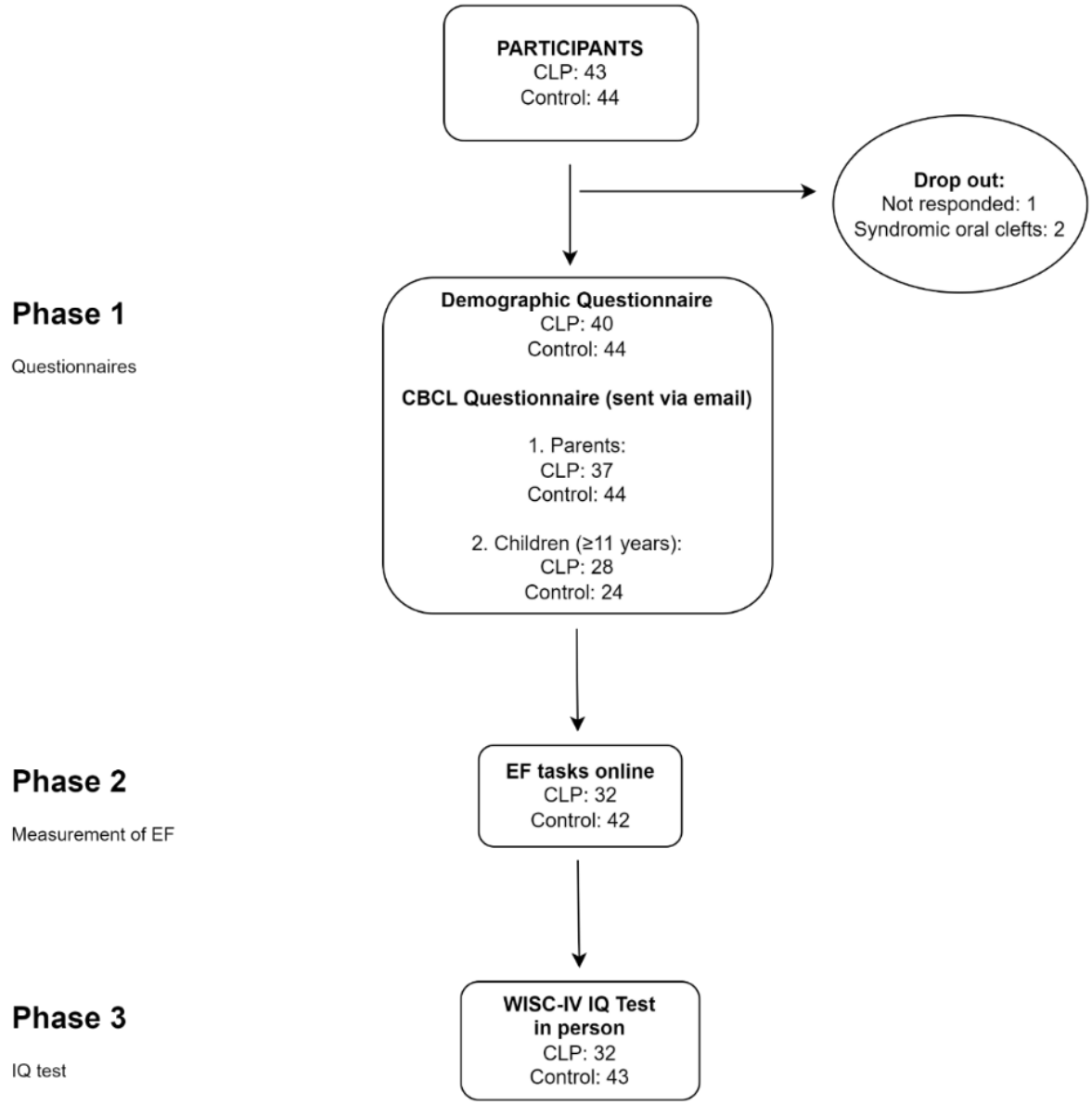

Figure 2. Study flow. The analyses were divided into three phases. The number of the participants are provided for each phase (CLP: cleft lip and/or palate group, EF: Executive function, IQ: Intelligence Quotient) (*Sándor-Bajusz, Dergez, Molnár, et al.,* Frontiers in Psychology, 2023).

Child Behavioral Checklist

Two dimensions of the Self-Report CBCL showed significant differences between the groups: Cleft children reported higher symptoms of affective problems (d= 0.24), while controls reported greater symptoms of externalization (d= 0.65), somatic (d=0.21), attention (d= 0.33) oppositional (d= 0.56), and behavioral problems (d=0.37). Parents of controls reported higher symptoms across all scales of the CBCL compared to parents of the cleft group, with small effect sizes.

Demographic data of children

Cleft status

Three subtypes of OFCs were present in the cleft group: 45% with cleft lip and palate (CLP), 37.5% with cleft lip (CL) and 17.5% with cleft palate (CP). Left-sided (32.5%) and bilateral (32.5%) OFCs were the most common. Overall, 29.16% of the cleft group reported their repaired OFCs as a current medical condition. All participants of the cleft group had repaired clefts, and none of these children had persistent hearing deficiency. More than half of the cleft group was represented by boys (56.6%), while controls had more girl participants (67.7%, $p = 0.031$, $\phi = 0.24$). There were no significant differences between the age of cleft versus controls (Table 1).

Table 1. Demographic data of the study groups. Data are presented as means and standard deviations (SD). The number of participants is provided for each variable (n). Units are provided for each measurement. Overall academic score was provided according to the 5-point grade system used in Hungary, which defines 1 as insufficient, 2 as sufficient, 3 as satisfactory, 4 as good, and 5 as excellent (*Sándor-Bajusz, Dergez, Molnár et al.,* Frontiers in Psychology, 2023).

Variable	**Cleft group (mean ± SD)**	***n***	**Control group (mean ± SD)**	***n***	***p* value**	**Cohen's *d***
Age	12.00±2.62	39	11.77±2.63	44	0.69	0.09
Education						
Academic year	6.17±2.38	39	6.06±2.75	44	0.99	0.04
Overall academic score	4.45±0.51	38	4.46±0.58	43	0.95	0.02
Birth						
Week of delivery	38.97±2.19	39	39.20±1.62	44	0.59	0.12
APGAR score 1	8.88±0.62	36	8.97±0.52	41	0.58	0.16
APGAR score 2	9.77±0.59	36	9.97±0.15	41	0.031*	0.48
Birth weight (g)	3414.87±614.58	39	3488.31±618.23	44	0.59	0.12
Birth height (cm)	51.76±4.08	38	50.43±3.32	44	0.11	0.36
Head circumference (cm)	34.75±1.51	16	34.43±1.90	30	0.57	0.19
Motor development						
Rolls over (months)	3.97±0.93	39	4.17±1.02	40	0.37	0.20
Sits (months)	6.50±1.55	38	7.29±2.00	41	0.06	0.44
Crawls (months)	8.61±1.74	38	8.47±1.80	41	0.73	0.08
Walks (months)	11.88±1.38	39	12.02±1.64	43	0.68	0.09
Potty-trained (years)	2.71±0.84	39	2.34±0.54	42	0.008*	0.53
Language development						
First words (months)	15.00 ±7.65	39	13.50±4.83	37	0.53	0.23
Two-word phrases (months)	24.43±9.77	38	19.52±6.11	34	0.039*	0.60
Coherent sentences (year)	2.50±0.75	38	2.22±0.59	38	0.055	0.41
Parental SES						
Gravidity of mother	2.44±1.37	39	2.66±1.94	44	0.99	0.13
Mother's age	42.79±4.43	39	44.67±4.57	43	0.063	0.42
Father's age	45.71±5.06	39	48.13±5.24	43	0.037*	0.47

Past psychiatric history and academic performance

We observed a higher proportion of psychiatric disorders in the cleft group (15%) compared to controls (4.5%; $p = 0.14$, $\phi = 0.18$). The cleft group received previous psychiatric therapy more often (15%) than controls (0%; $p = 0.009$, $\phi = 0.29$). The reported psychiatric diagnoses were ADHD (50%), borderline personality disorder (12.5%), learning disability (12.5%), depression (12.5%) and anxiety disorder (12.5%). Children in the cleft group required additional support for learning, psychological and physical well-being during their education more often than controls ($p < 0.001$, $\phi = 0.49$), specifically speech and language therapy ($p < 0.001$, $\phi = 0.51$). Overall, 4.5% of controls reported having a psychiatric comorbidity, which included dyslexia (50%) and ADHD (50%).

Preschool integration was significantly more difficult for the cleft group compared to controls ($p = 0.025$, $\phi = 0.26$). Both study groups did well later in preschool without requiring grade repetition ($p = 0.96$, $\phi = 0.005$). Children of the cleft group were examined by pedagogical professional services more often than controls ($p < 0.001$, $\phi = 0.49$). Participants in the cleft group required special education plans more often than controls ($p = 0.016$, $\phi = 0.29$). There were no differences in the rate of elementary grade repetition between clefts and controls ($p = 0.60$, $\phi = 0.073$). We observed no differences in the overall academic score; both clefts and controls achieved a good overall score in the current academic year (Table 1).

Pregnancy and developmental history

All participating children were born full-term via uncomplicated births. Apgar score at 5 min was lower in the cleft group ($p = 0.031$, $d = 0.48$, Table 1). No differences were observed in the total number of pregnancies, and natural and caesarian delivery ($p = 0.63$, $\phi = 0.05$). No differences were observed in the week of delivery, head circumference and birthweight between the two study groups (Table 1). The need for postnatal supportive care did not differ between clefts and controls (respiratory support, surfactant therapy, phototherapy, antibiotics, and transfusions; $p = 0.23$, $\phi = 0.13$).

Mothers of the cleft group reported feeding ($p = 0.007$, $\phi = 0.29$) and hearing ($p < 0.001$, $\phi = 0.51$) difficulties more often than mothers of controls. The cleft group developed motor skills (roll over, sitting) later than controls, however the effect sizes were small (Table 1). The cleft group was potty trained at an older age than controls ($p = 0.008$, $d = 0.53$, Table 1). Parents of the cleft group reported that their children were able to form two-word sentences at a later age compared to reports of parents of controls ($p = 0.039$, $d = 0,60$, Table 1). First words and coherent sentences were also spoken later by children in the cleft group (Table 1).

Demographic data of parents

Parents of the control group were older at the time of assessment than those of the cleft group (Table 1). Most parents of clefts (70.0%) and controls (69.8%) were married, and no differences were observed between the relationship statuses of parents of both groups ($p = 0.47$, $\phi = 0.08$). The employment statuses of fathers ($p = 0.42$, $\phi = 0.25$) and mothers ($p = 0.86$, $\phi = 0.19$) did not differ between the two groups.

Past psychiatric and academic history

The majority of reported psychiatric diagnoses in the family of the cleft group were depression (75%) and anxiety disorders (25%). History of psychiatric disorders was more often reported by parents of controls (27.3%) compared to clefts (7.5%; $p = 0.010$, $\phi = 0.39$). One parent of the control group reported to have history of anxiety, but most parents did not further specify

these conditions. Fathers of the control group achieved a higher degree of education than fathers of the cleft group who had lower secondary education ($p = 0.024$, $\phi = 0.25$). There were no differences in the mother's level of education between the two study groups ($p = 0.29$, $\phi = 0.12$). Most parents completed high school and/or had a university degree.

Cognitive functioning

The CPT revealed differences between the two groups: the cleft group scored lower on detectability (%) than controls ($p = 0.022$, $d = 0.55$, Table 2). They also missed more targets than controls ($p = 0.058$, $d = 0.46$, Table 2). We did not observed differences for the remaining cognitive test results (Stroop, TOL, Corsi). None of the participants scored below average in any of the dimensions of the WISC-IV; however, controls scored higher on the PRI ($d= 0.22$) and WMI ($d= 0.25$) subtests.

Table 2. Results of the CPT (Continuous Performance Task). Data are presented as means and standard deviations (SD) (*Sándor-Bajusz, Dergez, Molnár et al.,* Frontiers in Psychology, 2023).

Performance measures	Group	*n*	Mean±SD	*p* value	Cohen's *d*
Detectability (%)	control	41	59.46±14.90	0.022*	0.55
	cleft	32	51.03±15.66		
Omission errors (%) (missed targets)	control	41	59.54±13.00	0.058	0.46
	cleft	32	53.84±11.84		
Commission errors (%) (false response without target)	control	41	52.00±12.21	0.47	0.17
	cleft	32	54.28±14.49		

Subgroup analysis of the cleft group

We hypothesized that the more complex cleft subtypes would obtain lower scores on the IQ test, and present with a history of atypical neurodevelopment, psychiatric disorders, and academic difficulties. We further assumed that early interventions for speech and language would positively impact cognitive development, and the later would be reflected in the IQ score of these children.

A total of 10 girls and 30 boys were tested in the cleft group. Boys became potty-trained earlier (2.39 years) than girls (3.50 years; $p = 0.037$, $d = 0.79$). Hearing difficulties were in highest proportion for CP (57.1%) than for CL (13.3%) and CLP (44.4%) however with small effect size ($p = 0$ 0.063, $d = 0.36$). In the analysis according to types of clefts, CLP was the subtype that was most often referred to special education services: CLP in 72%, CL in 40%, and CP in 14% of the cases ($p = 0.023$, $d = 0.29$). CLP subtype was also diagnosed with psychiatric comorbidities in highest proportion (22.2%) compared to CL (13.3%) and CP (0%) ($p = 0.53$, $d = 0.22$). CLP subtype had additionally received previous psychiatric care in highest proportion (22.2%) compared to the rest of the cleft subtypes ($p = 0.61$, $d = 0.23$). Bilateral (30.8%) and left-sided clefts (15.4%) presented the highest proportion of psychiatric diagnoses ($p = 0.27$, $d = 0.35$).

Parental socioeconomic status and children's cognitive performance

We explored variables of parental SES that may influence the outcome of academic and cognitive performance of the OFC group. Children who had fathers with a high academic background reached a higher overall academic average ($p = 0.005$, $d = 1.02$). Children with mothers of a high academic background also reached a higher overall academic average ($p=<0.001$, $d= 1.88$). The same pattern was observed for the IQ scores: children who scored higher on the FS-IQ index had fathers (($p= 0.011$, $d= 1.04$) and mothers ($p= 0.015$, $d= 1.25$) with a higher academic background A total of 44.4% of cleft children with single parents had a psychiatric condition(s), while only 6.5% had psychiatric condition(s) when raised by married parents ($p = 0.016$, $d = 0.44$).

Speech and language therapy and the IQ score

We explored the effect of speech and language therapy on IQ scores and overall academic average. FS-IQ and VCI scores were higher for children who received therapy (Table 3). Overall academic average was higher for cleft participants who did not receive therapy, although with small effect size (Table 3). A one-way ANOVA was performed to compare the effect of the affected side of the cleft (left, right, bilateral and midline) on IQ scores. We observed differences for continuous variables in WMl when tested by the affected side ($p = 0.037$, $\eta 2 = 0.27$).

Table 3. Effect of speech and language therapy on IQ scores and overall academic average. FS-IQ: Full-scale IQ, VCI: Verbal Comprehension Index, PRI: Perceptual Reasoning Index, WMI: Working Memory Index, PSI: Processing Speed Index (*Sándor-Bajusz, Dergez, Molnár et al.,* Frontiers in Psychology, 2023).

Cognitive performance	**Speech and language therapy**	***n***	**Mean±SD**	***p value***	**Cohen's *d***
FS-IQ	No	16	107.06±10.77	0.077	0.66
	Received	15	114.13±10.68		
VCI	No	16	109.44±10.73	0.005*	1.10
	Received	15	121.20±10.63		
PRI	No	16	104.50±10.67	0.24	0.43
	Received	15	108.67±8.44		
WMI	No	16	102.38±13.88	0.55	0.22
	Received	15	105.13±11.54		
PSI	No	16	103.63±9.02	0.83	0.07
	Received	15	104.53±14.22		
Overall academic average	No	18	4.54±0.48	0.22	0.40
	Received	21	4.33±0.56		

Study III

Materials and methods

Materials

The current meta-analysis was registered in PROSPERO (International Prospective Register of Systematic Reviews; RRID:SCR_019061, identifier CRD42020167773), and is reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses 2020 (PRISMA 2020, RRID:SCR_018721) guideline. The data that was retrieved and analysed for this study was initially acquired by primary investigators who obtained informed consent from participants. Ethical approval was therefore deemed not necessary for the current study.

Database searches

MEDLINE, Scopus, Cochrane Central Register of Controlled Trials, Web of Science and Embase were systematically searched in September 2020 for case-control studies that reported structural brain MRI in individuals with non-syndromic OFCs and healthy controls.

Study selection and data extraction

The following criteria had to be met for inclusion into the study: (1) Case-control studies with humans; (2) Individuals with non-syndromic (isolated) OFCs, without restriction to age; (3) Healthy controls; (4) Structural brain differences of individuals with non-syndromic OFCs vs. their controls as a relevant outcome: structural differences had to be explored with brain MRI. No restrictions were applied for language. The publication was excluded if it had any of the following: (1) Animal studies (2) Individuals with syndromes (syndromic forms of OFCs, such as Pierre-Robin sequence or Velocardiofacial syndrome).

Two review authors independently screened studies for eligibility, extracted data and assessed risk of bias with the Newcastle-Ottawa Scale. Any differences between the two reviewers were settled by consensus after consulting a third author. Additional sources were also screened (hand searching, reference/citation lists) to identify articles that may potentially meet the inclusion criteria. Study setting (design, institution, country), patient demographics (number, age, sex, ethnicity, gender, type of OFC, brain imaging details, data processing) and outcome measurement details (general and regional brain MRI measurements) were collected. Any data that were not described in the article were calculated from existing data or were obtained by contacting the authors.

The primary outcome measures were structural differences of the brain of individuals with OFCs vs. individuals without OFCs (controls) investigated via MRI. Other sought outcomes included the correlation between observed structural brain differences and alterations in neurological and/or mental functioning.

Statistical analysis

Review Manager Software Version 5.4 was used for data synthesis (Cochrane, 2020). The random-effects model was chosen a priori as the primary method to estimate all pooled estimates for studies that were comparable in design, exposure, and outcomes. This model was used to account for the differences within study populations such as age, sex, and type of OFCs. Mean Differences (MDs) and their corresponding 95% confidence intervals (CI 95%) were used for continuous outcomes.

The extent and impact of between-study heterogeneity was assessed by inspecting the forest plots and by calculating the tau-squared and the I-squared statistics, respectively. The I-squared thresholds represented heterogeneity that may not be important (0–40%), moderate (30–60%), substantial (50–90%), or considerable (75–100%). Possible sources of heterogeneity in meta-analyses were sought through pre-specified mixed-effects subgroup analyses if at least two studies were included for a comparison (same intervention/outcome). Pre-defined subgroup analyses included: (i) age; (ii) sex; (iii) ethnicity; (iv) cleft form (non-syndromic vs. syndromic).

Results

Systematic literature review

A total of 257 records were identified following the database searches. Of this total, 245 records underwent title and abstract screening following duplicate removal and 32 records were retrieved and assessed for eligibility. Two records were additionally identified by handsearching, and only one met the inclusion criteria (Yang et al., 2012). Three records included individuals diagnosed with Van der Woude syndrome (Nopoulos et al., 2000, 2002a, 2005). These records were included in the current systematic review as none of the syndromic individuals exceeded 15% of total cleft participants.

Fifteen records seemed to meet the inclusion criteria; however, they were excluded during the full-text screening process. The reasons for exclusion were as follows: absence of a control group (n = 3) (Shen and Huang, 1996; Mueller et al., 2007; Zheng et al., 2019), conference abstracts or commentaries (n = 4) (Chollet et al., 2010; Tollefson and Sykes, 2010; DeVolder et al., 2014, 2015), wrong study population that only included syndromic cases of OFCs (n = 2) (Nopoulos et al., 2007c, 2007b), absence of neuroimaging (n = 5) (Čeponien≐ et al., 1999; Scott et al., 2005; Kummer et al., 2007; Conrad et al., 2008; Watkins et al., 2018), or neuroimaging other than brain MRI (n = 1 (Becker et al., 2008). The study size ranged between 24 and 234 participants. Most of the participants were males of Caucasian ethnicity, and the majority were children.

Risk of bias

The overall risk of bias ranged from medium to high. Selection of cleft participants, their comparators and the assessment of exposure were described in half of the studies. Information on recruitment and reasons for dropout were not available in most studies. Only one study reported blinding personnel of group status during MRI scanning (Nopoulos et al., 2007a).

Meta-analyses

Five studies were comparable in terms of study design, exposure, and outcome. Studies were pooled using a random-effect meta-analysis.

Studies investigating global measurements

These measurements included three anatomical groups: total brain volumes (including MRI volumes of the cerebrum and cerebellum), cerebral volumes (only MRI volumes of the cerebrum), and cerebellar volumes (only MRI volumes of the cerebellum).

The cleft group had lower total brain gray matter volume compared to controls (MD: −41.14 cm3; 95% CI: −57.36 to −24.92; n = 2; 172 participants; I2: 0%). The cerebellum was significantly smaller in OFCs compared to controls (MD: −12.46 cm3; 95% CI: −18.26, −6.67; n = 3; 354 participants; I2: 0%, n = 3).

Studies investigating regional measurements

Measurements included the frontal, temporal, parietal, and occipital lobes. Smaller temporal lobes were found for the cleft group compared to controls (MD: −10.53 cm3; 95% CI: −18.23 to −2.82; n = 2; 120 participants; I2: 0%). The cleft group had significantly smaller occipital lobes compared to controls (MD: −7.39 cm3; 95% CI: −12.80 to −1.99; n = 2; 120 participants; I2: 0%).

Studies investigating mental and cognitive functioning

Heterogeneity of methods and outcomes prevented statistical pooling for meta-analyses for most secondary outcomes, except for IQ scores. All studies used the Wechsler Intelligence Scale of different editions. Significantly lower FS-IQ scores were as observed in individuals with OFCs compared to controls (MD: −12.58; FS-IQ; 95% CI: −21.98 to -3.17; n = 2; 234 participants; I2 = 84%).

Subgroup analysis

Four meta-analyses demonstrated moderate to considerable levels of heterogeneity. We performed the analysis to identify possible sources of the heterogeneity observed in the main analyses. Subgroup analysis was feasible for only two meta-analyses (Figures 3 and 4). Subgroup analyses were performed for age, sex, ethnicity, non-syndromic, and mixed (syndromic and non-syndromic) OFCs.

The non-syndromic subgroup had significantly smaller total brain volume compared to controls. However, this significant difference was not seen in the mixed subgroup (syndromic and non-syndromic cases) (MD: −77.06 cm3; 95% CI: −115.47 to −38.64; n = 2; 202 participants; I2 = 0%; Figure 3). The same phenomenon was observed for age (children vs. adults), sex (male only vs. mixed) and ethnicity (Caucasian vs. mixed). These factors may be possible sources of the heterogeneity seen in the main analysis. A decrease in heterogeneity was found in the subgroup analysis of mixed OFCs for cerebral volume (MD: −0.80 cm3; 95%CI: −40.88 to 39.29; n = 2; 120 participants; I2 = 0%; Figure 4). The same phenomenon was observed for age (children vs. adults) and sex (male vs. male and female).

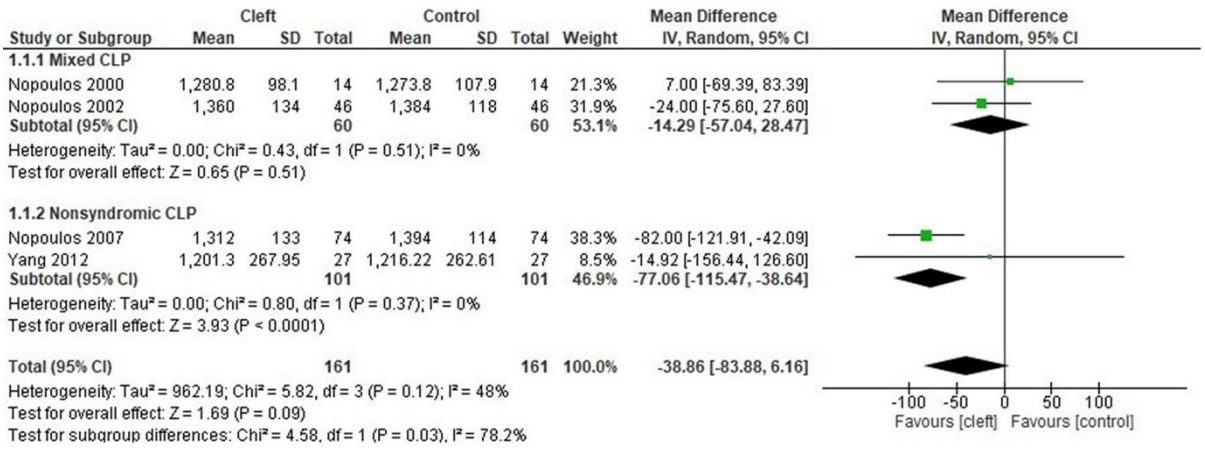

Figure 3. Forest plot for total brain volume (cm^3) with subgroup analysis (non-syndromic vs. mixed) (*Sándor-Bajusz, Sadi, Varga et al.,* Frontiers in Neuroanatomy, 2022).

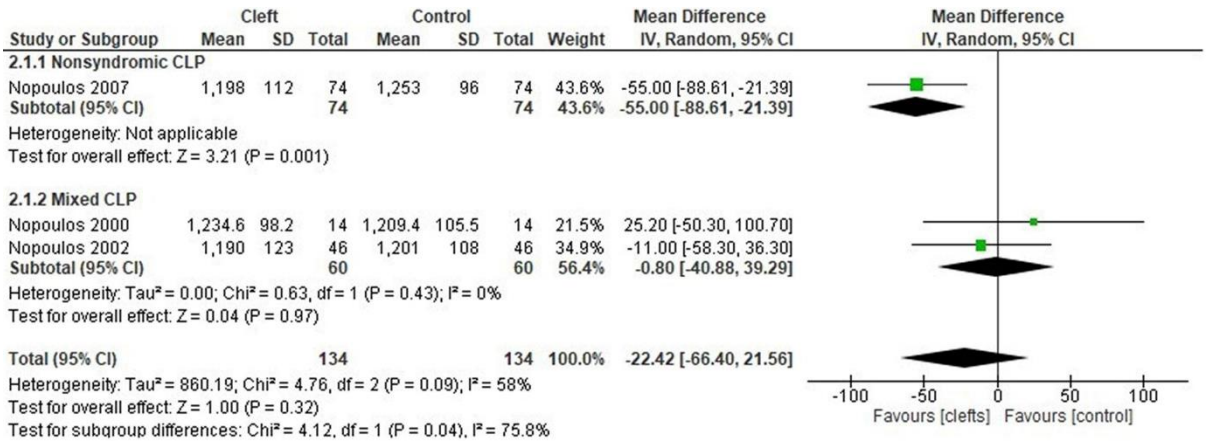

Figure 4. Forest plot for total volume of the cerebrum (cm^3) with subgroup analysis (non-syndromic vs. mixed) (*Sándor-Bajusz, Sadi, Varga et al.,* Frontiers in Neuroanatomy, 2022).

Summary of new findings and discussion

Study I

- The Cleft Team needed to modify the treatment algorithm for primary and/or secondary operations in the majority (81%) of the syndromic patients.
- The main causes of the delay in palatoplasty for PRS patients were airway issues and feeding problems. In other syndromic patients, cardiorespiratory and urogenital interventions had priority and therefore caused a delay in the timing of the primary cleft operations.
- The observed high rate (37.5%) of the secondary operations such as speech improvement surgery and ancillary procedures such as placement of tympanostomy tubes for the syndromic OFC patients is in accordance with the literature.
- Velopharyngeal insufficiency and speech problems were more common conditions in syndromic patients, especially in patients with PRS. This finding explains the high rate of pharyngoplasties and tympanostomies in these patients.
- The percentage of the syndromic OFC patients managed by the PCT was 2.6% during the study. This number is below the 10%-30% prevalence of syndromic OFC described in the literature.

Study II

- Apgar score at 5 min was lower for the cleft group than for controls, but clinically within the normal range.
- There was a tendency of a slower onset of developmental milestones in children with OFCs and experienced difficulties integrating into preschool; most required additional support for learning, psychological and physical well-being throughout their education. Based on our results, children with non-syndromic OFCs initially have a slower development and experience difficulties integrating into preschool; however, it seems that they go through a "catch-up phase" around school age and perform well−almost equal to their peers−throughout elementary and high school.
- The highest proportion of psychiatric diagnoses were observed in CLP and bilateral-sided clefts.
- We did not observe psychiatric comorbidities in CP children, which is in contrast with previous observations.
- Children with non-syndromic OFCs reported symptoms of internalizing disorders (affective, anxiety), in contrast to symptoms of externalizing disorders reported by controls (attention, oppositional, behavioral).
- Children with non-syndromic OFCs were clinically diagnosed with psychiatric disorders at a higher proportion and received psychiatric support more often than controls.
- Children with non-syndromic OFCs scored lower on the CPT and missed targets more often than controls (omission errors, Table 2).

- Children with non-syndromic OFCs who had parents with a higher educational background scored significantly higher on the IQ test, specifically reflected in perceptual reasoning and the FS-IQ score.
- Children with non-syndromic OFCs who received speech and language therapy achieved higher scores specifically reflected in the verbal component (VCI) of the WISC-IV (Table 3).
- Children with non-syndromic OFCs raised by single parents were diagnosed with psychiatric conditions more often than children raised by married parents.

Study III

- Subjects with OFCs had smaller total gray matter, cerebellum, temporal lobes, and occipital lobes on brain MRI compared to controls.
- Individuals with OFCs had lower FS-IQ scores compared to matched controls.
- The risk of bias for the included studies was moderate to high.
- Subgroup analysis revealed a significantly smaller brain and cerebrum in studies with exclusively non-syndromic OFC participants. These differences were not observed in studies with mixed syndromic participants (Figures 3, 4).

Future directions

The future will underscore the importance of dedicated cleft teams with multidisciplinary expertise and experience in cleft excellence. The application of early interventions, special educational programmes, and proper parental support will aim at the goal of attaining outcomes where most children with OFCs develop and perform as well as their peers. Future studies with increasing sophistication may greatly benefit the clinical field in establishing more refined timely therapeutic interventions. These include such possible approaches as robotic surgical platforms, simplified rapid genetic testing, and early screening of executive functions to carefully monitor neurodevelopmental trajectories as a part of the complex therapy applied to OFC patients.

Stem cell-based interventions are becoming increasingly recognized in the medical field. They may in the future, offer novel approaches to the clinical care of OFC patients. Stem-cells may be used for example, in reconstructions to replace missing orofacial hard and soft tissues in the defects left behind the wake of malformations caused by the presence of a cleft. Stem cells may also in the future, provide a new model for cleft research to monitor interneuronal development and identify key gene/protein pathways that are altered or dysregulated in these individuals.

Conclusions

Treating syndromic OFC patients is by nature, more complex than treating non-syndromic cleft patients. Syndromic patients require more attention and support for their multiple potential special needs from both the family and the health care facility, including the cleft teams. The surgical treatment of certain associated anomalies, such as heart defects and respiratory insufficiency, has priority over the timing of the reconstruction of the cleft lip and/or cleft palate in syndromic patients. The presence of a genetic syndrome may therefore notably affect the treatment algorithm of cleft repair surgeries.

Some Hungarian children with non-syndromic OFCs seem to be at risk for atypical cognitive and speech development compared to children not born with OFCs. Future studies with large sample sizes are needed to further explore this underlying etiology to identify this at-risk subpopulation, since not all children with non-syndromic OFCs present with such difficulties. Longitudinal studies are further needed to provide more evidence of baseline cognitive functioning to study early signs of atypical neurodevelopment and the effect of early interventions.

There may be structural brain differences between individuals with non-syndromic clefts and controls based on the available evidence, which may indicate a co-occurring brain involvement in orofacial clefts. Structural brain MRI studies may provide evidence on how the type and degree of clefts plays a role with later cognitive development and functioning. Improvement in study design, size, methodology, and participant selection may allow a more thorough analysis and decrease study heterogeneity.

List of own publications

Publications related to the Doctoral Thesis

Total impact factor: 8,845

1. **Sándor-Bajusz KA**, Dergez T, Molnár E, Hadzsiev K, Till Á, Zsigmond A, Vástyán A, Csábi G. *COGNITIVE FUNCTIONING AND CLINICAL CHARACTERISTICS OF CHILDREN WITH NON-SYNDROMIC OROFACIAL CLEFTS: A CASE-CONTROL STUDY*. Front Psychol 2023 Feb 28;14. doi: 10.3389/fpsyg.2023.1115304. **IF: 4,232**

2. **Sándor-Bajusz KA**, Sadi A, Varga E, Csábi G, Antonoglou G and Lohner Sz. *THE BRAIN IN ORAL CLEFTING: A SYSTEMATIC REVIEW WITH META-ANALYSES*. Front Neuroanat 2022 Jun 10;16:863900. doi: 10.3389/fnana.2022.863900. PMID: 35756498. **IF: 3,543**

3. **Sándor-Bajusz KA**, Maros T, Olasz L, Sándor G, Hadzsiev K and Vástyán A. *THE INFLUENCE OF GENETIC SYNDROMES ON THE ALGORITHM OF CLEFT LIP AND PALATE REPAIR – A RETROSPECTIVE STUDY*. Ann Maxillofac Surg. 2021 Nov 29. doi: 10.4103/ams.ams_77_21. Ahead of print. PMID: 35265497. PMID: 35833477. **IF: 1,07**

Conference abstracts related to the Doctoral Thesis

Total impact factor: 12,571

1. **Sándor-Bajusz KA**, Molnár E, Dergez T, Hadzsiev K, Vástyán A, Csábi G. *EARLY LANGUAGE INTERVENTION AND IQ OF CHILDREN WITH NON-SYNDROMIC ORAL CLEFTS.* 6th Hungarian Neuroscience Meeting for Undergraduate Students, Graduate Students and young Post-Docs (HuNDoC 2023). Budapest, January 31, 2023.

2. **Sándor-Bajusz KA**, Dergez T, Vástyán A, Csábi G. *NEUROPSZICHIÁTRIAI ZAVAROK AJAK- ÉS SZÁJPADHASADÉKKAL SZÜLETETT GYERMEKEKBEN* [Neuropsychiatric disorders in children born with oral clefts]. Hungarian Paediatric Association, Annual National Paediatric Congress, Kecskemét, Hungary. September 15-17, 2022. GYERMEKGYÓGYÁSZAT 73 : 5 pp. 394-394. , 1 p.

3. **Sándor-Bajusz KA**, Sadi A, Varga E, Csábi G, Antonoglou G, Lohner S. *THE BRAIN IN ORAL CLEFTING: PRELIMINARY RESULTS OF A SYSTEMATIC REVIEW WITH META-ANALYSES.* EPA 30th European Congress of Psychiatry, Budapest. June 4-7, 2022. EUROPEAN PSYCHIATRY 65(S1):S641-S641. doi: 10.1192/j.eurpsy.2022.1644. **IF: 7,156**

4. **Sándor-Bajusz KA**, Molnár E, Dergez T, Hadzsiev K, Vástyán A, Csábi G. *EXECUTIVE FUNCTIONS OF CHILDREN WITH ORAL CLEFTS: A PILOT STUDY.* 19th International Congress of ESCAP, Maastricht, The Netherlands. June 19-21, 2022.

5. **Sándor-Bajusz KA**, Varga E, Antonoglou GA, Lohner S. *BRAIN STRUCTURE OF INDIVIDUALS WITH ORAL CLEFTS: FIRST RESULTS OF A SYSTEMATIC REVIEW WITH META-ANALYSES.* 34th ECNP Congress, Lisbon, Portugal. October 2-5, 2021. EUROPEAN NEUROPSYCHOPHARMACOLOGY 53(S1): S147. doi: 10.1016/j.euroneuro.2021.10.194. **IF: 5,415**

6. **Sándor-Bajusz KA**, Molnár E, Dergez T, Hadzsiev K, Vástyán A, Csábi G. *KOGNITÍV FUNKCIÓK VIZSGÁLATA AJAK- ÉS SZÁJPADHASADÉKKAL SZÜLETETT GYERMEKEKBEN: ELŐZETES EREDMÉNYEK* (*COGNITIVE FUNCTION OF CHILDREN WITH ORAL CLEFTS: A PILOT STUDY).* Hungarian Paediatric Association, Annual National Paediatric Congress, Pécs, Hungary. May 24-26, 2021.

7. **Sándor-Bajusz KA**, Molnár E, Dergez T, Hadzsiev K, Vástyán A, Csábi G. *COGNITIVE FUNCTION OF CHILDREN WITH ORAL CLEFTS: A PILOT STUDY.* MedPECS (Medical Conference for PhD Students and Experts of Clinical Sciences), Pécs, Hungary. May 15, 2021

Other publications

Total impact factor: 21,164

1. **Sándor-Bajusz KA**, Kraut Andrea, Baasan O, Máarovics G, Berényi K and Lohner S. *PUBLICATION OF CLINICAL TRIALS ON MEDICINAL PRODUCTS: A FOLLOW-UP STUDY*. Trials. 2022 Apr 21;23(1):330. doi: 10.1186/s13063-022-06268-y. PMID: 35449017. **IF: 2,754**

2. Nagy D, **Sándor-Bajusz KA**, Bódy B, Decsi T, van Harsselaar J, Theis S, Lohner S (2021). *EFFECT OF CHICORY-DERIVED INULIN-TYPE FRUCTANS ON ABUNDANCE OF BIFIDOBACTERIUM AND ON BOWEL FUNCTION: A SYSTEMATIC REVIEW WITH META-ANALYSES.* Crit Rev Food Sci Nutr. 2022 Jul 14:1-18. doi: 10.1080/10408398.2022.2098246. **IF: 11,208**

3. Mendez-Echevarria A, **Sándor-Bajusz KA**, Calvo C. *SEVERE SINUS BRADYCARDIA ASSOCIATED WITH REMDESIVIR IN A CHILD WITH SEVERE SARS-COV-2 INFECTION-REPLY*. Eur J Pediatr. 2021 Jan 19:1–2. doi: 10.1007/s00431-021-03952-0. PMID: 33464367. **IF: 3,601**

4. Méndez-Echevarría A, Pérez-Martínez A, Gonzalez Del Valle L, Ara MF, Melendo S, Ruiz de Valbuena M, Vazquez-Martinez JL, Morales-Martínez A, Remesal A, **Sándor-Bajusz KA**, Cabañas F, Calvo C. *COMPASSIONATE USE OF REMDESIVIR IN CHILDREN WITH COVID-19*. Eur J Pediatr. 2020 Nov 16:1–6. doi: 10.1007/s00431-020-03876-1. PMID: 33200304. **IF: 3,601**

Acknowledgements

The research of the current thesis was carried out at the Department of Pediatrics of the University of Pécs, Hungary.

First and foremost, I would like to express my sincere gratitude to the University of Pécs, Prof. Dr. József Janszky and Prof. Dr. Tamás Tényi, and to Prof. Dr. Tamás Decsi, Clinical Director of the Department of Pediatrics, for providing this unique opportunity to learn and experience academic research in medicine.

I would like to express my deepest gratitude to my corresponding Supervisor, Prof. Dr. Györgyi Csábi, for always being there when I needed her support, reviewing my progress constantly, and guiding me through my PhD studies.

A special thanks to all the co-authors of the three publication that provided the basis for this thesis; Thank you Dr. Attila Vástyán, Dr. Kinga Hadzsiev, Prof. Dr. György Sándor, Dr. Barna Teodor, Dr. Anna Zsigmond, Dr. Ágnes Till, Edit Molnár, Dr. Tímea Dergez, Dr. Szimonetta Lohner, Dr. Georgios Antonoglou, Dr. Eszter Varga, and Dr. Asaad Sadi for the outstanding team work and diligence you provided for each project.

A very special thanks to the members of the Pécs Cleft Team, especially Dr. Attila Vástyán, for all your support and the amazing opportunity to be a part of your team. I would like to thank the members of the University Hospital Cleft Lip and Palate Centre in Oulu, Finland, for making it possible for me to visit.

I would like to thank the patients of the Pécs Cleft Clinic who despite their own challenges are willing to participate in research. Without your contribution, cleft research in Hungary would not be possible.

I would like to highlight the following truly exceptional people from the University of Pécs; Dr. Szimonetta Lohner, Dr. Tímea Dergez, Edina Mendl, and Ildikó Csölle, who have shown immense support and have given me guidance during the most challenging times as a PhD student.

A special thanks to Dr. Karolina Pircs, Kilian Gutiérrez, Dr. Raul Alelú-Paz, Dr. Ariel Cariaga Martinez, and Dr. Jacob Vorstman, who took their time to teach me the most important aspects of neuropsychiatric research, and have truly inspired me to continue future research as a clinician.

I want to give my deepest appreciation to my parents, Prof. Dr. György Sándor and Dr. Cecilia Bajusz, for providing me with the best parental support and guidance throughout this journey. I would like to thank my siblings Enikő Sándor and Hunor Sándor, and my significant other, Gergely Sámson, for their support and keeping me sane throughout the whole process. I would further like to thank all my dear friends, specifically Laetitia Verville, for her support and amazing art skills which have greatly increased the value of this thesis.

This work was financially supported by the University of Pécs and the ÚNKP-21-3 New National Excellence Program of the Ministry for Culture and Innovation from the Source of the National Research, Development, and Innovation Fund.