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Ventricular septal defects in function of maternal sociodemographic aspects

Research Article

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Abstract: The objective of our project is to reveal the possible etiological factors of different congenital cardiovascular abnormalities. In this study, we evaluated single ventricular septal defect (VSD) after surgical correction or with lethal outcome. The birth outcomes of these cases in the function of maternal socio-demographic features were evaluated. Data are based on 1,659 VSD cases, 2,534 matched controls and 38,151 all controls without any defects, in addition in the mothers of 19,393 malformed controls with other isolated defects in the population-based large dataset of the Hungarian Case-Control Surveillance of Congenital Abnormalities. VSD had mild female excess with a higher rate of preterm birth and mainly low birth weight indicating intrauterine growth restriction of affected fetuses, particularly in males and full-term or average weighted cases. The mothers of cases with VSD had lower socioeconomic status and higher rate of smoking and particularly drinking habit. The evaluation of medically recorded pregnancy complications showed an association of gestational diabetes with a higher risk of VSD. In conclusion, the association of small localized size of VDS and obvious fetal growth restriction needs further explanation in these cases, while gestational diabetes, lower socioeconomic status and adverse lifestyle of pregnant women may have a role in the origin of VSD.

Keywords: *Ventricular septal defect • Birth outcomes • Intrauterine growth restriction • Lower socioeconomic status • Alcohol drinking • Smoking • Gestational diabetes • Case-control population-based study*

1. Introduction

Among structural birth defects, i.e. *congenital abnormalities* (CAs), CAs of heart and great vessels, i.e. *congenital cardiovascularis abnormalities* (CCVAs) are the most frequent CA-group. The birth prevalence of cases with CCVA was between 4 and 50 per 1000 live-births in different studies because their occurrence is dependent on the age at examination and the sensitivity of the examination technique, the case definition and the types of cases included in the studies [1-5]. A Hungarian population-based study of 2,259 children based on the pediatric cardiologic examination and/or the evaluation of autopsy report of each individual child, the birth prevalence of CCVAs was 10.2 per 1000 [6]. CCVAs cause about 10 % of infant mortality, but are associated with about 25% of CAs related infant deaths [7,8].

Ventricular septal defect (VSD) belongs to the most frequent CCVAs with 2.0 per 1000 births prevalence [9], though when echocardiography was used in the diagnostic algorithm, a prevalence of up to 3.9 per 1000

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patients has been recorded [4]. In the vast majority of patients with VSD the underlying etiology is unclear [10]. The objective of our project is to estimate the possible etiological factors in the origin of different CCVA-types. VSD was evaluated in the first step; however, VSD is a heterogeneous CCVA including different developmental errors of ventricular septum [11-18] because it is composed from 4 parts: septum membranaceum, anterior septum, posterior smooth septum and posterior trabeculated septum. These 4 components of the ventricular septum are derived of 3 developmental processes: (i) the posterior septum forms from the ventricle, (ii) anterior septum from the conotruncal crest and (iii) the septum membranaceum from the endocardial cushions. VSD due to the failure of the union between the endocardial cushions causes defect of the interventricular muscular ridge and septum bulbi, and these defects are the consequences the defective growth and position of different components of the heart. Obviously VSD caused by the muscular or junctional defects have different embryonic and possible etiological origin, therefore only the membranous and muscular groups of VSD were included to the study. However, the severity of VSD is very wide from the spontaneous closure to the lethal outcome. Thus we decided to evaluate VSD based on well-established and homogeneous diagnosis; therefore only cases with single VSD after surgical correction and/or lethal outcome were included to the study.

Here the birth outcomes of these cases with VSD and the socio-demographic characteristics of their mothers are described in the population-based Hungarian Case-Control Surveillance of Congenital Abnormalities (HCCSCA) [19]. The possible effects of acute and chronic maternal diseases and related drug treatments in the origin of VSD are published in a separate paper [20].

2. Material and Methods

2.1. The Hungarian Case-Control Surveillance of Congenital Abnormalities

The *cases* with CA including VSD in the HCCSCA were selected from the *Hungarian Congenital Abnormality Registry* (HCAR) [21]. The reporting of cases with CA is mandatory for physicians to the HCAR, and most are reported by obstetricians (in Hungary practically all deliveries occur in inpatient obstetric clinics and birth attendants are obstetricians) and pediatricians (who are working in the neonatal units of inpatient obstetric clinics and various general and specialized, e.g. cardiologic inpatient and outpatient pediatric clinics). Autopsy was mandatory for all infant deaths, and pathologists sent a copy of the autopsy report to the HCAR if defects were identified. Since 1984 prenatal diagnostic centers were also asked to report malformed fetuses diagnosed prenatally with or without elective termination of pregnancy to the HCAR. The recorded total (birth + fetal) prevalence of cases with CA was 35 per 1000 *informative offspring* (live-born infants, stillborn fetuses and electively terminated malformed fetuses) between 1980 and 1996 [19], and about 90% of major CAs were recorded in the HCAR [22].

Only cases reported to the HCAR during the first 3 months after births or pregnancy termination (the rest, 23% of cases were affected mainly with mild CAs) were selected from the HCAR for the HCCSCA.

Controls were defined as newborn infants without CAs. The source of these controls was the National Birth Registry of the Central Statistical Office for the HCCSCA. In general two controls were matched to every case according to sex, birth week in the year when the case was born and district of parents' residence. If controls were twins, only one of these twin-pairs was randomly selected as controls for the HCCSCA.

A structured questionnaire with an explanatory letter and printed informed consent was mailed continuously to the address of mothers immediately after the selection of cases and controls for the HCCSCA. The questionnaire requested information on maternal characteristics (demographic data, history of previous pregnancies, etc.) and pregnancy complications. Mothers were also requested to send us the prenatal maternity logbook, discharge summary of their deliveries and every medical record of their child's CA.

The mean ± S.D. time elapsed between the end of pregnancy and return of the "information package" (including logbook, discharge summary, questionnaire and signed informed consent) in our prepaid envelope was 3.5 + 2.1 and 5.2 ± 2.9 months in cases and controls, respectively.

In addition regional district nurses were asked to visit all non-respondent case mothers and to help them to fill-in the same questionnaire used in the HCCSCA and to evaluate the available medical documents. Unfortunately district nurses could visit only 200 non-respondent and 600 respondent control mothers in two validation studies [23,24] because the ethics committee considered this follow-up to be disturbing for the parents of all healthy children. Another validation study showed the low reliability of retrospective maternal self-reported information regarding smoking and alcohol drinking during the study pregnancy [25]. The number of smokers and alcohol drinkers during the study pregnancy therefore were evaluated only in those mothers, who were visited and questioned at home, but these data

were completed on the basis of cross interview of family members living together, and finally the so-called family consensus was recorded. Two groups of drinking habit were differentiated: regular drinkers (from one drink per week to daily one drink), and hard drinkers (more than one drink per day).

The necessary information was available for 96.3% of cases (84.4% from replies and 11.9% from visits) and 83.0% of controls (81.3% from replies and 1.7% from visits). The signed informed consent was sent back by 98% of mothers, the name and address were deleted in 2% of subjects without signed informed consent. The flow of cases and controls in the HCCSCA was reported previously [26].

The data of birth outcomes were based on the Notification Form of Cases with CA in the HCAR confirmed by the discharge summary of delivery and maternal information in the questionnaire. The birth outcomes of controls were evaluated on the basis discharge summary of delivery and maternal information in the questionnaire. The gestational age was calculated from the first day of the last menstrual period. The rate of low birth weight (LBW) newborns (less than 2,500 gram) and preterm births (PB) (less than 37 completed gestation weeks), in addition post term births (42 completed weeks or more) and large birth weight (more than 4,500g) was estimated on the basis of gestational age at delivery and birth weight.

Among maternal characteristics, age and birth order (parity) were recorded in the HCAR but these variables were checked in the HCCSCA completed by pregnancy order, marital and employment status based on the prenatal maternity logbook and maternal questionnaire. The maternal employment is good indicator of socioeconomic status in Hungary [27]. The data of pregnancy complications were based on the medical records in the prenatal maternity logbook.

The method of data collection was changed in 1997 (since all case and control mothers are visited and questioned at home by regional nurses, but these data have not been validated at the time of this analysis), and it explains that here only the 17 years' dataset of the HCCSCA, 1980-1996 are evaluated.

2.2. Study design of cases with VSD

The major evaluation problem of CCVAs was that in general cases with CA were reported immediately after birth to the HCAR and about 50% of cases with CCVA were reported as unspecified CCVA, because the exact diagnosis of CCVAs needed further time consuming examinations. The collection of medical data of cases with CA in the HCCSCA was 3.5 $\pm$ 2.1 months later thus we were able to get specified CCVA diagnoses in further 20% of cases. However, the rest, i.e. 30% of CCVA cases had no specified diagnosis in the HCCSCA. Most cases with CCVA were cared or had surgical intervention in the pediatric cardiologic institutions in Hungary, therefore one of us (M. Cs-Sz.) visited these cardiologic in- and outpatients clinics in 2008. Medical records were reviewed and the previous diagnosis of specified CCVAs was checked (and corrected it if necessary) and unspecified CCVAs were modified to specified CCVA diagnoses. If cases were not found in the records of pediatric cardiologic institutions, we had a correspondence with their mothers to clarify the fate and/or diagnosis of these cases in 2009 and 2010. However, (i) if these cases were not found, (ii) CCVA-diagnosis was not specified, or (iii) not confirmed, and (iv) mothers refused the collaboration, they were excluded from the study.

At the evaluation of VSD we had 4 selection steps.

I. Cases with multiple/syndromic VSD due to major mutant genes such as CA-syndromes (e.g. Holt-Oram) or chromosomal aberrations (e.g. Down syndrome) in the HCCSCA and unclassified multiple CAs including VSD were excluded from the study.

II. Among cases with isolated VSD, two subgroups were differentiated. (i) Single VSD. (ii) Complex VSD includes partly specified CCVA-entities such as tetralogy of Fallot, partly unspecified combinations of VSD with other CCVA, e.g. atrial septal defect. Only cases with single VSD were included to the study. However, if patent ductus arteriosus was also reported during the first 3 postnatal weeks with a spontaneous closure later, these cases were also considered as single VSD.

III. VSD, as it was mentioned in the Introduction, is a heterogeneous CCVA including different developmental errors of ventricular septum [11-18], only the membranous and muscular groups of VSD were included to the study. However, cases with common ventricle (i.e. cor triloculare biatriatum or single ventricle) were also excluded from the study

IV. Spontaneous closure in cases with muscular VSD was estimated on 30-35%, while it occurred about 10-15% in cases with membranous [28]. Thus, some experts considered at least muscular VSD as "delayed physiological development" rather than as a CA [16]. These diagnostic problems explained that finally only cases with VSD with surgical correction and/or lethal outcomes based on autopsy record were included to the study.

Controls were differentiated into two groups in the study: (i) *controls without CA* matched to cases with VSD and (ii) *all controls* without CA in the dataset of the HCCSCA. However, we had a third control group

including all other isolated CAs, as *malformed controls* from the HCCSCA.

2.3. Statistical analysis

Statistical analysis of data was performed with the software SAS version 8.02 (SAS Institute, Cary, North Carolina, USA). At the evaluation of quantitative data of birth outcomes of newborn infants and mothers such as age and pregnancy/birth order, Student t test was used while categorical variables of mothers regarding as marital and employment status were analyzed by chi square test. At the evaluation of pregnancy complications and categorical birth outcomes, odds ratios (OR) with 95% confidence intervals (CI) were calculated in multivariable conditional regression model at the comparison of cases and their matched controls, and multivariable unconditional regression model at the comparison of cases and all controls and malformed controls.

3. Results

Our population-based dataset included 1,661 cases with VSD. Of these 1,661 cases, 2 (0.1%) were diagnosed in stillborn fetuses, they were excluded from the study. Of 1,659 live-born cases, 1,133 (68.3%) were identified as membranous type. There was no case with VSD born to mothers with assisted reproductive technology.

The groups of all controls included 38,151 newborn infants without CA and this sample represented 1.8% of all Hungarian newborns during the study period. Of these 38,151 controls, 2,534 were selected as matched controls of 1,659 live-born cases.

Of 19,833 malformed controls with other isolated CA, 340 (1.7%) occurred in stillborn fetuses and 97 (0.5%) had prenatally diagnosed defects followed by elective termination of pregnancy. Thus finally 19,396 live-born malformed controls were evaluated.

The rate of infant mortality was 4.0% (No: 66), 0.2%, 0.3% and 7.0% in the groups of cases, matched, all and malformed controls.

The medically recorded live-birth outcomes in the study groups are shown in Table 1. There was a females excess (51.7%) among cases with VSD because the expected female proportion among newborns is 48.7% in Hungary. Thus the expected number of females was 808 based on the Hungarian population figure instead of observed number, i.e. 856 (p = 0.70). However, this female excess is much more obvious at the comparisons of malformed controls and all controls matched to malformed cases in Table 1 due to their drastic male excess. The latter is explained mainly by the higher rate of CAs in male genital organs such as hypospadias and undescended testis.

The rate of twins was somewhat higher in cases with VSD compared to all controls, but was somewhat lower than in the group of malformed controls.

The mean gestational age at delivery was the shortest in the malformed controls, but it was also shorter in cases with VSD than in matched and all controls. These findings were in agreement with the higher rate of PB in the group of cases; this rate was 1.6 and 1.3 fold higher in cases than in their matched and all controls, but 0.85 fold lower than in malformed controls, respectively. There was no significant difference in the rate of post term birth among the study groups. The mean birth weight was 214 and 230 grams smaller in cases compared with matched and all controls, but 14 grams larger than in malformed controls. However, an obvious difference was found in the rate of LBW, because it was 2.8 and 2.9 fold higher in cases with VSD than in

Variables	Cases (N=1,659)		Matched controls (N=2,534)				All controls (N=38,151)				Malformed controls (N=19,396)			
Quantitative	Mean	S.D.	Mean	S.D.	t=	p=	Mean	S.D.	t=	p=	Mean	S.D.	t=	p=
Gestational age (wk)*	**39.1**	2.3	39.5	2.0	203.6	≤ 0.0001	39.4	2.1	625.9	≤0.0001	38.9	2.7	343.1	≤0.0001
Birth weight (g)**	**3,046**	600	3,260	509	5.1	≤ 0.0001	3,276	511	161.1	≤0.0001	3,032	658	0.8	0.4
Categorical	No.	%	No.	%	OR	95% CI	No.	%	OR	95% CI	No.	% I	OR	95% CI
Sex ratio (boy)	803	**48.3**	1,187	46.8	1.1	0.9-1.2	24,799	65.	**0 0.5**	**0.4-0.6**	12,995	67.0	**0.5**	**0.5-0.6**
Twins	29	1.7	33	1.3	1.4	0.8-2.2	410	1.1	**1.6**	**1.1-2.4**	362	1.9	0.9	0.7-1.4
Preterm birth*	204	**12.3**	200	7.9	**1.5**	**1.2-1.9**	3,496	9.2	**1.3**	**1.1-1.5**	2,797	14.4	0.9	0.7-1.0
Postterm birth*	11	0.7	15	0.6	1.1	0.5-2.4	151	0.4	1.7	0.9-3.1	160	0.8	0.8	0.4-1.5
Low birth weight**	274	**16.5**	150	5.9	**3.0**	**2.3-3.8**	2,167	5.7	**3.5**	**3.0-4.2**	3,358	17.3	1.0	0.9-1.1
Large birth weight**	3	**0.2**	21	0.8	**0.2**	**0.1-0.7**	315	0.8	**0.2**	**0.1-0.7**	114	0.6	0.3	0.1-1.0

**Adjusted for sex of cases/controls, in addition to the age, parity (birth order) and employment status of mothers*
*** Adjusted for sex of cases/controls, in addition to the age, parity (birth order), employment status of mothers and gestational age of newborns*
Bold numbers show significant associations

Table 1. Live-birth outcomes of cases with ventricular septal defect (VSD), matched and all controls, in addition malformed controls

Variables	Male cases (N=803)		Matched controls (N=1,187)				All controls (N=24,799)				Malformed controls (N=13,293)			
Quantitative	Mean	S.D.	Mean	S.D.	t=	p=	Mean	S.D.	t=	p=	Mean	S.D.	t=	p=
Gestational age (wk)*	**39.1**	2.4	39.5	2.0	4.03	<.0001	39.4	2.0	4.15	<.0001	38.9	2.6	2.1	.04
Birth weight (g)**	**3,123**	620	3,326	506	8.01	<.0001	3,323	514			3,090	647	3.3	.001
Categorical	No.	%	No.	%	OR	95% CI	No.	%	OR	95% CI	No.	%	OR	95% CI
Preterm birth	90	**11.2**	88	7.4	1.6	1.2-2.2	2,069	8.3	1.4	1.1-1.7	1,756	13.3	0.8	0.7-1.0
Low birth weight	127	**15.9**	54	4.5	3.9	2.8-5.5	1,238	5.0	3.6	2.9-4.4	1,191	15.1	1.9	1.6-2.3
Variables	Female cases (N=858)		Matched controls (N=1,347)				All controls (N=13,352)				Malformed controls (N=6,540)			
Quantitative	Mean	S.D.	Mean	S.D.	t=	p=	Mean	S.D.	t=	p=	Mean	S.D.	t=	p=
Gestational age (wk)*	**39.1**	2.3	39.5	2.0	4.3	<.0001	39.3	2.1	2.7	<.007	38.7	2.8	4.0	<.001
Birth weight (g)**	**2,974**	571	3,203	505	9.9	<.0001	3,187	494	12.12	<.0001	2,909	665	2.7	.006
Categorical	No.	%	No.	%	OR	95% CI	No.	%	OR	95% CI	No.	%	OR	95% CI
Preterm birth	114	**13.3**	112	8.3	1.7	1.3-2.2	1,427	10.7	1.3	1.0-1.6	1,041	16.7	0.8	0.7-1.0
Low birth weight	147	**17.1**	96	7.1	2.7	2.1-3.5	929	7.0	2.8	2.3-3.3	1,367	21.9	0.8	0.7-0.9

**Adjusted for sex of cases/controls, in addition to the age, parity (birth order) and employment status of mothers*
*** Adjusted for sex of cases/controls, in addition to the age, parity (birth order), employment status of mothers and gestational age of newborns*
Bold numbers show significant associations

Table 2. Live-birth outcomes of male and female cases with ventricular septal defect (VSD), matched and all controls, in addition malformed controls

matched and all controls, in addition it was nearly similar (only 0.95 fold lower) to the rate of LBW in malformed controls, respectively. The rate of large birth weight was the lowest in cases with VSD and matched controls.

In the next step, birth outcomes of cases were differentiated according to sex (Table 2). The mean gestational age was similar in males and females; nevertheless, the rate of PB was somewhat higher in female newborns both in case and control groups. The mean birth weight as in general was lower in females, but the rate of LBW was higher both in male and female controls than in matched and all controls. However, it is worth mentioning that this difference was 3.2-3.5 folds larger in male cases than in their matched and all controls while these differences were only 1.2-1.6 fold larger in female cases than in their matched and all controls. In addition the rate of LBW was higher in the group of male cases than in the group of malformed male controls while the rate of LBW in female cases was significantly lower than in malformed female controls. Thus male cases showed worse pattern of birth outcomes particularly LBW indicating their more obvious intrauterine growth restriction.

Study groups:	Cases		Matched controls				All controls				Malformed controls			
Variables/ Full-term	(N=1,444)		(N=2,319)				(N=34,655)				(N=16,435)			
Quantitative	Mean	S.D.	Mean	S.D.	t=	p=	Mean	S.D.	t=	p=	Mean	S.D.	t=	p=
Gestational age (wk)*	39.7	1.4	39.9	1.4	4.3	<.0001	39.8	1.4	2.7	.008	39.7	1.4	0.0	1.000
Birth weight (g)**	**3,146**	533	3,329	444	11.4	<.0001	3,354	445	17.3	<.0001	3,175	536	2.0	0.049
Categorical	No.	%	No.	%	OR	95% CI	No.	%	OR	95% CI	No.	%	OR	95% CI
Sex (boys)	704	**48.8**	1,090	47.0	1.07	0.94-1.22	22,632	65.3	0.50	0.45-0.56	11,301	68.8	0.43	0.39-0.48
Variables/Preterm	(N=204)		(N=200)				(N=3,496)				(N=2,790)			
Quantitative	Mean	S.D.	Mean	S.D.	t=	p=	Mean	S.D.	t=	p=	Mean	S.D.	t=	p=
Gestational age (wk)*	34.4	1.8	34.7	1.7	1.7	0.089	34.8	1.6	1.8	0.077	33.8	2.4	1.9	0.077
Birth weight (g)**	**2,322**	540	2,441	499	2.3	0.022	2,483	436	2.5	0.018	2,168	629	3.3	0.001
Categorical	No.	%	No.	%	OR	95% CI	No.	%	OR	95% CI	No.	%	OR	95% CI
Sex (boys)	90	**44.1**	88	44.0	1.00	0.68-1.49	2,069	59.2	0.54	0.41-0.72	1,751	62.8	0.47	0.35-0.62

**Adjusted for sex of cases/controls, in addition to the age, parity (birth order) and employment status of mothers*
*** Adjusted for sex of cases/controls, in addition to the age, parity (birth order), employment status of mothers and gestational age of newborns*
Bold numbers show significant associations

Table 3. Birth outcomes of full-term and preterm live-born babies in the groups of cases, malformed and all control, in addition malformed controls. (At the statistical analysis the age, parity (birth order) and employment status of mothers were considered as confounders)

Study groups:	Cases		Matched controls				All controls				Malformed controls			
Variables/2500-4000g	(N=1,382)		(N=2,363)				(N=35,669)				(N=15,920)			
Quantitative	Mean	S.D.	Mean	S.D.	t=	p=	Mean	S.D.	t=	p=	Mean	S.D.	t=	p=
Gestational age (wk)*	39.6	1.8	39.7	1.7	1.7	0.089	39.6	1.7	0.0	1.000	39.5	1.8	1.9	0.047
Birth weight (g)**	**3,232**	437	3,323	405	6.44	<.0001	3,354	412	10.8	<.0001	3,246	429	1.2	0.245
Categorical	No.	%	No.	%	OR	95% CI	No.	%	OR	95% CI	No.	%	OR	95% CI
Sex (boys)	672	**48.6**	1,119	47.4	1.14	1.00-1.30	22,300	65.3	0.61	0.55-0.69	11,080	69.6	0.45	0.40-0.50
Variables/LBW	(N=274)		(N=150)				(N=2,167)				(N=3,356)			
Quantitative	Mean	S.D.	Mean	S.D.	t=	p=	Mean	S.D.	t=	p=	Mean	S.D.	t=	p=
Gestational age (wk)*	**36.7**	3.1	36.2	3.4	1.86	0.063	35.6	3.2	10.1	<.0001	35.6	3.7	9.7	<.0001
Birth weight (g)**	**2,090**	308	2,094	358	0.12	0.904	2,106	335	0.8	0.453	1,962	426	4.9	<.0001
Categorical	No.	%	No.	%	OR	95% CI	No.	%	OR	95% CI	No.	%	OR	95% CI
Sex (boys)	127	**46.1**	54	36.0	1.54	1.02-2.32	1,238	57.1	0.65	0.51-0.83	1,989	59.3	0.59	0.46-0.76

**Adjusted for sex of cases/controls, in addition to the age, parity (birth order) and employment status of mothers*
*** Adjusted for sex of cases/controls, in addition to the age, parity (birth order), employment status of mothers and gestational age of newborns*
Bold numbers show significant associations

Table 4. Birth outcomes of average birth weigh (2500-4000 g) and low birth weight (LBW) live-born babies in the groups of cases, malformed and all control, in addition malformed controls. (At the statistical analysis the age, parity (birth order) and employment status of mothers were considered as confounders)

Variables	Case mothers (N=1,661)		Matched control mothers (N=2,534)				All control mothers (N=38,151)				Malformed control mothers (N=19,833)			
Quantitative	No.	%	No.	%			No.	%			No.	%		
Maternal age					X^2_3	p=			X^2_3	p=			X^2_3	p=
–19	155	9.3	202	8.0			3,277	8.6			2,193	11.1		
20 – 29	1,176	70.8	1,853	73.1	3.4	0.18	27,602	72.3	2.1	0.4	13,527	68.2	6.3	≤ 0.05
30–	330	19.9	479	18.9			7,272	19.1			4,113	20.7		
Mean, S.D.	25.6	5.2	25.5	4.9	t=0.7	p=0.51	25.5	4.9	t=0.8	p=0.42	25.4	5.3	t=1.5	p=0.13
Birth order					X^2_2	p=			X^2_2	p=			X^2_2	p=
1	734	44.2	1,181	46.6	2.4	0.13	18,209	47.7	8.0	0.01	9,328	47.0	5.0	≤ 0.05
2 or more	927	55.8	1,353	53.4			19,942	52.3			10,505	53.0		
Mean, S.D.	1.9	1.2	1.8	0.9	t=3.1	p≤ 0.01	1.7	0.9	t=8,7	p≤0.0001	1.9	1.1	t=0.0	p=1.0
Pregnancy order					X^2_2	p=			X^2_2	p=			X^2_2	p=
1	647	39.0	1,064	42.0	3.8	0.06	16,320	42.8	9.5	≤0.003	8,296	41.8	5.2	≤ 0.05
2 or more	1,014	61.0	1,470	58.2			21,831	57.2			11,537	58.2		
Mean, S.D.	2.1	1.4	1.9	1.1	t=5.2	p≤0.001	1.9	1.2	t=6.6	p≤0.0001	2.1	1.3	t=0.0	p=1.0
Categorical	No.	%	No.	%	X^2_1	p=	No.	%	X^2_1	p=	No.	%	X^2_1	p=
Unmarried	94	5.7	88	3.5	11.5	≤ 0.001	1,472	3.9	9.9	≤ 0.01	1,105	5.6	0.10	0.76
Employment status					X^2_6	p=			X^2_6	p=			X^2_6	p=
Professional	145	8.7	289	11.4			4,423	11.6			1,729	8.7		
Managerial	339	20.4	645	25.5			10,265	26.9			4,451	22.4		
Skilled worker	497	29.9	857	33.8			11,908	31.2			5,645	28.5		
Semiskilled worker	294	17.7	391	15.4	77.8	≤ 0.05	6,161	16.1	143.2	≤ 0.05	3,632	18.3	6.1	0.41
Unskilled worker	129	7.8	136	5.4			2,187	5.7			1,549	7.8		
Housewife	184	11.1	156	6.2			2,354	6.2			2,062	10.4		
Others	73	4.4	60	2.4			853	2.2			765	3.9		

Table 5. Main variables of mothers of cases with VSD, matched and all controls, in addition of malformed control mothers

Table 3 shows the data of full-term and preterm cases with VSD and their controls. Female predominance was stronger in preterm babies with VSD but the growing proportion of females was characteristic for controls as well. The mean gestational age was only 0.1-0.2 week shorter in full-term babies than in their matched and all controls, in addition was same with the figure of malformed controls. The difference of mean gestational age was more obvious between preterm cases and preterm controls, it was 0.3-0.4 week shorter than in matched and all controls but 0.6 week longer than in preterm malformed controls. The mean birth weight was lower in full-term cases than in matched (183g), all (208g) and malformed (29g) controls. This difference in mean birth weight was smaller between preterm cases and matched (119g) and all (161g) controls, in addition it was 157 g larger than in malformed controls. Thus intrauterine growth restriction was stronger in full-term cases with VSD.

The above trend was confirmed at the comparison of cases with average (2500-4499g) and LBW (Table 4). Their sex difference was limited (2.2%). There was no real difference in mean gestational age of cases and controls with average birth weight, but the mean birth weight of cases was lower by 95, 106 and 16g smaller than in matched, all and malformed controls. This difference was smaller between cases with LBW and matched (4g) and all controls (16g), but it was 128g larger than in malformed controls. Thus intrauterine growth restriction was stronger in cases with average birth.

Among maternal variables (Table 5), the mean maternal age was only 0.1 year higher in the mothers of cases than in the mothers of controls. However, the mean birth order (parity) and pregnancy order (live- and stillbirth, and miscarriages) was higher in the mothers of cases and malformed controls than in the mothers of matched and particularly all controls. However, the difference between mean birth and pregnancy order

Variables	Mothers of full-term cases (N=1,444)		Mothers of preterm cases (N=204)				Mothers of cases with average birth weight (N=1,382)		Mothers of cases with low birth weight (N=274)			
Quantitative	No.	%	No.	%			No.	%	No.	%		
Maternal age					X^2_3	p=					X^2_3	p=
–19	121	8.4	33	16.2			121	8.7	34	12.4		
20 – 29	1,052	72.8	118	57.8	22.0	<.0001	999	72.3	174	63.5	8.8	.012
30–	271	18.8	53	26.0			262	19.0	66	24.1		
Mean, S.D.	25.6	5.0	25.7	6.3	t=0.26	>.05	25.6	5.1	25.8	5.9	t=0.29	>.05
Birth order					X^2_2	p=					X^2_2	p=
1	639	44.3	89	43.6	0.03	>.05	611	44.2	122	44.5	0.01	>.05
2 or more	805	55.7	115	56.4			771	55.8	152	55.5		
Mean, S.D.	1.9	1.1	2.1	1.6	t=1.31	>.05	1.9	1.1	2.1	1.5	t=1.50	>.05
Pregnancy order					X^2_2	p=					X^2_2	p=
1	566	39.2	76	42.0	0.28	>.05	536	38.8	110	40.1	0.18	>.05
2 or more	878	60.8	128	58.2			846	61.2	164	59.9		
Mean, S.D.	2.1	1.3	2.5	2.1	t= 3.76	≤.0002	2.1	1.3	2.4	1.9	t=3.2	<.0014
Categorical	No.	%	No.	%	X^2_1	p=	No.	%	No.	%	X^2_1	p=
Unmarried	79	5.5	15	7.4	1.2	>.05	79	5.7	15	5.5	0.03	>.05
Employment status					X^2_7	p=					X^2_6	p=
Professional	132	9.1	13	6.4			136	9.8	8	2.9		
Managerial	304	21.1	32	15.7			291	21.1	47	17.2		
Skilled worker	445	30.8	47	23.0			420	30.4	77	28.1		
Semiskilled worker	250	17.3	44	21.6	25.3	<.001	236	17.1	57	20.8	29.3	<.0002
Unskilled worker	107	7.4	21	10.3			98	7.1	31	11.3		
Housewife	143	9.9	39	19.1			140	10.1	44	16.1		
Others	63	4.4	8	3.9			61	4.4	10	3.6		

Table 6. Main variables of mothers of cases with VSD, matched and all controls, in addition of malformed control mothers

was similar (0.2) in case mothers and malformed control mothers.

The rate of unmarried mothers was higher in the groups of cases and malformed controls than in matched and all controls. The employment status showed a lower socioeconomic status in the mothers of cases with VSD (58.0%) and malformed controls (59.6%) due the lower proportion of professional, managerial and skilled worker mothers than in the mothers of matched (70.7%) and all (69.7%) controls. Particularly the high rate of housewives is noteworthy in the mothers of cases because in general housewives belong to the lowest socioeconomic families in Hungary.

There was no difference in maternal variables between male and female cases with VSD. However, maternal variables are presented at the comparison of cases with full-term/PB and average/LBW (Table 6). There was a U-shaped distribution of maternal age-groups, i.e. higher proportion of younger and elder mothers in both PB and LBW. The mean birth order was the same in cases with PB and LBW and somewhat higher (0.2) than in cases with full-term and average birth weight. The difference of pregnancy order was larger between cases with PB and full-term babies (0.4) and between cases with LBW and average birth weight newborns (0.3), thus these data indicate a somewhat higher rate of miscarriage in previous pregnancies of mothers of cases particularly with PB. The proportion of the so-called low maternal employments: semi- and unskilled workers and housewives was higher both in the mothers of cases with PB (51.0% vs. 34.6%) and LBW (48.2% vs. 34.3%), these data indicated their lower socio-economic status. Particularly the high proportion of housewives is worth mentioning.

Of 220 mothers of cases visited at home, 68 (30.9%) had smoker mothers while this figure was 19.0% in 800 control mothers (OR with 95% CI: 1.9, 1.4-2.7). The distribution of 1-10, 11-20 and 21 or more cigarettes per day was 43 (19.5% vs. 13.0%), 23 (10.5% vs. 5.0%) and 2 (0.9% vs. 1.0%) in case mothers (the percentage figures of 800 controls visited at home are also shown in brackets as vs.). The number of occasional, regular and hard drinkers was 45 (20.5%), 12 (5.8%) and 8 (3.9%), while these figures were 152 (19.0%), 8 (1.0%) and 0 (0.0%) in 800 control mothers. Thus the number of regular and hard drinkers together was 20 (9.6%) in the mothers of cases with VSD while this figure was 8 (1.0%) in the mothers of 800 controls visited at home (OR with 95% CI: 9.9, 4.3-22.8). Of 20 mothers with regular or hard drinkers, 18 were smokers. Thus, the proportion of smokers and drinkers was 1.9 fold and 9.9 fold higher in case mothers than in control mothers, respectively. The rate of smokers in the mothers of cases with VSD was also higher than in the mothers of 2,640 malformed controls (22.2 %) visited at home (OR with 95% CI: 1.6, 1.2-2.1) but this difference was more obvious in regular/

Pregnancy complications	Case mothers (N=1,661)		Matched control mothers (N=2,534)				All control mothers (N=38,151)				Malformed control mothers (N=19,833)			
	No.	%	No.	%	OR	95% CI*	No.	%	OR	95% CI*	No.	%	OR	95% CI*
Threatened abortion	240	14.4	399	15.7	0.9	0.8-1.1	6,510	17.1	**0.8**	**0.7-0.9**	3,006	15,2	1.0	0.8-1.1
Nausea-vomiting, severe	141	8.5	303	12.0	**0.7**	**0.6-0.8**	3,856	10.1	**0.8**	**0.7-0.9**	1,501	7.6	1.1	1.0-1.4
Pre-eclampsia/eclampsia	46	2.8	79	3.1	0.9	0.6-1.3	1,156	3.0	0.9	0.7-1.2	584	2.9	0.9	0.7-1.3
Placental disorders**	21	1.3	47	1.9	0.7	0.4-1.1	593	1.6	0.8	0.5-1.3	253	1.3	1.0	0.6-1.6
Poly/oligohydramios	10	0.6	22	0.9	0,7	0.3-1.5	205	0.5	1.1	0.6-2.1	174	0.9	0.7	0.4-1.3
Anemia	201	12.1	352	13.9	0.9	0.7-1.0	6,358	16.7	**0.7**	**0.6-0.8**	2,849	14.4	0.8	0.7-1.0
Pregnancy related renal diseases	19	1.1	28	1.1	1.0	0.6-1.9	492	1.3	0.9	0.6-1.4	299	1.5	0.8	0.5-1.2
Gestational hypertension	51	3.1	74	2.9	1.1	0.7-1.5	1,100	2.9	1.1	0.8-1.4	494	2.5	1.2	0.9-1.7
Gestational diabetes	18	1.1	14	0.6	**1.8,**	**1.0-4.8**	229	0.6	**1.7**	**1.1-3.4**	100	0.5	**2.2**	**1.6-3.4**
Oedema with escessive weight gain without hypertension	34	2.0	56	2.2	0.9	0.6-1.4	912	2.4	0.9	0.6-1.2	373	1.9	1.1	0.8-1.6
Threatened preterm delivery***	199	12.0	362	14.3	0.8	0.7-1.0	5,447	14.3	0.8	0.7-1.0	2,264	11.4	1.1	0.9-1.2

*Adjusted for sex of cases/controls, in addition to the age, parity (birth order) and employment status of mothers
** including placenta previa, premature separation of placenta, antepartum hemorrhage
***including cervical incompetence as well
Bold numbers show significant associations

Table 7. Occurrence of medically recorded pregnancy complications in the mothers of cases with VSD, matched and all controls, in addition malformed controls

hard drinker mothers (9.6% vs. 1.9%, OR with 95% CI: 5.0, 1.7-8.8).

The birth outcomes of newborn infants in the subgroups of cases and all controls born to mothers visited at home were evaluated according to smoking during the study pregnancy. Mean gestational age (39.1 wk) was similar in 68 case mothers with smoking and 152 case mother without smoking, however, mean birth weight was lower in the newborns of smoker case mothers (2,960 g) than in the newborns of non-smoker case mothers (3,066). The mean gestational age was 39.3 wk and 39.4 wk in the newborns of 152 control mothers who smoked during the study pregnancy and 648 control mothers who did not smoke, respectively. However, there was difference in the mean birth weight in the newborns of smoker (3,110g) and non-smoker (3302g) control mothers.

Among pregnancy complications (Table 7), the incidence of gestational diabetes was higher in the mothers of cases than in the mothers of controls. The lower incidence of severe treated nausea and vomiting in pregnancy is also worth mentioning in the mothers of cases than in the mothers of matched and all controls groups. However, the rate of this pregnancy complication was somewhat higher in the mothers of cases than in the mothers of malformed cases. The rate of threatened abortion, pre-eclampsia and anemia (iron deficient) was the lowest in the mothers of cases, though this difference did not reach the level of significance at the comparison of mothers of cases and their matched controls.

4. Discussion

As far as we know this study is the first attempt to evaluate a homogeneous group of single VSD with well-controlled diagnosis based on surgical description or autopsy record. The main findings of the study were a mild female excess, a shorter (0.3 wk) mean gestational age at delivery, smaller (214 g) mean birth weight, in addition a higher rate (1.6 fold) of PB and mainly a higher rate (2.8 fold) of LBW in the cases with VSD than in their matched controls without any CA. The detailed analysis of birth outcomes indicated some intrauterine growth restriction of fetuses affected with VSD, particularly in males and full-term and or average birth weight babies. The mothers of cases with VSD had lower socioeconomic status, particularly in the subgroups of cases with PB and LBW. In addition there was a higher rate of smoking and particularly drinking habits, in addition gestational diabetes in the mothers of cases with VSD.

The somewhat higher proportion of females among cases with VSD confirmed the experiences of previous studies [14-17].

The rate of twins was also slightly higher in cases compared to the rate of all controls. The occurrence of twins was greater among infants with membranous type of VSD in the Washington-Baltimore Infant Study [17]. Previously a higher risk of twins and CCVAs particularly septal defects were found in the offspring of pregnant women with assisted reproductive technology [29,30], however, our cases had no mothers with the use of this method for the treatment of sterility.

The shorter gestational age at delivery in cases with VSD associated with a higher rate of PB births compared to the data of matched and all controls. The lower mean birth weight can be explained only partly by the shorter gestational age, however, the rate of LBW showed a more obvious increase in cases compared to matched and all controls. The mean birth weight was 200-300 g lighter in cases with VSD in previous studies [14-17], but this decrease depended on the size of defect in the study of the Washington-Baltimore Infant Study [17]. Thus an intrauterine fetal growth restriction in fetuses with VSD was found in other studies as well. However, our study is the first showing a more obvious intrauterine growth restriction in male fetuses than in female fetuses and mainly in full-term or average weighted babies.

The age of mothers did not show characteristic difference between the group of cases with VSD and controls, similarly to the findings of the Baltimore-Washington Infant Study [17]. However, a maternal age of 35 years or older associated with a somewhat higher risk (1.20, 1.06-1.36) of VSD in an urban area of the United States [31]. The mothers of cases with VSD were more likely to be unmarried and had somewhat lower employment status, these findings were also in agreement with the results of the Baltimore-Washington Infant Study [17]. Our study showed particularly the importance of low socio-economic status of mothers of cases with PB and LBW. However, there was no difference in the marital and employment status between case mothers and malformed control mothers, thus these findings seems to be characteristics for the mothers of cases with CA in general.

Our data confirmed the association of drinking habit during pregnancy with higher risk of VSD [32,33,17]. The cross interview of pregnant women and their close relatives regarding drinking of women during the study pregnancy is much more reliable than the unreliable self-reported maternal information in other studies. In addition a weaker association was found between maternal

smoking habit and a higher risk of VSD in their children in our study. Similar finding was published regarding all VSD [34], but not in the Baltimore-Washington Infant Study [17].

Among pregnancy complications only gestational diabetes occurred more frequently in the mothers of cases than in the mothers of different controls. Previously a higher risk of VSD was found in the children of diabetic pregnant women [35-37,17]. In addition a lower occurrence of severe nausea and vomiting during the study pregnancy of case mothers and malformed controls was observed compared to the mothers of matched and all controls. Our previous study showed some CA-preventive effect of severe nausea and vomiting in pregnancy [38] and now this finding was confirmed in the origin of VSD as well.

The question is why VSD is manifesting more frequently in newborns with PB and particularly LBW. Three options are worth discussing. (i) It is difficult to believe that this localized CA in the small region of ventricular septum itself can induce intrauterine fetal growth restriction. (ii) Possible confounding factors have to consider as well, e.g. poor socioeconomic status associates with lower birth weight. However, this confounder was considered at the calculation of adjusted risk figures. Obviously drinking and smoking habits are also important, and the adverse effect of smoking for birth weight was also shown in our study, but these adverse lifestyle factors cannot explain totally the fetal growth restriction of fetuses affected with VSD. Similar but milder intrauterine fetal growth restriction was observed in the fetuses with VSD of non-drinking and non-smoking mothers as well. Maternal diseases and related drug treatments are analyzed in another study [20] without any reasonable association with higher risk of intrauterine growth restriction, (iii) The VSD and intrauterine growth restriction as two developmental errors may have a common route, mainly in males and full-term or average weighted babies. Recently the association of some gene polymorphisms with fetal growth and birth weight was shown [39], thus it would be interesting to test the effect of these gene variants in the origin of VSD.

The strengths of our study are connected with the large population-based data set of the HCCSCA including 1,659 cases with single VSD after surgical correction, 2,534 matched and 38,151 all controls without CA and 19,393 malformed controls with other isolated CA in the ethnically homogeneous Hungarian (Caucasian) population. The diagnosis of VSD was controlled due to the follow-up of our cases in cardiologic institutes and by the help of their mothers. We did our best to work cases with VSD as homogeneous as possible, therefore syndromic, unclassified multiple and complex VSD cases, in addition VSD with spontaneous closure were excluded from the study, and only cases with membranous and muscular single VSD after surgical correction were evaluated. The analysis of birth outcomes and pregnancy complications was based on medically recorded data.

However, there were some weaknesses of our study. Our sample included only severe VSD with lethal outcome or surgical intervention, thus cannot represent the whole spectrum of VSD. On the other hand the data of smoking and drinking of alcohol beverages were available only in mothers visited at home due to the unreliable retrospective maternal information [25]. First the smoking habit was evaluated according to maternal information during the study pregnancy after the birth of 809 children with orofacial clefts. The distribution of cigarettes per day, i.e. 1-10, 11-20 and 21 or more was 13.8, 4.3, 3.1 %, thus totally 21.2% in these mothers while these figures were 11.0, 5.2, 2.7%, i.e. totally 18.9% in their 809 matched controls, respectively. However, when these mothers were visited at home and their family members were also asked by independent cross interviews on the smoking habit of pregnant women studied, the final family consensus data showed a different pattern. The previous distribution of quantitative smoking habit was 19.8, 9.9, 6.2%, respectively, i.e. totally 35.9% in the mothers of cases with orofacial clefts and 11.1, 6.2, 2.5, respectively, i.e. totally 19.8% in the mothers of matched controls without any CA. Similar results were found regarding drinking habit but this lifestyle factor was denied by the mothers of both cases and controls. Thus the mothers particularly of cases with CA might have a guilty feeling; therefore they did not want to confess their smoking and drinking habit. This information bias can modify the adjusted risk for CAs in a case-control study. Thus our dilemma was to evaluate unreliable data or delete these data from our analysis. The second option was accepted with our hope that the family consensus data after the home visit in a subsample of these families may reduce the magnitude of this problem.

In conclusion, our findings showed a small female excess in cases with VSD, a higher rate of PB and mainly LBW indicating some intrauterine growth restriction in fetuses affected with VSD, particularly in males and full-term or average weighted babies. There was a lower socioeconomic status of mothers of cases with VSD and it's associated with a higher rate of smoking and particularly drinking habit, in addition the incidence of gestational diabetes was higher in their mothers.

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