TITLE PAGE

Full Title: A Literature Review of the Causes of Congenital Limb Deficiencies Over the Last 20 Years

Short Title: Cong Limb Causes Lit Review

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Conflicts of Interest and Source of Funding: The authors declare that there are no conflicts of interest and no funding was received in relation to this work.

Funding Statement: No funding was received in relation to this work

ABSTRACT

Introduction

Despite the prevalence of congenital limb deficiencies (CLDs) occurring in around 7.9/10,000 births (Gold NB, Westgate M-N, Holmes LB. Anatomic and etiological classification of congenital limb deficiencies. American journal of medical genetics Part A. 2011;155A(6):1225-35.), there is still a gap in the knowledge regarding the aetiology of a large proportion of cases and literature addressing this topic is more sparse compared to other conditions.

Objective

The aim of this study is to assess the literature surrounding the causes of CLDs and use this to suggest the area in which a potential gap in the information on the causes of CLDs lies.

Study Design

A literature review on articles published from the year 2000 onwards.

Methods

A search was conducted on five databases (CINAHL, Scopus, PubMed, Embase, Cochrane Library) and the ProQuest platform resulting in 18 papers to be discussed, after inclusion and exclusion criteria were applied and critical appraisals were completed.

Results and Discussion

The main findings could be separated into four themes including, genetics, environment, drugs and vascular. Comparisons were made between similar literature, although within some

Funding Statement: No funding was received in relation to this work.

topics this was less effective given the specificity and reduced volume of research. Positive correlations were seen in all studies however, the biggest underlying factor for most causes appeared to originate from a vascular disruption.

Conclusion

Future studies should focus on an underlying vascular disruption as a base for further research to attempt to find reasoning for the proportion of CLDs with an unknown cause. Further attempts should be made into creating a consensus on classifying and recording CLDs to ensure a more uniform approach to this topic worldwide, allowing comparisons to be drawn more easily.

Key words

Congenital, limb deficiency, aetiology, upper limb

INTRODUCTION

Congenital limb deficiencies (CLDs) occur in around 7.9/10,000 births (1) but there is still a large proportion of these cases where the cause ~~of these CLDs~~ is ~~still~~ unknown. In a small prevalence study 32% of cases with a CLD had an unknown cause (2). A CLD is often described as an absence or incomplete development of a long bone, metacarpal, metatarsal or phalanx, and usually does not include curvatures or mild shortenings as seen in brachydactyly (1).

Upper limb buds begin to develop around day 24 after conception and development of lower limb buds occur ~~later~~ on approximately day 28 (3). ~~Between week 4 and the end of week 8~~ ~~a~~All ~~the~~ limb~~'s~~ components are formed by week eight (4). The limb develops across three axes: proximodistal, anteroposterior and dorsoventral each with their own signalling centre (3). In the proximodistal axis the Apical Ectodermal Ridge (AER) forms at the tip of the limb and is where signalling for limb formation arises (3). The AER uses Fibroblast Growth Factor (FGF) signalling as a key part of initiating growth (3). The AER is also implemented in aiding vascular network growth (3). In the anteroposterior axis the Zone of Polarising Activity (ZPA) is the signalling centre, with the key molecule being Sonic Hedgehog (SHH) (3). To form separate digits the tissue between them must go through apoptosis (3). Bone morphogenic proteins regulate this process (3). The AER and ZPA work in a positive feedback loop meaning the two processes rely on each other to ensure normal limb development (3) through the production of various FGF proteins (5). In the dorsoventral axis the entire dorsal ectoderm provides the signalling centre (3). The ventral and dorsal rami form together to make a plexus in week four ~~4~~ and then extend ~~proximal to~~ distally as the limbs grow (3). The musculoskeletal systems form from muscular blastema and chondrogenic blastema with bones forming in a proximal to distal fashion and superficial and distal muscles forming first before deep muscle tissue (3).

The classification of CLDs lacks consistency throughout the literature and has been the topic of some studies (1, 6). A variety of classification systems are used across studies to group cases. Three main groups are regularly used; ~~to group CLDs which are~~ terminal transverse, longitudinal and intercalary. Terminal transverse deficiencies are defined by the absence of distal structures after a specific point, longitudinal deficiencies are the absence of a bone that is parallel to the long axis and intercalary deficiencies are seen when there is an absence of the middle section of a long bone with normal distal structures (1). The International Statistical Classification of Diseases and Health Related Problems (ICD) is one such system using hierarchical coding, with the current codes in use being ICD-10 and aims to be the standard way of reporting health conditions globally (7). The European Surveillance of Congenital Anomalies (EUROCAT) focuses on epidemiological information and risk factors for congenital anomalies ~~across Europe~~ and utilises~~has~~ a different classification system alongside ~~although the~~ ICD codes ~~are used in the database with an extension~~ (8). EUROCAT excludes a list of minor anomalies (8). The International Federation for Societies for Surgery of the Hand (IFSSH) adopted a seven ~~7~~ category classification system specifically for upper limb anomalies with the categories being; failure of formation (A for transverse or B for longitudinal), failure of differentiation, polydactyly, overgrowth, undergrowth, amniotic band syndrome and generalised skeletal syndromes (9).

The aim of this review is to collate the literature surrounding the known or potential causes of CLDs, and ~~to use this~~ to assess where there may be a potential gap in the literature that could be investigated in the future to reduce the number of cases of CLD with an unknown cause.

METHODOLOGY

A literature review was conducted ~~with a broad search strategy~~ using the search ((congenital OR birth OR newborn) AND (cause* OR etiology OR aetiology) AND ("limb defect" OR "limb deficiency" OR "limb reduction" OR "limb absence")) within the titles and abstracts of articles. The following ~~F~~filters were applied to refine the search in line with the inclusion criteria~~:, comprising of:~~ "published in year 2000 to present", "humans" and "English language". The search was carried out in five databases: CINAHL, Embase, Scopus, Cochrane Library and PubMed in addition to all databases on the ProQuest platform~~, which includes databases like Medline.~~ The results were collated and duplications were removed, before the titles and abstracts were screened to apply the inclusion and exclusion criteria ~~as~~ seen in (Table 1).

The exclusion criteria were ~~chosen~~ selected to reduce the number of articles while retaining beneficial research with clear methods, covering a broad scope of causes. Reviews and papers on classification were removed as these were not presenting new findings and despite classification being an important aspect for comparisons, these papers were not showing cause and effect relationships. Case reports/series and papers with a study population less than ten were removed as these types of findings were a lower grade of evidence ~~due to only being based on one or few people which is similarly the reason for removing the papers with a study population of less than 10~~. ~~Although CLDs are rare, with the scope of literature surrounding this topic found in the search of a higher grade of evidence, it makes the removal of these papers reasonable.~~ Conference abstracts were excluded due to the lack of information given in them as were animal studies as this review is focused on CLDs in human infants. Any literature retrieved that was deemed irrelevant in answering the question of this review was removed. If the results of the study did not show a conclusive, significant correlation between the cause being investigated and CLDs, the study was removed to eliminate any exposures that were expressed to not be of concern regarding~~in regard to~~ CLDs.

Once the papers that did not meet the criteria had been filtered out, the remaining papers were critically appraised to identify the quality of the research and to exclude any weak papers if this was advised in the ~~checklists for the~~ appraisal checklists~~s~~. Any papers that could be, were critically appraised using the Scottish Intercollegiate Guidelines Network (SIGN) guidelines, following the appropriate checklist for each study design. Where ~~this was not possible as~~ the SIGN algorithm suggested that no checklist was needed for that study design, ~~the~~ papers were critically appraised using the Credibility, Accuracy, Reasonableness and Support (CARS) method. The papers that were ~~graded~~ analysed using the SIGN guidelines were graded between 2- and 2++ as all these papers were case-control studies. The papers evaluated through the CARS method were ~~given~~ assigned a grading of high, medium or low in terms of overall quality. The number of papers throughout the search process can be seen in (Figure 1) and resulted in 18 papers to be discussed. (Table 2) shows the critical appraisal grading for each study along with the number of participants and the classification system used within each paper.

This review includes discussions on results comprising of upper and lower limb reductions, of terminal transverse, intercalary and longitudinal descriptions along with split hand split foot (SHSF) malformation as multiple studies included these. ~~If separate r~~Results for deficiencies such as syndactyly or brachydactyly ~~were presented they~~ will not be discussed, as many authors excluded these deficiencies due to their less severe nature.

The search returned evidence of other potential causes for CLDs for example maternal fever or obesity, as well as known syndromes like Cornelia de Langes Syndrome. These were removed and will not be discussed due to the literature retrieved relating to these topics being limited and lacking quality or sufficient information, meaning they would not be comparable within a given theme.

RESULTS

The topics discussed in the literature were split into four themes to group similar information for comparison;. The themes include, drugs, the environment, genetics and vascular aetiologies. All the papers within the environmental theme were published in the last 6 years, possibly due to greater awareness of environmental changes and sustainable living. This could be an indication of a newer concept believed to have the potential to give a reasoning for some of the cases with an unknown cause. In contrast, Whereas some of the investigations into drug and vascular causes, like Chorionic Villus Sampling (CVS) for example, are from the the early 2000searlier years. included in this search showing how these have been accepted factors of CLDs for a longer time and therefore being included as part of the already known causes. (Table 3) presents the causes investigated within each study and the findings that the authors stated as being significant.

Drugs

The search returned four papers discussing the effects of different drugs, with various drugs now being known teratogens. Within the literature, the drugs found to be associated with CLDs were misoprostol, thalidomide and antiepileptic drugs.

Arpino investigated antiepileptic drugs, but only discovered a statistically significant association between valproic acid and CLDs with 5/299 infants exposed to antiepileptic drugs presenting with a CLD after being exposed to valproic acid (10). Although three3 other infants in this study exposed to various other antiepileptic drugs presented with CLDs, these were not statistically significant associations. It was also stated that the five5 infants that presented with a CLD were exposed to valproic acid only and not in combination with any

other antiepileptic drugs, which is more evidence for the teratogenic characteristics of valproic acid (10).

Two authors, Orioli (11) and Auffret (12) covered the association of misoprostol, a synthetic prostaglandin analogue (13) and CLDs. Misoprostol can be used in the treatment of ulcers but is also a uterine stimulant and thus can be used as part of a pregnancy termination or to induce labour (13). Orioli showed a significant association between misoprostol exposure and terminal transverse deficiencies when looking at the probability that this congenital anomaly would occur in a mother exposed to misoprostol compared to a non-exposed mother, with 8.82% of the malformed infants exposed to misoprostol having a CLD (11). This association is supported by the results described by Auffret (12). Although using a different classification system and not specifying exact numbers for each congenital anomaly, it was still shown that exposure to misoprostol increased the rate of major congenital malformations at 5.5%, which included infants with CLDs. However, this was only observed in the group of women that were exposed to misoprostol for a voluntary abortion rather than any other indications (12). This author's research also interpreted whether the dose of this drug impacted the occurrences of congenital anomalies. The infants presenting with CLDs were exposed to a dose of 200 micrograms of misoprostol followed by mifepristone (another drug used in the process of abortion), however, due to the wide range of dosages used by the rest of the group, no dose-effect could be discovered and instead this highlighted the teratogenic effects even a small dose could inflict (12).

Thalidomide is a drug that is known for its teratogenic effects after the epidemic in the 1960s resulting in its withdrawal from the market, with the limbs being the most commonly affected (14). This drug in the UK now tends to only be used for treating myelomas when high-dose chemotherapy is not feasible and there are precautions in place when a ~~women~~woman of child bearing potential is using thalodomide~~this drug~~ (15). The research presented by Vianna

(14) was based in Brazil where thalidomide is more commonly used for the treatment of erythema nodosum leprosum, an inflammatory condition commonly seen in people with leprosy which has a high prevalence in Brazil (14). This study defined a case as whether specific CLDs were present and compared this with the number of thalidomide tablets distributed. A reported 27.9% increase in cases of CLDs for every 100,000 thalidomide tablets dispensed was shown through this study (14) which supports the knowledge of thalidomide being a teratogen.

This section of the review presented evidence that there are significant correlations between multiple drugs and the presence of CLDs, with valproic acid, misoprostol and thalidomide being recognised causes of CLDs.

Environment

Three papers in this review explored the associations between environmental factors and CLDs. These factors included air pollution, chlorinated solvents and pesticides. The papers found ~~in this search~~ highlight in their discussions how there are few studies focusing on these variables as well as differences in the models used to conduct these types of studies. Some studies focus on single pollutants while others attempt to ~~make~~ adjust~~ments~~ for co-pollutant groups and therefore~~, comparisons of results for these types of studies can make~~ it can be difficult to find trends (16-18).

The study investigating the impact of air pollution ~~with CLDs~~ found a statistically significant association between three of the six pollutants observed with an increase in the chance of CLDs occurring when looking at a single pollutant model (16). When the odds ratios were adjusted for the co-pollutant model the association between two of those three pollutants were near null and thus only one pollutant, carbon monoxide was shown to increase the likelihood of CLDs occurring in both single and co-pollutant adjustments (16). With this

association being found the potential underlying cause of developmental disruption was suggested to be hypoxia, as less oxygen will be able to bind with haemoglobin to be transferred to the foetus if there is increased carbon monoxide binding (16). This gives rise to a link between environmental factors and an underlying vascular disruption.

The results from the studies by Carmichael (17) and Brender (18) show small associations between the environmental variables ~~they~~ tested and CLDs, however~~,~~ the authors ~~themselves point out~~acknowledge that~~how~~ the quality of this evidence may be low due to factors like false positive results. The results from Brender only concluded that one of the 14 chlorinated solvents (perchloroethylene) tested, showed significant results in association with transverse limb deficiencies (18) and therefore CLDs were not a result that was discussed at length in the paper, which could show that this risk factor is not as significant for CLDs compared with the other congenital defects. In the same respect the research conducted by Choi (16), only showed a robust association in one of the six air pollutants recorded which again does not correspond to this being a highly significant risk factor in comparison to other known causes of CLDs. Out of the 53 groups and 248 individual pesticides Carmichael recorded, a notable association was only observed for 3 groups and 6 individual pesticides (17) which again questions whether ~~these~~ associations with environmental pollutants are ~~major concerning~~noteworthy factors. Other causes presented much stronger correlations with a greater volume of comparable research and more consistent results, which these environmental papers did not. Having said that, the research surrounding this theme was relatively new and with some results showing positive correlations, if larger studies with similar methodologies, to allow for easier comparisons could be conducted in the future, these results may become more ~~beneficial~~convincing.

Varying environmental pollutants could be a cause of CLD and with further investigation, these studies show the potential for further correlations between environmental factors and CLDs to be discovered.

Genetics

Four papers retrieved in the search focused on genetics as a cause of CLDs.

Two papers presented research into CLDs caused by genetic variations by investigating single nucleotide polymorphisms (SNPs) (19, 20) with differing results. The study by Carmichael tested 29 SNPs (20) and 20 were tested by Browne (19). However, only ~~3~~ three SNPs were investigated in both studies meaning less comparison could be carried out than expected. The two studies had conflicting results with the F5 gene which was a significant result for Carmichael showing an over 2-fold increase in odds of CLDs occurring (20) whereas the odds ratio was not significantly impacted in the results shown by Browne (19). Carmichael suggested that although potential correlations were found, the knowledge of the role in embryo development that these variations play remains limited (20). The studies agreed in the observation of null results for the MTHFR SNP (19, 20) which showed some similarities in the literature.

The second study by Carmichael (21) investigating genetic causality showed an over 1.5-fold increased odds in CLDs in variations of NAT1 and NOS3 genotypes. The correlation between CLDs and NOS3 could have a link to an underlying vascular disruption as this kind of deficiency can be associated with reduced blood flow to the limb and a reduction in oxygen being supplied to the developing foetus.

Carter, who investigated genetic variations, specifically focused on the SHSF malformation (22). This research into copy number variants showed significant results in three different

chromosome regions however, there seems to be no similarities between the genes within these regions and the genes of significance in the previous studies. This could be due to the specificity of that study and the small study population of only 25 (22).

The range of genes that could impact foetal development emphasises the complexity of this process and how the CLD observed can differ depending on which gene mutations have occurred. Genetic implications can be seen to impact CLDs however comparing results is challenging due to methodological differences.

Vascular

Results regarding potential vascular causes of CLDs were found in seven papers within the search. This theme covers a variety of causes that stem from vascular disruptions and many of the research articles that are focused more towards another theme such as genetics or the environment link into this vascular theme as the potential underlying mechanism to why CLDs occur.

One association that was focused on by two authors, Hunter (23) and Ordal (24), was the link between thrombophilia and CLDs. A limitation of both these studies was the small study populations available however, the preliminary associations made within each of the studies could justify attempts for larger~~, more in-depth~~ studies based around these findings (23, 24). In both studies a comparison was made between the general and study population prevalence rates which in both showed a significantly higher prevalence of thrombophilia in the study populations where the infants presented with CLDs, than in the general population (23, 24). Despite these similarities in overall associations the two papers showed variation when it came to specific screening results. For example, Hunter showed a significant correlation between protein S deficiency and CLDs (23) however, Ordal stated that during pregnancy

there is normally a lower level of this protein meaning only mothers that had been diagnosed with a deficiency before or after pregnancy were included (24). Not only did Ordal (24) not present a significant correlation between protein S deficiency and CLDs but this could question whether the result presented by Hunter (23) was reliable. The assays that had statistically significant results presented by Hunter (23) did not match any of the thrombophilia mutations presented by Ordal (24), which decreases the ability for the studies to support each other. The lack of similarities could be down to the small study populations, as the study by Hunter only consisted of 24 mother-child pairs (23) and the study by Ordal only had 19 participants included in the analysis (24). With overall correlations being made to justify an attempt for larger studies in the future, there may be potential for more cross over between the different findings when a larger set of results can be analysed.

CVS has been shown by many authors to cause an increase in risk of terminal transverse limb defects, amongst other problems, with the main theory behind the cause of these deformities being a vascular disruption (25) particularly with early CVS procedures. One study observed the effects of early CVS both when the procedure was done trans-cervically and trans-abdominally, in a cohort who were aware of this 1-2% risk of CLD (26). According to Wapner previous literature showed evidence for an increased risk of CLDs through trans-abdominal CVS so the trans-cervical approach was used for most of cases in this study and resulted in only 1 infant presenting with a CLD although, many spontaneous abortions were recorded as well as mothers opting for abortions (26). The results from this study could show that CVS may be less dangerous when carried out early if a trans-cervical procedure was used. However, due to the small number of participants and known outcomes alongside the overriding evidence that early CVS should be avoided, the quality of these findings is debatable. This research focussed on early CVS for religious reasons thus for the general

population carrying out CVS later and only when necessary is safer and should continue to be the norm.

Amniotic band syndrome is another significant cause of CLDs that affects vascular processes. 181/240 infants presenting with CLDs in the study regarding Amniotic Deformity, Adhesion and Mutilation (ADAM) sequence also presented with either ring constrictions, band cohesions or both compared with only 34/52 in infants that did not present with CLDs, showing an increased frequency between the 3 parts of this condition with the biggest association being between ring constrictions and CLDs at 46.6% (27). This study showed a statistically significant association between skin evidence of constriction rings and the presentation of CLDs giving evidence for amniotic bands to be a cause of CLDs (27). Koskimies looked at constriction band syndrome showing 12% of infants with CLDs having constriction band syndrome (28). This figure is consistent with the prevalence study carried out by McGuirk which also showed 12% of the infants presenting with CLDs having amniotic band syndrome as a cause (2). Another prevalence study conducted by Bedard shared a similar prevalence of 12.7% of infants presenting with CLDs in the study being caused by amniotic band sequence (29). The birth prevalence of constriction band syndrome presented in the study by Koskimies was 1/13,900 (28) whereas the prevalence of ADAM in the previous study was 1/11,200 (27). The two prevalence studies presented similar prevalence rates of amniotic band syndrome in total births with McGuirk showing a prevalence rate of 0.8/10,000 (2) and Bedard presenting 0.7/10,000 (29). These studies although used variations of wording were observing very similar things and the prevalence rates were in the same region building a good basis of evidence for amniotic bands being a cause of CLDs and being easily identified. The wording differences in this set of literature highlights another situation where the lack of consistency on definitions and classifications can make it harder for studies to be compared.

The increased amount of research into the implications of vascular disruptions on CLDs highlights this as a prominent cause of CLDs, stemming from various roots including genetic and environmental factors.

DISCUSSION

The literature surrounding this subject varies in how research is conducted which authors have often highlighted when trying to compare their own studies with other findings. Arguably one of the main issues in this kind of research is the lack of clarity around ~~what kind of~~ classification ~~should be used~~ and who determines~~cides~~ ~~the~~ classification ~~of a CLD~~. Within the literature studied in this review, authors used various classification systems including the ICD codes (10, 14, 16, 22, 30), EUROCAT (12), ~~one study using the~~ IFSSH (28), a combination of different systems (28) or mainly using one system with additions specific to the results observed (24). Other studies did not use a specific classification system ~~and just~~but rather ~~split~~ divided the results into general groupings (17-21, 23, 27) or developed their own groupings based on results or previous literature (11, 29). Having multiple ways of classifying the same CLD and, as discussed in multiple papers the risk of infants being misclassified, can make results less accurate and cause issues when trying to compare results across various studies.

Another factor that can affect study populations and further comparisons of statistics between studies is the inclusion criteria used. One of the main differences between study populations ~~regardless of the exposure being investigated~~ was whether the population included still births and terminations as well as live births. Only seven~~7~~ authors included all live or still born and terminations (2, 10, 12, 16, 18, 24, 29). Two authors included live and still born but not terminations (27, 28) and a large group of authors just included live births (14, 19-23, 30). In

the remaining studies it was not clear what birth outcomes were included (11, 17, 26). The exclusion of terminations for example, could alter prevalence statistics of CLDs, as if severe CLDs are made aware to the mother, usually during the 20 week scan when detailed anatomy is observed (24), some mothers may choose to terminate this pregnancy. ~~Therefore, if~~If terminations were excluded in this case, the prevalence of CLDs would seem lower.

A potential problem with a portion of the research in this area (11, 16, 17, 20, 21, 23, 27), is how some of the information being collected by the researchers is self-reported by the mothers and ~~difficulties in recollection often in the studies included, due to the~~the ~~collection of this~~ information ~~could~~ being collected ~~collected~~arried out ~~months after the birth, possibly making recall harder~~. This could be less reliable compared to factors that can be measured directly especially if information is recalled after a long period. However, some studies discussed this and argue that most of the information needed is simple and often based on very habitual routines ~~and~~ thus ~~may be~~making it less subjected to recall bias. Although, in some cases recall bias may not be the concern, the problem may arise when events are suspected by researchers, such as drug or physical abuse but are ~~strongly~~ denied by the mother in question (23). Issues like this are rare to occur however, could impact results if other exposures could have been the cause of foetal damage.

Many of the studies included were using controls for comparison however, the different studies varied on what kind of controls to use. Two authors (18, 19) stated the controls used in the study were frequency matched but differed on how this matching occurred. Brender matched the controls by year of delivery and region (18) whereas Browne matched the controls by race/ethnicity (19). Others matched controls of the same sex and used the next subsequent live, non-malformed birth within the hospital to select the controls (11, 27). Arpino on the other hand, used two sets of controls by including and excluding infants with malformations already associated with antiepileptic drugs (10). This approach was adopted to

see whether there may be an underestimation of the effect of the drug in question (10). Numerous studies did not use matched controls and instead randomly selected controls with no congenital anomalies (16, 17, 21). Some authors although randomly selected controls, specifically state they were chosen within the same time period of the study (22) and in the same geographical location (20). Having matched controls would make the comparison between case and control groups more effective and can help in reducing bias from confounders as a lot of the studies commented on how trends appeared in certain aspects like race/ethnicity. Race/ethnicity was a variable that odds ratios were often adjusted for in multiple studies to reduce any bias effect.

Across ~~all~~ the papers another question when drawing comparisons is what each author decided was a significant result. In one paper both the p value had to be less than 0.05 and the confidence intervals could not overlap one~~1~~ (10), whereas in other papers it could be either of those options but did not have to be both at the same time (17, 29) with the majority of the authors showing a p value less than 0.05 as significant (11, 12, 14, 19, 22-24, 27, 28). With other papers the confidence intervals and odds ratios were the main presentation of the results without stating the p value (18). Other authors saw a notable result simply if the odds ratio increased over a certain limit, for example 1.5 or 2 fold (20, 21). The definitions of each of those analysis tools are very different and therefore, results should be interpreted in very different ways. This can cause confusion when attempting to compare results from similar studies as what would be a significant result in one study may not be seen as significant to another author meaning that trends may be missed or may not exist at all.

LIMITATIONS

A limitation of this review is that despite all the papers included fitting easily into the separate themes, within ~~each~~ some themes there is a lack of comparison ~~for some topics~~ as only one paper discussed specific aspects such as specific genes or the ~~various~~ environmental factors. This means there is less conviction when it comes to discussing whether trends can be seen across multiple sources of literature, as a limited number of sources ~~have been~~were found ~~through this search~~ regarding certain potential causes.

The broad scope~~ness~~ of the search strategy used~~, although~~ achieves the aim of retrieving information on a wide variety of potential causes~~,~~ but, may lack more specific~~, in depth~~ literature into some ~~of the~~ topics ~~that may be available~~. Similarly, the exclusion of papers regarding known syndromes and other potential causes that were not comparable within one of the themes presented, limits the scope of this review as it could mean ~~some valuable~~ information is missing from discussions. However, it is ~~may be~~ worth pointing out that often the authors removed participants with a known genetic or chromosomal disorder hence why these known syndromes do not present as part of the results ~~in the~~within studies included in this review.

CONCLUSION

~~Looking across the themes discussed, a~~An underlying vascular disruption seems to be the most likely cause of CLDs and therefore a good base for future research with the potential ~~to find more causes of CLDs~~ to fill the gap in the knowledge that can be provided to families now. The more recent research into the environmental factors is attempting to do this.~~, with~~ Despite limited success in finding strong, positive correlations ~~but showing noteworthy~~ preliminary results indicate ~~it may be beneficial to have~~that larger, ~~more thorough~~robust

studies are required ~~in these areas~~ to truly assess whether these impacts should be considered as a regular cause of CLDs.

As well as this, the main ~~difference~~ change that would ~~seem to~~ be beneficial ~~and has been discussed in the literature already is~~ is a consensus on the classification used to define results regarding CLDs and potentially ~~a way of~~ making the methodology regarding controls and study population characteristics ~~more~~ universal. This ~~potentially~~ could be ~~done~~ achieved by standardising procedures for data input. Registries are a useful resource for researchers studying CLDs, and a universal approach to data entry would facilitate the collection of better quality data. ~~making information inputted into registry systems more universal as these were often used by authors to retrieve information about the study populations.~~ Registries such as the "Limb Loss and Prevention Registry" (30) ~~(1)~~ ~~(1)~~ in the USA and the "National Congenital Anomaly and Rare Disease Registration" (NCARDRS) in England (31) are potential sources of data for future studies in this area. ~~This~~ Standardised procedures would allow comparisons to be drawn ~~more easily~~ from larger data sets,~~the data~~ and could ~~increase the~~improve our understanding of trends across this vast topic. ~~to accelerate future research in the right direction to reduce the number of unknown causes of CLDs.~~

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Figure 1

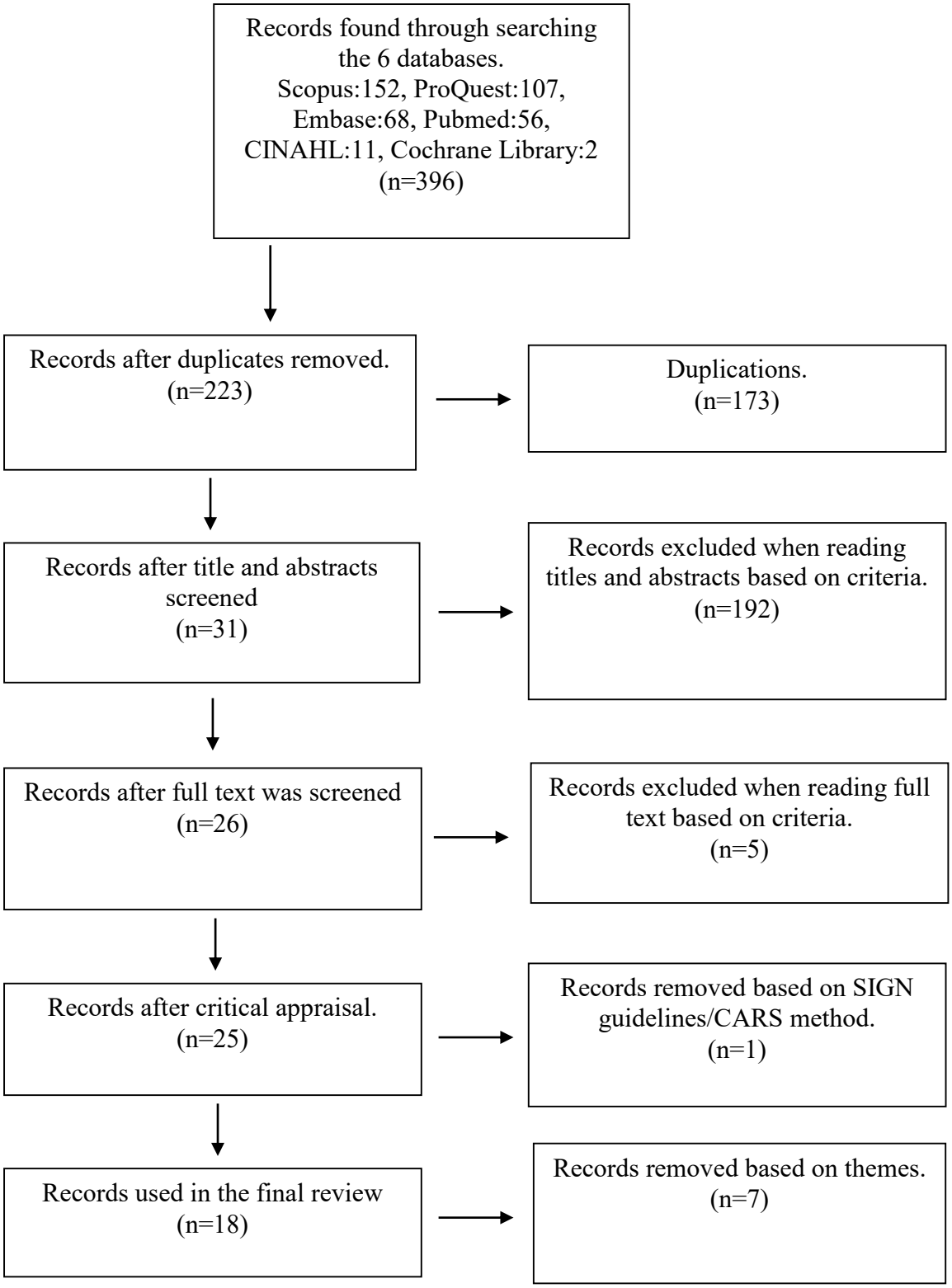

Figure 1 Flow Diagram showing Records in Each Phase of the Search Process

Table 1 Inclusion and Exclusion Criteria Applied

Inclusion Criteria	**Exclusion Criteria**
English language	Animal studies
Human	Reviews
Published in year 2000 onwards	Case reports/series
From peer reviewed journals	Conference abstracts
	Papers discussing classification
	Papers where the results showed that what was tested was either not a cause or were inconclusive for being a cause
	Irrelevant to the question
	Less than 10 participants

Table 2

Table 1 Study Design, Number of Participants and Classification Used with Critical Appraisal Results

Article (Author and Year)	Study Design	SIGN Grading	CARS Evaluation	Number of Participants	Classification system used
Arpino.C 2000 (10)	Case-Control Study	2++	NA	8005	ICD-9
Auffret.M 2016 (11)	Before-After Study	NA	Medium	265	EUROCAT
Bedard.T 2015 (12)	Cross-Sectional Study	NA	Medium	795	Developed own groupings based on Gold (1)
Brender.J 2014 (13)	Case-Control Study	2++	NA	60613 (2046 with limb deficiency) cases, 244927 controls	Split into Transverse and Longitudinal
Browne.M 2012 (14)	Case-Control Study	2++	NA	389 cases, 980 controls	Longitudinal, transverse and intercalary
Carmichael.S 2006 (15)	Case-Control Study	2+	NA	92 cases, 201 controls (chose to only use 201 out of the 437 eligible)	Longitudinal, transverse and amniotic band limb deficiency defects
Carmichael.S 2006 (16)	Case-Control Study	2-	NA	96 cases, 437 controls	Longitudinal, transverse and amniotic band limb deficiency defects

Carmichael.S 2016 (17)	Case-Control Study	2-	NA	467 cases, 785 controls	Transverse limb deficiency only
Carter.T 2017 (18)	Cross-Sectional Study	NA	Medium	25	ICD codes specific to SHSF
Choi.G 2019 (19)	Case-Control Study	2+	NA	615 cases, 5701 controls	ICD-9
Hunter.A 2000 (20)	Before-After Study	NA	Low	24 mother-child pairs	Terminal limb defects only
Koskimies. E 2015 (21)	Before-After Study	NA	Medium	419 upper limb, 171 lower limb, only 71 cases of amniotic band	ICD-9 then IFSSH upper limb and EUROCAT lower limb
McGuirk.C 2015 (2)	Cross-Sectional Study	NA	Medium	110	ICD-9
Ordal.L 2016 (22)	Before-After Study	NA	Low	19 included in analysis	System developed by Gold (1) based on ICD- 9 with some additions
Orioli.I 2000 (23)	Case-Control Study	2+	NA	4673 cases, 4980 controls, only 57 exposed to misoprostol	Groups based on findings in previous literature

Orioli.I 2003 (24)	Cross-Sectional Study	NA	Medium	292 phenotypic analysis	Split into limb reductions and syndactyly
Vianna.S 2015 (25)	Cross-Sectional Study	NA	Medium	2802 with limb reduction defects	ICD-10
Wapner.R 2002 (26)	Before-After Study	NA	Low	82	Not stated

Table 3

Table 1 Causes and Significant Results

Article (Author and Year)	Potential Cause Investigated	Odds Ratio	Confidence intervals for notable results (95%)	P Values
Arpino.C 2000 (10)	Antiepileptic drugs	3.45 (valproic acid) 5.08 (monotherapy valproic acid)	1.3-9.1 (valproic acid), 1.8-14.1 (monotherapy valproic acid)	p=0.008 (valproic acid), p<0.001 (monotherapy valproic acid)
Auffret.M 2016 (11)	Misoprostol drug	5.5 (major malformations including limb reduction)	2.65-9.82 (major malformations including limb reduction)	Not presented
Bedard.T 2015 (12)	Range	0.7 (amniotic band)	0.6-0.9 (amniotic band)	Not presented
Brender.J 2014 (13)	Chlorinated Solvent Emissions	1.21 (perchloroethylene)	1.01-1.45 (perchloroethylene)	Not presented for limb defects
Browne.M 2012 (14)	Genetic variants (20 SNPs)	1.49 (FGF10 rs10805683 heterozygous), 1.88 (FGF10 rs10805683 homozygous), 1.47 (FGF10 rs13170645 heterozygous) 1.83 (FGF10 rs13170645 homozygous) 1.66 (EN1 rs893574 heterozygous)	1.16-1.92 (FGF10 rs10805683 heterozygous), 1.13-3.12 (FGF10 rs10805683 homozygous), 1.10-1.79 (FGF10 rs13170645 heterozygous), 1.30-2.59 (FGF10 rs13170645 homozygous), 1.16-2.38 (EN1 rs893574 heterozygous)	P=0 FGF10 rs10805683 heterozygous), p=0.01508 (FGF10 rs10805683 homozygous), p=0.00890 (FGF10 rs13170645 heterozygous), p=0.00060 (FGF10 rs13170645 homozygous), p=0.00590 (EN1 rs893574 heterozygous)

Carmichael.S 2006 (15)	Genetic variants	2.3 (NAT1 T1088A homozygote), 1.6 (NAT1 C1095A homozygote), 1.7 (NOS3 A(-922)G heterozygote) 2.1 (NOS3 A(-922)G homozygote), 1.8 (NOS3 G894T heterozygote)	1.0-5.2 (NAT1 T1088A homozygote), 0.7-3.3 (NAT1 C1095A homozygote), 1.0-3.0 (NOS3 A(-922)G heterozygote), 1.0-4.7 (NOS3 A(-922)G homozygote), 1.1-3.1 (NOS3 G894T heterozygote)	p<0.001 (NOS3)
Carmichael.S 2006 (16)	Genetic variants (29 SNPs)	2.5 (F5 Arg506Gln heterozygosity), 2.1 (TNF(-376)G>A heterozygosity), 4 (NPPA2238T>C homozygosity)	1.0, 6.5 (F5 Arg506Gln heterozygosity), 0.7,6.2 (TNF(-376)G>A heterozygosity), 1.1,15.4 (NPPA2238T>C homozygosity)	Not presented
Carmichael.S 2016 (17)	Pesticides	2.5 (dichlorophenoxy acid/ester), 2.1 (petroleum derivative), 2.3 (triazine), 2.2 (oxyfluorfen), 3.9 (copper sulphate), 3.8 (oryzalin), 2.9 (imidacloprid), 2.3 (petroleum oil), 2.6 (oxyethylene)	1.1-6.0 (dichlorophenoxy acid/ester), 1.1-3.9 (petroleum derivative), 1.1-5.0 (triazine) 1.1-4.3 (oxyfluorfen), 1.5-10.1 (copper sulphate), 1.4-9.8 (oryzalin), 1.1-7.4 (imidacloprid), 1.1-5.0 (petroleum oil), 1.2-5.7 (oxyethylene)	Not presented

Carter.T 2017 (18)	Copy Number Variants	Not presented	Not presented	P=0.011 (10q24 duplication, 17p13.3 duplication and 17q25 deletion)
Choi.G 2019 (19)	Air Pollution	1.11 (CO), 1.10 (NO2), 1.10 (SO2)	0.99-1.24 (CO), 0.96-1.26 (NO2), 0.97-1.25 (SO2)	Not presented
Hunter.A 2000 (20)	Thrombophilia	Not presented	Not presented	P<0.004 (Protein S), P=0.027 (anticardiolipin G), P<0.001 (heterozygote MTHFR)
Koskimies. E 2015 (21)	Amniotic Band Syndrome	Not presented	Not presented	Only provide P values for birth weight
McGuirk.C 2015 (2)	Range	Not presented	Not presented	Not presented
Ordal.L 2016 (22)	Thrombophilia	Not presented	Not presented	P<0.01 (inherited thrombophilia in study population compared to general population)
Orioli.I 2000 (23)	Misoprostol drug	12.04 (terminal transverse limb reductions), 40.72 (limb constriction ring or skin scars)	3.42-41.12 (terminal transverse limb reductions), 10.83-153.12 (limb constriction ring or skin scars)	p=0.003 (terminal transverse limb reductions), p=0.0001 (limb constriction ring or skin scars)

Orioli.I 2003 (24)	ADAM Sequence	9.6 (skin evidence of constriction band and limb reduction)	Not presented	P<0.01 (skin evidence of constriction band and limb reduction)
Vianna.S 2015 (27)	Thalidomide drug	1.279 (limb reduction defect)	1.268-1.290 (limb reduction defects)	p<0.001 (limb reduction defects)
Wapner.R 2002 (26)	CVS	Not presented in relation to CLDs	Not presented in relation to CLDs	Not presented in relation to CLDs