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ORIGINAL ARTICLE

Ear anomalies and hearing loss in patients with VACTERL association and the effect of maternal diabetes

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Abstract

VACTERL association is typically defined as the presence of three components among these birth defects: vertebral anomalies, anal atresia, cardiac anomalies, esophageal atresia/tracheoesophageal fistula (EA/TEF), renal anomalies, and limb defects. There is increasing recognition that VACTERL and other recurrent constellations of embryonic development often overlap clinically and might share pathogenesis. We conducted a comprehensive chart review of a large patient population with VACTERL association from two tertiary care centers in California. We included patients with incomplete VACTERL expression, which we denoted as "partial VACTERL" (pVACTERL). We assessed the occurrence of craniofacial (CF) findings in these two groups and the combined cohort. We collected data on potential risk factors and demographic information such as sex, Hispanic ancestry, pregnancy complications, and maternal age. The study included 409 participants, of whom 263 had VACTERL and 146 pVACTERL. CF abnormalities were found in 17.3% of VACTERL patients and 9.4% of pVACTERL patients. In the VACTERL group, ear anomalies were found in 10.2%, microtia in 5.9%, hearing loss (HL) in 13.90%, and orofacial clefts in 3.1%. In the pVACTERL group, ear anomalies were found in 7.2%, microtia in 5.0%, HL in 9.3%, and orofacial cleft in 2.2%. Maternal diabetes significantly increased the risk for HL in VACTERL (odds ratio [OR]: 3.71, 95% confidence interval [CI]: 1.5–7.3) and pVACTERL patients (OR: 6.7, 95% CI: 1.70–23.4). Poorly controlled maternal diabetes significantly increased the risk for all the outcomes in VACTERL patients including CF anomalies (OR: 4.2, 95% CI: 1.9–9.6), ear anomalies (OR: 4.7, 95% CI: 1.8–11.8), microtia (OR: 5.4, 95% CI: 1.7–16.6), and HL (OR: 8.1, 95% CI: 3.4–19.4). Twin status was significantly associated with the occurrence of microtia ($p = 0.038$) in VACTERL patients. Occurrence of CF features, particularly ear anomalies, microtia, and HL, might be considered as part of phenotypic diversity of VACTERL association. Diabetes and twinning might appear to play a role in increasing the risk for this phenotype in VACTERL association.

KEYWORDS

craniofacial anomalies, ear anomalies, hearing loss, maternal diabetes, microtia, VACTERL

1 | INTRODUCTION

The typical definition of VACTERL/VATER association involves a minimum of three congenital malformations among the following: vertebral defects, anal atresia, cardiac anomalies, esophageal atresia/tracheoesophageal fistula (EA/TEF), renal defects, and limb abnormalities (Quan & Smith, 1973; Solomon, 2011; Temtamy & Miller, 1974). Despite significant advancements in genomics research, the underlying causes of this condition have remained elusive. Only a small proportion of patients have a confirmed genetic etiology (Thiem et al., 2022), while the etiology remains unknown in most cases. It is likely that environmental factors and gene–environment interactions play a role in pathogenesis (Solomon, 2018; Stevenson & Hunter, 2013; van de Putte et al., 2020).

Recently, there has been growing recognition of more extensive phenotypic diversity in VACTERL association (de Jong et al., 2008; Husain et al., 2018). While the association of ear anomalies and VACTERL has been reported (Brademann et al., 2011; Duncan & Shapiro, 1993; Rudic et al., 2017), it has not been extensively studied or systematically investigated.

During a retrospective study of patients with EA/TEF in 2020, we incidentally found some patients with VACTERL association, including those with incomplete presentations, exhibited ear anomalies, and or hearing loss (HL; Galarreta et al., 2020). To explore the systematic occurrence of this pattern of malformation, we conducted a comprehensive retrospective chart review of a large patient sample of VACTERL association patients from two tertiary care medical centers in California.

2 | METHODS

2.1 | Study design

This retrospective study was conducted at two centers, Rady Children's Hospital San Diego (RCHSD) and Valley Children's Hospital (VCH) in Madera, California. The data for RCHSD were collected from patients seen from January 1st, 2010, to October 16th, 2021, while data for VCH corresponded to patients seen from January 1st, 2010, to April 25th, 2021.

To identify patients with VACTERL association, we queried the electronic medical record systems at both hospitals using the ICD-10 codes Q87.2 (multiple malformation syndrome with limb defect as a major feature) and Q87.89 (multiple system malformation syndrome). Additionally, varying combinations of codes were used to identify patients with at least two core birth defects (Table S1).

2.2 | Study population

2.2.1 | Inclusion criteria

Patients with VACTERL association were included, defined as the presence of at least three core defects (vertebral anomaly, anal atresia, cardiac defect, EA/TEF, renal anomaly, or limb anomaly). Patients with "partial VACTERL" were also included, defined as cases with two core VACTERL-type defects.

2.2.2 | Exclusion criteria

The following cases were excluded: patients with alternative diagnoses such as those with known genetic syndromes; patients with suspected unknown or undiagnosed syndromes, even when VACTERL features were present, including those with growth retardation, intellectual disability; patients with known teratogenic exposures other than maternal diabetes; patients with isolated birth defects; patients with caudal regression sequence were excluded when there were no anomalies above the pelvis; and patients with insufficient records and cases ascertained in error.

2.2.3 | Participants

The search process yielded 1952 cases for RCHSD and 1724 from VCH. After a review of these cases, 1493 cases from RCHSD were excluded because of an alternative or second diagnosis, while 97 were excluded as they were suspected to have an unknown syndrome. Additionally, 56 were excluded for having isolated malformations, 39 cases were excluded for having insufficient records, and 21 cases were excluded for being ascertained in error, resulting in a total of 246 cases being included in the study.

Similarly, from VCH, 1408 were excluded due to an alternative or second diagnosis, while 49 cases were excluded as an unknown syndrome was suspected. Additionally, 44 cases were excluded for having isolated malformations, 9 for having insufficient records, and 51 for being ascertained in error, resulting in a total of 163 cases being included in the study.

2.3 | Data collection

A detailed review of the electronic charts from both centers was conducted by the investigators; article records from the pre-electronic charting era were also reviewed when available. This review included an examination of clinical notes, imaging studies reports, and laboratory results. In addition to basic demographic information, the data collected encompassed a wide range of variables, such as maternal complications, teratogenic exposures, birth history, major and minor malformations, as well as the presence of HL, neurogenic bladder, tethered cord, and other relevant factors.

The data were reviewed by a second supervisory investigator to define diagnostic categories, ensuring the accuracy of case classification.

2.4 | Study definitions

"Maternal diabetes" was defined as the presence of history of maternal diabetes documented on the neonatal records. "Poorly controlled

maternal diabetes" was defined as the presence of history of maternal diabetes documented at the neonatal records, insulin use during pregnancy, and documentation of poor control such as the presence of one or more of the following: record of maternal hemoglobin A1C equal or above 8% during pregnancy, mother had history of complications related to diabetes such as diabetic neuropathy, mother was hospitalized during pregnancy for complication related to diabetes such ketoacidosis, the words "poor control," "uncontrolled" or "non-compliant" related to maternal diabetes control was documented in the neonatal records.

2.5 | Statistical analysis

The demographic and clinical characteristics were summarized by presenting the means and standard deviations (SDs) for continuous variables, and percentages along binomial 95% confidence intervals (CIs) for binary and categorical variables. The comparison of proportions between groups was done using χ^2 (chi-square) test and/or Fisher's exact test, and quantified through odds ratios (ORs) and percentages, together with binomial 95% CI. When the sample was small for some subcategory analysis, Fisher's exact test was used. All tests were conducted two-sided, with p values less than 0.05 considered statistically significant. IBM SPSS Statistics (version 25.0) was used to perform the statistical analyses.

2.6 | Ethical considerations

The project was reviewed and approved by the University of California San Diego Human Research Protection Program (IRB # 200444) and Valley Children's IRB Program (IRB# HSC2267). Given its retrospective and minimal-risk nature, a waiver of informed consent was granted by the IRB. This study adheres to the Declaration of Helsinki principles.

3 | RESULTS

The study included a total of 409 patients, of whom 246 (60.1%) were from RCHSD and 163 (39.9%) were from VCH. The demographic and birth information of the study population are summarized in Table 1.

A total of 263 patients (64.3%) were classified as having VACTERL association, while an additional 146 individuals (35.7%) exhibited only two of the core VACTERL-type birth defects, which we referred to as "partial VACTERL" (pVACTERL).

Craniofacial (CF) anomalies were detected in 57 of the 394 patients, with a documented facial exam accounting for 14.5% of the cases (95% CI: 11.1%–18.4%). Table 2 provides the prevalence of specific CF birth defects and HL in patients with VACTERL, pVACTERL, and the entire cohort. With the exception of the general category of CF anomalies, there were no significant differences in the prevalence of specific CF defects or HL when comparing VACTERL versus pVACTERL patients.

Figure 1 displays the frequency distributions of the CF birth defects found in the whole cohort. Structural ear anomalies were present in 36 patients (9.1%; 95% CI: 6.5%–12.4%), with 22 cases of microtia/anotia (5.6%; 95% CI: 3.5%–8.3%), 8 cases of ear tags (2.0%; 95% CI: 0.9%–4.0%), and 2 cases of preauricular ear pits (0.5%; 95% CI: 0.1%–1.8%). Four patients had a combination of ear tags and microtia (1%; 95% CI: 0.3%–2.6%). Orofacial clefting was observed in 11 patients (2.8%; 95% CI: 1.4%–4.9%). Other less frequent CF findings included facial palsy (four cases), coloboma (two cases), bifid uvula (two cases), facial tag (one case), choanal atresia (one case),

TABLE 1 Demographic and birth characteristics of study population.

	Mean	SD	N	%
Sex				
Male			192	47.1
Female			216	52.9
Hispanic ethnicity				
Hispanic			230	58.7
Not Hispanic			162	41.3
Twin status				
Singletons			318	91.9
Twin pregnancy			28	8.1
Birth weight (kg)	2.64	0.7		
Gestational age (weeks)	36.6	3.3		
Maternal age (years)	27.4	6.3		

TABLE 2 Frequency of craniofacial anomalies and hearing loss in patients with VACTERL association, partial VACTERL, and the combined cohort.

	VACTERL		pVACTERL			Combined cohort	
	%	95% CI	%	95% CI	$\chi 2$ p value[a]	%	95% CI
Craniofacial anomalies	17.30	12.8–22.5	9.40	5.1–15.5	0.033	14.50	11.1–18.3
Ear-related anomalies	10.20	6.8–14.6	7.20	3.5–12.8	0.323	9.10	6.5–12.4
Microtia	5.90	3.3–9.5	5.00	2.0–10.0	0.715	5.60	3.5–8.3
Hearing loss	13.90	9.8–18.9	9.3	4.9–15.7	0.196	12.30	9.2–16.1
Orofacial clefts	3.10	1.4–6.1	2.20	0.4–6.2	0.573	2.80	1.4–4.9

Abbreviation: CI, confidence interval.
[a]Comparing VACTERL and pVACTERL cases.

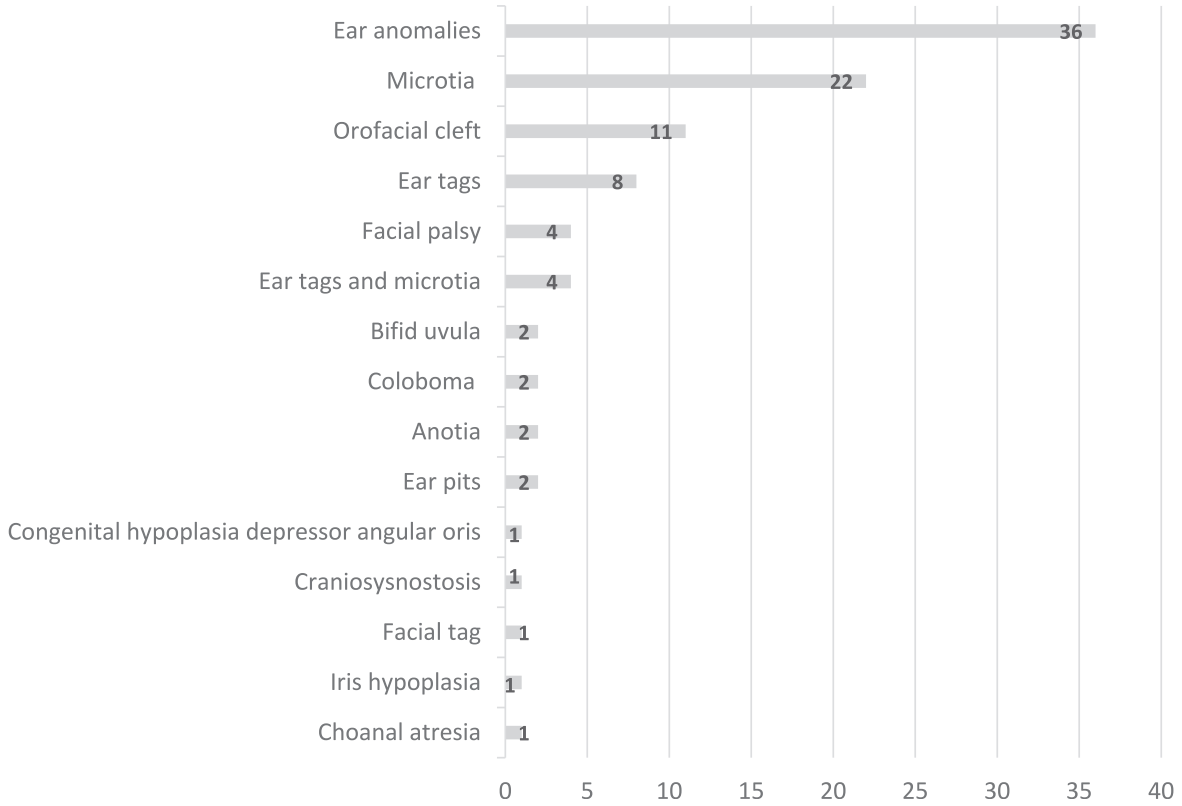

FIGURE 1 Frequency of craniofacial defects in 394 subjects with VACTERL association and partial VACTERL.

facial asymmetry (one case), iris hypoplasia (one case), orbital dystopia (one case), broad nose (one case), metopic craniosynostosis (one case), micrognathia (one case), facial sinus (one case), epibulbar dermoid (one case), optic nerve hypoplasia (three cases), congenital optic disc anomaly (one case), Duane anomaly (two cases), cataract (one case), congenital hypoplasia of depressor angularis oris (one case), and short palpebral fissures (one case).

Hearing status was known in 373 patients with enough records and follow-up time available to determine this, HL identified in 46 patients from the total cohort (12.3%; 95% CI: 9.2%–16.1%). The HL type distribution was sensorineural HL (SNHL) in 11 patients (23.9%), conductive HL in 24 cases (52.2%), mixed HL in 3 patients (6.5%), and unknown in 8 patients (17.4%). The severity distribution of HL revealed that 4 patients (8.7%) had mild HL, 7 (15.2%) had moderate HL, 14 (30.4%) had severe HL, and 6 (13%) had profound HL; 3 patients (6.5%) with unspecified HL severity required hearing aids, and for 12 patients (26.1%), the HL severity was unknown. Seven patients (15.2%) had left-sided involvement, 13 patients (28.3%) had right-sided HL, 17 (37.0%) exhibited bilateral HL, and for 9 patients (19.6%), the HL laterality was unknown.

The maternal diabetes status was known for 338 patients. Of these, 85 patients (25.1%; 95% CI: 10.6%–30.1%) had a history of maternal diabetes during pregnancy, while 53 (15.7%; 95% CI: 12.0%–20.0%) met the study criteria for poorly controlled maternal diabetes. The frequency of maternal diabetes in the VACTERL group was 23.4% (52/222; 95% CI: 18%–29.6%), whereas in the pVACTERL group, it was 28.4% (33/116; 95% CI: 20.5%–37.6%).

We investigated the impact of diabetes on the study outcomes. Table 3 displays the prevalence of the most representative CF findings among patients with VACTERL and pVACTERL, comparing the prevalence of these CF features based on the presence of maternal diabetes. Note that patients with a history of maternal diabetes were found to have a significantly higher risk of HL across all diagnostic categories, including VACTERL (OR: 3.271, 95% CI: 1.472–7.269), pVACTERL (OR: 5.763, 95% CI: 1.476–22.502), and the combined cohort (OR: 3.739, 95% CI: 1.892–7.390).

Table 4 displays the prevalence of the CF findings among patients with VACTERL and pVACTERL, comparing the prevalence of these CF features based on the presence of poorly controlled maternal diabetes. For the VACTERL association patient cohort, the additional presence of poorly controlled maternal diabetes significantly increased the risk of CF anomalies (OR: 4.266, 95% CI: 1.858–9.614), as well as specific features such as ear anomalies (OR: 4.636, 95% CI: 1.814–11.847), microtia (OR: 5.37, 95% CI: 1.701–16.575), and HL (OR: 8.081, 95% CI: 3.370–19.377). In the case of pVACTERL patients, the presence of poorly controlled maternal diabetes did not elevate the risk of any structural CF anomalies, but it did significantly increase the risk of HL (OR: 6.148, 95% CI: 1.457–25.951). For the combined cohort, poorly controlled maternal diabetes was also associated with significantly increased risk for all CF outcomes including HL (see Table 3).

Our study revealed no evidence of an association between either Hispanic ethnicity or sex and increased risk for any outcome variable including CF anomalies, ear anomalies, microtia, HL, or orofacial clefting among all diagnostic categories. Furthermore, we did not identify

TABLE 3 Frequency of craniofacial defects and hearing loss in patients with VACTERL association, pVACTERL, and combined cohort based on the presence of maternal diabetes.

	VACTERL					
	No maternal diabetes	Maternal diabetes	χ2	χ2 *p* value	Fisher's exact *p* value	Odds ratio (95% CI)
Craniofacial anomalies	27/166 (16.3%)	13/50 (26%)	2.413	0.12	0.092	1.809 (0.851–3.846)
Ear anomalies	15/166 (9.0%)	9/50 (18%)	3.126	0.077	0.07	2.210 (0.902–5.411)
Microtia	8/166 (4.8%)	6/50 (12%)	3.269	0.071	0.075	2.693 (0.888–8.172)
Hearing loss	17/156 **(10.9%)**	14/49 **(28.6%)**	9.075	**0.003**	**0.004**	**3.271 (1.472–7.269)**
Orofacial cleft	4/166 (2.4%)	3/50 (6.0%)	1.58	0.209	0.203	2.585 (0.559–11.960)
	pVACTERL					
	No maternal diabetes	Maternal diabetes	χ2	χ2 *p* value	Fisher's exact *p* value	Odds ratio (95% CI)
Craniofacial anomalies	8/81 (9.9%)	5/30 (16.7%)	0.976	0.323	0.249	1.825 (0.546–6.096)
Ear anomalies	5/81 (6.2%)	4/30 (13.3%)	1.507	0.22	0.197	2.338 (0.584–9.371)
Microtia	3/81 (3.7%)	3/30 (10.0%)	1.697	0.193	0.198	2.889 (0.550–15.179)
Hearing loss	4/77 **(5.2%)**	6/25 **(24.0%)**	7.547	**0.006**	**0.013**	**5.763 (1.476–22.502)**
Orofacial cleft	1/81 (1.2%)	2/30 (6.7%)	2.457	0.117	0.177	5.714 (0.499–65.477)
	Combined cohort					
	No maternal diabetes	Maternal diabetes	χ2	χ2 *p* value	Fisher's exact *p* value	Odds ratio (95% CI)
Craniofacial anomalies	35/247 (14.2%)	18/80 (22.5%)	3.087	0.079	0.06	1.759 (0.932–3.318)
Ear anomalies	20/247 **(8.1%)**	13/80 **(16.3%)**	4.427	**0.035**	**0.033**	2.202 (1.041–4.660)
Microtia	11/247 **(4.5%)**	9/80 **(11.3%)**	4.861	**0.027**	**0.031**	2.720 (1.084–6.824)
Hearing loss	21/233 **(9.0%)**	21/74 **(27.0%)**	15.75	**<0.0001**	**<0.0001**	**3.739 (1.892–7.390)**
Orofacial cleft	5/247 (2.0%)	5/80 (6.3%)	3.64	0.056	0.069	3.227 (0.909–11.448)

Note: Bold values are statitically significant.
Abbreviation: CI, confidence interval.

any statistically significant difference among the two centers for any of the evaluated outcomes. CF anomalies were present in 22/150 (14.7%) VCH patients, and in 35/244 (14.3%) RCHSD patients. Ear anomalies were present in 15/150 (10.0%) VCH patients, and in 21/244 (8.6%) RCHSD patients. Microtia was present in 12/150 (8.0%) VCH patients, and in 10/244 (4.1%) RCHSD patients. HL was present in 19/149 (12.8%) VCH patients, and in 27/224 (12.1%) RCHSD patients. Orofacial clefting was present in 4/150 (2.7%) VCH patients, and in 7/244 (2.9%) RCHSD patients.

Table 5 presents the relationship between twinning history and our variables of interest. Due to the limited number of patients in certain categories, we used the Fisher exact to obtain the *p* value, and the OR was not calculated. Only patients with available birth records were included. VACTERL twin patients had significantly higher prevalence of microtia (23.1%) compared to VACTERL singleton patients (5.2%; see Table 5).

4 | DISCUSSION

We conducted a comprehensive and detailed chart review of a large sample of 409 patients affected with VACTERL/pVACTERL association from two tertiary centers in California over a span of approximately 10 years. Our objective was to collect data on the presence and characteristics of CF findings in these patients. We found that patients with VACTERL and pVACTERL present CF anomalies at a rate higher than the general population. We present the frequencies of CF defect subcategories and HL in these cohorts. We analyzed the contribution of maternal diabetes, poorly controlled maternal diabetes, Hispanic ancestry, sex, and history of twinning to the risk of our outcome variables. Our analysis revealed that poorly controlled maternal diabetes was a significant risk factor for the co-occurrence of CF anomalies in VACTERL patients, specifically ear anomalies, microtia, orofacial clefts, and HL. We also found that maternal diabetes by itself, even without considering level of control, increased the risk of HL in VACTERL patients and those with pVACTERL phenotypes. Twinning was associated with microtia in VACTERL patients.

There has been limited focus on investigating the occurrence of CF anomalies, anotia/microtia, and HL in patients with VACTERL association. This is likely because CF anomalies or HL are not typically considered part of the overall spectrum of VACTERL association presentation (Quan & Smith, 1973). Nevertheless, some authors have observed an overlap between VACTERL phenotypes and CF malformations. For example, Duncan and Shapiro (1993) reported an overlap between hemifacial microsomia and VACTERL phenotypes, hypothesizing a shared embryologic pathogenic mechanism. Botto et al.

TABLE 4 Frequency of craniofacial defects and hearing loss in patients with VACTERL association, pVACTERL, and combined cohort based on the presence of poorly controlled maternal diabetes.

	VACTERL					
	No history of poorly controlled maternal diabetes	History of poorly controlled maternal diabetes	χ2	χ2 p value	Fisher's exact p value	Odds ratio (95% CI)
Craniofacial anomalies	27/185 **(14.6%)**	13/31 **(41.9%)**	13.153	**<0.0001**	**0.001**	**4.226 (1.858–9.614)**
Ear anomalies	15/185 **(8.1%)**	9/31 **(29.0%)**	11.77	**0.001**	**0.002**	**4.636 (1.814–11.847)**
Microtia	8/185 **(4.3%)**	6/31 **(19.4%)**	9.896	**0.002**	**0.007**	**5.37 (1.701–16.575)**
Hearing loss	17/174 **(9.8%)**	14/31 **(45.2%)**	25.031	**<0.0001**	**<0.0001**	**8.081 (3.370–19.377)**
Orofacial cleft	4/185 (2.1%)	3/31 (9.7%)	4.782	0.029	0.063	4.848 (1.030–22.818)
	pVACTERL					
	No history of poorly controlled maternal diabetes	History of poorly controlled maternal diabetes	χ^2	χ2 *p* value	Fisher's exact *p* value	Odds ratio (95% CI)
Craniofacial anomalies	9/93 (9.7%)	4/18 (22.2%)	2.295	0.13	0.134	2.667 (0.722–9.850)
Ear anomalies	6/93 (6.5%)	3/18 (16.7%)	2.112	0.146	0.16	2.90 (0.653–12.871)
Microtia	4/93 (4.3%)	2/18 (11.1%)	1.368	0.242	0.25	2.781 (0.470–16.474)
Hearing loss	6/89 **(6.7%)**	4/13 **(30.8%)**	**7.406**	**0.007**	**0.022**	**6.148 (1.457–25.951)**
Orofacial cleft	1/93 (1.1%)	2/18 (11.1%)	5.776	0.016	0.068	11.50 (0.984–134.398)
	Combined cohort					
	No history of poorly controlled maternal diabetes	History of poorly controlled maternal diabetes	χ2	χ2 *p* value	Fisher's exact *p* value	Odds ratio (95% CI)
Craniofacial anomalies	36/278 (12.9%)	17/49 (34.7%)	14.503	**<0.0001**	**<0.0001**	**3.571 (1.801–7.081)**
Ear anomalies	21/278 (7.6%)	12/49 (24.5%)	13.169	**<0.0001**	**0.001**	**3.969 (1.804–8.733)**
Microtia	12/278 (4.3%)	8/49 (16.3%)	10.464	**0.001**	**0.004**	**4.325 (1.668–11.219)**
Hearing loss	23/263 **(8.7%)**	18/44 **(40.9%)**	36.566	**<0.0001**	**<0.0001**	**7.625 (3.675–15.824)**
Orofacial cleft	5/278 (1.8%)	5/49 (10.2%)	9.928	**0.002**	**0.009**	**6.205 (1.725–22.312)**

Note: Bold values are statitically significant.
Abbreviation: CI, confidence interval.

(1997) reported a frequency of 2.1% of severe ear defects and 5.2% of cleft lip ± palate in patients with VACTERL association, based on data compiled from global birth defects registries. While our study found a higher frequency of ear anomalies and a lower frequency of orofacial clefts compared to that report, this could be partly attributed to limitations in registry methodology, potentially leading to incomplete identification of milder birth defects.

Conversely, several studies have explored the co-occurrence of other malformations in individuals with structural ear defects (Mastroiacovo et al., 1995; Paul et al., 2021; Shibazaki-Yorozuya & Nagata, 2019; van Nunen et al., 2014), particularly through birth registry studies. For instance, Cabrejo et al. (2019) conducted a large-scale examination of 1563 individuals with microtia and found a significantly higher risk for congenital anomalies compared to the general population, including 51 times more likely to have cardiac anomalies, 40 times more likely to have EA/TEF, 30 times more likely to have renal agenesis, and 19 times more likely to have genitourinary anomalies (Cabrejo et al., 2019). Similar findings were reported by Stoll et al. (2016), who observed a prevalence of cardiac and genitourinary anomalies in a group of 146 patients with microtia or anotia, all of whom underwent geneticist evaluations (Stoll et al., 2016). Schraw et al. (2023) also reported increased risk of cardiac, musculoskeletal, and nervous system malformations in a large sample of patients with microtia/anotia based on data from a birth registry in Texas (Schraw et al., 2023).

Several case reports have described patients with VACTERL association and ear defects, further supporting the notion of an association between VACTERL and CF anomalies. These reports include VACTERL patients with bilateral vestibulocochlear nerve abnormality (Rudic et al., 2017), unilateral vestibulocochlear nerve absence (Brademann et al., 2011), and a case of a patient with EA/TEF with anotia (Upadhyaya et al., 2012). There are also case reports of patients

TABLE 5 Craniofacial findings in VACTERL, pVACTERL patients, and the combined cohort based on twinning status.

All cases				Excluding poorly controlled maternal diabetes cases			
	VACTERL group				VACTERL group		
	Singleton	Twin	*p* Value		Singleton	Twin	*p* Value
Craniofacial anomalies	34/213 (16.0%)	5/13 (38.5%)	0.053	Craniofacial anomalies	22/166 **(13.3%)**	5/11 **(45.5%)**	**0.014**
Ear anomalies	19/213 (8.9%)	3/13 (23.1%)	0.12	Ear anomalies	12/166 (7.2%)	3/11 (27.3%)	0.054
Microtia	11/213 **(5.2%)**	3/13 **(23.1%)**	**0.038**	Microtia	5/166 **(3.0%)**	3/11 **(27.3%)**	**0.009**
Hearing loss	27/202 (13.4%)	4/13 (30.8%)	0.099	Hearing loss	12/155 **(7.7%)**	4/11 **(36.4%)**	**0.013**
Orofacial cleft	6/213 (2.8%)	1/13 (7.7%)	0.343	Orofacial cleft	3/166 (1.8%)	1/11 (9.1%)	0.228
	pVACTERL group				pVACTERL group		
	Singleton	Twin	*p* Value		Singleton	Twin	*p* Value
Craniofacial anomalies	7/94 (7.4%)	3/14 (21.4%)	0.12	Craniofacial anomalies	5/70 (7.1%)	2/11 (18.2%)	0.24
Ear anomalies	6/94 (6.4%)	2/14 (14.3%)	0.277	Ear anomalies	3/70 (4.3%)	2/11 (18.2%)	0.134
Microtia	4/94 (4.3%)	2/14 (14.3%)	0.173	Microtia	2/70 (2.9%)	2/11 (8.21%)	0.087
Hearing loss	8/84 (9.5%)	1/14 (7.1%)	0.622	Hearing loss	5/67 (7.5%)	1/11 (9.1%)	0.611
Orofacial cleft	1/94 (1.1%)	1/14 (7.1%)	1.697	Orofacial cleft	1/70 (1.4%)	0/11 (0%)	0.864
	Combined cohort				Combined cohort		
	Singleton	Twin	*p* Value		Singleton	Twin	*p* Value
Craniofacial anomalies	41/307 (13.4%)	8/27 (29.6%)	**0.029**	Craniofacial anomalies	27/236 **(11.4%)**	7/22 **(31.8%)**	**0.015**
Ear anomalies	25/307 (8.1%)	5/27 (18.5%)	0.081	Ear anomalies	15/236 **(7.6%)**	5/22 **(22.7%)**	**0.019**
Microtia	15/307 **(4.9%)**	5/27 **(18.5%)**	**0.016**	Microtia	7/236 **(4.3%)**	5/22 **(22.7%)**	**0.002**
Hearing loss	35/286 (12.2%)	5/27 (18.5%)	0.251	Hearing loss	17/222 (8.7%)	5/22 (22.7%)	0.35
Orofacial cleft	5/307 (2.3%)	2/27 (7.4%)	0.159	Orofacial cleft	4/236 (1.8%)	1/22 (4.5%)	0.362

Note: Bold values are statitically significant.

with overlapping VACTERL and OAVS phenotypes (Aftimos & Winship, 1999; Bergmann et al., 2003). A study also reported the presence of VACTERL association in a review of pediatric patients with unilateral SNHL (Haffey et al., 2013).

There is growing recognition that the phenotypic diversity in VACTERL association might be more extensive than previously believed (Husain et al., 2018). Furthermore, several authors have noted an overlap between recurrent malformation associations including VACTERL and OAVS (Adam et al., 2020; Duncan & Shapiro, 1993; Mark, 2022). Adam et al. (2020) proposed re-conceptualizing this group of disorders, introducing the term "recurrent constellations of embryonic malformations" (RCEM), acknowledging the phenotypic overlap, and suggesting shared pathogenesis (Adam et al., 2020).

Some authors have also implicated nicotinamide adenine dinucleotide (NAD) deficiency as a potential underlying mechanism in these RCEM-shared pathways, highlighting the pleiotropic effects of NAD deficiency on organ system development (Mark, 2022). Shi et al. (2017) reported the first four patients with pathogenic biallelic variants in genes in the NAD de novo synthesis pathway. Notably, two of the patients with biallelic pathogenic variants in *HAAO* presented ear defects along with VACTERL-type defects (Shi et al., 2017). Cuny et al.'s (2020) remarkable study provided further evidence of the teratogenic effects of NAD deficiency by demonstrating its effects in wild-type mice. These mice exhibited variable phenotypes, including isolated or combined birth defects such as skeletal malformations (rib and vertebral anomalies), congenital heart defects, underdeveloped eyes, hypoplastic kidneys, and cleft palate (Cuny et al., 2020). Moreover, Szot et al. (2020) proposed that congenital NAD deficiency can impact the development of multiple organ systems development, extending beyond the vertebra, kidneys, and limbs. This suggests a broader scope of organ involvement in the context of NAD deficiency during embryonic development.

Although we did not study NAD deficiency in our study, we observed that poorly controlled maternal diabetes increased the risk for CF anomalies, specifically ear-related anomalies, HL, and orofacial clefts in VACTERL association patients. We also found that maternal diabetes alone, without considering poor control, was sufficient to increase the risk of HL in both VACTERL and pVACTERL patients. We hypothesize that diabetes may act as an environmental factor influencing the phenotypic expression in patients with the VACTERL association spectrum. A possible explanation of this association is the known relationship between diabetes and dysregulation of NAD metabolism (Allegri et al., 2003; Fan et al., 2020; Mark & Dunwoodie, 2022). Diabetes may also increase the risk of vascular disruption which has been proposed as a mechanism in VACTERL (Stevenson & Hunter, 2013). Of note, the prevalence of gestational

diabetes in our cohort (25.1%; 95% CI: 10.6%–30.1%) was much higher compared to the population prevalence of gestational diabetes in California (8.1%; 95% CI: 8.0–8.2; Gregory & Ely, 2022).

We can hypothesize that the concept of pleiotropy could be applied to the manifestation of CF features in a subgroup of patients with VACTERL association. This association might be linked to the presence of additional risk factors that affect embryogenesis, drawing parallels to the findings of the Cuny et al. (2020) study. In their research, mice exhibited more numerous and severe congenital malformations when exposed to multiple factors, such as mild hypoxia, NAD deficiency, and maternal heterozygous variants in the NAD synthesis pathway gene *Haao* (Cuny et al., 2020).

There is evidence of an association between microtia and diabetes, independent of the context of VACTERL (Correa et al., 2008; Mastroiacovo et al., 1995; Van Bennekom et al., 2013), and diabetes has been shown to be associated with increased risk of HL (Lee et al., 2020; Padmadasan et al., 2022; Sharma et al., 2023; Tsao et al., 2023). Although the pathogenicity of diabetes in hearing impairment is not well understood, some studies proposed vascular disruption as a possible mechanism (Kelemen, 1955, 1960).

Our study suggests that maternal diabetes, particularly when poorly controlled, could exacerbate the phenotype of infants affected with VACTERL association, increasing the likelihood of CF features including HL, even in patients with pVACTERL phenotypes. These findings have significant implications, considering the prevalence of diabetes in the general population (https://www.cdc.gov/diabetes/data/statistics-report/index.html), and it suggests this particular phenotype could be a target of prevention.

Regarding the influence of sex and Hispanic ancestry on our outcomes, our study found no significant effects. Previous studies have reported an association between male sex and anotia, but mainly for the isolated form of microtia (Stallings et al., 2018). Canfield et al. (2009) reported in a Texas registry study that Hispanic ethnicity, particularly Mexican Hispanic ancestry, was a risk factor for microtia, along with male sex, and maternal Hispanic ethnicity (Canfield et al., 2009). This difference in findings is likely due to methodological differences, as the aforementioned studies focused on microtia diagnosis and included isolated cases. Another possibility is that our sample size was relatively smaller compared to large registry studies, which have more power to detect weaker associations.

Our study has several limitations. We were unable to assess other potential maternal risk factors such as nutrition, weight, presence of genetic risk factors, or exposure to teratogens other than diabetes. A prospective study with standardized intake could address these limitations. Additionally, only a limited number of patients underwent genetic testing, an expected finding since those with known or suspected syndromic causes were excluded from the study. Furthermore, despite collecting detailed data from a relatively large sample, our study lacks the power to assess the influence of other factors with smaller effects.

In summary, our study suggests that considering maternal diabetes status, particularly when poorly controlled, is important in understanding the presentation of birth defects in VACTERL association patients, with a higher risk for ear anomalies and HL. While we did not systematically analyze the outcomes of newborn hearing screening, we advocate for maintaining a proactive approach for conducting hearing assessments in patients with VACTERL and pVACTERL with overlapping CF anomalies, especially when there is a history of maternal diabetes. Our results highlight the importance of diabetes diagnosis and control during pregnancy. Future areas of research should include hearing assessment in VACTERL patients and prospective studies to assess other maternal risk factors such as NAD deficiency status and obesity.

AUTHOR CONTRIBUTIONS

Carolina I. Galarreta: Conceptualization; visualization; methodology; data collection; data curation; formal analysis; writing—original draft. **Laura Forero**: Data collection; writing—review and editing. **Erin Hoyt**: Data collection; writing—review and editing. **Cynthia J. Curry**: Conceptualization; resources; writing—review and editing. **Lynne Bird**: Conceptualization; methodology; data collection; data curation; writing—review and editing; supervision.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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