article

Malformations reported in chorionic villus sampling exposed children: A review and analytic synthesis of the literature

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Purpose: To determine whether the frequency of vascular disruption defects, other than limb defects, is increased in reports of chorionic villus sampling (CVS) exposed children compared with an unexposed population. **Methods:** Only studies that reported the total number of CVS-exposed pregnancies and details of pregnancy outcome, including all the malformations, were included. Twenty-five articles met these criteria. **Results:** The frequencies of gastroschisis, intestinal atresias, and clubfoot were significantly increased among the CVS-exposed infants as compared with the baseline unexposed population. The frequencies of other vascular disruption defects, including Poland sequence, amniotic band sequence, and cleft lip/cleft palate, were not increased. **Conclusion:** CVS–exposed children have an increased frequency of intestinal atresia, gastroschisis, and clubfoot compared with the nonexposed population. The fact that an increased frequency of other defects attributed to vascular disruption was not found may be due to under–ascertainment, misclassification, or "lumping" of the defects identified in previous studies. ***Genetics in Medicine*, 1999:1(7):315–322.**

Key Words: *Chorionic villus sampling, vascular disruption, gastroschisis, intestinal atresia*

Several studies have suggested that the limb defects reported in chorionic villus sampling (CVS) exposed children may result from vascular disruption.[1,2] Support for this proposed mechanism comes from vascular anomalies, such as aberrant vessels,[3] and evidence of emboli in the fetal vasculature or placenta,[4] reported in similar defects in nonexposed children. Other malformations hypothesized to be secondary to vascular disruption include: gastroschisis,[5] intestinal atresias,[6] hemifacial microsomia,[7] Poland sequence,[8] Klippel-Feil anomaly,[8] Moebius sequence and oromandibular-limb hypogenesis (OMLH) sequence,[8,9] horse-shoe kidneys, unilateral renal agenesis, unilateral urethral obstruction sequence,[10] porencephaly,[11] and talipes equinovarus.[10] Many of these defects in monozygotic twins have been reported to have had vascular etiologies.[12] Intestinal atresias have been associated with two exposures thought to cause vascular disruption, maternal cocaine abuse[13] and intra-amniotic injections of methylene blue.[14] Similarly it is hypothesized that premature ablation or occlusion of the omphalomesenteric artery results in gastroschisis.[5]

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If the basis of the limb defects reported in CVS-exposed children is vascular in origin, one would expect to see a similar increase in other defects caused by the same mechanism. We have reviewed the published literature on CVS-associated malformations to determine whether there is an increased frequency of defects associated with vascular disruption in comparison with the frequency in an unexposed population of newborn infants.

MATERIALS AND METHODS

We sought to identify all articles published in the English literature from 1987 to 1997 on CVS in which malformations were reported. These articles were identified from multiple sources: databases, reference lists, review of abstracts, and recommendations of investigators in the field. The databases searched were MEDLINE, EMBASE, Biosis Serial Sources, Conference Papers Index, Inside Conferences, Dissertation Abstracts Online, Current Contents/Life Sciences, and the Cochrane Library. The search was conducted using the keyword/phrase 'chorionic villus sampling' or 'chorionic villus biopsy' along with other spellings (chorionic villous sampling, chorionic villi sampling, chorion villus sampling, chorion villous sampling, chorion villi sampling, and CVS). The references listed at the end of the articles that were obtained were reviewed for additional references. All of the abstracts from meetings published in the American Journal of Human Genetics, American Journal of Obstetrics and Gynecology, and Teratology were reviewed for additional citations. The list of the

compiled articles was reviewed by members of the CVS Birth Defects Registry Advisory Committee and participants of the 1992 NICHD Workshop on CVS and Limb and Other Defects[15] who added additional references.

In all, 92 published articles were identified and analyzed for description of any malformations identified in CVS-exposed fetuses (both liveborns and abortuses). Table 1 describes the range of birth defects reported.

To determine the frequency of specific defects among CVS-exposed infants, the subset of articles providing sufficient information to permit calculation of rates were reviewed. Articles were included in this analysis only if the following were present:

1. The total number of women who had the CVS procedure in the series;
2. descriptions of nonlimb malformations; and
3. documentation of pregnancy outcome (abortion, stillbirth, and liveborn).

Articles were excluded for the following reasons:

1. Case reports;
2. case-control studies;
3. reviews;
4. did not report the total number of women who underwent CVS in their population;
5. descriptions of the malformations were inadequate (i.e., listed solely the total number of malformations or type of malformation, such as gastrointestinal malformation without the specific details); and
6. registry data in which the data collection relied on voluntary reporting of the malformations (as under-reporting of malformations is a problem with such methods of data collection).

If multiple articles included the same cases, only the most recent article was used in the compilation. For example, for articles written by the same group of authors, the time frame during which the cases were included was examined carefully to make sure that there was no overlap.

Twenty-five publications from 1987-1997 met the inclusion criteria.[16-40] All of these studies included more than 100 patients who underwent CVS. Malformations due to single gene defects, such as achondroplasia, Apert syndrome, short rib polydactyly syndrome, and those due to chromosome defects (such as trisomies, duplications, and deletions), and recognized malformation syndromes (other than vascular disruption syndromes) were excluded in calculating the rates of malformations. Information on defects seen in abortuses were included, if a detailed description of the defects in the abortuses was provided. In many articles, the details about the cases that were terminated due to a fetal defect were not given, only noting that the fetal defect was present. Only eight papers[17,19,21,23,25,32,37,39] detailed the structural malformations in the terminations with some being confirmed by autopsy, others only by prenatal ultrasonography, all of which were included in our calculations. Most of these cases included major defects such as neural tube defects, renal agenesis, and body stalk anomaly.

The transcervical method only was used in eight studies,[16,17,22,23,28,29,31,39] the transabdominal method only in eight studies,[18,19,21,24,30,32,33,36] whereas both methods were used in six studies.[20,25,34,37,38,40] Three studies did not report the method employed.[26,27,35] The timing of the CVS across studies varied widely. Seven studies included procedures performed before 9 weeks.[18,19,22,25,27,30,38] Four studies did not report the timing of the CVS procedures.[26,28,34,35]

The baseline data for the prevalence of each defect in non-exposed infants and fetuses were derived from two sources:[1] the Brigham and Women's Hospital (BWH) Active Malformation Surveillance Program and[2] published prevalence data.[41-42] The BWH Active Malformation Surveillance Program, which is on-going, has collected data from 161,252 livebirths and abortuses at this hospital for the years 1972-1974 and 1979-1994, the sample used for the comparison. Index cases are identified from a daily review of the medical records of all newborn infants and other reports, such as autopsies and diagnostic studies.[43] Interviews with the mothers and the review of the mother's medical record confirmed that she did not undergo CVS during that pregnancy. Infants whose mothers had planned to deliver elsewhere, but were transferred to the BWH after the prenatal detection of a fetal abnormality or because of a complication of pregnancy, were excluded from the analysis. In addition, infants whose malformations were part of a recognized malformation syndrome (other than vascular disruption syndromes), genetic disorder, associated with a chromosome abnormality or were associated with a recognized teratogen were excluded. Published prevalence data were used for all of the defects other than the terminal transverse limb defects with nubbins. Published prevalence data for limb defects did not separate out those terminal transverse limb defects with nubbins.

The frequency of the defects in the CVS-exposed population attributed to vascular disruption was calculated by dividing the number of cases with the defect in question by the total number of CVS-exposed babies in all of the studies. The frequency of several defects not thought to be secondary to vascular disruption were also calculated for comparison.

Analysis was performed using a Poisson random effects model, as described by Brumback et al.[44] This model incorporates study to study variability by assuming that defect rates among unexposed infants vary randomly according to a gamma distribution. *P* values and confidence limits associated with the risk-ratio corresponding to CVS exposure can be computed using standard likelihood-based methods after integrating over the distribution of the random effects. We applied the method using parameter values that yielded prior means corresponding to the values observed in the historical series of 161,252 infants described earlier. Analyses were repeated under varying assumptions about the degree of study-to-study variability. The standard deviation was varied from being only one-quarter of the mean up to two times the mean. This ensured that the assumed prior distribution corresponded to the ranges of rates reported in the literature. For ex-

Table 1
Spectrum of reported malformations in CVS-exposed infants

Category	Specific malformation	No. of reported cases
1. Cutaneous	Hemangioma	34
	Port wine stain	1
	Scalp defect	1
2. Craniofacial	Microcephaly	1
	Craniosynostosis	3
	Facial clefts	26
	Auricular abnormalities	16
3. Central Nervous System	Hydrocephalus	13
	Agenesis of corpus callosum	3
	Neural tube defect	8
	Strabismus	2
4. Cardiac	Ventricular septal defect	18
	Atrial septal defect	3
	Tetralogy of Fallot	1
	Aortic stenosis	1
	Coarctation of aorta	1
	Total anomalous pulmonary venous return	2
	Unspecified	25
5. Abdominal/Intestinal	Diaphragmatic hernia	4
	Gastroschisis	6
	Omphalocele	5
	Hirschsprung disease	3
	Intestinal Atresia	10
	Imperforate Anus	1
	Pyloric stenosis	5
6. Genitourinary/Renal	Renal agenesis	4
	Hypospadias	16
	Obstruction	8
	Hydrocele	8
	Cryptorchid	1
	Inguinal hernia	5
7. Extremities	Amniotic band sequence	1
	Poland sequence	0
	Oromandibular hypogenesis	3
	Clubfoot	33
	Transverse digital deficiency with nubbins	5
	Transverse digital deficiency	24
	Transverse proximal deficiency	7
	Syndactyly	15
	Hip dislocation/defect	23
8. Other	Thyroid aplasia	1
	Limb body wall defect	1

See references 16–40.

ample, intestinal atresia was observed at a rate of 0.062 per 1000 in our control series of 161,252 infants, whereas the rate reported in the literature is approximately 0.2 per 1000. We, thus, chose a gamma prior with parameters 0.25 and 4.17, which yielded a range of defect rates from 0.002 to 0.413 per 1000. The 95% confidence interval on the log risk ratio associated with CVS exposure was (1.1,3.45) with associated p value of 0.001 (Table 2).

RESULTS

Information was available for 25,104 CVS-exposed pregnancies from the 25 studies.[16-40] The frequencies of the following defects, thought to be secondary to vascular disruption, were calculated: cleft lip and/or cleft palate, club feet, intestinal atresia, gastroschisis, Poland anomaly, and amniotic band syndrome. For comparison, the frequency of two defects not at-

Table 2
Analysis of the frequency of the malformations using the poisson random effects model

Outcome	Mean rate/1000 (95% range) among the unexposed (using BWH data)*	Estimated log risk ratio and 95% confidence intervals associated with CVS exposure	p value (one–sided)	Literature mean rate/ 1000 and range)
Terminal transverse limb defects with nubbins	0.056 (0.001, 0.206)	1.58 (0.5, 2.45)	0.009	**
	0.056 (0.015, 0.12)	1.36 (0.4, 2.15)	0.015	
	0.056 (0.032, 0.087)	1.30 (0.3, 2.05)	0.017	
OMLH	0.006 (0, 0.023)	2.61 (0.8, 3.85)	0.013	<0.05
	0.006 (0.002, 0.014)	2.57 (0.8, 3.65)	0.020	
	0.006 (0.004, 0.010)	2.56 (0.8, 3.65)	0.020	
Gastroschisis	0.074 (0.002, 2.84)	1.32 (0.3, 2.15)	0.015	0.09
	0.074 (0.02, 0.162)	1.22 (0.3, 1.9)	0.016	
	0.074 (0.043, 0.115)	1.19 (0.3, 1.9)	0.016	
Intestinal Atresia	0.062 (0.002, 0.231)	1.98 (1.2, 2.75)	<0.001	0.2
	0.062 (0.017, 0.137)	1.89 (1.2, 2.45)	<0.001	
	0.062 (0.035, 0.096)	1.87 (1.2, 2.45)	<0.001	
Club foot	0.657 (0.017, 2.459)	0.94 (0.4, 1.5)	0.002	1 (0.64, 1.24)
	0.657 (0.179, 1.442)	0.801 (0.4, 1.2)	0.001	
	0.657 (0.382, 1.031)	0.773 (0.3, 1.1)	0.001	
Amniotic bands	0.093 (0.002, 0.343)	−0.91 (−3, 1.4)	NS***	0.76
	0.093 (0.025, 0.204)	−0.85 (−3, 1.3)		
	0.093 (0.053, 0.144)	−0.84 (−3, 1.3)		
Poland anomaly	0.031 (0.001, 0.114)	−0.99 (−3, 1.7)	NS***	0.03 (0.018, 0.15)
	0.031 (0.008, 0.068)	−0.99 (−3, 1.6)		
	0.031 (0.018, 0.048)	−0.99 (−3, 1.6)		
Diaphragmatic hernia	0.062 (0.002, 0.229)	0.54 (−0.9, 1.6)	NS***	0.18 (0.16, 0.21)
	0.062 (0.017, 0.136)	0.62 (−0.7, 1.6)		
	0.062 (0.035, 0.096)	0.36 (−1.3, 1.8)		
Cleft lip/cleft palate	0.657 (0.017, 2.425)	1.00 (−0.5, 1.2)	NS***	1
	0.657 (0.179, 1.441)	1.00 (−0.4, 1.1)		
	0.657 (0.376, 1.02)	0.29 (−0.6, 1.3)		
Neural tube defect	0.943 (0.024, 3.477)	−0.96 (−1.8, −0.2)	NS***	1
	0.943 (0.257, 2.06)	−0.99 (−1.8, −0.4)		
	0.943 (0.539, 1.458)	−0.99 (−1.8, −0.5)		

*Analysis repeated with variable ranges assumed for the defect rate among unexposed.
**No published prevalence data available.
***Not of statistical significance (one-sided $p > 0.05$).

tributable to vascular disruption, diaphragmatic hernia and neural tube defects, was also calculated. These two were selected because they were reported commonly in the studies. The frequencies of terminal transverse limb deficiencies and oromandibular hypogenesis (OMLH), two types of limb defects reported to be associated with CVS, were also calculated. The frequencies of unilateral urethral obstruction sequence and renal agenesis could not be calculated accurately, because most cases did not distinguish between bilateral and unilateral cases. The frequencies of the CVS-associated malformations were compared with the frequency of the same malformations identified by the surveillance program at BWH in 161,252 nontransferred mothers and to published prevalence data (Tables 2 and 3). Our analyses revealed significant ($p < 0.05$) associations between CVS and the occurrence of terminal transverse limb defects with nubbins, OMLH, gastroschisis, intestinal atresia, and club foot. No association was found with the presence of amniotic bands, Poland anomaly, diaphragmatic hernia, cleft lip/cleft palate, or neural tube defect.

Table 2 summarizes the analyses for the 10 different types of birth outcomes considered. The second column in the table shows the assumed mean rate per 1000 unexposed births used in our Poisson random effects model. Also shown is the range of values corresponding to the central 95% of the random effects distribution. For the purpose of a sensitivity analyses, each analysis was repeated three times using the same mean, but changing the assumed variability among unexposed births. For example, the first row (nubbins) reports an analysis based on an assumed prior mean of 0.056 per 1000, but with three possible ranges. The first range (0.001, 0.206) is the one that allows the most variability and has the widest confidence interval for the true risk ratio associated with CVS exposure (Column 3 of Table 2). The fourth column of the table shows the p value associated with the null hypothesis of no effect of CVS exposure. For the first 5 outcomes in Table 2, these p values remain significant even when the prior distribution allows substantial variability in control rates. For the last five outcomes, the p values all remain substantially > 0.05, and the confidence intervals all contain 0, regardless of the prior distribution assumed. The last column of the table shows the mean and ranges of control defect rates reported in the literature. In some cases (e.g., nubbins), no data are available from the literature. For outcomes, where such data are available, more confidence should be placed on prior distributions whose 95% ranges include those values.

More malformations were reported in the studies in which only the transabdominal method was used as compared with the transcervical method (Table 4). However, the number of total malformations reported was less than would be expected regardless of the method used (1% in the transcervical method studies, 1.6% in the transabdominal method studies, and 1% in studies using either method versus an expected baseline 2-3%).[43,45] This may be a consequence of under-reporting of all the malformations.

Several studies reported on the timing of the CVS and the malformations seen (Table 5). No clear pattern between the malformations and the timing was noted.

DISCUSSION

The frequencies of gastroschisis, intestinal atresia, clubfoot, terminal transverse limb defects, and OMLH, defects postulated to be due to vascular disruption, were increased among the CVS-exposed infants in the published reports. However, other defects associated with vascular disruption, such as Poland syndrome, amniotic band syndrome, and cleft lip/cleft palate, were not increased. In addition, not all of the defects postulated to be due to vascular disruption were reported in

Table 3
Frequency of malformations

Malformation	CVS-exposed frequency (rate/1000) (n = 25,104)*	Published prevalence data (41,42) (rate/1000)	BWH** frequency (rate/1000) (n = 161,252)
Terminal transverse		***	
Limb defects with nubbins	0.20		0.056
Amniotic bands	0.04	0.76	0.093
Poland anomaly	0.0	0.03	0.031
OMLH	0.12	0.05	0.006
Gastroschisis	0.24	0.16	0.074
Intestinal atresia	0.40	0.2	0.062
Clubfoot	1.3	1	0.657
Cleft lip/cleft palate	1.0	1	0.657
Diaphragmatic hernia	0.16	0.18	0.062
Neural tube defect	0.32	1	0.943

*Reported in the 25 articles analyzed.
**Active Malformation Surveillance Program, Brigham and Women's Hospital, 1972–1974, 1979–1994.
***No prevalence data available that separates the transverse limb defects with nubbins from the rest of the transverse limb defects.

Table 4
Route of CVS and reported malformations

Malformation	Studies using transcervical method only* No. CVS performed n = 3391 Rate/1000	Studies using transabdominal method only** No. CVS performed n = 4402 Rate/1000
Total No. malformations reported	11.2	16.1
Neural tube defects	0.59	0
Hydrocephalus	0.30	0.91
Omphalocele	0.59	0.23
Gastroschisis	0.30	0.68
Intestinal atresia	1.2	0.23
Imperforate anus	0.30	0
OMLH	0	0.23
Clubfoot	1.5	2.0
Facial clefts	1.2	1.6
Oligodactyly	0	1.6
Transverse digital deficiency	0	0.68
Transverse proximal deficiency	0	0.91
Renal malformations/obstruction	0.30	0.91
Hydrocele	0.59	1.13
Cryptorchid	0	0.23
Hypospadias	1.5	0.23
Hip dislocation	0.59	1.13
Congenital heart disease	1.5	3.86

*Only studies in which only the transcervical method was used (n = 8).
**Only studies in which only the transabdominal method was used (n = 8).

these papers. For example, no cases of hemifacial microsomia, horse-shoe kidneys, porencephaly, or Klippel-Feil anomaly were reported in this series of reviewed articles.

There could be several reasons for these findings. First, there may have been under-reporting of defects in the reports. In fact, when the number of malformations reported was analyzed by the method used (Table 4) the malformation rate was lower than would have been expected. Furthermore, not all of the descriptions in the reports were as detailed as in others. In some of the reports, for example, the emphasis was on limb defects, and the other malformations identified were not described completely.[29] This would result in an under-estimate of the defects in the CVS-exposed population. Second, in some studies, a number of the defects were lumped into one category,[35] such as facial clefts and did not distinguish between cleft palate alone and cleft lip ± cleft palate, which may differ in their etiology. In our tabulation, we counted these as unspecified facial clefts. Third, malformations found in terminated fetuses were not included in every report. This would result in an under-estimation of the defects in the exposed population. Fourth, there was some variability in classification of the defects in the various studies. For example, amniotic band syndrome could be classified simply as a transverse limb deficiency. This would result in under-estimation of the frequency of amniotic band syndrome and over-estimation of transverse limb deficiency. Fifth, the diagnosis may be inaccurate. For example, a child with a limb defect could also have had a hypoplastic pectoralis muscle that was not detected and, therefore, would not have been classified as having Poland sequence. This would also result in a lower reported frequency of Poland sequence.

For some of the defects in question, the etiology may be heterogeneous, with vascular disruption being only one causative factor. In such cases it may be that there was not enough statistical power to detect an increase above the baseline. For example, cleft lip ± cleft palate is a very diverse group with genetic heterogeneity and accordingly, it is not surprising that there was not a significant association with CVS.

The BWH database is very detailed, includes pregnancies terminated electively because of fetal abnormalities identified prenatally, and classifies defects precisely, using an extension of the ICD-9 codes.[43] By having more precision in phenotype and the apparent etiology, affected infants with malformations due to apparent single mutant genes, chromosome abnormalities, and specific syndromes can be excluded or included as needed in establishing birth prevalence rates. Because the ex-

Table 5
Timing of CVS procedure and reported malformations

Defect	Gestational age (weeks) at time of procedure (range)	References
Terminal transverse limb deficiency	9–14	25, 27, 29, 34, 40
Proximal transverse limb deficiency	9.7	37
Oromandibular hypogenesis	8.4	34
Intestinal atresia	9–11.1	25
Gastroschisis	not reported	0
Poland anomaly	not reported	0
Clubfoot	8–12.7	25, 27
Facial clefts	9.8–14	25, 37
Syndactyly	10–14.3	21, 25, 27, 34, 37
Hydrocephalus	9–12.7	25, 27, 37
Neural tube defects	9–9.5	27, 37
Hemangioma	10	27
Diaphragmatic hernia	9.9–14	25, 37
Hypospadias	9.3–11.9	27, 37
Pyloric stenosis	10.4–11	25, 27
Congenital heart disease	9–13	25, 27, 37

amining pediatricians' findings in each newborn infants' record are reviewed, the ascertainment may be more complete than was the case for the data used in some of the 25 studies reviewed. This could result in higher prevalence rates in the BWH comparison group, which would decrease the probability of detecting an increase among CVS-exposed infants. Nevertheless, this makes the comparison more precise and less subject to errors caused by the lumping of similar, but etiologically different, malformations. The prevalence figures in the literature may lump malformations that are etiologically different together, resulting in higher frequencies.

All of the above limitations would result in an underestimation of the frequency of the various defects in the CVS-exposed population. Nonetheless, despite these limitations, this analysis has demonstrated that there was an increased frequency of three additional defects attributed to vascular disruption, intestinal atresia, gastroschisis, and clubfoot, among the CVS-exposed infants. Complete data from a huge randomized trial would be the ideal way to address these limitations. In the absence of this, an analysis such as we have done, is the only way to address the present data.

CONCLUSION

Previous studies have reported that infants born from pregnancies in which the mother underwent chorionic villus sampling have an increased frequency of terminal transverse limb defects and cavernous hemangiomas.[1,2,20] This review of 25 published reports of CVS-associated malformations suggested that these infants also have an increased frequency of intestinal atresia, gastroschisis, and clubfoot. The fact that an increased frequency of other defects attributed to vascular disruption was not seen could be due to under-ascertainment, misclassification, or 'lumping' of the defects identified in previous studies.

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