Undetected anomalies in foetuses with a prenatal diagnosis of isolated cleft


Abstract. The aim of this study was to determine the rate of undetected additional anomalies following a prenatal diagnosis of isolated oral cleft. Data of all infants with a prenatal diagnosis of isolated oral cleft born between 2000 and 2015 were studied retrospectively. Additional anomalies detected after birth were categorized as minor or major and included structural and chromosomal anomalies. Isolated clefts of the lip (CL), lip and alveolus (CLA) and lip, alveolus, and palate (CLAP) were diagnosed prenatally in 176 live-born infants. The type of cleft was more extensive after birth in 34/176 (19.3%) and less extensive in 16/176 (9.1%) newborns. Additional anomalies were diagnosed in 24 infants (13.6%), of which 12 (6.8%) were categorized as major. The latter included two submicroscopic chromosome anomalies and two gene mutations. Postnatal additional anomalies occurred more frequently in CLA and CL AP than in CL, and more in bilateral than in unilateral clefts. Major anomalies are still found in infants with a prenatal diagnosis of an isolated oral cleft. The prevalence of additional anomalies seems to be related to the type and bilaterality of the cleft, and this should be considered during prenatal counselling.

Key words: genetic testing; prenatal diagnosis; cleft lip; cleft palate; prenatal ultrasonography.

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Oral cleft (OC) is the most common congenital craniofacial anomaly, with an incidence of 1:700. The following phenotypes are distinguished: cleft lip (CL), cleft lip and alveolus (CLA), cleft lip, alveolus, and palate (CLAP), and cleft palate (CP). The likelihood of the presence of other structural anomalies and chromosomal anomalies increases when an oral cleft is diagnosed. The presence of additional anomalies may result in a challenging start to life and may have a substantial impact on the (psychosocial) health of the child and parent. Prenatal assessment is important to determine the type of OC and the presence of other anomalies in order predict the outcome. Counselling may enable parents to process disappointment and prepare for adjusted care during the pregnancy and after birth, rather than being confronted with difficulties when a child is born.

The introduction of the routine prenatal anomaly scan in the Netherlands in 2007 has increased the prenatal detection rate of CL, CLA, and CLAP substantially, from 5% in the 1980s to over 86% in the past decade. Prenatal detection rates of isolated CP are low and remain challenging. This could be explained by the absence of obvious facial clues suggesting the presence of a CP when no other anomalies are suspected.

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Invasive prenatal testing is offered when an OC is diagnosed\textsuperscript{11}. In our laboratory, foetal karyotyping was replaced by microarray analysis starting in 2011\textsuperscript{14,15}. Despite advanced prenatal diagnostic methods, not all additional anomalies may or can be detected before birth. In 17 postnatal studies including 28,953 infants with OC, the prevalence of additional anomalies varied between 17% and 60\%\textsuperscript{16}. The rate of additional anomalies following a prenatally diagnosed isolated OC has been reported in only three studies in the recent literature (including 344 foetuses), with this rate varying between 10\% and 30\%\textsuperscript{17–19}. These studies did not include data on chromosomal anomalies detected by means of microarray analysis.

The aim of this study was to evaluate the rate and severity of postnatally detected additional chromosomal aberrations and/or structural anomalies in infants with a prenatal diagnosis of isolated oral cleft in the South-West region of the Netherlands. The ultimate aim was to enable comprehensive prenatal counselling.

### Materials and methods

This was a retrospective cohort study of all consecutive pregnancies with foetuses diagnosed with an isolated OC, live-born between January 2000 and May 2015 in the South-West region of the Netherlands. When an OC was suspected during the pregnancy, the prospective mother was referred to Erasmus MC, a tertiary referral hospital, for a prenatal expert ultrasound examination, including two-dimensional and three-dimensional ultrasound. When

### Table 1. Prenatal diagnosis and postnatal outcome of 12 cases with prenatal apparent isolated cleft and minor postnatal additional anomalies.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Birth year</th>
<th>Prenatal diagnosis</th>
<th>Postnatal diagnosis</th>
<th>Side cleft</th>
<th>Prenatal genetic investigation</th>
<th>Postnatal structural anomalies</th>
<th>Postnatal syndromic anomalies</th>
<th>Postnatal genetic investigation</th>
<th>Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>2014</td>
<td>CLA</td>
<td>CLA</td>
<td>L</td>
<td>x</td>
<td>Pre-auricular fistula</td>
<td>None</td>
<td>None</td>
<td>x</td>
</tr>
<tr>
<td>M</td>
<td>2014</td>
<td>CLA(P)</td>
<td>CLA</td>
<td>L</td>
<td>Normal microarray</td>
<td>ASD type II, mild peripheral pulmonary stenosis, syndactyly</td>
<td>None</td>
<td>Van der Woude</td>
<td>IRF6 gene mutation (target mutation analysis)</td>
</tr>
<tr>
<td>M</td>
<td>2012</td>
<td>CLAP</td>
<td>CLA</td>
<td>B</td>
<td>Normal microarray</td>
<td>Cleft earlobe</td>
<td>None</td>
<td>None</td>
<td>x</td>
</tr>
<tr>
<td>F</td>
<td>2012</td>
<td>CLA</td>
<td>CLA</td>
<td>L</td>
<td>x</td>
<td>None</td>
<td>Van der Woude</td>
<td>None</td>
<td>x</td>
</tr>
<tr>
<td>M</td>
<td>2010</td>
<td>CLAP</td>
<td>CLA(P)</td>
<td>L</td>
<td>Normal karyotype</td>
<td>Perimembranous VSD, ASD type II</td>
<td>None</td>
<td>Van der Woude</td>
<td>No</td>
</tr>
<tr>
<td>F</td>
<td>2010</td>
<td>CLAP</td>
<td>CLAP</td>
<td>R</td>
<td>x</td>
<td>Accessory auricle</td>
<td>None</td>
<td>None</td>
<td>x</td>
</tr>
<tr>
<td>M</td>
<td>2009</td>
<td>CLA(P)</td>
<td>CLA</td>
<td>B</td>
<td>Normal karyotype</td>
<td>Syndactyly</td>
<td>None</td>
<td>Amniotic band constriction left hand</td>
<td>x</td>
</tr>
<tr>
<td>M</td>
<td>2008</td>
<td>CLAP</td>
<td>CLA</td>
<td>L</td>
<td>x</td>
<td>None</td>
<td>Van der Woude</td>
<td>CDH1 gene mutation (target mutation analysis)</td>
<td>No</td>
</tr>
<tr>
<td>M</td>
<td>2003</td>
<td>CLA(P)</td>
<td>CLA</td>
<td>L</td>
<td>x</td>
<td>Syndactyly</td>
<td>None</td>
<td>BCD</td>
<td>No</td>
</tr>
<tr>
<td>F</td>
<td>2002</td>
<td>CLA(P)</td>
<td>CLA</td>
<td>B</td>
<td>x</td>
<td>Congenital ectropion</td>
<td>None</td>
<td>None</td>
<td>No</td>
</tr>
</tbody>
</table>

ASD, atrial septal defect; B, bilateral; BCD, blepharocheilodontic syndrome; CLA, cleft lip and alveolus; CLAP, cleft lip, alveolus, and palate; CLA(P), cleft lip, alveolus, and (probably) palate; F, female; L, left; M, male; R, right; VSD, ventricular septal defect; ‘x’, not performed/not available.
Table 2. Prenatal diagnosis and postnatal outcome of 12 cases with prenatal apparent isolated cleft and major postnatal additional anomalies.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Birth year</th>
<th>Prenatal diagnosis</th>
<th>Postnatal diagnosis</th>
<th>Side cleft</th>
<th>Prenatal genetic investigation</th>
<th>Postnatal structural anomalies</th>
<th>Postnatal syndromic anomalies</th>
<th>Postnatal genetic investigation</th>
<th>Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>2015</td>
<td>CLAP</td>
<td>CLAP</td>
<td>L</td>
<td>x</td>
<td>Subglottic stenosis</td>
<td>None</td>
<td>x</td>
<td>No</td>
</tr>
<tr>
<td>M</td>
<td>2014</td>
<td>CLAP</td>
<td>CLAP</td>
<td>L</td>
<td>Normal microarray</td>
<td>Microcephaly, hepatosplenomegaly,</td>
<td>None</td>
<td>x</td>
<td>No</td>
</tr>
<tr>
<td>M</td>
<td>2014</td>
<td>CLAP</td>
<td>CLAP</td>
<td>L</td>
<td>x</td>
<td>Choanal atresia, choiroretinal coloboma,</td>
<td>CHARGE</td>
<td>x</td>
<td>Yes, at 4.5 months (infectious respiratory failure)</td>
</tr>
<tr>
<td>F</td>
<td>2013</td>
<td>CL</td>
<td>CLAP</td>
<td>L</td>
<td>x</td>
<td>Radioulnar synostosis</td>
<td>None</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>2012</td>
<td>CLAP</td>
<td>CLAP</td>
<td>L</td>
<td>x</td>
<td>Anterior ectopic anus, peripheral pulmonary stenosis, developmental delay</td>
<td>None</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>2010</td>
<td>CLA(P)</td>
<td>CLAP</td>
<td>L</td>
<td>x</td>
<td>Unilateral microtia/unilateral external auditory canal atresia, accessory auricle</td>
<td>None</td>
<td>Wolf–Hirschhorn</td>
<td>Yes, at 31 months (infectious respiratory failure)</td>
</tr>
<tr>
<td>M</td>
<td>2009</td>
<td>CLA(P)</td>
<td>CLAP</td>
<td>R</td>
<td>x</td>
<td>Developmental delay</td>
<td>None</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>2008</td>
<td>CLA(P)</td>
<td>CLA</td>
<td>B</td>
<td>x</td>
<td>Ectopic posterior pituitary glands, persisting cavum septi pellucidi, ASD type II (clinically insignificant), mild glandular hypospadias, psychomotor retardation, congenital dysplasia of the hip</td>
<td>None</td>
<td>Derivative chromosome 3 der(3)del(3)(p25.3) inv dup(3)(p22.3p25.3) arr[hg18] 3p21.31p14.1 (48,012,380-64,294,973)x3 16.3 Mb 3p21 duplication</td>
<td>No</td>
</tr>
<tr>
<td>F</td>
<td>2008</td>
<td>CLAP</td>
<td>CLAP</td>
<td>B</td>
<td>x</td>
<td>Congenital nasal cyst with extension into the intracranial space, congenital filamentous adhesion of the upper and lower eyelids</td>
<td>BCD</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>2004</td>
<td>CLA(P)</td>
<td>CLA</td>
<td>R</td>
<td>x</td>
<td>Craniofacial microsomnia, microtia, external auditory canal atresia, scoliosis</td>
<td>Goldenhar</td>
<td>x</td>
<td>No</td>
</tr>
<tr>
<td>M</td>
<td>2002</td>
<td>CLA(P)</td>
<td>CLAP</td>
<td>B</td>
<td>x</td>
<td>Oesophageal atresia, hypertrophic pyloric stenosis</td>
<td>None</td>
<td>x</td>
<td>No</td>
</tr>
</tbody>
</table>

ASD, atrial septal defect; B, bilateral; BCD, blepharocheilodontic syndrome; CHARGE, coloboma, heart defects, choanal atresia, growth retardation, genital abnormalities, and ear abnormalities; CL, cleft lip; CLA, cleft lip and alveolus; CLAP, cleft lip, alveolus, and palate; CLA(P), cleft lip, alveolus, and (probably) palate; F, female; L, left; M, male; R, right; VSD, ventricular septal defect; ‘x’, not performed/not available.
Based on ultrasound reports, the type of cleft was categorized as CL, CLA, CLAP, or CLAP/CP. The CLAP(P) type was assigned when following the diagnosis of CLA, the presence of a cleft palate was noted as ‘probable’ because it could not be ascertained on the sonographic images obtained. Isolated cleft palates were not detected prenatally and hence were not included in this study. The postnatal data were reviewed by a clinical geneticist, who categorized any additional anomalies into minor or major. This was based on the clinical relevance; abnormalities resulting in any permanent functional impairment were considered major. The cases with chromosomal aberrations were re-evaluated by a laboratory specialist. Terminated pregnancies (n = 5) and premature deliveries (n = 1) were excluded because phenotypic data were lacking.

Statistical analysis
The statistical analysis was performed using IBM SPSS Statistics version 21.0 software (IBM Corp., Armonk, NY, USA). Associations between the prenatal and postnatal type of OC and postnatal additional anomalies were tested by logistic regression analysis. Probabilities less than 0.05 were regarded as being significant.

Results
The data of 176 live-born infants diagnosed prenatally with an isolated OC were included. The postnatal follow-up period ranged from 1.4 to 16.2 years (median 6 years).

Prenatal data
During prenatal expert ultrasound examination, 32 (18.2%) foetuses were diagnosed with CL, 35 (19.9%) with CLA, 52 (29.5%) with CLAP, and 57 (32.4%) with CLAP(P) (Fig. 1).

Postnatal data
Postnatal clinical assessment of the OC confirmed the ultrasound diagnosis in 126/176 (71.6%) infants, while 50 (28.4%) diagnoses were revised (Fig. 1): 34 (19.3%) to a more extensive type and 16 (9.1%) to a less extensive type of cleft. A submucous cleft of the palate was determined in two infants with a prenatal diagnosis of CL and in two with CLA. (Bi) laterality was revised in 13/176 (7.4%) infants; seven prenatally unilateral clefts were diagnosed as bilateral and six prenatally bilateral clefts were diagnosed as unilateral after birth. A unilateral cleft was seen in 143 (81.3%) infants (15 CL, 39 CLA, 89 CLAP) and a bilateral cleft in 33 (18.8%) infants (1 CL, 6 CLA, 26 CLAP). Ninety-seven of the unilateral clefts (67.8%) were left-sided and 46 (32.2%) were right-sided.

Postnatal assessment of associated structural and syndromic anomalies
Additional anomalies were found in 24/176 (13.6%) infants with a prenatally apparent isolated oral cleft; 12 (6.8%) were categorized as minor (0 CL, 3 CLA, 9 CLAP) and 12 (6.8%) were categorized as major (0 CL, 2 CLA, 10 CLAP) (Tables 1 and 2). A higher prevalence of additional anomalies was noted with increasing severity of the type of cleft in reference to cleft lip only (CL: odds ratio (OR) 2.91, 95% confidence interval (CI) 0.29–29.45; CLAP: OR 8.32, 95% CI 1.02–67.9). Additional anomalies were diagnosed in 16/143 (11.2%) infants with a unilateral oral cleft and in 8/33 (24.2%) infants with a bilateral oral cleft (OR 2.54, 95% CI 0.98–6.57). No statistical significance was found.

All infants with additional anomalies categorized as major had multiple anomalies. Eleven of the 12 infants with anomalies categorized as minor had a single anomaly. Fig. 2 shows the distribution of the additional structural anomalies by organ system. Chromosomal aberrations and gene mutations associated with known syndromes were diagnosed in 8/176 (4.5%) infants (one Van der Woude syndrome, two blepharocheilodontic syndrome (BCD), one CHARGE syndrome, and four pathogenic chromosomal aberrations, of which one was associated with Wolf–Hirschhorn syndrome). Syndromic disorders were clinically diagnosed in 3/176 (1.7%) cases (two Van der Woude and one Goldenhar syndrome). Two patients died due to respiratory failure as a result of a viral pulmonary infection, one at 4.5 months after birth and the other at 31 months. Both had been diagnosed with a syndromic disorder (CHARGE syndrome and Wolf–Hirschhorn syndrome).

Chromosomal aberrations and prenatal cytogenetic diagnosis
Invasive prenatal testing was performed in 87/176 (49.4%) pregnancies; 54/176 (30.7%) by karyotyping and 33/176 (18.7%) by microarray analysis. Eight of the 87 infants who had undergone amniocentesis (9.2%) were diagnosed with additional anomalies during the postnatal period, of which four were major. One infant with a prenatal normal karyotype was diagnosed postnatally with a submicroscopic 4p deletion associated with

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Wolf–Hirschhorn syndrome identified by microarray analysis. Prenatal microarray analysis was not routinely available at the time of pregnancy.

Amniocentesis was declined by 89 (50.6%) pregnant women. Postnatal genomic microarray revealed chromosomal aberrations in the offspring of three of these women (3.4%), which were categorized as major: two microscopically visible (3p21 duplication and a derivative chromosome 3) and one submicroscopic
aberration associated with developmental delay and likely causal for CLAP (3.4 Mb 6p21.1 deletion)\textsuperscript{11,22}. In two of these cases, additional structural anomalies were found, and developmental delay was detected in all three during follow-up. Furthermore, postnatal targeted mutation analysis identified two gene mutations in the major group (a CHD7 mutation associated with CHARGE syndrome and a CTNNB1 mutation associated with BCD syndrome) and two gene mutations in the minor group (an IRF6 mutation associated with Van der Woude syndrome and a CDH1 mutation associated with BCD syndrome).

**Discussion**

Additional congenital structural anomalies and/or constitutive chromosomal aberrations were found in 13.6% of patients, half of which were considered as major. The prenatal diagnosis of the type of cleft was revised to a more extensive type in 34 (19.3%) infants and to a less extensive type in 16 (9.1%).

No false-positive prenatal cleft diagnoses were noticed, confirming earlier studies\textsuperscript{18,23,24}. The prenatal diagnosis of the type of cleft was revised in 28.4% of infants, which compares well with the 30% and 35% found in previous studies\textsuperscript{12,18}. The revisions were most common in the CL group. The prenatal diagnosis of CL was revised in 19/32 (59.4%) cases to a more extensive type (CLA or CLAP), and one infant with CLAP was also revealed to have a radioulnar synostosis. These revisions could be explained by the challenging detection of mild alveolar notches and (submucous) cleft palate, as reported previously by several authors and shown in Fig. 5\textsuperscript{25-27}. No additional structural anomalies or chromosomal aberrations were found in infants with a postnatal diagnosis of CL.

The type and extent of the OC is related to the prevalence of additional anomalies\textsuperscript{2,3,16,24}. The present study data confirm these findings, as the frequency of additional anomalies increased with involvement of the alveolus and the palate. Only this study and the study by Depla et al.\textsuperscript{18} have reported the prenatal diagnoses of CL, CLA, and CLAP subdivisions, suggesting differences in the prevalence of additional anomalies.

Additional anomalies were diagnosed more frequently in infants with a bilateral cleft compared to a unilateral cleft, as also reported by Hagberg et al.\textsuperscript{29} and Fleurke-Rozema et al.\textsuperscript{12}.

In the majority of cases, the prenatal diagnosis was determined prior to 24 weeks of gestation. The additional structural anomalies not detected until after birth were anomalies that may only become evident late in gestation (microcephaly and severe pulmonary stenosis)\textsuperscript{30,31} or anomalies known to be difficult to diagnose due to the variable presentation of abnormal structures. Examples of the latter are Ebstein anomaly, ventricular septal defect, anterior ectopic anus, and oesophageal atresia\textsuperscript{31-34}. Similar anomalies were not detected before birth in two recent studies, indicating the limitations of ultrasound in pregnancy\textsuperscript{18,28}.

Two of the four patients with an aberrant chromosome status carried a large chromosomal aberration (3p duplication and derivative chromosome 3); the other two carried submicroscopic aberrations (6p21 deletion and 4p deletion). Novel high-resolution genetic tests such as microarray-based genomic analysis can detect all of these chromosomal aberrations, in contrast to conventional karyotyping, which has a limited resolution\textsuperscript{35}. The use of this technique reduces the rate of undetected chromosomal aberrations associated with structural and syndromic malformations\textsuperscript{30}. Contrary to past opinions\textsuperscript{15,37,38}, we believe that invasive genetic testing for all types of OC is justified in view of the occasional inconsistencies in the pre- and postnatal diagnosis of the type of cleft in relation to the prevalence of additional anomalies, the introduction of microarray analysis, and the low additional risk of foetal loss following amniocentesis\textsuperscript{12,23,38}. Microarray testing may reveal chromosomal aberrations associated with features that cannot be detected on ultrasound, such as developmental delay or hypotony, but which strongly influence the foetal prognosis. Although microarray testing is offered in all pregnancies with anomalies detected on ultrasound, parents often decline genetic testing, as revealed in the present study and a previous Dutch study\textsuperscript{12}. Prior to the microarray era, the risk of an abnormal karyogram associated with OC was low\textsuperscript{39}. This might have influenced counselling concerning invasive procedures. Cultural attitudes in the Netherlands, in addition to parental fear of miscarriage could also have played a role, despite the low risk of pregnancy loss\textsuperscript{37,40}.

In addition to the chromosomal aberrations detected postnatally by microarray analysis, gene mutations were identified using targeted mutation analysis, of which two were major anomalies (CHARGE and BCD with intracranial extension). If prenatal diagnosis using microarray and whole exome sequencing (WES) was performed in all cases, the diagnosis of additional genetic anomalies would have been possible before birth in an additional 4.5% (8/176).

The strengths of this study are the large sample size, the subdivision of cleft types (CL, CLA, CLAP) and extent (unilateral/bilateral), and the reporting on the additional value of microarray analysis. A main limitation is the retrospective nature. The shorter follow-up period of infants born in the last 2 years of the study might have resulted in an underestimation of the rate of associated anomalies (especially milder developmental delay, speech disorders, and intellectual disability revealed later in life). Moreover, submicroscopic aberrations and gene mutations were not tested and excluded in all of the cases during the follow-up period.

In conclusion, additional anomalies were seen in almost one in every 7.5 infants diagnosed prenatally with an isolated OC. The introduction of microarray analysis has increased the diagnosis of chromosomal aberrations. Involvement of the alveolus and the palate and a bilateral cleft appear to be related to a higher risk of additional anomalies. The diagnosis of only a CL during prenatal screening does not rule out the presence of associated anomalies postnatally, taking into account the revision rate of the type of cleft after birth. Possible inconsistencies with the final postnatal diagnosis of the type of cleft and the rate of undetected additional anomalies should be discussed during prenatal parental counselling.

**Patient consent**

Not required.

**Funding**

None.

**Ethical approval**

Data collection and protection took place according to the privacy regulations of the Erasmus MC. Approval was not required according to the judgement obtained from the Ethics Review Board of the Erasmus MC, Rotterdam, the Netherlands (MEC-2016-576).
Competing interests

The authors have no conflicts of interest to declare.

References


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