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Etiological diagnosis in limb reduction defects and the number of affected limbs: A population-based study in the Northern Netherlands

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Abstract

Limb reduction defects (LRDs) that affect multiple limbs are considered to be more often heritable, but only few studies have substantiated this. We aimed to investigate if an etiological diagnosis (genetic disorder or clinically recognizable disorder) is more likely to be made when multiple limbs are affected compared to when only one limb is affected. We used data from EUROCAT Northern Netherlands and included 391 fetuses and children with LRDs born in 1981–2017. Cases were classified as having a transverse, longitudinal (preaxial/postaxial/central/mixed), intercalary, or complex LRD of one or more limbs and as having an isolated LRD or multiple congenital anomalies (MCA). We calculated the probability of obtaining an etiological diagnosis in cases with multiple affected limbs versus one affected limb using relative risk (RR) scores and Fisher's exact test. We showed that an etiological diagnosis was made three times more often when an LRD occurred in multiple limbs compared to when it occurred in one limb (RR 2.9, 95% CI 2.2–3.8, $p < 0.001$). No genetic disorders were found in isolated cases with only one affected limb, whereas a genetic disorder was identified in 16% of MCA cases with one affected limb. A clinically recognizable disorder was found in 47% of MCA cases with one affected limb. Genetic counseling rates were similar. We conclude that reduction defects of multiple limbs are indeed more often heritable. Genetic testing seems less useful in isolated cases with one affected limb, but is warranted in MCA cases with one affected limb.

KEYWORDS

bilateral, etiology, genetic, limb deficiency, limb malformation, unilateral

1 | BACKGROUND

Limb reduction defects (LRDs) are congenital defects with absence or severe hypoplasia of skeletal structures of the limb (Stoll, Mastroiacovo, de Wals, Weatherall, & Garne, 2004). Various causes have been identified for LRD, including disorders with Mendelian inheritance, chromosomal abnormalities, known associations, and sequences, teratogenic exposures and presumed vascular disruption defects. However, in many cases no cause can be identified (Firth & Hurst, 2017). Depending on which skeletal structures are affected,

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LRDs can be classified into different subcategories: transverse, longitudinal (preaxial, postaxial, central, or mixed), intercalary or complex. Although several classification systems have been described, there is no consensus about which is best (Lowry & Bedard, 2016).

In 2019, the media reported an unusual high number of children being born with congenital hand anomalies in France and Germany (Gant, 2019; Sankt Marien-Hospital Buer, 2019). In both countries, the defects were classified as isolated transverse LRDs of one arm. It is still unclear whether these were true clusters and the cause also remains unknown after investigations carried out in France (Benachi et al., 2019).

LRDs of one limb less often have a genetic cause, compared to LRDs of multiple limbs (Cobben, Hiemstra, & Robinson, 1994; Firth & Hurst, 2017; Harper, 2010), but only few studies have substantiated this. Previous epidemiological studies regarding LRDs have reported prevalence rates (general and per subcategory), the affected side (left: right ratio), symmetry, associated malformations, and diagnoses (Alberto, Barbero, Liascovich, Bidondo, & Groisman, 2020; Bedard, Lowry, Sibbald, Crawford, & Kiefer, 2018; Bedard, Lowry, Sibbald, Kiefer, & Metcalfe, 2015; Evans, Vitez, & Czeizel, 1994; Klungsoyr et al., 2019; Koskimies, Lindfors, Gissler, Peltonen, & Nietosvaara, 2011; Vasluian et al., 2013). To our knowledge there has only been one retrospective cohort study, which analyzed diagnosis rates in patients with uni- versus bilateral preaxial longitudinal defects of the upper limb (n = 119) (James, Green, McCarroll, & Manske, 2004). The authors reported that the probability of diagnosing a syndrome in patients with bilateral involvement was twice as high (relative risk, RR 2.3) as that for patients with unilateral involvement.

Since LRDs are being diagnosed prenatally at greater rates than ever before, information about the associated anomalies and underlying syndromes may affect the pregnancy outcome. Stoll et al. performed a study with data from 20 congenital anomaly registries from 12 European countries and found that termination of pregnancy for fetal anomaly (TOPFA) was performed in 9.4% (13/138) of cases with an isolated LRD, in 39.6% (23/58) of cases with multiple congenital anomalies (MCA, including an LRD) without an etiological diagnosis, and in 50.0% (27/54) of cases with a recognized syndrome (Stoll et al., 2000). This study did not provide data on the number of the affected limbs, a factor which could also influence the choice to terminate a pregnancy, particularly if defects of multiple limbs are more often syndromic. Clearly, accurate knowledge about the number of affected limbs according to the etiology is important in a prenatal setting, but this knowledge is valuable postnatally as well. If an etiological diagnosis is (almost) never obtained in patients with a non-familial isolated LRD of one limb, physicians could refrain from genetic testing in these cases, which would reduce healthcare costs.

The aim of this study was to investigate if an etiological diagnosis is obtained more often when multiple limbs are affected compared to when there is an LRD of just one limb. To assess this, we gathered data about these cases from the European Concerted Action on Congenital Anomalies and Twins Northern Netherlands (EUROCAT-NNL) for the period 1981–2017, resulting in the most detailed dataset on congenital LRDs to date. Within this dataset, we differentiated between genetic disorders and clinically recognizable disorders and between cases with isolated LRD and MCA. Analyses were performed for the total cohort and for the different LRD subcategories. We also examined the rates of genetic counseling and testing.

2 | METHODS

2.1 | Ethical considerations

Ethics approval for this study was not necessary because anonymous data were used for the analyses and parents had given consent to use their data in studies concerning the etiology of congenital anomalies.

2.2 | Data resource

Cases were selected from the database of EUROCAT-NNL. This population-based congenital anomaly registry has been monitoring births from an expanding area since 1981 and has covered the entire provinces of Groningen, Drenthe, and Friesland since 1989. The total number of births in the EUROCAT-NNL region has fluctuated between 7,500 (early years) and 20,000 (around 2000). Currently, around 15,000 births per year are covered. In addition to live births, EUROCAT-NNL also registers stillbirths (gestational age 24 weeks or more), miscarriages (gestational age less than 24 weeks), and TOPFAs (irrespective of gestational age). Since 1992, cases are only registered if parents give written informed consent, and the consent rate was around 70–80%, independent of the type of congenital anomaly. Since 2010, Eurocat NNL can also register data of parents who do not respond to repeated requests to participate, these are called “non-responders”. Parents are informed about this procedure by letter, in compliance with applicable guidelines (code of conduct by the Dutch biomedical research community, 2004). This procedure was initiated to allow for more complete registration of prevalence data. Complete information on the type of congenital anomalies and genetic testing is registered, but only limited data regarding risk factors is recorded. Therefore, all non-responder cases were also included in this study. From 2010 onwards, approximately 70% of parents give consent, 23–25% are non-responders and 5–7% refuse registration. EUROCAT-NNL personnel search medical files from various sources, including genetics and pathology reports, to ensure accurate case registration. Since 1997, parents have been asked to fill out a questionnaire that includes questions about the pregnancy, exposure to risk factors, and family history. Case finding, coding, and classification of congenital anomalies are performed as described previously (EUROCAT, 2013; Greenlees et al., 2011). Congenital anomalies are coded according to the International Classification of Diseases version 9 (ICD9) with British Pediatric Association (BPA) extension codes until birth year 2001 and the ICD10-BPA thereafter. Cases are updated in the database when new information becomes available until the child reaches the age of 11 years.

2.3 | Study population

For this retrospective cohort study, we used EUROCAT-NNL data of all cases with birth years between 1981 and 2017. Cases were identified by the ICD codes for LRDs: ICD9 7552–7554 and ICD10 Q71–73 and Q8725. This was supplemented with ICD9 codes for split hand or split foot (755511–755514 and 755612–755615) and monodactyly of hand or foot (755515–755518 and 755616–755619) because these congenital anomalies were not included in the ICD9 codes for LRDs. Cases with sirenomelia ($n < 5$) were excluded from the study, because in the EUROCAT guide 3 (Stoll et al., 2004), sirenomelia is not classified as an LRD. In total, we identified 404 cases with LRD codes. In addition, we checked whether cases with the following syndromes had an LRD that was not coded separately: Poland syndrome ($n = 21$), Holt-Oram syndrome ($n = 9$), Okihiro syndrome ($n < 5$), Cornelia de Lange syndrome ($n = 14$), Fanconi anemia ($n = 9$), oculoauriculovertebral spectrum (OAVS, $n = 44$), amniotic band syndrome/limb body wall complex (ABS/LBWC, $n = 60$), Vertebral, Anorectal, Cardiac, Tracheo-Esophageal, Renal and Limb anomalies (VACTERL association, $n = 73$), Adams-Oliver syndrome ($n < 5$), Ulnar-mammary syndrome (UMS, $n < 5$), Roberts syndrome ($n < 5$), and oromandibular-limb-hypogenesis syndrome ($n = 0$). This led to the identification of five additional cases with an LRD (OAVS, LBWC, VACTERL, and UMS). When a diagnosis was made in fewer than five cases, the exact number of cases is not reported due to confidentiality problems with low numbers.

We reviewed whether the cases had an LRD as described in EUROCAT guide 3 (Stoll et al., 2004) and the latest updates made by the EUROCAT coding and classification committee. When relevant information was missing (e.g., number of affected limbs, classification of the LRD), we checked the medical files and the paper archive of EUROCAT-NNL for additional information. In cases with a prenatal diagnosis, the LRD was confirmed postnatally. We also checked if cases were ever counseled by a clinical geneticist and if the etiological diagnosis could be updated. We excluded cases that did not fit the definition of an LRD ($n = 14$), cases where the description was too general to determine if it was an LRD ($n = 2$), incorrectly coded cases ($n = 1$), and cases without a known number of affected limbs ($n = 1$). This resulted in a study population of 391 cases.

2.4 | Classification

We used the EUROCAT classification for the classification of LRDs (Stoll et al., 2004). As the EUROCAT definition of longitudinal central defects only contains a split hand-split foot group, we broadened this group to include all longitudinal central defects. Because the EUROCAT classification of the longitudinal defects lacks information about absence or hypoplasia of digits other than digits 1 and 5, we used Bedard et al.'s definition for a more detailed description (Bedard et al., 2015). The classification used for this study (a combination of the classification of EUROCAT and Bedard et al.) is described in Table 1. All cases were classified by KL (clinical geneticist in training) and difficult-to-classify cases were discussed with two clinical geneticists (PR and JEHB).

2.5 | Definitions

For all cases, we studied the number of affected limbs, whether an etiological diagnosis had been obtained in the clinic, and if the LRD occurred in isolation or was part of MCA. Etiological diagnoses were divided into (a) genetic disorders and (b) clinically recognizable disorders (e.g., VACTERL association, ABS, LBWC, femur-fibula-ulna

TABLE 1 Classification of limb reduction defects in this study

Category	Description
Transverse	Absence of distal structure of the limb with proximal structures more or less normal, including amelia
Longitudinal preaxial	Absence or severe hypoplasia of preaxial structures of the limb: Thumb, first metacarpal, radius, hallux, first metatarsal, tibia. In addition, the second digit of the hand or foot may be involved[a]
Longitudinal postaxial	Absence or severe hypoplasia of postaxial structures of the limb: Little finger, fifth metacarpal, ulna, fifth toe, fifth metatarsal, fibula. In addition, the fourth digit of the hand or foot may be involved[a]
Longitudinal central	Absence or severe hypoplasia of central digits with or without absence or severe hypoplasia of central metacarpal/ metatarsal bones, including typical and atypical types of split hand-split foot, monodactyly Absence or severe hypoplasia of digits 2 through 4, digits 3 and 4, digits 2 and 3, or digits 2, 3 or 4 alone[a]
Longitudinal mixed	Absence or severe hypoplasia involving more than one axis, for example, absent thumb and absent digit 5[a]
Longitudinal unknown	Longitudinal limb reduction defect without sufficient information to classify it into any of the other longitudinal categories[a]
Intercalary	Absence or severe hypoplasia of a proximal-intercalary part of a limb when the distal structures (the digits), whether normal or malformed, are present, that is, absence of humerus and/or radius and ulna with a (near) normal hand or absence of femur and/or tibia and fibula with a (near) normal foot
Complex	Different types of limb reduction defects of multiple limbs
Unknown	Limb reduction defect without sufficient information to classify into any of the above categories[a]

[a]Added from Bedard et al. (2015).

complex, or valproate embryopathy). Genetic disorders include both molecularly confirmed diagnoses, where there were abnormal findings on molecular or cytogenetic testing requested by pediatricians or clinical geneticists, and clinical diagnoses, where the clinical geneticist diagnosed a genetic disorder based on the phenotype or a positive family history. Clinically recognizable disorders were diagnosed by the treating physician in the clinic, mainly clinical geneticists and pediatricians. Isolated LRDs were defined as reduction defects without major structural anomalies outside the musculoskeletal system. MCA cases had an LRD and at least one major congenital anomaly outside the musculoskeletal system.

2.6 | Data analyses

The characteristics of several subgroups of cases were described with frequencies and percentages. We examined the number of affected limbs and the probability of obtaining an etiological diagnosis using relative risk (RR) scores and Fisher's exact test. The RR score indicates if an etiological diagnosis is made more or less often when multiple limbs are affected as compared to when one limb was affected.

Subgroup analysis was performed of the transverse, longitudinal preaxial, longitudinal postaxial, and longitudinal central defects. The other subgroups contained too few cases (defined as $n < 10$) for statistical analysis (longitudinal mixed [$n = 6$] and intercalary [$n = 8$]), or the analysis had no clinical relevance (longitudinal unknown and unknown). Separate analyses were performed for the cases with isolated LRDs and the MCA cases. Due to low numbers, we did not calculate RRs for the different LRD subcategories in these groups.

A p-value below 0.05 was considered to be statistically significant. Data were analyzed by using SPSS version 23.

3 | RESULTS

3.1 | Characteristics of cases with limb reduction defects

The total study population consisted of 391 cases. The prevalence of LRDs in the Northern Netherlands from 1981 to 2017 was estimated at 6.4/10,000 births. Case characteristics are summarized in Supplementary Table 1.

The majority of cases were live born (83.4%) and had one affected limb (59.9%). The upper limb was most often affected (75.2%). Longitudinal LRDs were most prevalent (59.8%), followed by transverse LRDs (27.6%). Most of the cases had an isolated LRD (63.4%). The LRD was diagnosed prenatally in 16.5% of the isolated cases, whereas a congenital anomaly was seen prenatally in 51.0% of the MCA cases ($p < 0.001$, data not shown). Overall, an etiological diagnosis was obtained in 148 (37.9%) cases: 105 MCA cases and 43 cases with an isolated LRD. Table 2 provides an overview of the most common diagnoses per LRD subcategory.

3.2 | Affected number of limbs and probability of obtaining an etiological diagnosis

Table 3 shows the probability that an etiological diagnosis was obtained in the clinic in cases with multiple affected limbs versus one affected limb (RRs and p-values). Analysis of all LRD subcategories together showed a higher probability of obtaining an etiological diagnosis when multiple limbs were affected (RR = 2.9, 95% CI 2.2–3.8, $p < 0.001$). The probability of diagnosing a genetic disorder is much higher when multiple limbs were affected (RR = 7.2, 95% CI 3.7–13.7, $p < 0.001$). The probability of diagnosing a clinically recognizable disorder was almost twice as high in cases with multiple affected limbs compared to those with only one affected limb (RR = 1.9, 95% CI 1.3–2.7, $p = 0.001$).

In cases with isolated LRDs, the probability of obtaining an etiological diagnosis was seven times higher for cases with multiple affected limbs than for those with one affected limb (RR = 7.2, 95% CI 3.7–13.8, $p < 0.001$). Most diagnoses were clinically recognizable disorders and were made in cases with complex LRDs (Supplementary Table 2). There were no genetic disorders diagnosed in the 170 isolated cases with only one affected limb (128 of these 170 cases were evaluated by a geneticist).

TABLE 2 Diagnoses ($n > 1$) made per subcategory of reduction defect

	Genetic Disorders	Clinically Recognizable Disorders
Transverse		ABS ($n = 7$) LBWC ($n = 6$)
Longitudinal preaxial	Cytogenetic disorder ($n = 14$; 11 with trisomy 18) Holt-Oram syndrome ($n = 5$) Fanconi anemia ($n < 5$) Okihiro syndrome ($n < 5$)	VACTERL association ($n = 17$) Valproate embryopathy ($n < 5$) Poland syndrome ($n < 5$) OAVS ($n < 5$)
Longitudinal postaxial	Cornelia de Lange syndrome ($n < 5$)	FFU complex ($n = 10$) ABS ($n < 5$) LBWC ($n < 5$)
Longitudinal central	Cytogenetic disorder ($n = 5$) Cornelia de Lange syndrome ($n < 5$) Split hand-split foot malformation ($n = 7$)	
Complex	Cornelia de Lange syndrome ($n < 5$)	ABS ($n = 17$) LBWC ($n < 5$)

Note: $n < 5$, when a diagnosis was made in fewer than five cases, the exact number of cases is not reported due to confidentiality problems with low numbers.

Abbreviations: ABS, amniotic band syndrome; FFU complex, Femur-Fibula-Ulna complex; LBWC, limb body wall complex; OAVS, oculoauriculovertebral spectrum; VACTERL association, Vertebral, Anorectal, Cardiac, Tracheo-Esophageal, Renal and Limb anomalies.

TABLE 3 Number of diagnoses in total and per subcategory of reduction defect

All cases (Isolated and MCA)	Total Number of Cases		All Diagnoses						Genetic Disorders						Clinically Recognizable Disorders					
	1	> 1	1		>1				1		>1				1		>1			
Affected number of limbs	*n*	*n*	*n*	%	*n*	%	RR (95% CI)	*p*-value	*n*	%	*n*	%	RR (95% CI)	*p*-value	*n*	%	*n*	%	RR (95% CI)	*p*-value
Transverse	93	15	11	11.8	9	60.0	5.1 (2.5–10.1)	**<0.001**	0	0.0	4	26.7			11	11.8	5	33.3	2.8 (1.1–7.0)	**0.045**
Longitudinal																				
Preaxial	35	38	18	51.4	33	86.8	1.7 (1.2–2.4)	**0.002**	4	11.4	23	60.5	5.3 (2.0–13.8)	**<0.001**	14	40.0	10	26.3	0.7 (0.3–1.3)	0.319
Postaxial	41	24	11	26.8	12	50.0	1.9 (1.0–3.6)	0.068	1	2.4	4	16.7	6.8 (0.8–57.7)	0.058	10	24.4	8	33.3	1.4 (0.6–3.0)	0.567
Central	40	26	3	7.5	11	42.3	5.6 (1.7–18.3)	**0.001**	2	5.0	10	38.5	7.7 (1.8–32.3)	**0.001**	1	2.5	1	3.8	1.5 (0.1–23.5)	1.000
Mixed	3	3	2	66.7	3	100	–	–	0	0.0	3	100	–	–	2	66.7	0	0.0	–	–
Unknown	13	11	5	38.5	5	45.5	–	–	3	23.1	1	9.1	–	–	2	15.4	4	36.4	–	–
Intercalary	8	0	0	0.0	-	-	–	–	0	0.0	-	-	–	–	0	0.0	-	-	–	–
Complex	–	39	–	–	24	61.5	–	–	–	–	3	7.7	–	–	–	–	21	53.8	–	–
Unknown	1	1	0	0.0	1	100	–	–	0	0.0	0	0.0	–	–	0	0.0	1	100	–	–
Total	234	157	50	21.4	98	62.4	2.9 (2.2–3.8)	**<0.001**	10	4.3	48	30.6	7.2 (3.7–13.7)	**<0.001**	40	17.1	50	31.8	1.9 (1.3–2.7)	**0.001**
Isolated cases																				
Total	170	78	10	5.9	33	42.3	7.2 (3.7–13.8)	**<0.001**	0	0	11	14.1	–	–	10	5.9	22	28.2	4.8 (2.4–9.6)	**<0.001**
MCA cases																				
Total	64	79	40	62.5	65	82.3	1.3 (1.1–1.6)	**0.013**	10	15.6	37	46.8	3.0 (1.6–5.5)	**<0.001**	30	46.9	28	35.4	0.8 (0.5–1.1)	0.176

Note: p-Values <0.05 are shown in bold.

Data are grouped by the number of affected limbs, the type of diagnosis and isolated or multiple congenital anomaly (MCA) cases, with corresponding relative risk scores and *p*-values for the differences between the number of affected limbs.

Subgroup analysis was performed of the transverse, longitudinal preaxial, longitudinal postaxial and longitudinal central categories.

Number of cases and diagnoses per subcategory for the isolated and MCA cases are shown in Supplementary Table 1.

Abbreviations: MCA, multiple congenital anomalies; *n*, number; RR, relative risk score, probability of obtaining an etiological diagnosis in cases with more than one affected limb compared to cases with one affected limb.

In MCA cases, the probability of obtaining an etiological diagnosis was 1.3 times higher in cases with multiple affected limbs than in cases with one affected limb (RR = 1.3, 95% CI 1.1–1.6, p = 0.013), but the probability of diagnosing a genetic disorder was three times higher (RR = 3.0, 95% CI 1.6–5.5, p < 0.001).

When subgroup analysis was performed for the different LRD subcategories, the probability of obtaining any kind of etiological diagnosis in cases with longitudinal postaxial defects of multiple limbs versus one affected limb was similar. In cases with longitudinal preaxial defects, the probability of diagnosing a genetic disorder was five times higher in cases with multiple affected limbs compared to those with one affected limb (RR 5.3, 95% CI 2.0–13.8, p < 0.001). In cases with longitudinal central defects, the probability of diagnosing a genetic disorder was seven times higher in cases with multiple affected limbs versus one affected limb (RR 7.7, 95% CI 1.8–32.3, p = 0.001). In cases with a transverse defect the probability of diagnosing a clinically recognizable disorder was almost three times higher in cases with multiple affected limbs versus one affected limb (RR 2.8, CI 95% 1.1–7.0, p = 0.045).

3.3 | Genetic counseling and testing

The probability of obtaining an etiological diagnosis might also depend on whether the case was evaluated by a clinical geneticist and on whether genetic testing was performed. Most cases in this study were counseled by a clinical geneticist (77.7%), and the counseling rates did not differ between cases with one affected limb and cases with multiple affected limbs (77.8 and 77.7%, respectively). The counseling rate did vary per LRD subcategory, from 63.4% in cases with a longitudinal postaxial defect of one limb to 91.4% in cases with a longitudinal preaxial defect of one limb. The counseling rates for isolated and MCA cases were similar.

Once counseling had taken place, genetic testing was performed in half of the cases (52.3%). The genetic testing rate was significantly higher in cases with multiple affected limbs (73.0%) compared to the cases with one affected limb (38.5%, p < 0.001). It was also dependent on the LRD subcategory, from 15.5% in cases with a transverse defect of one limb to 100% in cases with a longitudinal preaxial LRD of multiple limbs. Genetic testing was performed least often in isolated cases with a transverse defect of one limb (9.5%). The genetic testing rate in MCA cases was much higher (85.5%), and there was no significant difference in genetic testing rates between the numbers of affected limbs (p = 0.300).

Table 4 shows the types and yields of genetic tests that were performed in the cases that were counseled by a geneticist. Karyotyping was the most-used type of test (68.6%), followed by array analysis (44.0%) and sequencing of single genes (28.9%). Mitomycin C test was only performed in cases with longitudinal preaxial LRDs, and it detected all cases with Fanconi anemia included in this study. Next-generation sequencing panel analysis or whole exome sequencing was performed in a minority of the cases (9/159, 5.7%). The genetic tests led to a molecularly confirmed diagnosis in 22.0% of the cases. The yield was significantly higher when multiple limbs were affected versus when one limb was affected (30.3% vs 11.4%, p = 0.006), with the highest yields for longitudinal preaxial defects (48.4%) and longitudinal central defects (38.5%) when multiple limbs were affected. A list of all diagnoses that were made in this study including the involved genes, is given in Supplementary Table 3.

4 | DISCUSSION

4.1 | Main findings

In this study, we show that an etiological diagnosis was obtained three times more often when an LRD occurred in multiple limbs than when it occurred in only one limb. Particularly of note, no genetic disorders were diagnosed in isolated cases with only one affected limb, that is, the yield of genetic testing was limited in isolated LRD cases with one affected limb. However, this result should be regarded with caution, as less genetic testing was performed in these cases. In contrast, a genetic disorder was found in 16% of MCA cases with one affected limb and a clinically recognizable disorder was identified in 47% of these cases. This finding shows that MCA cases with one affected limb should undergo genetic testing and emphasizes the importance of considering a clinically recognizable disorder in these cases. In our study, the highest yield of genetic testing was found for longitudinal preaxial (48%) and longitudinal central (38%) defects of multiple limbs.

4.2 | Strengths and limitations

The strengths of this study include the use of population-based registry data of EUROCAT-NNL. First, there is no bias in including patients, as all patients with an LRD who lived in the registration area at the time of birth were included. Second, the data are of high quality because we could review the medical files when relevant information was missing (e.g., the number of affected limbs, classification of the LRD). In addition, we updated the genetic tests that were done and the etiological diagnosis of all cases. As far as we know, this produced the largest cohort to date for which there is detailed data on LRDs and genetic test results. The recent LRD clusters in Germany and France (Gant, 2019; Sankt Marien-Hospital Buer, 2019) have emphasized the importance of continuous monitoring of rare anomalies like LRDs by registries. Our study further emphasizes the need to separate cases into isolated LRD cases and MCA cases in order to determine etiology. Another strength of this study comes from the fact that the genetic counseling rates were similar between cases with one affected limb and cases with multiple affected limbs. If evaluation by a clinical geneticist increases the chance of obtaining a diagnosis, this did not bias our results. The similar counseling rates we observe may be a result of the involvement of the clinical geneticist in the multidisciplinary outpatient clinic for Congenital Upper Limb Abnormalities at the University Medical Center Groningen, the only university

hospital in the EUROCAT-NNL region. This outpatient clinic was established in 1987 and is visited by patients with abnormalities of the upper limb.

This study also has some limitations. First, there is no consensus about the classification of LRD, which makes it difficult to compare data from our study to data from previously published studies (Lowry & Bedard, 2016). Second, we studied a retrospective clinical cohort, which means that, while some patients were evaluated by a geneticist and underwent extensive genetic testing, others underwent limited investigations. This might have biased the data because we showed that the frequency with which clinical geneticists performed genetic testing was higher in cases with multiple affected limbs, especially in cases with isolated LRDs. The genetic testing frequency also differed between LRD subcategories, with a high frequency for longitudinal preaxial defects, but a much lower frequency for transverse defects. The likelihood of diagnosing a genetic disorder in a patient also depends on their birth year; karyotyping was the only test available in the early years and next-generation sequencing was only broadly implemented after 2013. Third, informed consent was obligatory from 1992 onwards for inclusion of cases in the registry and therefore not all LRD cases could be registered. However, since 2010 data of non-responders could be registered, leading to a much lower percentage of cases that could not be registered (5–7% of parents refuse registration). Fourth, EUROCAT-NNL can only update the diagnosis when cases visit a hospital in the EUROCAT-NNL region. If a case moves outside the EUROCAT-NNL catchment region, we are no longer able to access their medical information. It is therefore possible that an etiological diagnosis was obtained after initial registration, but that we are not aware of it. Finally, the inclusion of the cases in our study was based on the ICD codes for LRD. It is possible that in some cases with an etiological diagnosis, the LRD was not coded separately and therefore these cases would not be included in the study. For this reason, we checked all cases registered with syndromes and associations in which LRDs are common, which led to the identification of five additional cases. Nonetheless, some cases might still have been missed, but we expect this number to be small.

4.3 | Interpretation

Our finding that an etiological diagnosis was obtained three times more often when multiple limbs were affected compared to when only one limb was affected is in agreement with the recommendations in genetic textbooks to consider a genetic diagnosis when multiple limbs are affected (Firth & Hurst, 2017; Harper, 2010). Our finding is also in agreement with a previous study that obtained an etiological diagnosis 2.3 times more often in cases with a longitudinal preaxial defect of both upper limbs versus one affected limb (James et al., 2004). In the cases with an etiological diagnosis, LRDs of multiple limbs were twice as common as LRDs of one limb in our study. Within the group of genetic disorders, LRDs of multiple limbs were 4.8 times as common. These results are in agreement with three previous studies (Furniss et al., 2009; James et al., 2004; Stoll et al., 2001). Stoll et al. (2001) described 149 syndromic cases (excluding associations and sequences, for example, VACTERL and Poland syndrome) out of a retrospective cohort of 815 infants with LRDs and one or more major unrelated birth defects. The laterality was known for half of these cases. In the group of syndromic cases, bilateral defects were 2.3 times as common as unilateral defects. In a retrospective cohort of 119 patients with a longitudinal pre-axial LRD of the upper limb, James et al. (2004) found 55 syndromic cases (including VACTERL). In this group of syndromic cases, bilateral defects were 3.6 times as common as unilateral defects. Furniss et al. (Furniss et al., 2009) performed a prospective cohort study of 202 individuals with congenital limb malformations, including 29 cases with LRDs (laterality not described). They obtained karyotypes and screened 13 genes, amongst them *SALL1*, *SALL4*, and *TBX5*, which are known to be involved in LRD syndromes. In six cases with an LRD, a genetic cause was found, and all these cases had bilateral defects. We also found a significantly higher yield of genetic tests after counseling by a clinical geneticist when multiple limbs were affected ($p = 0.006$).

The difference in the number of diagnoses between cases with one affected limb and multiple affected limbs is greater in isolated cases than MCA cases, as illustrated by higher RRs in the isolated group. In isolated cases with one affected limb ($n = 170$), only 10 diagnoses were obtained, and all of them were clinically recognizable disorders (ABS, Femur-Fibula-Ulna complex and Poland syndrome). No genetic disorders were diagnosed in the isolated cases with one affected limb, which suggests that genetic testing in this group of patients might not be useful. However, as genetic testing after counseling was not performed in most of these cases (102/128, 79.7%), it is possible that a genetic cause is present but was not investigated. We do not have information about the reasons why genetic testing was not performed. It is possible that the parents of cases with isolated LRDs of one limb do not wish genetic testing. Nevertheless, it seems that clinical geneticists consciously and deliberately refrain from genetic testing in this group, possibly based on assumptions from the literature and on the clinical picture and development of the child.

In MCA cases, the probability of diagnosing a genetic disorder was three times higher in cases with multiple affected limbs compared to cases with one affected limb (RR 3.0, 95% CI 1.6–5.5, $p < 0.001$). However, in MCA cases with one affected limb, a genetic diagnosis was still obtained in 15.6% (10/64), a clinically relevant percentage. In MCA cases, the probability of diagnosing a clinically recognizable disorder was similar in cases with multiple affected limbs versus one affected limb, which indicates that one should consider this kind of disorders in MCA cases even when only one limb is affected. The most commonly diagnosed clinically recognizable disorders in MCA cases with one affected limb were VACTERL, LBWC, and Poland syndrome.

It is noticeable that the probability of obtaining any kind of etiological diagnosis is similar in cases with longitudinal postaxial LRDs of multiple affected limbs versus one affected limb. The number of genetic disorders in this LRD subcategory is low (5/65, 7.7%). This aligns with the general view in literature that postaxial LRDs usually are sporadic (Stevenson, Hall, Everman, & Solomon, 2015), which is also reflected in the low yield of genetic testing in this category. However, rare genetic disorders with longitudinal postaxial LRDs, like Cornelia de Lange syndrome and UMS, should not be overlooked.

TABLE 4 Type and yield of genetic tests performed in the cases counseled by a geneticist

Category of Reduction Defect	Affected Number of Limbs	Number of C ounseled Cases	Counseled Cases Who Underwent Genetic Testing	Type of Genetic Test[a]														Yield (Genetic Disorders with Molecular Confirmed Diagnosis)		
				Karyotyping		Array Analysis[b]		Single Gene Sequencing		NGS Panel		WES		Mitomycin C Test		FISH[c]				
		n Total (*n* Isolated Cases)	*n* Total (*n* Isolated Cases)	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*p*-Value
Transverse	1	84 (74)	13 (7)	10	76.9	6	46.2	0	0.0	0	0.0	0	0.0	0	0.0	3	23.1	0	0.0	
	>1	13 (6)	7 (2)	4	57.1	2	28.6	3	42.9	0	0.0	0	0.0	0	0.0	1	14.3	2	28.6	0.111
Longitudinal																				
Preaxial	1	32 (7)	29 (7)	23	79.3	12	41.4	11	37.9	2	6.9	5	17.2	18	62.1	4	13.8	2[d]	6.9	
	>1	31 (4)	31 (4)	20	64.5	9	29.0	11	35.5	1	3.2	1	3.2	13	41.9	8	25.8	15	48.4	**<0.001**
Postaxial	1	26 (19)	9 (3)	7	77.8	3	33.3	1	11.1	0	0.0	0	0.0	0	0.0	1	11.1	1	11.1	
	>1	19 (14)	15 (10)	10	66.7	4	26.7	5	33.3	1	6.7	1	6.7	0	0.0	2	13.3	2	13.3	1.000
Central	1	28 (22)	12 (6)	6	50.0	9	75.0	3	25.0	1	8.3	1	8.3	0	0.0	1	8.3	2	16.7	
	>1	22 (18)	13 (10)	10	76.9	8	61.5	6	46.2	0	0.0	0	0.0	0	0.0	2	15.4	5	38.5	0.378
Complex	>1	29 (15)	15 (5)	9	60.0	8	53.3	3	20.0	4	26.7	1	6.7	2	13.3	5	33.3	1	6.7	
Total	1	182 (128)	70 (26)	50	71.4	34	48.6	16	22.9	3	4.3	6	8.6	19	27.1	9	12.9	8[d]	11.4	
	>1	122 (59)	89 (33)	59	66.3	36	40.4	30	33.7	6	6.7	3	3.4	16	18.0	20	22.5	27[e]	30.3	**0.006**

Note: Data are shown per subcategory of reduction defect and affected number of limbs. The subcategories that are not shown were not analyzed because they contained too few cases (longitudinal mixed and intercalary) or the analysis had no clinical relevance (longitudinal unknown and unknown). In 25 cases genetic testing was performed without counseling by a geneticist: karyotyping *n* = 24, array analysis *n* = 1, FISH *n* = 3. These cases are not included in this table.

Abbreviations: FISH, fluorescence in situ hybridization; NGS, next generation sequencing; WES, whole exome sequencing.

[a]Per case more than one genetic test can be performed.

[b]Includes array CGH (105k and 180

[a]k) and SNP array.

[c]Includes subtelomeric FISH.

[d]All are MCA cases.

[e]22 MCA cases, 5 isolated cases (3 longitudinal central, 2 longitudinal preaxial).

We found relatively high yields of genetic testing for longitudinal preaxial LRDs (15/31, 48.4%) and central LRDs (5/13, 38.5%) of multiple limbs. This agrees with the literature view that preaxial LRDs are seen in a variety of syndromes and that the most common type of central LRD, the split hand-split foot malformation, is considered to be genetic in its typical form (Cobben et al., 1994; Stevenson et al., 2015). Genetic testing therefore seems to be valuable for these LRD subcategories. In contrast, the high genetic testing rate (90.6%) but low yield (2/29, 6.9%) for preaxial LRDs of one limb suggests that genetic testing seems less useful for this category.

5 | CONCLUSIONS

In this study, we confirm that an etiological diagnosis is obtained more often in cases with an LRD of multiple limbs as compared to those with only one affected limb. For clinical practice, it seems less useful to perform genetic testing in isolated LRD cases with one affected limb. However, genetic testing is warranted in MCA cases with one affected limb. In MCA cases, a clinically recognizable disorder should be considered, irrespective of the number of affected limbs. Because of the high yield in longitudinal preaxial and longitudinal central LRDs of multiple limbs, genetic testing seems especially valuable in these subcategories. Future studies could investigate the yield of current genetic testing, particularly for isolated LRDs of one limb. Ideally, a prospective diagnostic study should be performed in which all patients are counseled by a geneticist and receive complete genetic testing using modern next-generation sequencing techniques. It has now been shown that the yield of these techniques in rare (pediatric) diseases is substantially higher than traditional techniques like single gene sequencing (Wright, FitzPatrick, & Firth, 2018).

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

KL checked the medical records and classified the LRD with help of JEHB and PR. KL and JEHB analyzed the data. All authors participated in the interpretation of the results and writing of the manuscript.

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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