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Risk factors, prevalence, associated anomalies and treatment

Johanna Syvänen

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ABSTRACT

In the 1950's congenital limb deficiencies became well-recognized birth defect because of the use of thalidomide treating morning sickness in pregnant women. Despite of many studies the causation of many congenital limb deficiencies is mainly unknown. Upper and lower limb develop simultaneously during the organogenetic phase in early pregnancy (fourth to eight weeks of gestation). This is rapidly differentiating phase and therefore it is the phase most sensitive to teratogens causing major birth defects.

Congenital lower limb reductions are rare but they are usually visible at birth and thus are well documented by registries. There are just a few population-based studies about the epidemiology of lower limb deficiencies. There are no population-based studies about the burden of hospital care of lower limb deficiencies to the healthcare system.

The aim of this study was to describe risk factors for congenital limb reduction defects and also explore maternal medication use just before pregnancy and during the first trimester. The purpose was to find out features of congenital lower limb reduction defects and to determine the prevalence of congenital lower limb deficiencies and associated mortality and to identify patterns of associated anomalies. The aim was also to explore the impact, that children with lower limb reduction pose to health-care system. The data is based on registries maintained by the Finnish institute for health and welfare.

We found that maternal pregestational diabetes, nulliparity and young and old maternal age increased the risk of congenital limb deficiencies. The use of progesterone and antiepileptics before pregnancy and during the first trimester had impact on the risk of congenital limb reductions. The total prevalence of congenital lower limb deficiencies was 2.8 per 10 000 births and the perinatal mortality was 78 per 1000 births. Almost half the cases with lower limb reductions had associated major anomalies The need of hospital care and the number of orthopedic procedures was markedly increased in the patients with congenital lower limb deficiency compared to whole pediatric population.

KEYWORDS: population-based, congenital limb reduction, congenital limb deficiency, congenital lower limb deficiency, risk factor, prevalence, mortality, associated anomaly, hospital treatment

TURUN YLIOPISTO
Lääketieteellinen tiedekunta
Kliininen laitos
Lastenkirurgia
JOHANNA SYVÄNEN: Synnynnäiset alaraajapuutokset Suomessa: riskitekijät, esiintyvyys, liitännäisanomaliat ja hoito
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TIIVISTELMÄ

1950-luvulla alettiin käyttää raskaudenaikaiseen pahoinvointiin talidomidia, jonka seurauksena syntyi pahasti vaurioituneita lapsia, joilla oli mm. raajapuutoksia. Raajapuutoksien riskitekijöistä on tehty paljon tutkimuksia, mutta suurimmassa osassa tapauksia syy jää epäselväksi. Ylä- ja alaraajat muodostuvat raskauden aikana samanaikaisesti ns. organogeneettisen vaiheen aikana. Siinä vaiheessa tapahtuu nopeaa elinten erilaistumista, ja tämä vaihe on herkin erilaisten teratogeenien vaikutukselle. Teratogeenit ovat tekijöitä, jotka aiheuttavat synnynnäisiä poikkeavuuksia.

Synnynnäiset alaraajapuutokset ovat harvinaisia, mutta usein ne ovat helposti tunnistettavia syntymän jälkeen. Niiden rekisteritiedot ovat luotettavia. On olemassa vain muutamia väestöpohjaisia tutkimuksia alaraajapuutosten epidemiologiasta. Kirjallisuudessa ei tunneta synnynnäisten alaraajapuutosten hoidon tarvetta eikä vaikutusta terveydenhuollolle.

Tämän tutkimuksen tavoite oli selvittää synnynnäisten raajapuutosten liittyviä riskitekijöitä. Lisäksi selvitettiin äidin lääkkeiden käyttöä ennen raskautta ja raskauden ensimmäisen kolmanneksen aikana. Tutkimuksessa kartoitettiin myös synnynnäisten alaraajapuutosten tyypit, esiintyvyys, mortaliteetti sekä liitännäisanomaliat. Tavoitteena oli myös selvittää synnynnäisten alaraajapuutospotilaiden sairaalahoidon sekä kirurgisen hoidon tarve. Aineisto perustuu Terveyden- ja hyvinvoinnin laitoksen rekistereihin.

Äidin ennen raskautta todettu diabetes, ensiraskaus ja äidin nuori, sekä korkea ikä lisäsivät raajapuutosten riskiä. Lisäksi äidin käyttämä progesteroni sekä epilepsialääkkeet nostivat raajapuutosten riskiä. Alaraajapuutosten kokonaisesiintyvyys oli 2.8 per 10 000 syntynyttä ja perinataalikuolleisuus 78 per 1000 syntynyttä. Melkein puolella alaraajapuutospotilaista todettiin jokin muu merkittävä synnynnäinen anomalia kuin raajapuutos. Alaraajapuutospotilaiden sairaalahoidon tarve ja ortopedisten toimenpiteiden määrä oli merkittävästi korkeampi verrattuna muuhun lapsipopulaatioon.

AVAINSANAT: populaatiopohjainen, synnynnäinen raajapuutos, synnynnäinen alaraajapuutos, riskitekijä, esiintyvyys, kuolleisuus, liitännäisanomalia, sairaalahoito

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Abbreviations

ABS-LBWC	Amniotic band syndrome-limb-bodywall complex
ADHD	Attention-deficit/hyperactivity disorder
AER	Apical ectodermal ridge
AK	Acterman-Kalamchi classification
ART	Artificial reproductive technologies
CI	Confidence interval
CVS	Chorionic villus sampling
EUROCAT	European network of population-based registries for the epidemiological surveillance of congenital anomalies
FHDR	The Finnish National Hospital Discharge Register
ICBDSR	International Clearinghouse for Birth Defects Surveillance and Research
ICSI	Cryopreserved intracytoplasmic sperm injection
IVF	In vitro fertilization
KELA	Social Insurance Institution of Finland
MCA	Major congenital anomaly
NA	Not applicable
NBDPN	National Birth Defects Preventive Network
NOMESCO	Nordic Medico-Statistical Committee
PFFD	Proximal femoral focal deficiency
PGDM	Pre-gestational diabetes mellitus
PIC	Personal identification code
SHORDT	Shortening osteotomy realignment distal tibia
SNP	Single nucleotide polymorphisms
SUPERhip	Systematic utilitarian procedure for extremity reconstruction
SUPERknee	Systematic utilitarian procedure for extremity reconstruction
THL	Finnish institute for health and welfare
TOPFA	Termination of pregnancy for fetal anomaly
WHO	World Health Organization
VATER	Vertebral defects, anal atresia, tracheo-esophageal fistula and renal anomalies

VACTERL	Vertebral defects, anal atresia, cardiac defects, tracheo-esophageal fistula, renal anomalies and limb abnormalities

List of original publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I Syvänen J, Nietosvaara Y, Ritvanen A, Koskimies E, Kauko T, Helenius I. High risk for major nonlimb anomalies associated with lower-limb deficiency: a population-based study. *J Bone Joint Surg Am*. 2014;19:1898–904.

II Syvänen J, Helenius I, Koskimies-Virta E, Ritvanen A, Hurme S, Nietosvaara Y. Hospital admissions and surgical treatment of children with lower-limb deficiency in Finland. *Scand J Surg*. 2019;108:352–360

III Syvänen J, Nietosvaara Y, Hurme S, Perheentupa A, Gissler M, Helenius I. Diabetes and first trimester medication use associated with increased risk of congenital limb deficiency. A population-based, case-control study. Manuscript.

1 Introduction

The International Clearinghouse of Birth Defects Surveillance and Research defines that limb deficiency is a congenital malformation characterized by total or partial absence or severe hypoplasia of skeletal structures of the limbs. (ICBDSR). They are rare anomalies. After the thalidomide catastrophe in the 1950's and 1960's they became a well-known group of congenital anomalies (Smithells and Newman 1992, Vargesson 2015, 2019). It is estimated that nearly 12 000 children were born with limb anomalies (phocomelia) and other congenital defects involving the eyes, ears, heart, gastrointestinal canal and urogenital organs (Moore et al. 2016, Smithells and Newman 1992, Vargesson 2015, 2019).

Outside thalidomide, the causes of limb anomalies remain mainly unknown, especially the isolated ones (Bedard et al. 2015). The causes can be environmental or genetic or they may follow a multifactorial inheritance pattern in which genetic and environmental factors act together (Moore et al. 2016). Low birth weight, short gestation duration, chorionic villus sampling, alcohol, maternal obesity, infertility treatments and lack of multivitamins and antioxidants have an impact on the development of congenital limb reductions (Aro et al. 1983, Polednak and Janerich 1985, Caspers et al. 2013, Firth et al. 1994, Martinez-Frias et al. 2004, Oddy et al. 2009, Källen et al. 2010, Davies et al. 2012, Botto et al. 2004). Certain maternal diseases may be connected to congenital limb deficiencies, e.g., pre-eclampsia, chronic hypertension, thyroid disease, influenza, goiter and rheumatoid arthritis (Polednak and Janerich 1985, Rogala et. al. 1974, Bellizzi et al. 2016, Källen 1989, Aro 1983, Luteijn et al. 2014). Pregestational diabetes and maternal hyperglycemia are associated with major birth defects (Reece 2012), but to what extent this is related to congenital limb deficiencies is mainly unknown (Aberg et al. 2001, Källen 1989, Polednak and Janerich 1985, Froster and Baird 1993b, Nielsen 2005). In a recent population-based study, pregestational diabetes increased the risk of limb deficiency three-fold (Klungsoyr et al. 2019).

In the 1960's and 1970's researchers reported an association between sex steroids used therapeutically by the mother and congenital limb reductions (Janerich et al. 1974, Czeizel et al. 1983, Heinonen et al. 1977). Newer studies have demonstrated that these associations may not be true (Brent 2005). Valproate,

phenytoin and phenobarbital use has been connected to limb anomalies (Jentink et al. 2010b, Holmes et al. 2001, Harvey et al. 2003) and antibiotics increase the risk of limb deficiency (Crider et al. 2009).

Congenital lower limb defects are obvious at birth and they are well documented within registers. The Finnish Register of Congenital Malformations includes data on congenital anomalies from other national registers, such as the Medical Birth Register, the Care Register for Health Care and Statistics Finland cause-of-death statistics, all with high accuracy and 100% country coverage (Greenlees et al. 2011, Register of Congenital malformations, Sund 2012).

There are just a few population-based studies on limb deficiencies (upper and lower limbs) (Froster-Iskenius and Baird 1989, Aro et al. 1982, Kallen et al. 1984, Calzoari et al. 1990, Evans et al. 1994, Forrester and Merz 2003, Koskimies et al. 2011, Klungsoyr et al. 2019). These studies have reported a prevalence of limb reduction defects that varies between 4.4 and 12.8 per 10,000 births. During their first month of life, 4.2% of the patients with limb deficiency died and 5.5% during their first full year (Klungsoyr et al. 2019).

The proportion of associated anomalies has varied between different studies from 12% (Calzoari et al. 1990) to 83% (Forrester and Merz 2003). A population-based study from Norway reported an increasing trend of associated anomalies from 1970 to 2016; 7% had a chromosomal syndrome. Cardiac defects were the largest group of associated anomalies (12.4%) followed by anomalies in the digestive and central nervous system (both 9.3%). (Klungsoyr et al. 2019)

Congenital lower limb deficiencies include a wide variety of different manifestations from one toe missing to an entire lower limb missing. The treatment of these disorders varies by deformity (Gillespie and Torode 1983, Fernandez-Palazzi et al. 1998, Spiegel et al. 2003, Westberry and Davids 2009, Birch et al. 2011, Paley 2016 a and b, Kulkarmi et al. 2019). There are no population-based studies on hospital admissions and the need for surgical treatment.

Today, different organizations monitor birth defects (e.g., ISBDSR, EUROCAT, Register of Congenital Malformations) in an effort to conduct national and worldwide surveillance and research on the occurrence and causes of congenital anomalies. The ultimate purpose is to prevent defects and to moderate the consequences of birth defects to the individuals and society (ICBDSR, EUROCAT, Register of Congenital Malformations).

2 Review of the literature

2.1 Human prenatal development

Cell division, migration, programmed cell death (apoptosis), differentiation, growth and cell rearrangement transform the fertilized oocyte (zygote) into a multicellular human being. The phase from fertilization to birth is divided into two periods: embryonic and fetal. The embryonic period lasts from the third to the eighth week of gestation and during that period most developmental advances take place. The fetal period consists of differentiation and growth of tissues and organs and the rate of body growth increases. Development is also divided to phases of growth, morphogenesis and differentiation. (Moore et al. 2016)

2.1.1 Organogenetic development

During the first three weeks of gestation major developmental events occur, such as the incipient development of the nervous and cardiovascular systems. However, all major external and internal structures become established during the fourth to the eighth week. This period is called the organogenetic period. At the end of this phase, the embryo has a human appearance and all major organ systems begin to develop but their function is minimal, with the exception of the cardiovascular system which functions already at that time. (Moore et al. 2016)

The organogenetic phase is a phase of rapid differentiation and therefore it is the phase when the developing organism is most sensitive to teratogens. (Moore et al. 2016)

2.1.2 Limb development

The appendicular skeleton (upper and lower limbs, pelvic and shoulder girdle) develops from the lateral plate mesoderm, which is split into a paraxial and a somatic part. The mesenchymal cells in the lateral mesodermal plate are activated and limb buds start to grow. At the same time, each tissue (cartilage, bone and muscle) starts to develop and differentiate. The mesenchyme, derived from the somatic layer of the lateral plate mesoderm, is the source of the skeletal components which form the

bones and connective tissues of the limb. The paraxial mesenchyme is the source of the muscular component. The mesenchyme in the limb bud also gives rise to ligaments and blood vessels. (Moore et al. 2016, Bermejo-Sanzhez et al. 2011a).

The limb buds form deep and thick band of ectoderm called the apical ectodermal ridge (AER). The buds first appear as small bulges on the ventrolateral body wall. The upper limb buds become visible first, by day 24, and the lower limb buds appear one or two days later. Each limb bud consists of a mesenchymal core of mesoderm covered by a layer of ectoderm. (Moore et al. 2016)

When the mesenchyme proliferates, the limb buds elongate. The earliest stages of limb development are the same for the upper and lower limbs. Differences between the limbs start to arise later in agreement with their specific future functions. The upper limb buds develop opposite the caudal cervical segments and the lower limb buds opposite the lumbar and upper sacral segments. By six weeks, hand and foot plates appear and over the next two weeks (week 7 and 8), digital rays and digits can be seen. At the tip of each digital ray, a part of the AER induces development of the mesenchyme into the mesenchymal primordia of the bones (phalanges). The areas between the digital rays are occupied by loose mesenchyme which will soon break down to form notches between the digital rays. (Moore et al. 2016, Bermejo-Sanzhez et al. 2011a)

Chondrification starts in week 5 as chondrification centers appear. By the end of the sixth week, the entire limb is cartilaginous. First, there is a hyaline cartilage precursor forming the skeleton of the limbs. The precursor ossifies by the end of the embryonic period. Osteogenesis of long bones begins in the seventh week from primary ossification centers. Ossification centers are present in all long bones by week 12. (Moore et al. 2016, Bermejo-Sanzhez et al. 2011a)

Early in the seventh week, the limbs extend ventrally. Initially, the flexor aspect of the limbs is ventral and the extensor aspect is dorsal. The upper limbs rotate laterally through 90 degrees on their longitudinal axis and the lower limbs rotate medially through almost 90 degrees. Developmentally, the radius and tibia are homologous bones, as are the ulna and fibula. The thumb and the big toe have also the same developmental field. (Moore et al. 2016)

2.2 Teratologic terms

2.2.1 Malformation, deformation, disruption and dysplasia

Deviations in morphogenesis can lead to abnormal development. There are four main types of birth defects. (Moore et al. 2016)

Malformation is a morphologic defect of an organ, part of an organ or larger region of the body due to an intrinsically abnormal developmental process.

Disruption is a morphologic defect of an organ, part of an organ or a larger region of the body due to extrinsic factors (e.g. exposure to teratogens) which interfere with what originates as a normal developmental process.

Deformation is an abnormal form, shape or position of a part of the body. The embryo or fetus is normal but mechanical forces (e.g. uterine constraint) alter morphogenesis.

Dysplasia is an abnormal organization of cells in tissues resulting in abnormal tissue morphology. Dysplasia is the process and the consequence of abnormal tissue formation.

Other descriptive terms used to describe infants with multiple anomalies are (Moore et al. 2016):

Polytopic field defect is a pattern of defects. It is due to a disturbance of a single developmental field.

Sequence is a pattern of multiple defects due to a single known or presumed structural defect or mechanical factor.

Syndrome is a pattern of multiple defects. All defects are thought to be pathogenetically related and are not known to represent a single sequence or a polytopic field defect.

Association is not a polytopic defect, a sequence or a syndrome, but denotes multiple defects which occur nonrandomly in two or more individuals.

2.3 Definition of birth defect and congenital limb deficiency

The International Clearinghouse of Birth Defects Surveillance and Research (ICBDSR) defines a birth defect as any structural or functional anomaly which is manifested at any age. It is environmental or genetic by nature (inherited or not) and is due to causes acting before birth.

The ICBDSR defines a limb deficiency as a congenital malformation characterized by total or partial absence or severe hypoplasia of skeletal structures of a limb. It includes femoral hypoplasia (Q72.5) but excludes mild hypoplasia with retained normal shape of skeletal parts, brachydactyly, finger or toe reduction directly associated with syndactyly (Q70.-), skeletal dysplasia (Q77.-) and sirenomelia (Q87.2). (ICBDSR)

2.4 Birth defect monitoring systems

In Finland, the Register of Congenital Malformations at the Finnish institute for health and welfare (THL) was established in 1963 as a consequence of the thalidomide catastrophy. The Register continuously monitors the prevalence and

types of congenital anomalies. The goal is to identify early any new teratogens (environmental factors) that could cause birth defects. Another task is to prevent congenital anomalies by influencing these factors. The Register uses statistical data for monitoring congenital anomalies nationally and regionally and for planning prenatal screening and diagnostics of congenital anomalies. The data can also be used for planning the treatment of congenital anomalies, and for conducting research on congenital anomalies. The data on congenital anomalies is collected to the Register of Congenital Malformations pursuant to the Act on Nationwide Health Care Registers (566/1989, Sections 2 and 3) and the subsequent Statute (774/1989, Sections 1 and 8) as well as to the Act on the National Institute for Health and Welfare (668/2008, Section 2). (Register of Congenital Malformations)

The ICBDSR is a voluntary non-profit International Organization under World Health Organization (WHO). ICBDSR brings together birth defect surveillance and research programs globally. The organization was established in 1974 in Helsinki and has now 42 member organizations worldwide. ICBDSR conducts worldwide surveillance and research of birth defects. The aim is to prevent defects and to decrease their impact. (ICBDSR)

EUROCAT (European network of population-based registries for the epidemiological surveillance of congenital anomalies) is a European network of population-based registries for epidemiologic surveillance of congenital anomalies. It was founded in 1979. It surveys more than 1.7 million births per year in Europe and includes 39 active registries with 33 full and six associate members in 21 countries; and 29% of the European birth population is covered. It is also a WHO collaborating Centre. EUROCAT provides epidemiologic information, facilitates early warning of new teratogenic exposures and evaluates the effectiveness of primary prevention and prenatal screening. (EUROCAT)

2.5 Human birth defects

Congenital birth defects may be single or multiple and be of major or minor clinical significance. Minor single defects occur in approximately 14% of all neonates and may be indicators of associated major defects. When the child has three or more minor defects, 90% have also one or more major defect. Major defects are much more common in young embryos (10–15%) and most of them abort spontaneously. (Moore et al. 2016)

The annual prevalence of anomaly cases in Finland in 2010–2014 was, on average, 543/10,000 births or 3,231 cases annually. The total prevalence of anomaly cases (per births and terminations of pregnancy performed for fetal indications) was 546/10,000 births in 2014. Major congenital anomalies occurred in 2,822 stillborn and live born infants in 2014. Anomalies occurred in around 5% of live born and

18% of stillborn infants. Of the stillborn infants and infants who died during their first year of life, 36% were diagnosed with a congenital anomaly.

The causes of birth defects can be divided into three categories: genetic factors (e.g., chromosomal abnormalities), environmental factors and multifactorial inheritance in which genetic and environmental factors act together. In over half of the cases the cause is unknown. (Moore et al. 2016)

Mutations cause approximately one third of all defects. Two kinds of changes occur in chromosomes: numeric (aneuploidy, polyploidy) and structural (translocations, deletions). These changes may affect the sex chromosomes as well as the autosomes. Approximately 8% of all birth defects are caused by gene defects. The mutation rate is apparently influenced by a number of environmental agents. Mutations or alterations in signaling pathways can also lead to human birth defects. (Moore et al. 2016)

In 7–10% of the cases, an environmental factor is identified as the cause of the birth defect. A teratogen is an agent that can produce a birth defect or increase the incidence of a defect in the population. Environmental factors may mimic genetic conditions and it is important to understand that not everything that is familial is genetic. When considering the possible teratogenicity of a drug or chemical there are three things to consider: critical periods of development, dose of the drug or chemical and the genotype of the embryo. (Moore et al. 2016)

2.6 Etiology and risk factors for congenital limb deficiencies

There may be a number of different causes for congenital limb deficiencies. Amniotic bands or disruption of the arterial supply (e.g., thrombi from the maternal placenta) may affect the limb buds and cause limb amputation *in utero* (Holmes et al. 2018). Limb development might also be affected by environmental factors and errors in the genetic control of limb development may occur (Ghanem 2008). Teratogens, notably such as thalidomide, may cause vessel failure and are linked causally to limb malformations through vascular loss (Therapontos et al. 2009, Vargesson 2015).

2.6.1 Thalidomide

Thalidomide was invented in the 1950's and it was used as a non-addictive, non-toxic, non-barbiturate sedative. In at least 46 countries pregnant mothers used it to treat morning sickness (Vargesson 2015, 2019). There were soon reports of limb abnormalities (phocomelia, dysmelia, amelia, bone hypoplasticity) and other congenital defects (ear, heart and internal organs) after a single dose of thalidomide

during gestation (Smithells and Newman, 1992, Vargesson 2015, 2019). The risk for teratogenic effects of thalidomide was highest 3–8 weeks after conception, and half of the pregnancies with thalidomide exposure resulted in malformed children. (Mellin and Katzenstein 1962, Lenz 1988, Smithells and Newman 1992) It is estimated that almost 12,000 children worldwide were born with malformations caused by thalidomide (Moore et al. 2016). The teratological mechanism of thalidomide is still unresolved. It is a complex drug and it has active metabolites, which affect the inflammatory pathways, the immune system and the vascular system (Franks et al. 2004, Vargesson 2015, Vargesson 2019). Thalidomide and its analogs are currently used to treat leprosy, several autoimmune diseases and multiple myeloma, but the use of these drugs is strictly controlled (Gordon and Goggin 2003; Vargesson 2015).

2.6.2 Genetics

Human genetics has identified or localized many genes connected to the development of limbs (Manouvrier-Hanu et al. 1999), but still only little is known about the genetic etiology of non-syndromic congenital limb deficiencies (Cleves et al. 2011, Bedard et al. 2018). It is, nevertheless, known that birth defects are likely to recur in families. In families there are shared genetic factors but also shared environmental factors. The relative risk of congenital limb deficiency among children to mothers with a limb defect was 5.6 ($p=0.05$) in a population-based study from Norway. (Skjaerven et al. 1999).

Many studies have explored the contribution of genetic factors to the development of congenital limb deficiencies (Hwang et al. 1998, Carmichael et al. 2004, Carmichael et al. 2006a and b, Carli et al. 2013, Furniss et.al 2009, Vergult et al. 2013, Carter et al. 2017, Zhang et al. 2017). The possibility of a genetic etiology for upper limb defects (Furniss et al. 2009, Carli et al. 2013) and radial ray defects (Vergult et al. 2013) has been explored. In the study of Carli et al. (2013) 41% of the cases had a genetic etiology. Carter et al. (2017) explored SNP (single nucleotide polymorphisms) mutations in split hand and foot cases. Zhang et al. (2017) reported genetic variations in subjects with the VATER / VACTERL syndrome. Single nucleotide polymorphisms (SNP) raises the risk of limb deficiency (Carmichael et al. 2006a). Cleves et al. (2011) reported changes in SNPs in patients with congenital limb deficiency. Maternal smoking and / or lack of multivitamin supplement intake increased the risk of limb deficiencies in genetic variants. (Carmichael et al. 2006b, Cleves et al. 2011) There are also reports of lower limb involvement (tibial and femoral deficiency) in VACTERL syndrome (Castori et al. 2008, Santos et al. 2013).

2.6.3 Other risk factors related to pregnancy and the mother

2.6.3.1 Birth weight, duration of pregnancy, mortality, maternal parity and gender distribution

Various pregnancy-related and maternal factors may be associated with congenital limb deficiencies (Froster and Baird 1993b, Polednak and Janerich 1985, Aro 1983, Aro et al. 1983, Källen 1989, Rogala et al. 1974). Polednak and Janerich (1985) studied the pregnancy characteristics in a case-control study. The mean birth weight and mean duration of gestation were significantly lower in the group of subjects with limb deficiency. The results in the study of Aro et al (1983) were similar. In that study, the duration of gestation was significantly shorter in the whole study population and in isolated cases especially in cases with associated anomalies. A low birth weight was associated with limb deficiency. Stoll et al. (1996) reported that the mean weight of newborns with multiple malformations was statistically significantly lower than the mean birth weight of the children with an isolated congenital limb deficiency. There was also a statistically significantly higher rate of prematurity in the group carrying multiple malformations than in the group with isolated malformations (Stoll et al. 1996). Low birth weight and preterm delivery were more common among newborns with limb deficiency in the study of Klungsoyr et al. (2019).

The frequency of neonatal or perinatal death has been reported to be significantly higher among subjects with congenital limb deficiency than among non-malformed controls. (Polednak and Janerich 1985, Aro et al. 1983). Aro reported (1983) there was no statistical difference in isolated limb deficiency cases. The study of Klungsoyr et al. (2019) reported that the risk of stillbirth was 7.4 times higher, neonatal mortality around 10 times higher and infant mortality 8.6 times higher among infants with congenital limb deficiency compared to infants without.

Klungsoyr et al. (2019) reported that there were significantly more primiparous women giving birth to children with congenital limb deficiency than multiparous women. Werler et al. (2009) reported that nulliparity increased the risk for amniotic bands, but not for terminal transverse reductions. The data of Duong et al. (2012) suggested that with increasing parity the risk of limb deficiencies decreases.

Male preponderance seems to be the rule concerning malformations in both the upper and lower limbs. (Bedard et al. 2018, Vasluian et al. 2013, Stoll et al. 1996, Forrester and Merz 2003, Rosano et al. 2000, Klungsoyr et al. 2019)

2.6.3.2 Invasive fetal investigations

Amniocentesis and chorionic villus sampling (CVS) increase the risk of miscarriage (Tabor et al. 1986, Smidt-Jensen et al. 1992). If amniocentesis is done very early in

pregnancy (before 14 weeks) fluid leakage may result. The amniotic fluid find its way between the membranes because the amnion and chorion have not fused at this early stage of pregnancy. This could affect the development of the limbs. (Cederholm et al. 2005) An increased risk of musculoskeletal anomalies including clubfoot and hip dislocation after amniocentesis prior to 14 weeks of gestation has been reported, but no increase in limb reduction defects (Cederhom et al. 2005).

Firth et al. (1994) reported an association between very early chorionic villus sampling (performed earlier than 90 days of gestation) and limb reductions. In the data of Olney et al. (1995), there was a six-fold increase in the risk for transverse digital deficiency (Olney et al 1995) after early CVS. Other studies have not found a similar correlation (Froster and Jackson 1996, Cederholm et al. 2005). According to the review of Brambati and Tului (2005) there was no increased risk of limb reduction after CVS. In a report on CVS safety, Kuliev et al. (1996) noted that when CVS is performed between 9 and 12 weeks of gestation, it is safe with regards to limb reduction defects. Golden et al. (2003) suggested that the abnormalities observed in connection with CVS are similar to those reported in children with amniotic-band deformities of unknown etiology. They concluded that at least some limb deficiency cases may result from disruption of the amniotic membrane. (Golden et al. 2003)

2.6.3.3 Maternal overweight and age

Maternal obesity is associated with pregnancy complications and adverse reproductive outcomes. (Galtier-Dereure et al. 2000, Prentice and Goldberg 1996, Watkins et al. 2003, Stothard et al. 2009). Pregestational maternal diabetes is associated with structural birth defects. A similar mechanism related to alterations in glycemic control may cause the associations between obesity and birth defects. (Becerra et al. 1990, Stothard et al. 2009)

Oddy et al. (2009) reported a two-fold increase in limb deficiencies in the pregnancies of women with pregestational obesity. In the same study, underweight was also associated with a non-significantly increased odds ratio for congenital limb deficiency. The estimated proportion of congenital limb deficiencies attributable to maternal obesity was 12%. Persson et al. (2017) reported that all adjusted risk ratios for malformations of limbs and other organ systems increased with increasing maternal BMI.

Waller et al. (2007) divided limb reduction defects into isolated and multiple. In the multiple group, the risk for a limb reduction defect was almost two-fold in obese (BMI >30) women compared to normosomic women. The systematic review and meta-analysis made by Stothard et al. (2009) concluded that obese mothers have a 1.3-fold risk of giving birth to a child with congenital limb deficiency.

Rogala et al. (1974) found that young parental age is associated with transverse limb reduction defects and Klungsoyr et al. (2019) reported that young as well as old maternal age (adjRR 1.3/adjRR 1.6) is associated with limb reduction defects.

2.6.3.4 Maternal diabetes

Poorly controlled PGDM (pre-gestational diabetes mellitus) implies maternal hyperglycemia before conception and during the first trimester. This condition is associated with major birth defects in 5–10% and with spontaneous abortions in 15–20% of all pregnancies. (Reece et al. 2012) The risk of infant malformations is increased three- to four-fold in women with PGDM or gestational diabetes and fasting hyperglycemia (Sheffield et al. 2002). A meta-analysis from 2015 showed that the RR for congenital malformations is 2.4 in PGDM and 1.11 in gestational diabetes (Zhao et al. 2015).

In a few studies diabetes has been associated with congenital limb deficiencies (Polednak and Janerich 1985, Åberg et al. 2001, Froster and Baird 1993b, Källen 1989, Correa et al. 2008, Klungsoyr et al. 2019, Dukhovny et al. 2018, Liu et al. 2015). On the other hand, while there was no association between diabetes and limb defects in the study of Nielsen et al. (2005), there were strong associations with renal agenesis, obstructive abnormalities of the urinary tract, cardiovascular anomalies and multiple congenital anomalies. Earlier studies (Polednak and Janerich 1985, Froster and Baird 1993b) have not provided statistical calculations on the association between diabetes and limb reductions. Becerra et al. (1990) reported a significant association between limb flexion contractures and diabetes. In the study of Correa et al. (2008) PGDM increased the risk of longitudinal limb deficiency six- to seven-fold. In a newly published study of Klungsoyr et al. (2019), maternal PGDM increased the risk of congenital limb deficiency three-fold and in the data of Dukhovny et al. (2018) the same corresponding figure was almost five-fold. In addition to these studies there are case reports which imply a connection between congenital limb deficiency and maternal diabetes (Hitti et al. 1994, Boyukkayhan et al. 2007, Lynch and Wright 1997).

2.6.3.5 Maternal hypertension

Maternal hypertension is related to the risk of birth defects, e,g., congenital heart defects and hypospadias (van Gelder et al. 2015, Bateman et al. 2015). There is a 20–30% increase in the risk of congenital malformations with maternal hypertension (both treated and untreated) (Bateman et al. 2015). An important but not fully resolved question is whether it is the disease or the medications which increase the risk of birth defects (Bateman et al. 2015, Fisher 2017).

There are reports of patients with limb defects being delivered by toxemic women (Polednak and Janerich 1985, Rogala et. al. 1974). Bellizzi et al. (2016) reported significantly increased odds ratios for limb anomalies in chronic hypertension and in pre-eclampsia superimposed on chronic hypertension.

2.6.3.6 Other maternal diseases

Polednak and Janerich (1985) reported that maternal thyroid conditions were associated with congenital limb deficiencies. Källen (1989) found no association. A Finnish study (Aro 1983) reported that maternal influenza during the first trimester is significantly associated with isolated as well as with all limb reduction defects. The OR for fever was 1.6 and for influenza 1.9. Goiter and rheumatoid arthritis were weakly associated with limb reductions. Maternal influenza exposure during the first trimester was analyzed in a recent systematic review and meta-analysis: Exposure was associated with an increased risk of any congenital anomaly, including limb reduction defects (OR 2.03, 95%CI 1.27–3.27). (Luteijn et al. 2014)

2.6.3.7 Maternal medications

In Finland Lahesmaa-Korpinen et al. (2014) reported that maternal drug use was common during pregnancy; half of the women giving birth had purchased at least one prescribed drug just before pregnancy or during the pregnancy and a quarter during the first trimester.

With maternal medications it is often impossible to determine whether the birth defect is associated with the medication or with the condition which the medication is prescribed for (Crider et al. 2009, Bateman et al. 2015, Fisher 2017).

Antibiotics are the most used medications during pregnancy (Lahesmaa et al. 2014). Crider et al. (2009) found that women who have children with intercalary limb defect were three times more likely to report use of penicillins. In the same study erythromycin and sulfonamides were associated with higher risk for transverse limb deficiency.

Fetuses exposed to antiepileptic drugs are at risk for major congenital malformations (Tomson et al. 2018). There is a dose dependency for lamotrigine, carbamazepine, valproate and phenobarbital (Tomson et al. 2018). The teratogenicity of carbamazepine is quite specifically related to spina bifida (Jentink et al. 2010a). Valproic acid monotherapy is also associated with spina bifida and many other congenital malformations, including limb defects (Jentink et al. 2010b). Prenatal exposure to phenytoin and phenobarbital may increase the risk of midface hypoplasia and digit hypoplasia (Holmes et al. 2001, Harvey et al. 2003), but digit hypoplasia does not usually affect the toes (Bokhari et al. 2002).

In the 1960's and 1970's, many studies reported an association between sex steroids and congenital limb reductions and other congenital malformations (Janerich et al. 1974, Czeizel et al. 1983, Heinonen et al. 1977). There were also contrary reports (Lammer and Cordero 1986). These associations have been reported to be unreliable (Brent 2005). Charlton et al. (2016) found no association between maternal contraceptive use and birth defects in a prospective, nationwide cohort study.

Hernandez et al. (2012) reported that aspirin and ibuprofen exposure was associated with amniotic band and limb body wall complex. Before that, also Werler et al. (2009) published similar results. Hernandez et al. also reported that there may be a two-fold increased risk of transverse limb deficiencies after naproxen use in pregnancy.

There is an increasing trend of ADHD among in adults (Anderson et al. 2018 and 2020) and ADHD medication is used during pregnancy. (Anderson et al. 2018 and 2020) In one study the use of ADHD medication was associated with an increased risk for transverse limb deficiencies (Anderson et al. 2018 and 2020).

2.6.3.8 Smoking

The association between active maternal cigarette smoking and various birth defects has been studied (Shi et al. 2009, Räisänen et al. 2014). Aro (1983) found a significant association between limb reduction defects and maternal smoking of more than five cigarettes per day. A similar result was reported by Källen et al. (1997). Czeizel et al. (1994) reported that in terminal transverse defects, the adjusted rate of maternal smoking during pregnancy was significantly higher in isolated congenital limb deficiencies than in matched controls. Maternal active smoking and exposure to passive cigarette smoke are potential teratogens which impact limb and digit formation (Caspers et al. 2013). The use of folic acid supplementation does not reduce the teratogenic effect of smoking (Caspers et al. 2013). There is also evidence that genetic susceptibility may modify the teratogenic effect of smoking (Hwang et al. 1998, Carmichael et al. 2004).

Maternal exposure to secondhand smoke has also been studied. A significant positive association was found between any passive cigarette smoke and amniotic band syndrome-limb-bodywall complex (ABS-LBWC), but there was no significant association between limb deficiencies and secondhand smoke. (Hoyt et al. 2016)

2.6.3.9 Alcohol

There are some studies on the influence of alcohol consumption on limb reduction defects. (Aro et al. 1983, Froster and Baird 1992, Caspers Conway et al. 2014,

Martinez-Frias et al. 2004). In the Finnish study from 1964 to 1977, there was a significant association between alcohol consumption and limb deficiencies (Aro et al. 1983). In the study by Martinez-Frias (2004) the occurrence of limb reduction defects was statistically significantly associated with high, sporadic doses of alcohol (OR 7.16 (95% CI, 0.89 –155.3; p=0.03). Caspers Conway et al. (2014) on the other hand found no teratogenic associations between alcohol and limb reductions.

2.6.3.10 Infertility treatments

The rate of congenital malformations is increased among infants born after *in vitro* fertilization (IVF) (Hansen et al. 2013). Belva et al. (2008) reported doubling of the malformation rate after cryopreserved intracytoplasmic sperm injection (ICSI). Hansen et al. (2013) concluded that after ART (artificial reproductive technologies) there was increased risk of birth defects compared to natural conceptions. On the other hand, more recent evidence suggests that there is no difference in perinatal outcomes or congenital malformation in ICSI children compared to naturally conceived children (Pereira et al. 2017).

A population-based study from Sweden found an increased risk for limb reduction defects after IVF (OR 1.86; 95% CI; 1.04–3.07) (Källen et al. 2010). In the study of Davies et al. (2012), the odds ratios for musculoskeletal abnormalities were almost two and a history of infertility either with or without assisted conception was significantly associated with birth defects. (Davies et al. 2012). It is not known whether ART treatment or parental subfertility is the cause for birth defects (Hansen et al. 2013, Berntsen et al. 2019)

2.6.3.11 Antioxidants

Folic acid supplementation prevents neural tube defects (Czeizel and Dudas 1992, Botto et al. 2004). The risk reduction is around 20 per cent according to a study by Erickson (1991) and 47 per cent in a Hungarian randomized trial (Czeizel 1998). Maternal use of multivitamin supplements containing folic acid may also modify the associations between other risk factors for congenital anomalies (e.g., maternal fever, smoking and diabetes) (Shaw et al. 2002, Correa et al. 2003).

Yang et al. (1997) reported that the periconceptional use of multivitamins lowers the estimated risk of having a child with limb deficiency. They also noted that the degree of protective effects varies between different types of limb defects. The lowest risk was associated with transverse limb defects. Shaw et al. (1995) and Klungsoyr et al. (2019) reported that women who take multivitamins or folate supplements have a 30 per cent lower risk for delivering a child with limb defect. Reduction of the incidence of upper limb defects after folic acid fortification has also

been reported by Canfield et al. (2005). Botto et al. (2004) calculated the number of cases potentially preventable by periconceptinal multivitamin use. They used three parametres: the highest and lowest published point estimates and an estimate of defect occurrence rates per 1,000 births. They arrived at the following figures for the OR for limb defects: 0.64, 0.19 and 0.5/1,000, respectively.

Rich dietary intake of antioxidants is also associated with a reduced risk for limb deficiencies (Pace et al 2018).

2.6.3.12 Environmental pollutants

Some teratogens cause oxidative stress which may be the reason for birth defects (Hansen 2006). Brender et al. (2014) reported an association between maternal residential proximity to industrial air emissions of chlorinated solvents and limb deficiency but the odds ratios were close to one. There is also evidence that exposure to outdoor air SO_2 during the first trimester may increase the risk of limb reduction defects (Lin et al. 2014). Despite a low statistical power in the study of Vinikoor-Imler (2013), there might be an association between air pollution and lower limb deficiencies. Other studies have not found any increased risk between limb defects and air pollutants (Rankin et al. 2009, Dolk et al. 2010).

2.7 Classification and prevalence of lower limb deficiencies

2.7.1 Common classifications

Frantz and O'Rafilly (1961) published the first classification system in the 1960's and introduced the terms terminal transverse, terminal longitudinal, intercalary transverse and intercalary longitudinal. In the original system there were also descriptive terms: hemimelia, peromelia, ectromelia, phocomelia, and dysmelia.

Nowadays the term transverse defect is used for complete absence of the distal structures with the proximal structures intact. It involves all axes of the limb, whereas a longitudinal defect involves the preaxial, postaxial or central axes of a limb. The term is used by many classifications (Calzolari et al. 1990, Evans et al. 1994, EUROCAT guide 3 2004, Gold et al. 2011, NBDP guidelines 2017).

The term intercalary deficiency is included in many systems (EUROCAT guide 3 2004, Gold et al. 2011, Bedard et al. 2015, NBDPN Guidelines 2017) and describes that bones proximally and distally from the defect are normal or nearly normal.

A preaxial defect usually means tibia, first metatarsal and first phalanx with analogy for the hand. Gold et al. (2011) also included digit two or even digit three with preaxial defects. Postaxial defects, on the other hand, are those of fibula, fifth

metatarsal and fifth phalanx (with analogy for the hand). Stoll et al. (1998) also included digits or toes two to five with postaxial defects and in Gold's system (2011) digits or toes three to five are included.

There are only a few studies that define split foot or hand: absence of central toes or digits with or without absence of central metatarsal or metacarpal bones, possibly associated with syndactyly of other toes or digits. (Calzolari et al. 1990, Czeizel et al. 1991, Lin et al. 1993, and EUROCAT guide 3 2004). There are two subtypes of split malformations. The first one is a foot or a hand divided by a cone shaped cleft. The mildest form is that the third toe or digit is missing but the third metatarsal or metacarpal bone is intact. The second type of split malformation is monodactyly, where the foot or hand lacks the central and tibial /radial toes/digits, there is no cleft and there is only one toe/digit left (usually the fifth). (Lowry and Berard 2016) Other studies classify second, third and fourth ray defects as central longitudinal deficiencies (Berard et al 2015, Gold et al. 2011). Split hand or foot malformations have a strong genetic backround and some classifications use this term only when there is a positive family history. (Berard et al. 2015, Lowry and Berard 2016)

Comparing the subtypes of anomalies between the studies is difficult due to different classifications (Bedard 2018, Lowry and Bedard 2016). Most cases had longitudinal deficiency (longitudinal preaxial, postaxial and central) in Bedard study (2018). Preaxial longitudinal has been the most frequent class in other studies (Rosano et al. 2000, Evans et al. 1994). In the EUROCAT classification (EUROCAT guide 3 2004) terminal transverse reductions have been the most common ones (Table 1).

Upper extremities are more commonly affected than the lower extremities (Aro et al. 1982, Calzoari et. al. 1990, Vasluian et al. 2013, Koskimies et al. 2011, Bedard et al. 2015).

Table 1. Percentages and prevalences of lower limb anomalies subclasses according to the EUROCAT classification (EUROCAT guide 3 2004 system (2004).

ANOMALY	CALZOARI ET AL. 1990	VASLUIAN ET AL. 2013	LIN ET AL. 1993
TERMINAL TRANSVERSE REDUCTION	14 (48%) 0.8 per 10,000 births	75 (50%) 1.5 per 10,000 total births	25 (22%) 0.3 per 10,000 live births
POSTAXIAL REDUCTION	5 (17%) 0.3 per 10,000	39 (26%) 0.8 per 10,000	19 (16%) 0.3 per 10,000
PREAXIAL REDUCTION	0 (0%) 0 per 10,000	16 (11%) 0.3 per 10,000	11 (9%) 0.2 per 10,000
INTERCALARY REDUCTION	6 (21%) 0.3 per 10,000	22 (15%) 0.4 per 10,000	18 (16%) 0.2 per 10,000
MULTIPLE REDUCTIONS	3 (10%) 0.2 per 10,000	17 (11%) 0.3 per 10,000	10 (9%) 0.1 per 10,000
SPLIT-FOOT REDUCTIONS	1 (3%) 0.1 per 10,000	16 (Central) (11%) 0.3 per 10,000	33 (28%) 0.5 per 10,000
UNKNOWN REDUCTION	-	2 (1%) 0.04	-
TOTAL	29 (lower limbs + both)	149 (lower limbs)	116 (lower limb + both)
NUMBER OF BIRTHS IN THE STUDY	173109 1.7 per 10,000 births	497751 3.0 per 10,000 births	720047 1.6 per 10,000 live births

2.7.2 Prevalence of congenital lower limb deficiencies

There are only a few population-based studies on the prevalence of limb (upper and lower) deficiencies (Froster-Iskenius and Baird 1989, Aro et al. 1982, Källen et al. 1984, Froster and Baird 1993a, Evans et al. 1994, Forrester and Merz 2003, Calzoari et al. 1990, Lin et al. 1993, Vasluian et al. 2013, Berard et al. 2015, Klungsoyr et al. 2019). Based on these studies, the prevalence of limb defects (upper and lower) varies from 4.4 to 12.8 per 10,000 births. In addition, there are also non-population-based studies on limb deficiencies which are based on clinical visits and hospital-based estimates (Jones and Lipson 1991, Stewart and Jain 1995, Rijnders et al. 2000, McGuirk et al. 2001). Some studies have published results after combining several registers (Stoll et al. 1996, Rosano et al. 2000. (Table 2)

There has been a decline in the prevalence of congenital limb deficiencies, according to EUROCAT registers. (Morris et al. 2018). On the other hand, a study from Norway reported a slight increase in prevalence of congenital limb deficiencies (upper and lower limbs) from 1970–1998 (4.4 per 10,000 births) to 1999–2016 (4.6 per 10,000 births) (Klungsoyr et al. 2019).

Table 2. Prevalence of congenital lower limb deficiencies.

STUDY	TOTAL PREVALENCE PER 10 000	BIRTH PREVALENCE PER 10,000	LIVE BIRTH PREVALENCE PER 10,000	STUDY YEARS	
ARO ET AL. 1982*		1.4		1964–1977	Cases with chromosomal or other syndromes excluded
FROSTER-ISKENIUS AND BAIRD 1989*			1.7	1952–1984	
FROSTER AND BAIRD 1993A*			1.1	1952–1984	Cases with amniotic bands or chromosomal or other syndromic cases excluded. Cases with both upper and lower limb reductions excluded
KÄLLEN ET AL. 1984*		1.6		1965–1979	
CALZOARI ET AL. 1990*		1.7		1978–1987	
WRIGHT ET AL. 1995			1.3	1985–1992	Only isolated limb reduction defects included
LIN ET AL. 1993*			1.6	1983–1987	
FORRESTER AND MERZ 2003*			1.4	1986–2000	
RINJDERS ET AL. 2000			2.1	1981–1996	
MCGUIRK ET AL. 2001	2.0			1972–1974 1979–1994	
EUROCAT (WWW.EUROCAT-NETWORK.EU)	1.8			2007–2011	
VASLUIAN ET AL. 2013*	3.0 (lower limbs 4.2 (lower limbs + both)			1981–2010	
BEDARD ET AL. 2015*		1.4		1980–2012 (early fetal deaths and terminations from 1997)	
KLUNGSOYR ET AL. 2019*	0.9 (lower limbs) 1.3 (lower limbs + both)			1970–1998	

* Population-based studies

2.7.3 Long bone deficiencies

2.7.3.1 Femur, proximal femoral focal deficiency (PFFD)

Proximal femoral focal deficiency is a rare congenital anomaly with a birth prevalence of 1 in 50,000. (Fixsen and Lloyd-Roberts 1974). PFFD is characterized by failure of the normal development of the proximal parts of the femur. (Aitken 1969, Gillespie and Torode 1983, Fixsen and Lloyd-Roberts 1974). The femur is short, and this accompanied with other deformities of the proximal femur (coxa vara, pseudoarthrosis, acetabular dysplasia). The mildest form is a minimal shortening of the femur and the most severe form is complete absence of the femur. (Westberry and Davids 2009) Fibular deficiency is associated with PFFD in 50% of the cases (Koman et al. 1982)

The most widely used classification is the one published by Aitken (1969). (Figure 1) It divides PFFD into four categories based on radiography. In Aitken class A, the femur is short and there is a defect in the subtrochanteric region. The acetabulum is well-formed. Class B is characterized by a more extensive defect or the absence of the proximal femur; the proximal femur is situated above the acetabulum. In Aitken class C, the femoral head is absent and the acetabulum is severely dysplastic, while in class D the femur is extremely short and the lateral pelvic wall is flat. (Aitken 1969)

Another classification is the one of Gillespie. Here, class A is a congenitally short femur with a normal hip. Class B includes Aitken classes A, B and C. Gillespie class C corresponds to Aitken' class D. (Gillespie and Torode 1983) Pappas (1983) also introduced his own system to classify these malformations.

The literature acknowledges also the existence of distal femoral deficiency (Gilsanz 1983, Tsou 1982, Taylor et al. 2009)

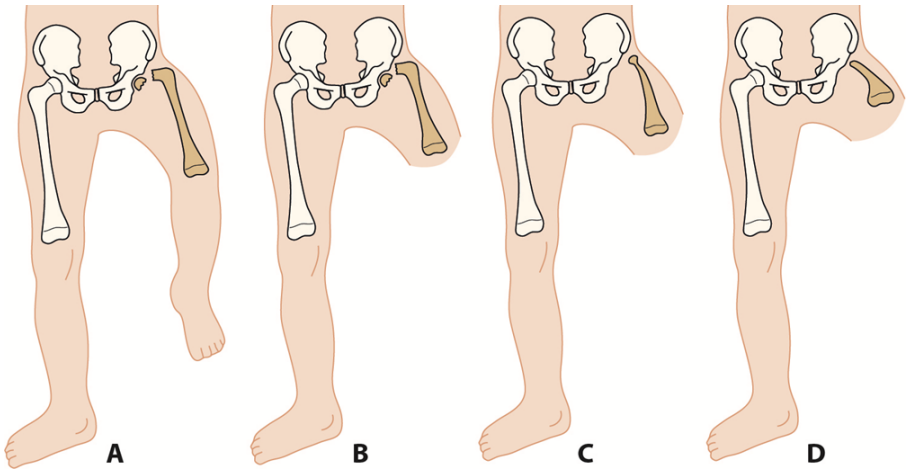

Figure 1. Aitken classification of proximal femoral focal deficiency. In the Eurocat classification system, femoral deficiencies are classified as intercalary defects.

2.7.3.2 Tibial deficiency

Congenital tibial deficiency is a rare condition with prevalence of 1 in 1,000,000 live births (Fernandez-Palazzi et al. 1998). It is characterized by partial or complete absence of the tibia associated with an intact but usually overgrown fibula. There may also be a variable degree of knee deformity and dysfunction, and frequently an equinovarus foot. (Litrenta et al. 2018)

Tibial deficiencies can be classified according to Kalamchi and Dave (1985) who divide deficiencies into complete absence, to absence of the distal tibia and to diastasis of the distal tibiofibular region. In the Jones (1978) classification (Figure 2), type 1 indicates complete absence of the tibia. In type 2 the distal tibia is missing and in type 3 there are diaphyseal and distal remnants of the tibia but no proximal tibia. Jones type 4 describes distal tibiofibular diastasis. (Jones et al. 1978)

A newer classification system with therapeutic relevance was published by Weber in 2008. It takes into account the cartilaginous anlage. Weber observed that 15% of the patients with tibial malformations had deformities which did not fit into the Jones classification system. Weber's system divides tibial deficiencies into seven different classes (Weber 2008). However, Weber's system has not been widely accepted (Clinton and Birch 2014). Recently, Paley published a classification system in which there are five types and eleven subtypes of tibial deficiency (Paley 2016b).

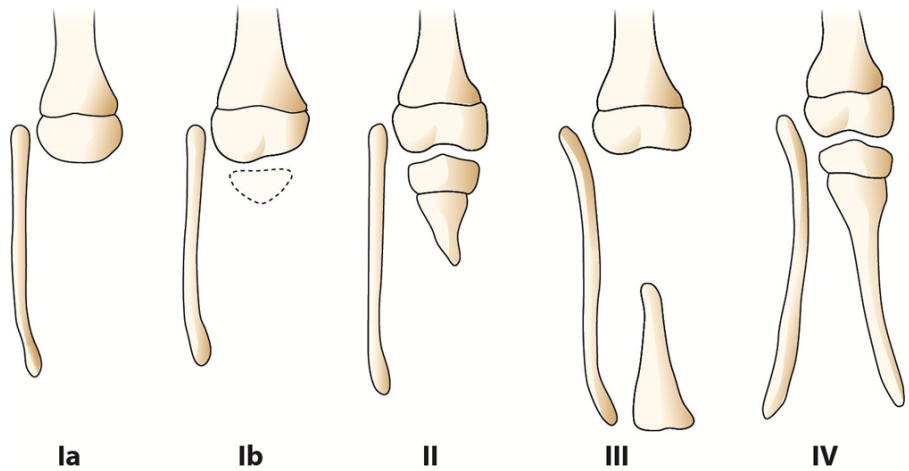

Figure 2. Jones classification system of tibial deficiencies. In the Eurocat classification system, the tibial deficiencies are classified as preaxial defects.

2.7.3.3 Fibular deficiency

Fibular deficiency is the most common long bone deficiency. The prevalence has been estimated to be between 7.4 and 20 per 1,000,000 live births (Foster and Baird

1993, Rogala et al. 1974). It is characterized by total or partial absence of the fibula. The condition has a wide range of clinical manifestations from mild lower limb shortening to significant short-leggedness with deformities of the foot and ankle. (Achterman and Kalamchi 1979, Hamdy et al. 2014)

Numerous classification systems have been used for patients with fibular deficiency. Acterman and Kalamchi introduced a classification system in 1979 based on radiological findings (Acterman and Kalamchi 1979). (Figure 3) Maffulli and Fixen (1991 and 1996) described total aplasia of the fibula and a *forme fruste* fibula which has varying degrees of shortness of the fibula and tibia.

Birch et al. (2011) proposed a new classification system for congenital fibular deficiency, based on the clinical status of the foot and on the magnitude of limb shortening as a percentage of the contralateral limb on radiographs. The system can be applied in infancy and it also provides a ground for assessing treatment. The foot is divided into two types, based on whether the foot can be saved or not. At least three rays should be present for a foot to be considered as salvageable. (Birch et al. 2011, Hamdy et al. 2014). Recently, Paley (2016a) published a new classification system to these patients.

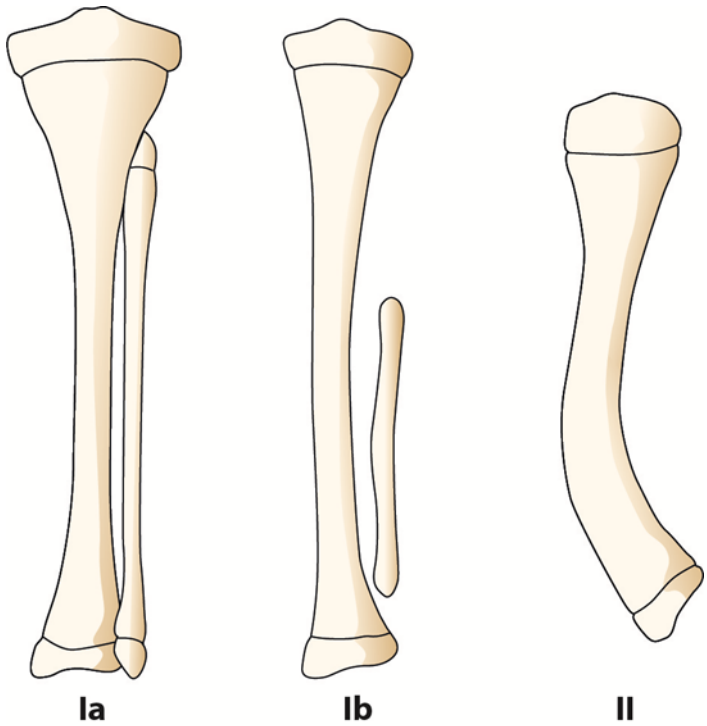

Figure 3. Acterman and Kalamchi classification system of fibular deficiency. In the Eurocat classification system, fibular deficiencies are classified as postaxial defects.

2.7.4 Cleft foot

Split hand and foot malformation affect approximately one in 18,000 individuals (Elliot et al. 2005). Cleft foot can be classified according to the classification system of Blauth and Borisch (1990). It divides cleft foot into six different types. Outside the system remains the monodactylous foot with a single ray and a tibial diastasis with central ray deficiency of the foot (Mosca 2014)

2.7.5 Amelia, phocomelia

Amelia is a congenital anomaly describing the complete absence of one or several limbs. It is a terminal transverse reduction defect (Franz and O'Rahilly 1961, Swanson 1976, EUROCAT guide 3 2004).

Phocomelia is a congenital anomaly in which the proximal part of the limb is absent or markedly hypoplastic, with a normal or nearly normal hand or foot (Bermejo-Sanzhez et al. 2011b). Phocomelia is classified as an intercalary transverse defect according Franzt and O'Rahilly (1961). The NBDPN guidelines (2017) uses it as a general term for any type of intercalary limb defect. The EUROCAT classification does not include this term. Because of the thalidomide catastrophe it has been extensively used. (Lowry and Berard 2016).

2.7.6 Sirenomelia

Sirenomelia is a limb anomaly in which the normally paired lower limbs are replaced by a single midline limb. It is also associated with defects of the sacrum, anal atresia, abnormal genitalia and absence of kidneys (Orioli et al. 2011, EUROCAT guide 3 2004). Sirenomelia is classified as a lower postaxial defect by Gold et al. (2011). In the EUROCAT system (EUROCAT guide 3 2004) it is a sequence. The Congenital Anomalies Surveillance System in Canada also classifies it as a separate entity (Bedard et al. 2015). It has also been classified separately by Stocker and Heifetz (1987) who classified the anomaly into seven types: I, all thigh and leg bones present; II, single fibula; III, absent fibulae; IV, partially fused femurs, fused fibulae; V, partially fused femurs, absent fibulae; VI, single femur, single tibia; VII, single femur, absent tibiae.

2.8 Mortality of lower limb deficiencies

In the study of Källen et al. (1984) the infant-mortality rate was 128 per 1,000 births for all limb deficiency cases. The infant mortality rate for congenital limb defects was 12.9% in the study of Froster-Iskenius and Baird (1989). In the same data, 85% of the subjects dying within the first year of life had additional malformations. More

recent data suggests that the mortality rate has decreased. In the study of Klungsoyr et al. (years 1970–2016), 82% of all subjects with limb deficiency were alive at the end of the study. They reported neonatal mortality rate of 4.2% and infant mortality rate of 5.5%. (Klungsoyr et al. 2019). Wang et al. (2011) studied the 25-year survival probability of children (years 1983–2006) with birth defects and in the case of lower limb defects the probability was 86.7% (95% CI 84.4–88.6).

2.9 Associated anomalies with lower limb deficiencies

The proportion of associated anomalies has been reported in several studies and it varies between 12% and 83%. (Aro et al. 1982, Froster-Iskenius and Baird 1989, Källen et al. 1984, Froster and Baird 1993a, Evans et al. 1994, Forrester and Merz 2003, Calzoari et al. 1990, Stoll et al. 1996, Rosano et al. 2000, Vasluian et al. 2013, Bedard et al. 2018, Klungsoyr et al. 2019) Usually, the proportion has varied between 20% and 30% (Bedard et al. 2015, Evans et al. 1994, Källen et al. 1984, Stoll et al. 2010, Vasluian et al. 2013). Patients born with congenital limb deficiency have very high relative risks of non-limb reduction malformations compared to all births in the population. (Stoll et al. 2010)

Stoll et al. (2010) included chromosomal and other syndromes in the calculations and reported that 58% of the upper and lower limb reduction cases had associated anomalies. A similar percentage was recorded by Froster-Iskenius and Baird (1989). Froster and Baird (1993) excluded all chromosomal and other syndromes after which 20% of the live births with lower limb reduction defects had associated major malformations. Källen et al. (1984) reported that there was at least one non-limb malformation in 27% among the lower limb reduction cases in unilateral reductions and the percentage was higher in bilateral cases and in cases with three to four limbs involved. A newer study and study population of upper and lower limb deficiencies reported that 77.6% of the cases had associated anomalies outside the musculoskeletal system (Bedard et al. 2018). The chromosomal abnormalities or known single gene or recognized conditions have been excluded from this study. Klungsoyr et al. (2019) found an increasing trend of associated anomalies during the study years from 1970–1998 (26%) to 1999–2016 (53%).

In the study of Bedard et al. (2018), musculoskeletal anomalies were present in 28.6%, cardiovascular in 16.8% and gastrointestinal in 12.6% of limb deficiency cases. Similar results were reported by Vasluian et al. (2013): digestive, cardiovascular and urinary tract anomalies were the most frequent anomalies associated with limb deficiencies. A significant association was found only between digestive anomalies and limb reductions. Similar results have been reported by Evans et al. (1994) and Klungsoyr et al. (2019).

Bedard et al. (2018) reported that the preaxial group had the most significant associations, as had been stated also previously (Rosano et al. 2000, Vasluian et al. 2013).

In the study of Vasluian et al. (2013) concerning lower limb reduction defects, 44% were isolated, 17% were MCA (multiple congenital anomalies) and 39% had a recognized condition (syndrome or chromosomal condition). Stoll et al. (2010) reported on upper or lower limb defects that 30% were associated with a recognized condition and 6% had chromosomal abnormalities. Klungsoyr et al. (2019) registered chromosomal abnormalities in 7% of the cases. Less recent figures vary between 5.6% and 6.4% (Stoll et al. 1992, Robert et al. 1997) for associated chromosomal defects. Limb reduction defects were associated with known hereditary disorders in 15%, with chromosome abnormalities in 6%, with specific malformation syndromes in 5% and with unclassified but familial phenotypes in 4% in the study by McGuirk et al. (2001). Trisomy 13 and 18 has been the most frequent chromosomal defects (upper and lower limbs together) among patients with limb deficiency. (Vasluian et al. 2013, Stoll et al. 2010, Stoll et al. 1996). (Table 3)

Table 3. Isolated, MCA and chromosomal or syndromic cases in different lower limb deficiency subclasses (Vasluian et al. 2013).

ANOMALY	ISOLATED	MULTIPLE CONGENITAL ANOMALIES	CONGENITAL LIMB DEFECT PART OF A RECOGNIZED CONDITION
TRANSVERSE REDUCTION	29	15	31
POSTAXIAL	21	2	16
PREAXIAL	4	3	9
INTERCALARY	7	5	10
MULTIPLE	7	2	8
SPLIT FOOT	13	1	2
UNKNOWN	1	1	0

Vasluian et al. (2013) reported that with limb deficiency amniotic bands were associated in 25% of the subjects. Most had transverse reduction defects. Evans et al. (1994) reported an association with amniotic bands in 16% of the cases. Smaller numbers have been registered by Stoll et al. (2010) (1.8%) and Lin et al. (1993) (7.7%).

2.10 Treatment of lower limb deficiencies

2.10.1 Hospitalizations for congenital birth defects

Colvin and Bower (2009) linked population-based birth registry and hospital discharge data in Australia. They reported that over 80% of the children with congenital birth defects were admitted to hospital compared to children without birth defects (54%). Admissions for children with birth defects accounted 12% of all admissions, while only 4.6% of all live born neonates had associated major birth defect. In the same study, a child with a major birth defect was 2.5 times more likely to be admitted to hospital compared to control group.

The burden of genetic diseases to hospital care has also been evaluated (McCandless et al. 2004) and some have studied hospital admissions related to specific congenital anomalies and syndromes (Radcliff et al. 2012, Fitzsimons et al. 2014, Fitzgerald et al. 2013, Robbins et al. 2014, Simeone et al. 2015, Islam et al. 2018, Razzaghi et al. 2015, Peterson et al. 2013). In a report on the burden of surgically treated congenital anomalies, Wu et al. (2013) reported that the burden was the highest for spina bifida.

2.10.2 Hospitalizations due to congenital lower limb deficiencies

There are no previous population-based studies on hospital admissions or hospital days due to congenital lower limb deficiencies. In the paper of Robbins et al. (2006), hospitalization rates of neonates with limb reductions declined after fortification of the foods with folic acid. The effect of childhood limb loss (congenital, malignancy or trauma) on healthcare utilization has been studied (Weir et al. 2010). A child with congenital limb deficiency had a mean of 0.3 annual hospital stays with amputation and 0.1 stays without. The total number of hospital nights was 7.0 with amputation and 2.5 without amputation.

2.10.3 Surgical treatment

There are no previous population-based studies on the need for surgical treatment among subjects with congenital lower limb deficiencies, although the surgical treatment of various subtypes of congenital lower limb defects have been studied and reported (Birch et al. 2011, Clinton et al. 2015, Ackman et al. 2013, Westberry and Davids 2009, Paley 2016a and b, Tani et al. 2000)

2.10.3.1 Proximal femoral focal deficiency

The problems with patients with PFFD are instability of the hip, rotational malalignment, poor hip musculature, hip contractures and leg length discrepancy (Fowler et al. 1996, Aitken 1969, Ackman et al. 2013). Traditionally, the treatment plan has relied on the predicted limb length inequality and PFFD has been treated with limb lengthening, rotationplasty or amputation. In cases when there is over 50% of shortening or over 20 cm predicted discrepancy, the treatment of choice has been surgery to facilitate prosthetic fitting. Patients have been treated either conservatively or with distal amputations, knee fusions, rotationplasties or femoro-pelvic arthrodesis. Milder deformities may be candidates for lengthening procedures. Stability of the hip and knee joint must be achieved prior to consideration for limb lengthening strategies. (Westberry and Davids 2009) Historically, in Gillespie's and Torode's system group 1 patients (congenital short femur) limb lengthening has been considered, Group 2 (Aitken types A, B, and C) patients have generally been treated with prosthesis after surgical limb modification and Group 3 (Aitken type D) patients have been managed prosthetically (Gillespie and Torode 1983, Torode and Gillespie 1991)

Acetabular dysplasia and proximal femoral deformity can be managed surgically, if there is developmental hip dysplasia. The deficiency in the acetabulum lies usually posteriorly in limb defect patients (Dora et al. 2004). (Westberry and Davids 2009) Iliofemoral fusion may be used in severely deformed hips (Aitken C and D) (Steel et al. 1987). A combination of resection, rotationplasty and femoro-pelvic arthrodesis has been reported in three patients with severe congenital femoral deficiency (Brown 2001). Van Nes described rotationplasty for patients with congenital femoral deficiency in 1950 (Van Nes 1950). The goal was to fuse the residual knee and rotate the limb 180° so that the foot is pointed backward and the ankle could function as a knee joint. This procedure has been reported to give good long-term outcomes (Ackman et al. 2013).

Paley has evolved his own classification system and strategy to treat these patients. This classification is based on lengthening and reconstruction strategies. Prior to lengthening, it is important to determine joint stability (hip, knee and ankle). Pelvic osteotomy and surgery of coxa vara may be needed. There may also be associated hip deformities of retroversion, hip flexion contracture and hip abduction contracture. The reconstructive procedure for these deformities is called the SUPERhip procedure (Systematic Utilitarian Procedure for Extremity Reconstruction). Knee instability may also require surgical treatment (SUPERknee procedure). After these procedures, serial lengthening surgeries are required (Paley and Guardo 2014).

2.10.3.2 Tibial deficiency

The principle of surgical treatment of tibial deficiency is to determine the stability of the knee and the functionality of the extensor mechanism (Litrenta et al. 2019). Traditionally, Jones type Ia deficiencies have no tibia and this has been treated with knee disarticulation. In the other types there is theoretically a functional knee and the goal of treatment is to preserve the knee joint by reconstructing the proximal tibia and fibula. Distally, the affected limb is managed by amputation *modo* Syme or Boyd or Chopart. In Jones class 1b and 2 deficiencies, a proximal tibiofibular synostosis is usually used. In Jones type 3 defects, a Syme or Chopart amputation is usually made. A modified Syme amputation has also been used in type 4 deficiencies with or without reconstruction of the ankle and correction of limb length discrepancy. Amputation has been recommended when the child is 6 to 12 months of age. (Schonecker et al. 1989, Christini et al. 1993, Clinton and Birch 2014, Litrenta et al. 2019)

The Brown procedure (Brown 1965) means fibular centralization, but the longterm results have been poor in some patients ending in knee disarticulation and knee flexion contractures (Clinton and Birch 2014), but satisfactory results have also been published (Simmons et al. 1996). Other methods for knee reconstruction have been proposed (Weber 2002). Brown recommended surgery when the patient turns one year of age (Brown 1965). The Brown procedure has mostly been used to treat Jones type 1a patients (Litrenta et al. 2019).

In the series of Fernandez-Palazzi et al. (1998) all patients with tibial deficiency were treated surgically. Clinton and Birch (2014) reported a series of 125 limbs with tibial deficiency. Most limbs with the type Ia deficiency were treated primarily or after a Brown procedure with knee disarticulation. Two patients were treated with knee fusion and Syme amputation. Five of type 1a patients had no surgery. Type Ib and II were mainly treated with the Syme amputation and tibiofibular synostosis. Patients with type 4 deficiency underwent the Syme amputation or reconstructive procedures to save their feet. 11% of the patients did not meet Jones's classification criteria. (Clinton and Birch 2014)

Some patients benefit from lengthening and reconstruction by which they obtain a plantigrade foot. There are a few reports on foot centralization procedures (Hosny 2005, Wada et al. 2015). Wada et al. (2015) had a mean follow-up of 10 years. Foot centralization was made by calcaneofibular arthrodesis. There were 19 feet of the Jones I or II type and 15 required secondary surgery due to loss of correction, recurrent foot deformities or fibular angular deformity.

Paley has proposed a new classification system and accompanying reconstruction and lengthening options for each type of defect. (Paley 2016b) Long-term follow-up of these patients is still lacking. The first goal is to reconstruct a stable ankle and plantigrade foot and, after that, a one-bone leg and knee are

reconstructed. These procedures are followed by serial lengthening periods. (Paley 2016b)

2.10.3.3 Fibular deficiency

Problems with a child with fibular deficiency are limb length discrepancy, foot deformities and ankle instability. The aim of treatment is to restore functional lower limbs. (Hamdy et al. 2014) In the study of Birch et al. (2011), 82.5% of the children maintained their limb length discrepancy proportion throughout the study years.

Traditionally, the severity of fibular deformity and length inequality in the lower limbs have been the main points considered (Coventry et al. 1952, Acterman and Kalamchi 1979, Letts et al. 1993). In the past few years, reports have been published on treating the patients with the goal to to obtain a functional foot and equal lower limb length (Statinitski and Statinitski 2003, Birch et al. 2011)

If the length discrepancy is less than 6% and the foot is plantigrade, the child can be managed conservatively with foot orthoses and shoe lifts (Birch et al. 2011). With more severe foot deformities and more pronounced limb length discrepancies, treatment is either amputation or lengthening procedures. Guidelines favor amputation if the patient lacks three or more rays in the foot and if the predicted length discrepancy exceeds 30% or more than 5 cm at birth. (Maffulli and Fixen 1996, Birch et al. 1999, Changulani et al. 2010). If the upper limb is also involved, the foot should be preserved (Birch et al. 2011). Amputations *modo* Syme and Boyd are carried out and the results are generally good also in the longterm (Birch et al. 1999, Hamdy et al. 2014).

Distraction osteogenesis is the method to lengthen the limb. The first lengthening is performed when the discrepancy is 5–6 cm. A contralateral epiphyseodesis can be used in some patients who are near skeletal maturity. (Hamdy et al. 2014) The rate of complications usually increases with serial lengthening (McCarthy et al. 2000, Birch et al. 2011). There is also a problem of genu valgus in patients with fibular deficiency, but this can be managed with hemiepiphyseodesis or osteotomy of the femoral or proximal tibia (Boakes et al. 1991). Anterior cruciate ligament deficiency has to be taken into account in these patients (Gabos et al. 2005), especially when lengthening is undertaken (Hamdy et al. 2014). There are numerous techniques published for foot and ankle reconstruction (Thomas and Williams 1987, Weber et al. 2002, El-Tayeby and Ahmed 2012)

There are several reports on the outcomes of amputation (Walker et al. 2009, Choi et al. 1990, McCarthy et al. 2000) which favor amputation over lengthening procedures, but here the foot deformation has been more severe in the amputation group (Choi et al. 1990, McCarthy et al. 2000). On the other hand, there are also reports which favor lengthening (El-Sayed et al. 2010, Gibbons et al. 1996).

Paley (2016a) has designed a new classification of these patients to guide reconstructive surgery. Each type in this classification system has a specific preferred surgical treatment independent of the number of rays and limb length inequality. Paley type I patients do not require foot surgery. Type 2 patients are treated by shortening realignment osteotomy of the distal tibia to correct the valgus and stabilize the ankle. The procedure is called SHORDT (shortening osteotomy realignment distal tibia). After or together with this procedure, the tibia is lengthened. Types 3 and 4 are candidates for the SUPERankle procedures (systematic utilitarian procedure for extremity reconstruction) which was presented by Paley in 1996. It is performed when the patient is 18–24 months old. Lengthening is usually done together with this procedure. (Paley 2016a) There is one report about the results of the SUPERankle procedure (Kulkarmi et al. 2019): results were excellent in over 50% of the patients.

2.10.3.4 Cleft foot

The goals of the cleft foot treatment are comfort and good function in standard shoes (Mosca 2014). The treatment may be observation alone (Wood et al. 1997). If surgery is needed, the options are to close the cleft to a certain level, to maintain symmetrical feet and to preserve the position of the border rays to prevent collapse and valgus deformities of the toes (Tani et al. 1997). The indications for surgical treatment are to improve shoe wear and foot cosmesis (Abraham and Waxman 1999).

Different surgical procedures have been described. These include, for example, simple closure (Tani et al. 1997), the use of flaps (Wood et al. 1997) and insertion of a silicone block to prevent foot collapse. (Tani et al. 1997)

3 Aims of the study

1. To calculate the population-based prevalence of congenital lower-limb deficiencies and to determine the associated mortality and anomalies. The goal was also to evaluate lower-limb deficiencies by defect type. (I)
2. To explore the impact of children with lower-limb deficiencies on the healthcare system and to compare different subcategories of lower-limb deficiencies and their surgical treatment. (II)
3. To examine risk factors for congenital limb deficiencies related to pregnancy and maternal variables. (III)

4 Materials and methods

For all studies (I, II and III), data on limb deficiencies was collected from the National Register of Congenital Malformations, maintained by the Finnish institute for health and welfare. The registry contains data from the year 1963 onward on congenital anomalies in Finland. Since 1993 the registry has upheld a database in electronic form for data management.

The Register contains data on all live births, stillbirths and spontaneously aborted fetuses and pregnancy terminations on fetuses with at least one major congenital anomaly. Major structural anomalies and chromosomal defects are coded according to an extended version of the 9th Revision of the International Classification of Diseases (ICD-9) of the World Health Organization. Minor anomalies are excluded according to the system of the European Surveillance of Congenital Anomalies, EUROCAT (EUROCAT). The Register also draws data with the help of the unique personal identification code (PIC) from other national health registers: Medical Birth Register, Register on Induced Abortions, FHDR (hospital discharge data on congenital anomalies from the first two calendar years after birth), The Register of Visual Impairment, all maintained by THL, as well as from the Cause-of-Death Statistics, maintained by Statistics Finland. All notified diagnoses are evaluated, classified and coded by a medical geneticist at the Register. If the diagnosis is equivocal, more information (e.g., patient records, photographs, X-ray images, specialist opinions) is asked from the hospitals concerned.

4.1 Classifications

For all studies (I, II and III) major congenital (fetal) anomalies were defined as anomalies or malformations that create significant medical problems or require specific management. Minor anomalies were described as features that vary from those seen in the normal population but not causing increased morbidity. (EUROCAT, Register of Congenital Malformations)

Limb deficiency was defined as total or partial absence or severe hypoplasia of skeletal structures of limbs, including femoral hypoplasia (ICBDSR). Lower and upper limb deficiencies were classified as terminal transverse, longitudinal (pre- and postaxial), intercalary and multiple types and split foot malformations according to

the EUROCAT classification system (EUROCAT guide 3 2004). In cases of syndactylies of toes or amniotic bands, the limb anomaly was classified as a limb deficiency only if at least one bone was clearly missing or severely hypoplastic. If the reduction could not be classified according to the EUROCAT classification system (EUROCAT guide 3 2004), the reduction was named unknown.

The term isolated limb deficiency was used when there were no major malformations involving other organ systems than limbs. Cases with isolated limb reductions could have abnormalities in one or more limbs, including upper and lower extremities. Cases with associated limb reductions had major structural anomalies in both limb(s) and non-limb structures. The associated limb deficiency could also be part of a syndrome. Associated structural anomalies were classified to cardio-respiratory, urogenital, central nervous system, abdominal and axial skeleton defects (Study I). Associated upper limb deficiencies were also investigated in study I.

In Study II the final study population was subdivided into terminal amputations and long bone, foot and toe deficiencies. Long bone deficiencies were further divided into femoral, tibial and fibular deficiencies according to the classification systems of Aitken (1969), Jones et al. (1978) and Achterman and Kalamchi (AK) (1979).

4.2 Studies I and II

For Study I and Study II all cases with the ICD-9 -codes 75XX (= congenital anomalies) and 65XX (=normal delivery, and other indications for care in pregnancy, labor and delivery) were collected from the Malformation Register. Live births, stillbirths, fetuses from spontaneous abortions and terminations of pregnancy for fetal anomalies between the years 1993 and 2008 were included. Diagnoses and medical records of the cases with a possible lower limb defect were re-evaluated by a pediatric and orthopedic surgeon and a medical geneticist (J.S and A.R). Problematic cases were further evaluated by two pediatric orthopedic surgeons (I.H. and Y.N.). In the beginning of data collection, there were 518 cases and after evaluation 247 cases (46.7%) were excluded because there was no lower limb reduction. Additional information was requested from the hospitals concerned in 142 of the cases (27% out of the 518). Based on this new information a diagnosis of lower limb deficiency was censored in eleven cases. The lower limb deficiency diagnosis became more accurate in 22 cases. To find additional cases with lower limb reduction diagnosed or treated later, the study data was cross-linked with the FHDR data. Forty-one possible new cases were identified. After evaluation of the medical records of these 41 patients, 13 new limb reduction cases were found. Thus, there was a total of 273 cases with lower limb deficiencies.

In Study II the study population included all live births (194) with lower limb deficiency born in 1993–2008. Nine children who died during their birth admission

were excluded. This population (185 cases) was cross-linked with the FHDR data by the PIC. The basic variables collected in FHDR include PIC (including date of birth and sex), area of residence, hospital ID, admission and discharge days and operation days, as well as the diagnoses. Diagnoses were recorded according to the ICD-9 during 1987−1995, and according to the ICD-10 since 1996. Operations were registered according to the classification of the National League of Hospitals during 1986−1996, and since 1996 according to the Finnish version of Nordic Medico-Statistical Committee (NOMESCO) procedure classification. The total number of hospital admissions (excluding the birth episode), surgical and non-surgical admissions (hospital admission has operation code), days spent in hospital, as well as number and type of surgical operations covering the time from from January 1st 1993 to December 31st 2009 were analyzed concerning 1) the 185 patients with lower limb deficiencies and 2) the whole live-born pediatric population covering the time from January 1st 1993 to December 31st 2008 (N = 942,692).

4.2.1 Statistics

For Study I, the birth and total prevalence were given per 10,000 births, live birth prevalence per 10,000 live births. Associations with major anomalies in other organ systems were compared using the $\chi 2$-test. Relative risks (RR) for associated other major anomalies and their 95% confidence intervals (CI) compared with the general malformation figures (including live births, still births and terminations of pregnancy for fetal anomalies) from the National Register of Congenital Malformations in 1993−2008 were calculated. P-values below 0.05 were considered statistically significant. Analyses were performed using R 3.0.0 (R Foundation for Statistical Computing, Vienna, Austria). Spontaneous abortions with lower limb reductions were not included, and thus there were 266 cases in the study material.

For Study II, continuous variables were described as means and ranges. Categorical variables were described as frequencies and relative proportions (percentages). Counts of events were modelled by Poisson's regression using the logarithm of the patient count per group as an offset parameter. The probability of cases remaining free of all operations, all orthopedic operations and all operations involving a lower limb deficiency were estimated using the method of Kaplan-Meier, and the differences between subgroups were tested using the log-rank test. Cox's regression model was used to calculate hazard ratios (HR) with 95% confidence intervals (CI). Also here, P-values less than 0.05 were considered statistically significant. All analyses were conducted using R version 3.0.2 (R Foundation for Statistical Computing, Vienna, Austria) and SAS System for Windows, version 9.4 (SAS Institute Inc., Cary, NC, USA).

4.3 Study III

The data of all limb deficiency cases (from 1996 to 2008) and controls was obtained from the National Register of Congenital Malformations, the Medical Birth Register and the Register on the Induced Abortions, all maintained by the Finnish institute for health and welfare (THL). Information on maternal prescription medicine use and drug reimbursements was based on the Register on Reimbursed Drug Purchases and the Register on Medical Special Reimbursements, both maintained by KELA (Social Insurance Institution of Finland). The information from the Social Institution of Finland was collected from 1995 to 2008.

The frequency of long-term illnesses among pregnant women was studied on the basis of the Special Reimbursement Entitlements granted by Kela effective during pregnancy. The frequency of drug use was determined by examining the number of people who bought drugs. Drug prescription data has high coverage (97% of all reimbursed drugs). In Finland, almost all prescription-only drugs deemed necessary for treatment of an illness are reimbursable. Some over-the-counter drugs are also reimbursable when prescribed by a physician. Over-the-counter medicines that are not prescribed by the phycisian are not involved in the present study. Every person entitled to special refunds is recorded by KELA. (Malm et al. 2003, Klaukka 2001) Drug purchases are recorded at all levels of the international Anatomical Therapeutic Chemical (ATC) classification.

The data on upper limb deficiencies (Koskimies et al. 2011) was expanded to include the years from 2006 to 2008 and to involve also cases from the elective terminations of pregnancy and spontaneous abortions from 1996 to 2008 (n=63). All cases with ICD-9 codes 75XX and 65XX from 1996 to 2008 were reviewed and other than limb reduction defects were excluded. There were 610 cases with limb deficiencies.

Each congenital limb deficiency case was assigned 5 controls (children and their mothers), picked from the Medical Birth Register (3050 controls). The controls were healthy with respect to any limb defects (but some had other congenital anomalies). They were standardized by university hospital district and the date of fertilization (year and month).

The impact of drug use during pregnancy on congenital limb deficiencies was analyzed during the first trimester of pregnancy and a month before pregnancy. Maternal long-term diseases (diabetes mellitus, asthma, psychotic mental conditions, depression, epilepsy and inflammatory bowel diseases) and their connection to limb deficiencies was examined by analysis on data from the Register on Reimbursed Drug Purchases and the Register on Medical Special Reimbursements. Other risk factors (maternal age, body-mass index (BMI), smoking, maternal parity, miscarriages, multiple pregnancy, child's gender, pregestational diabetes and infertility treatments including *in vitro* fertilization) were examined by analysis of

data from the Medical Birth Register and from the National Register of Congenital Malformations.

4.3.1 Statistics

All children (n=610) with congenital limb deficiencies and their 3050 controls born in Finland between 1996 and 2008 were included in the statistical evaluation.

Identifying the risk factors for congenital limb deficiencies was the primary study aim. Subgroup results (isolated limb deficiencies, multiple congenital anomalies and syndromes) were interpreted as explanatory.

Categorical variables were summarized with counts and percentages.

Potential risk factors for limb deficiencies were analyzed for live births, stillbirths and elective terminations of pregnancy (smoking and infertility treatment was not available for terminated pregnancies). Separate analyses were performed on the primary population (all cases) and subgroups (isolated limb deficiencies, multiple congenital anomalies and syndromes). Crude odds ratios (ORs) were estimated, together with the 95% confidence intervals (CI) using conditional logistic regression models for potential risk factors: maternal age (<25 years, 25–34 years and >35 years), BMI (body mass index; classified <18.5, 18.5–30, ≥30), smoking (yes/no), previous (0,1,2, 3 or more), multiple pregnancy, child's gender, infertility treatment (yes/no), maternal parity (nulliparous *vs* multiparous) and pregestational diabetes (yes/no). After univariate analyses, any possible risk factors ($p<0.05$ in the univariate models) and confounders were selected and appropriate multivariable conditional logistic regression models were built. The multivariable model for the live births and stillbirths included age as classified, smoking, infertility treatment, maternal parity and pregestational diabetes. In the multivariable model also including smoking and infertility treatment, the analysis population covered the live births and stillbirths.

After these evaluations for live and stillbirths, each medicine (at least 10 exposed mothers) was studied by univariate logistic regression analysis and significant risk factors in these analyses along with maternal risk factors were included the multivariable analyses to yield adjusted odds ratios (OR) and 95% confidence intervals (CI). P-values (two-tailed) below 0.05 were considered statistically significant. The analyses were performed using the SAS Software, version 9.4 for Windows (SAS Institute Inc., Cary, NC, USA).

5 Results

5.1 Population-based prevalence, gender distribution, mortality, prevalence of different lower limb deficiencies and associated anomalies (I)

5.1.1 Prevalence

According to the national Medical Birth Register (2013), there were 946,295 births in Finland (942,724 live births) from 1993 to 2008. During the study years, 273 cases with lower limb reduction defects were identified from the Register (194 live births/ 71.1%, 10 stillbirths/ 3.7%, 7 spontaneous abortions/ 2.6% and 62 terminations of pregnancy for fetal anomalies/ 22.7%). The total prevalence was 2.8 per 10,000 births and the birth prevalence 2.2 per 10,000 births. The live birth prevalence was 2.1 per 10,000 live births.

5.1.2 Gender distribution

Over half of the cases were males (53.0% [141/266]), 44.7% were (119/266) females and 6 (2.3%) were of unknown/undetermined gender. This corresponds the general gender distribution in the Malformation Register.

5.1.3 Mortality

Ten stillbirths and six deaths during the first week of life were recorded. The perinatal mortality was 78 per 1000 births. Additionally, three children died during the first year of life, giving an infant mortality rate of 46 per 1000 live births. Associated congenital malformations and chromosomal or other syndromes were noted in all nine infant deaths (Table 4).

Table 4. Associated major anomalies in infant deaths with lower limb deficiencies in 1993–2008. (Original publication I, copyright with permission)

CASE	ASSOCIATED ANOMALIES	CHROMOSOMAL OR OTHER SYNDROME	LOWER LIMB DEFICIENCY
1	AVSD, TAPVD, atresia of pulmonary artery Hydronephrosis, ureterocele Cleft hard palate Hypoplasia of the second finger		One toe missing
2	VSD Hypoplasia of lungs, thoracic cage anomaly Dysplasia of kidney Cleft hard palate	Trisomy 18	Cleft feet
3	Hydrocephaly Amnion constriction band Bilateral pes equinovarus Reduction deformity of fingers		Reduction defect of toes
4	Atresia of pulmonary artery, dilated aortic valve and ascending aorta Lisencephaly, agenesis of corpus callosum		Reduction defect of second and third toes
5	Muscular VSD Gastroschisis Pes equinovarus Congenital scoliosis, hypoplastic pelvis	Congenital arthrogryposis	Left toes missing
6	VSD Hypoplasia of lungs Hydronephrosis, hydroureter Diaphpragmatic hernia Congenital scoliosis	Trisomy 18	Reduction defect of first toe bilateral
7	Reduction of fingers	Lamellar ichthyosis	Reduction defect of toes
8	Truncus arteriosus, AVSD, DORV Agenesis of right lung Agenesis of left kidney, agenesis of ureters		Total aplasia of right lower limb
9	Anomaly of thoracic cage Hypoplasia of lungs Non-rotation of intestine Phocomelia of upper limbs	Unknown syndrome	Phocomelia of lower limbs

5.1.4 EUROCAT classification of lower limb deficiencies

Terminal transverse reductions were the most common (44.7%) lower limb deficiency type (Table 5). Nearly half of the cases were toe reductions (48.1% [128/266]).

Table 5. Number, proportion and total prevalence of subgroups of lower limb deficiencies. Live births, stillbirths and terminations of pregnancy for fetal anomalies with lower limb deficiencies in 1993−2008. Unknown reduction is a reduction which could not be classified according to the EUROCAT classification system. (Original publication I, copyright with permission)

TYPE OF LOWER LIMB REDUCTION	CASES	PERCENTAGE	TOTAL PREVALENCE /10,000
TERMINAL TRANSVERSE REDUCTION	119	44.7	1.26
POSTAXIAL REDUCTION	46	17.3	0.49
PREAXIAL REDUCTION	15	5.6	0.16
INTERCALARY REDUCTION	21	7.9	0.22
MULTIPLE REDUCTIONS	22	8.3	0.23
SPLITFOOT REDUCTION	12	4.5	0.13
UNKNOWN REDUCTION	31	11.7	0.33

5.1.5 Associated anomalies

Associated major anomalies were present in 47.7% of the 266 cases. Upper limb reductions were found in 32.0% of all cases. Both lower and upper limb reductions were noted in 31 cases of the isolated group (31/139, 22.3%). (Table 6) In 21% of the cases (56/266, 19 isolated cases and 37 associated cases), the amniotic band syndrome was thought to be the reason for the limb deficiency.

Table 6. Associated major anomalies in cases with lower limb deficiencies. Live births, stillbirths and terminations of pregnancy for fetal anomalies with lower limb deficiencies in 1993−2008. (Original publication I, copyright with permission)

ASSOCIATED ANOMALY	NUMBER OF ALL CASES	NUMBER OF NON-CHROMOSOMAL / NON-SYNDROMIC CASES	PERCENT OF ALL 266 CHILDREN
CARDIO-PULMONARY	33	15	12.4
UROGENITAL	41	15	15.4
CENTRAL NERVOUS SYSTEM	34	22	12.8
ABDOMINAL	46	18	17.3
AXIAL SKELETON	36	7	13.5
UPPER LIMB REDUCTION DEFECT	85	59	32.0

The lower limb deficiency cases were compared with the general malformation figures from the Malformation Register in 1993−2008. Cases with lower limb

reduction defects had a significantly increased relative risk (RR = 12.54, 95 % CI 11.06−14.23, p < 0.0001) for non-limb malformations. (Table 7)

Table 7. Relative risk (RR) and 95%CI for associated major anomalies compared with general malformation figures from the National Register of Congenital Malformations. Live births, stillbirths and terminations of pregnancy for fetal anomalies with lower limb deficiencies in 1993−2008. (Cases with lower limb deficiencies = 266, all births = 946 295). (Original publication I, copyright with permission)

MAJOR ORGAN ANOMALY	NO OF CASES WITH LIMB REDUCTION	NO OF CASES IN GENERAL POPULATION	RR (95%CI) OF ORGAN ANOMALY COMPARED WITH GENERAL POPULATION	P
CONGENITAL HEART DEFECT	27	12,967	7.42 (5.19–10.61)	<0.0001
URINARY	32	3,720	30.86 (22.26–42.78)	<0.0001
CENTRAL NERVOUS SYSTEM	34	2,863	42.74 (31.16–58.63)	<0.0001
DIGESTIVE	23	2,579	32.00 (21.61–47.39)	<0.0001
ABDOMINAL WALL DEFECT	28	747	138.50 (96.83–198.10)	<0.0001

Twelve of the cases with lower limb reduction (4.5%) had a chromosomal syndrome and 38 (13.5 %) had other known syndromes. 7% (19/266) were classified as limb-body-wall complex, body-stalk anomalies or caudal regression syndrome. The remaining 199/266 cases (74.8 %) had a non-syndromic, non-chromosomal lower limb reduction. Of them, 60/266 (22.6 %) had other major malformations besides limb deficiencies.

The number of cases without associated anomalies varied significantly (p=0.0018) between the different types of lower limb reductions (Table 8). Over 50% of the patients within the transverse, postaxial, multiple and cleft defect groups had isolated lower limb defects, while the preaxial deficiencies were strongly associated with other major anomalies (RR 1.75; 95% CI 1.2 – 2.53, p = 0.028).

Table 8. Types of lower limb deficiency and relative risk (95% CI) for associated major anomalies compared with the terminal transverse defect type. Calculated among live births, stillbirths and terminations of pregnancy for fetal anomalies (N=266) with lower limb reductions in 1993−2008 MCA=multiple congenital anomalies. (Original publication I, copyright with permission)

TYPE OF LOWER LIMB REDUCTION	ISOLATED	KNOWN SYNDROME / CHROMOSOMAL ANOMALY	MCA WITHOUT KNOWN SYNDROME / CHROMOSOMAL ANOMALY	RR (95% CI)	P
TERMINAL TRANSVERSE	69	25	25	1	
POSTAXIAL	31	7	8	0.78 (0.49–1.24)	0.29
PREAXIAL	4	3	8	1.75 (1.20–2.53)	0.028
INTERCALARY	10	9	2	1.25 (0.79–1.97)	0.47
MULTIPLE	11	6	5	1.19 (0.75–1.90)	0.49
SPLIT FOOT	7	3	2	0.99 (0.49–2.00)	1.00
UNKNOWN	7	14	10	1.84 (1.39–2.45)	0.00050

5.2 Hospital admissions, days spent in hospital and surgical treatment of lower limb deficiencies and their subclasses (II)

5.2.1 Hospital admissions

During the study years, the whole live born pediatric population had 1,524,481 hospital admissions and 4,194,675 hospital days. The mean number of hospital admissions was 0.10 and the mean duration of in-patient care 0.3 days per child per year.

Children with lower limb deficiencies (185) had altogether 1,140 hospital admissions and 5,170 days spent in hospital (Table 9). Thus, the mean annual number of hospital admissions was 0.6 (range 0−5), and days spent in hospital 2.9 (range 0−124) per child. Compared to the whole live born pediatric population in Finland this signifies a six-fold and ten-fold excess, respectively.

5.2.1.1 Hospital admissions by subclass

There were seven patients with terminal amputations (lower leg 4 [bilateral 1], foot 3 [bilateral 2]). They had an annual mean of 1.5 (range 1−4) hospital admissions and of 5.6 (range 1−25) days spent in the hospital. This was 15- and 19-fold compared to the whole live born pediatric population, respectively.

There were 11 PFFD patients, five tibial deficiency and 37 fibular deficiency patients (53 patients with long bone deficiency). They had an annual mean of 1.1 (range 0−5) hospital admissions and 3.9 (range 0−10) days of hospital care. Compared to the whole pediatric population in Finland, these numbers were 11- and 13-fold, respectively.

Both the number of hospital admissions and days spent at the hospital were six-fold compared to the whole pediatric population in patients with foot deficiencies. There were nine split-feet cases. The annual mean of hospital admissions was 0.6 (range 0−2) and 1.9 (range 0−6) of days in hospital care.

The patients with toe deficiencies had an annual mean of 0.4 (0−3) hospital admissions and of 2.6 (range 0−47) hospital days, which was 4- and 9-fold compared to the whole live born pediatric population in Finland, respectively. (Table 9)

Table 9. Number of patients who required hospital treatment in four groups of patients with lower limb deficiency (N=185). (Original publication II, copyright with permission)

	HOSPITAL ADMISSIONS % OF PATIENTS RANGE OF ADMISSION / PATIENT	HOSPITAL DAYS % OF PATIENTS RANGE OF ADMISSION / PATIENT	NUMBER OF PATIENTS
TERMINAL AMPUTATIONS	100% 2–50	100% 1–47	7/7
LONG BONE DEFICIENCIES	81.1% 0–81	81.1% 0–118	43/53
FOOT DEFICIENCIES	83.3% 0–28	83.3% 0–53	20/24
TOE DEFICIENCIES	71.3% 0–38	71.3% 0–352	72/101

5.2.2 Surgical admissions

Almost half (49%) of the admissions were surgical. Sixty-four per cent (119) had operations and the total number of operations was 561. Over half of the operations (54%) were orthopedic: 226 of the lower limbs, 70 of the upper limbs and 8 of the spine. The Kaplan-Meier estimates of patients having at least one operation, one orthopedic operation and one operation to treat the deficiency were 64%, 44% and 32% after 10-year follow-up (Figure 4)

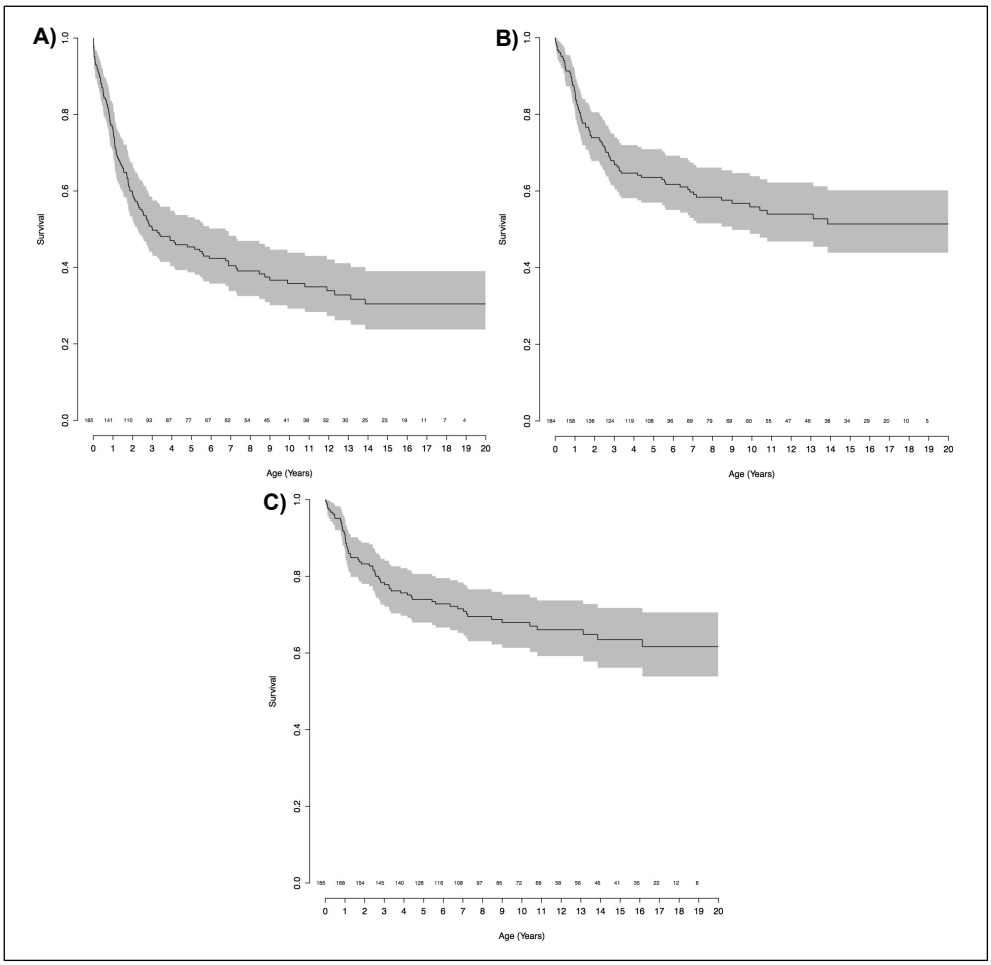

Figure 4. Five and ten-year operation-free survival: A) all operations, B) orthopedic operations and C) operations due to lower-limb deficiency. Number of patients at risk for operation in x scale. (Original publication II, copyright with permission)

5.2.2.1 Surgical admissions by subclass

The age of the patients in the in terminal amputation group requiring an orthopedic procedure was between 0.4 and 2.4 years. (Table 10)

The proportion (Kaplan-Meier estimate) of patients (n=53) with a long bone deficiency requiring at least one operation, one orthopedic operation or one operation due to lower limb deficiency by age ten years was 77%, 67% and 70%, respectively (Figure 5).

In the group of patients with foot deficiency, the median number of operations was 2 (range 1−23). Over half (55%, 46/84) of these operations were orthopedic procedures.

They were equally divided into lower (n=24) and upper limb (n=22) surgery and were performed when the patients were between 0 and 16 years of age. (Table 10)

The proportion (Kaplan-Meier estimate) of patients with foot deficiency having at least one operation, one orthopedic operation or one operation due to foot deficiency by 10 years of age were 82%, 60% and 25%, respectively (Figure 5).

Toe deficiency patients had a median of two operations (range 1−18) per child. Forty-two per cent were orthopedic procedures, most of which or 62% (47/76) were directed to treat the lower limbs. The mean age of the patients was 3.3 years (range 0.1−13.9). (Table 10)

The proportion (Kaplan-Meier estimate) of patients (n=101) with toe deficiencies requiring at least one operation, one orthopedic operation or one operation concerning toe deficiency by age ten was 52%, 26% and 19%, respectively (Figure 5).

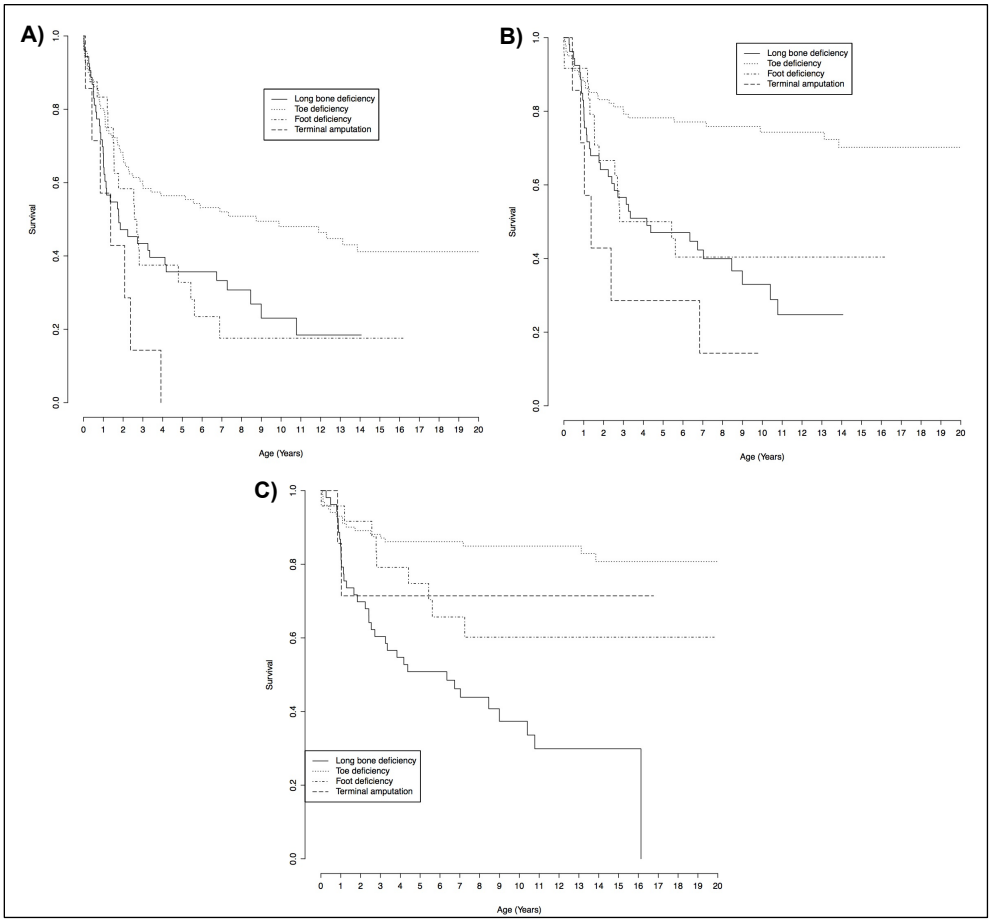

Figure 5. Five- and ten-year operation-free survival: A) all operations, B) orthopedic operations and C) operations due to lower-limb deficiency by patient group. (Original publication II, copyright with permission)

Table 10. Percentage of patients and range of surgical sessions per patient that had surgical treatment in four groups of patients with lower limb deficiency. (ENT = ear, nose and throat procedures, GI-surgery = gastrointestinal surgery). (Original publication II, copyright with permission)

	ALL SURGERY % PATIENTS RANGE OF TREATMENT/ PATIENT	ORTHOPEDIC	GI-SURGERY	UROLOGIC SURGERY	CARDIAC SURGERY	ENT PROCEDURES	CASTING OR BANDAGE PROCEDURES	WOUND AND SCAR SURGERY	OTHER PROCEDURES
TERMINAL AMPUTATIONS N=7	100% (7/7) 1–13	71.4% (5/7) 0–3	0%	5.9% (6/101) 0–5	0%	28.6% (2/7) 0–1	14.3% (1/7) 0–1	28.6% (2/7) 0–1	0%
LONG BONE DEFICIENCIES N=53	73.6% (39/53) 0–34	62.3% (33/53) 0–21	9.4% (5/53) 0–5	11.3% (6/53) 0–3	1.9% (1/53) 0–2	34.0% (18/53) 0–7	22.6% (12/53) 0–4	9.4% (5/53) 0–4	11.3% (6/53) 0–3
FOOT DEFICIENCIES N=24	79.2% (19/24) 0–23	58.3% (14/24) 0–11	8.3% (2/24) 0–1	4.2% (1/24) 0–1	0%	25.0% (6/24) 0–8	22.6% (12/53) 0–4	20.8% (5/24) 0–3	4.2% (1/24) 0–1
TOE DEFICIENCIES N=101	53.5% 54/101) 0–18	24.8% 25/101) 0–11	5.9% (6/101) 0–5	4.0% (4/101) 0–6	4.0% (4/101) 0–4	26.7% (27/101) 0–7	5.0% (5/101) 0–2	10.9% (11/101) 0–3	4.0% (4/101) 0–2

Patients with toe deficiency had a lower risk than the other groups to get operated during the follow-up time (Table 11).

Table 11. Risk of getting operated by patient group compared to patients with toe deficiency (Cox regression model). (Original publication II, copyright with permission)

	ANY OPERATION HR (95% CI)	ORTHOPEDIC OPERATION HR (95% CI)	OPERATION CONCERNING LOWER LIMB DEFICIENCY HR (95% CI)
TERMINAL AMPUTATIONS	3.6 (1.6–8.0)	6.0 (2.4–15.0)	2.0 (0.5–8.6)
LONG BONE DEFICIENCIES	1.8 (1.2–2.8)	3.4 (2.0–5.6)	5.6 (3.0–10.0)
FOOT DEFICIENCIES	1.8 (1.1–3.1)	2.7 (1.4–5.2)	2.5 (1.1–5.6)

5.2.3 Occurrence and treatment of long-bone deficiencies in Finland between 1993 and 2009

The live birth prevalence of PFFD was 0.12 per 10,000 live births in Finland. Seventy-two per cent (8/11) with PFFD had a median of 5.5 operations (range 1−25) per child, most often an orthopedic procedure (66%, 41/62) involving lower limbs. The age range of the patients at the time of the orthopedic procedures was 2.3 to 6.6 years. (Table 12)

The live birth prevalence of tibial deficiency was 0.05 per 10,000 live births. All five children with tibial deficiency (1 Jones II, 2 Jones III and 2 unclear types) needed hospital admissions and surgical procedures. Most (71%, 39/55) of the procedures were orthopedic operations of the lower limb (n=31) and spine (8). Two patients required amputation (at age 1.8 and 3.6 years). One limb lengthening operation was performed in a patient aged under 4 years with an unclassified type of tibia deficiency. (Table 12)

The live birth prevalence of patients with fibular deficiency was 0.39 per 10,000 live births. Twenty-six of the 37 patients with fibular deficiency (10 bilateral) had a median of three operations (range 1−34) per child. About two-thirds (94/155, 61%) of these operations were orthopedic procedures of the lower (n=83) and upper (n=11) limbs. Ten patients with a median age of 1.1 years (0.5−2.4) needed an amputation. Six children had lengthening operations. (Table 12).

Table 12. Long bone deficiencies. Number of different subtypes and number of related orthopedic procedures (lower limb procedures subcategorized into osteotomies, lengthenings, amputations, epiphyseodeses, soft tissue surgeries, hardware removals and other procedures). PFFD=proximal femoral focal deficiency, AK=Acterman-Kalamchi classification. (Original publication II, copyright with permission)

	CASES	ORTHOPEDIC PROCEDURES	OSTEOTOMY	LIMB LENGTHENING	AMPUTATION	EPIPHYSEODESIS	SOFT TISSUE SURGERY	REMOVAL OF HARDWARE	OTHER	SPINAL SURGERY	HAND SURGERY
PFFD	11	41	9	4	0	4	1	9	14		0
AITKE A	5		7	4		4		9	12		
AITKE B	1		2								
AITKE C	2										
AITKE D	2										
CONGENITAL SHORT FEMUR	1										
TIBIAL DEFICIENCY	5	39	2	1	2	1	2	5	18	8	0
JONES II / TIBIAL + FIBULAR HYPOPLASIA OTHER LEG	1										
JONES III	2				2			1	4		
UNCLASSIFIED	2		2	1		1	2	4	14		
FIBULAR DEFICIENCY	37	94	4	11	11	3	6	19	29	0	11
AK 1A	11		2	4		1	1	65	5		
AK1B	1					1					
AK1	7			3				3	2		
AK2	8				7		1	1	3		
AK2 / AK1B	1		1	1			1	2	5		1
AK2 / AK2	6				4	1	2	7	11		8
AK2 / AK1	1										
AK1 / AK1	1		1	3					3		2
AK1B / AK1	1						1				

5.2.4 Patients with no hospital admissions

In nearly one fourth of the cases (43/185, 23%) no hospital admissions were registered. Most (29/43) of these patients had a toe deficiency, seven Achterman-Kalamchi type IA fibular deficiency (follow-up 1.4−8.1 years), three PFFD (Aitken A, C and D one each, follow-up 4.3−11.1 years), three had one missing metatarsal and one had bilateral cleft feet (follow-up 1.3–11.5 years).

5.3 Risk factors (III)

The whole study population consisted of 610 children with limb deficiency and 3050 controls. There were 385 (63.1%) upper limb deficiencies, 151 (24.8%) lower limb deficiencies and 74 children (12.1%) with both upper and lower limbs affected. Isolated limb deficiencies counted for 295 (48.4%) cases. Of the children, 107 (17.5%) had anomalies in other organ systems and a syndromic or chromosomal background was reported in 208 cases (34.1%).

Univariate analyses

In univariate analyses, maternal smoking (OR 1.31, 95% CI 1.02–1.68), young age (<25 years, OR 1.42, 95% CI 1.14–1.77) and high maternal age (>35 years, 1.33, 1.04–1.70), primiparity (1.55, 1.30–1.85) and pregestational diabetes (5.00, 2.17–11.5) were identified as significant potential maternal risk factors for congenital limb deficiencies. Previous miscarriages, multiple pregnancy or abnormal BMI (below 20 or above 30) did not increase this risk ($p>0.10$ for all comparisons). Infertility treatment increased only the risk of limb deficiency associated with other major anomalies (OR 5.00, 95% CI 1.61–15.5). (Table 13, Table 14) First trimester use of insulin and analogues (4.55, 1.93–10.70), beta blocking agents (3.12, 1.42–6.89), estrogens (2.73, 1.14–6.54), progesterone (1.83, 1.22–2.75), gonadotropins (1.61, 1.01–2.57), muscle relaxants (2.41, 1.10–5.28) and antiepileptics (4.09, 1.70–9.87) increased the risk of limb deficiencies. In contrast, gastrointestinal medication (proton pump inhibitor, propulsives, H2 antagonists), antimicrobial treatment (antibiotics, antifungal, antiviral; systemic or local gynecological), glucocorticosteroids (systemic or topical), analgesics (nonsteroidal anti-inflammatory, paracetamol) or asthma medication (inhaled corticosteroids, B2-agonists), antidepressants, antipsychotics, anxiolytics, antihistamines and thyroid or hypothalamic hormones did not increase the risk for congenital limb deficiencies ($p>0.10$ for all comparisons).

Table 13. Univariate analysis of pregnancy-related and maternal risk factors for congenital limb deficiencies in all cases (live births, stillbirths and elective terminations of pregnancy) (N=610). Every limb deficiency case was assigned five controls. *Analysis does not contain elective terminations of pregnancy. (Original publication III)

RISK FACTORS	NO CASES ANALYZED	ALL CASES 0R (95% CI)	P	ISOLATED OR (95%CI)	P	MULTIPLE ANOMALIES OR (95%CI)	P	SYNDROMIC OR (95% CI)	P
MATERNAL AGE (25–34 YEARS = REF)	610		<0.001		0.11		0.020		0.030
<25 YEARS		1.42 (1.14–1.77)		1.39 (1.02–1.89)		2.04 (1.23–3.27)		1.19 (0.80–1.77)	
35 OR MORE		1.33 (1.04–1.70)		1.08 (0.73–1.59)		1.30 (0.72–2.35)		1.66 (1.13–2.42)	
MATERNAL PARITY (MULTIPARITY AS REFERENCE)	607	1.55 (1.30–1.85)	<0.001	1.63 (1.27–2.10)	<0.001	1.87 (1.22–2.86)	0.0040	0.080 (2.80–0.09)	0.090
PREGESTATIONAL DIABETES	610	5.00 (2.17–11.53)	<0.001	6.25 (1.68–23.28)	0.0060	Only 1 diabetic mother		3.57 (1.13–11.25)	0.030
INFERTILITY TREATMENT*	492	1.52 (0.91–2.55)	0.11	0.92 (0.41–2.08)	0.84	5.00 (1.61–15.50)	0.0050	1.59 (0.63–4.03)	0.33
SMOKING*	474	1.31 (1.02–1.68)	0.030	1.38 (1.00–1.91)	0.050	1.02 (0.50–2.08)	0.95	1.30 (0.81–2.11)	0.28
MOTHER'S BMI (18.5–29.9 AS REFERENCE)	153		0.64		0.57		0.95		0.16
<18.5		1.42 (0.67–2.98)		1.21 (0.46–3.19=		0.71 (0.08–6.17)		3.06 (0.73–12.84)	
≥ 30		0.96 (0.55–1.70)		0.68 (0.31–1.50)		1.04 (0.28–3.95)		1.94 (0.66–5.69)	
MULTIPLE PREGNANCY	610	1.37 (0.91–2.07)	0.14	0.80 (0.39–1.63)	0.54	3.18 (1.29–7.81)	0.010	1.59 (0.83–3.01)	0.16
MALE SEX (FEMALE = REF)	597	1.35 (1.13–1.61)	<0.001	1.47 (1.14–1.89)	0.0030	1.20 (0.79–1.83)	0.39	1.27 (0.93–1.73)	0.13
PREVIOUS MISCARRIAGES (0=REF)	604		0.53		0.37		0.18		0.98
1		1.17 (0.92–1.48)		1.22 (0.87–1.72)		1.27 (0.73–2.22)		1.05 (0.69–1.59)	
2		0.89 (0.55–1.42)		1.10 (0.57–2.12)		0.17 (0.02–1.28)		1.14 (0.54–2.43)	
3 OR MORE		0.83 (0.37–1.84)		0.26 (0.04–1.94)		2.19 (0.56–8.52)		0.95 (0.28–3.25)	

Table 14. Univariate analysis of special reimbursements approved for medication to treat maternal chronic diseases in all cases (live births, stillbirths and elective terminations of pregnancy) (N=610). Every limb deficiency case was assigned five controls.

SPECIAL REIMBURSEMENT	ALL CASES P 0R (95% CI)	ISOLATED P OR (95%CI)	MULTIPLE ANOMALIES P OR (95%CI)	SYNDROMIC P OR (95% CI)
DIABETES, INSULIN TREATMENT	0.00020 5.00 (2.17–11.53)	0.0063 6.25 (1.68–23.28)	NA	0.030 3.57 (1.13–11.25)
THYROID INSUFFICIENCY	0.43 1.41 (0.60–3.27)	0.53 1.43 (0.47–4.34)	0.26 5.00 (0.31–79.94)	1.00 1.00 (0.21–4.68)
EPILEPSY	0.19 1.90 (0.74–4.93)	0.51 0.50 (0.06–3.91)	0.11 5.00 (0.70–35.50)	0.070 4.32 (0.85–21.86)
MENTAL HEALTH	0.66 0.63 (0.08–5.00)	0.87 0.83 (0.10–6.92)	NA	NA
AUTOIMMUNE DISEASES	0.70 0.79 (0.23–2.67)	0.58 0.56 (0.07–4.39)	0.84 1.25 (0.14–11.18)	0.87 0.83 (0.10–6.92)
CHRONIC ASTHMA AND OBSTRUCTIVE PULMONARY DISEASES	0.89 0.96 (0.56–1.65)	0.60 1.22 (0.59–2.55)	0.35 0.50 (0.12–2.14)	0.94 0.96 (0.37–2.52)
CHRONIC HYPERTENSION	0.53 1.52 (0.41–5.68)	0.84 1.25 (0.14–11.18)	0.45 2.50 (0.23–27.57)	0.84 1.28 (0.13–12.78)
MULTIPLE SCLEROSIS	0.060 10.00 (0.91–110.28)	NA	NA	0.26 5.00 (0.31–79.94)

Multivariate analyses

After adjusting for maternal age and smoking, primiparity increased the risk for all limb deficiencies (OR 1.42, 95% CI 1.14–1.76) and isolated limb deficiencies (1.61, 1.21–2.13); pregestational diabetes the risk for all (3.59, 1.32–9.74) and isolated limb deficiencies (5.16, 1.27–21.0); and infertility treatment for limb deficiencies associated with multiple congenital anomalies (6.35, 1.88–21.4) in the group of live or stillborn children (N=492). (Table 15)

After adjusting for all maternal risk factors mentioned above, first trimester usage of progesterone increased the risk for all limb deficiencies (OR 1.85, 95% CI 1.06–3.22) and antiepileptics increased the risk for all (5.66, 2.16–14.87) and syndromic limb deficiencies (16.6, 1.71–160.46). (Table 16)

Table 15. Multivariable analysis of maternal risk factors in group of live births and stillbirths (N=492). Every limb deficiency case was assigned five controls. (Original publication III)

	ALL CASES OR (95% CI)	P	ISOLATED OR (95% CI)	P	MULTIPLE ANOMALIES OR (95%CI)	P	SYNDROMIC OR (95% CI)	P
NO CASES ANALYZED	474		274		73		127	
MATERNAL AGE (25–34 YEARS = REF)								
<25 YEARS	1.26 (0.97–1.63)	0.060	1.23 (0.88–1.73)	0.44	1.57 (0.80–3.06)	0.32	1.16 (0.69–1.96)	0.14
>35 YEARS	1.34 (0.99–1.80)		.15 (0.76–1.75)		1.47 (0.67–3.26)		1.68 (1.01–2.82)	
SMOKING	1.40 (0.99–1.80)	0.19	1.23 (0.88–1.74)	0.23	1.03 (0.48–2.17)	0.95	1.25 (0.76–2.07)	0.38
INFERTILITY TREATMENT	1.34 (0.78–2.32)	0.29	0.64 (0.26–1.56)	0.33	6.35 (1.88–21.43)	0.0030	1.48 (0.56–3.90)	0.43
PRIMIPAROUS VS MULTIPAROUS (AS REFERENCE)	1.42 (1.14–1.76)	0.0010	1.61 (1.22–2.14)	<0.001	1.63 (0.91–2.90)	0.10	1.02 (0.66–1.56)	0.94
PREGESTATIONAL DIABETES	3.59 (1.32–9.74)	0.010	5.16 (1.27–21.00)	0.020	Not included (only 1 diabetic mother)		2.78 (0.64–12.11)	0.17

Table 16. Multivariable analysis on the risk of medications used during the first trimester of pregnancy adjusted for maternal age, smoking, infertility treatment, parity and pregestational diabetes in live and stillbirths (N=492). Every limb deficiency case was assigned five controls. (Original publication III)

	ALL CASES OR (95% CI)	P	ISOLATED OR (95% CI)	P	MULTIPLE ANOMALIES OR (95% CI)	P	SYNDROMIC OR (95%CI)	P
NO CASES ANALYZED	474		274		73		127	
BETA BLOCKING AGENTS	2.40 (0.89–6.47)	0.080	2.15 (0.65–7.09)	0.21	NA		3.54 (0.58–21.46)	0.17
ESTROGENS	0.090 (0.23–3.39)	0.85	NA		0.47 (0.03–8.40)	0.61	1.50 (0.23–9.77)	0.67
PROGESTERON	1.85 (1.06–3.22)	0.030	1.94 (0.87–4.36)	0.11	1.89 (0.53–6.77)	0.33	1.85 (0.63–5.40)	0.26
GONADOTROPINS AND OTHER OVARIAN STIMULATING MEDICATION	1.70 (0.89–3.26)	0.11	1.91 (0.68–5.42)	0.22	0.86 (0.17–4.27	0.86	2.12 (0.70–6.37)	0.18
MUSCLE RELAXANTS, CENTRALLY ACTING AGENTS	1.86 (0.74–4.67)	0.19	1.70 (0.42–6.90)	0.46	0.84 (0.070–10.69)	0.89	2.32 (0.53–10.22)	0.27
ANTIEPILEPTICS	5.66 (2.16–14.87)	<0.001	2.81 (0.81–9.79)	0.10	NA		16.56 (1.71–160.46)	0.020

When adding elective pregnancy terminations to the multivariable analyses, young (<25 years) (OR 1.25, 95% CI 1.00–1.57) and high (> 35 years) maternal age (1.50, CI 1.16–1.93), primiparity (1.55, 1.29–1.88) and pregestational diabetes (4.78, 2.06–11.13) were significant risk factors for all congenital limb deficiencies. (Table 17, Figure 6).

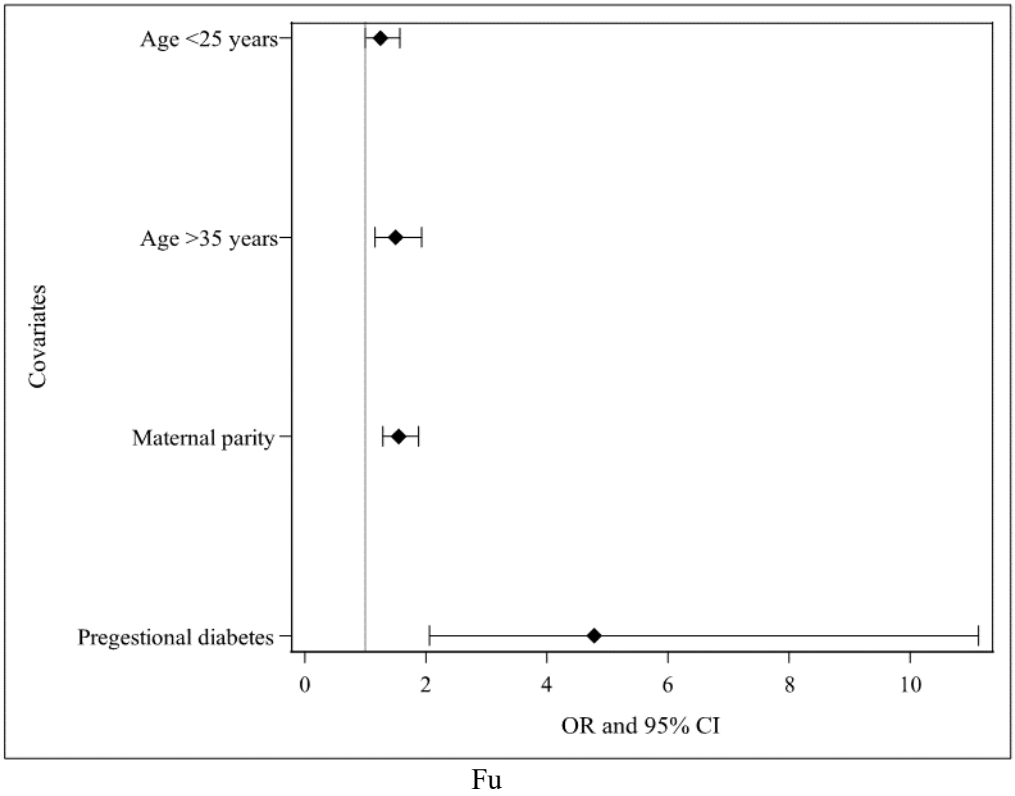

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Figure 6. Multivariable analysis of maternal risk factors, all cases (live births, stillbirths and elective terminations of pregnancy) (N=610). Every limb deficiency case was assigned five controls.

Table 17. Multivariable analysis on maternal risk factors with all cases (live births, stillbirths and elective terminations of pregnancy) analysed (N=610). Every limb deficiency case was assigned five controls. (Original publication III)

	ALL CASES OR (95% CI)	P	ISOLATED OR (95% CI)	P	MULTIPLE ANOMALIES OR (95%CI)	P	SYNDROMIC OR (95% CI)	P
NO CASES ANALYZED	607		295		106		206	
MATERNAL AGE (25–34 YEARS = REF)								
<25 YEARS	1.25 (1.00–1.57)	0.0030	1.27 (0.93–1.74)	0.24	1.67 (0.98–2.84)	0.12	1.06 (0.70–1.60)	0.0080
>35 YEARS	1.50 (1.16–1.93)		1.23 (0.83–1.83)		1.43 (0.77–2.68)		1.84 (1.25–2.72)	
PRIMIPAROUS VS MULTIPAROUS (AS REFERENCE)	1.55 (1.29–1.88)	<0.001	1.59 (1.22–2.07)	<0.001	1.73 (1.09–2.76)	0.020	1.42 (1.02–1.97)	0.040
PREGESTATIONAL DIABETES	4.78 (2.06–11.13)	<0.001	6.03 (1.60–22.73)	0.0080	Not included (only 1 diabetic mother)		3.78 (1.19–12.00)	0.020

After adjusting for the above risk factors, first trimester progesterone use added the risk of congenital limb deficiency in the whole study population (OR 1.71, 95% CI 1.13–2.59) and in multiple congenital anomaly group (3.52, 1.37–9.03). Other statistically significant associations were found in the whole study population with antiepileptics (4.03, 1.66–9.79), beta blocking agents (2.74, 1.22–6.18) and centrally acting muscle relaxants (2.25, 1.01–4.99). (Table 18, Figure 7)

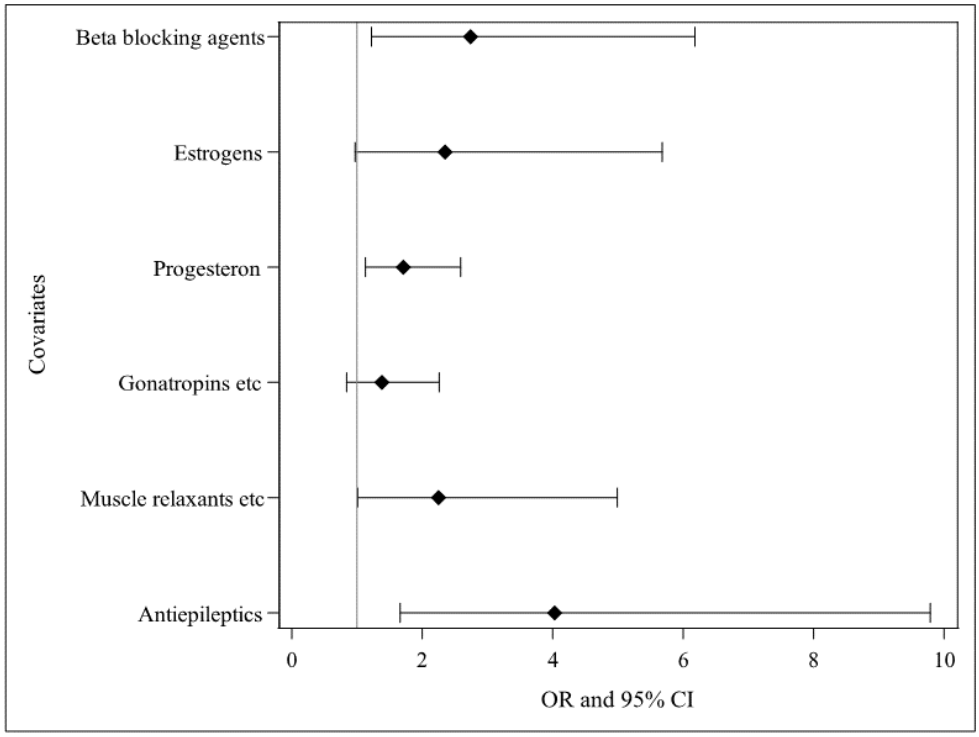

Figure 7. Multivariable analysis of the risk of maternal use of medication during the first trimester of pregnancy adjusted for maternal age, parity and pregestational diabetes in all cases (live births, stillbirths and elective terminations of pregnancy) (N=610). Every limb deficiency case was assigned five controls.

Table 18. Multivariable analysis of the risk of maternal use of medication used during first trimester adjusted for maternal age, parity, and pregestational diabetes in all cases (live births, stillbirths and elective terminations of pregnancy) (N=610). Every limb deficiency case was assigned five controls. (Original publication III)

	ALL CASES OR (95% CI)	P	ISOLATED OR (95% CI)	P	MULTIPLE ANOMALIES OR (95% CI)	P	SYNDROMIC OR (95%CI)	P
NO CASES ANALYZED	607		295		106		206	
BETA BLOCKING AGENTS	2.74 (1.22–6.18)	0.020	2.34 (0.74–7.38)	0.15	NA		3.05 (0.84–11.11)	0.090
ESTROGENS	2.35 (0.97–5.68)	0.060	0.74 (0.09–6.29)	0,78	6.94 (1.13–42.73)	0.04	2.65 (0.73–9.67)	0.14
PROGESTERON	1.71 (1.13–2.59)	0.010	1.21 (0.61–2.39)	0.59	3.52 (1.37–9.03)	0.0090	1.79 (0.94–3.41)	0.080
GONADOTROPINS AND OTHER OVARIAN STIMULATING MEDICATION	1.38 (0.84–2.26)	0.21	1.18 (0.50–2.82)	0.70	1.52 (0.49–4.75)	0.47	1.45 (0.71–2.96)	0.31
MUSCLE RELAXANTS, CENTRALLY ACTING AGENTS	2.25 (1.01–4.99)	0.050	2.01 (0.56–7.22)	0.29	1.35 (0.14–13.25)	0.79	2.78 (0.88–8.78)	0.080
ANTIEPILEPTICS	4.03 (1.66–9.79)	0.0020	2.97 (0.86–10.29)	0.090	NA		4.68 (0.94–23.41)	0.060

6 Discussion

6.1 Validity of the data

Congenital upper and lower limb reductions are usually identified visually after delivery. Thus, they are also well registered in different registers. Our data (from 1993 to 2008) is collected from the National Register of Congenital Malformations, and it includes data on congenital anomalies from other national registers, such as the Medical Birth Register, The Register of Induced Abortions, the Care Register for Health Care and the Statistics Finland cause-of-death statistics. Information on maternal prescription medicine use was obtained from the Register on Reimbursed Drug Purchases and the Register on Medical Special Reimbursements (Social Insurance Institution of Finland) from 1996 to 2008. All these registers report data with high accuracy and 100% country coverage (Greenlees et al. 2011, Congenital Malformatios 2015, Sund 2012, Pakkasjärvi et al. 2006, Leoncini et al. 2010, Gissler et al. 1995, Finnish Statistics on Medicines). Drug prescription data has high coverage (97% of all reimbursed drugs). In Finland, almost all prescription-only drugs necessary for treatment of an illness are reimbursable. Some over-the-counter drugs are also reimbursable when prescribed by a physician. Every person entitled to special refunds is recorded in KELA. (Malm et al. 2003, Klaukka 2001) We have further validated the limb deficiency data by examining all medical records and available radiographs and confirming the diagnosis of each affected subject. In the data on congenital lower limb deficiencies, we found only eleven false-positive cases and thirteen false-negative cases, both less than five per cent of the total amount of cases. Additionally, none of our country-specific diseases or syndromes (Norio 2003a, b and c) include limb reductions and there are no marriages between first-degree relatives due to restrictions of our legislation. Because congenital anomalies are more common in pregnancies ending in elective terminations, we analyzed our risk factor data (III) separately with and without elective terminations. All these circumstances increase the reliability of our data.

A limitation of this study was the secondary nature of the data collected in the registers. The data were not collected to specifically study the epidemiology of congenital lower limb deficiencies and the maternal risk factors related to congenital limb reductions. We started to collect data in 2011, and by then the data collection

of the last study year (2008) had been completed and entered electronically into the Malformation Register. It is feasible that at that point some children with a very mild lower limb deficiency may not have been entered into the register and would thus be missing from our study population. Also, in the study of hospital admissions and hospital days, the follow-up time for reductions born during the last study years was very short, because the data of the Care Register for Health Care was available only until 2009. The information from Care Register for Health Care was partially unsuitable concerning some surgical operation codes, and some codes were too rough to inform of what actually was done to the patient (e.g., code NHK99, other operation on bone of ankle or foot).

The data concerning the treatment of lower limb deficiencies is historical. The treatment plan of these patients has changed profoundly over the past years. New surgical approaches have been introduced and technical development has been rapid during the past years (Paley and Guardo 2014, Paley 2016a and b, Kulkarni 2019, Laaksonen et al. 2019)

The case-control design was selected for Study III because of very low prevalence figures of congenital limb deficiencies reported in the literature (Froster-Iskenius and Baird 1989, Aro et al. 1982, Källen et al. 1984, Froster and Baird 1993a, Evans et al. 1994, Forrester and Merz 2003, Calzoari et al. 1990). The lack of data of maternal folic acid supplementation might have influenced the results. However, it is known that over 70% of Finnish mothers do use dietary supplements (Meinilä et al. 2015, Erkkola et al. 1998). A weakness of Study III is also that although we know the maternal prescriptions, we do not know if the mothers really used the medicine as prescribed nor when the prescription medicine was used. This can cause both finding and not finding correlations. Based on previous studies we know that especially mothers with long term illnesses use their medication also under pregnancy (Olesen et al. 2001, Malm et al. 2003).

6.2 Pregnancy-related and maternal risk factor for congenital limb deficiencies

Pregestational diabetes increased the risk of limb deficiency to four to six-fold and this was especially noticed in the isolated group of children (Study III). In previous studies, the association between pregestational diabetes (Polednak and Janerich 1985, Åberg et al. 2001, Froster and Baird 1993b, Källen 1989, Correa et al. 2008, Klungsoyr et al. 2019, Dukhovny et al. 2018, Liu et al. 2015) and gestational diabetes (Correa et al. 2008) and birth defects has been established. A meta-analysis from 2015 showed that pregestational diabetes carries a RR of 2.4 and gestational diabetes of 1.11 for congenital malformations. Congenital limb deficiencies have also been associated with pregestational diabetes (Åberg et al. 2001, Froster and Baird 1993b,

Källen 1989, Correa et al. 2008, Klungsoyr et al. 2019, Dukhovny et al. 2018). Klungsoyr et al. (2019) reported a three-fold increased risk to the child for limb deficiency if the mother had pregestational diabetes. They excluded terminations of pregnancy due to fetal anomaly, because the Norwegian Registry in TOPFA cases does not contain information on maternal chronic diseases in most of the TOPFA cases. The comparable figure is a four-fold risk in our study. Dukhovny et al. (2018) excluded chromosomal abnormalities and single gene disorders and, in some states, induced pregnancy terminations. They studied metformin medication and its impact on birth defects in a telephone interview study. In their study, if there was maternal exposure to insulin during the first trimester of pregnancy, the risk of limb reduction defects was almost five-fold compared to no exposure and if there was exposure to metformin for diabetes in the first trimester, the risk increase was nearly three-fold for limb reduction defects. They speculated if it is the medication or the disease itself that generates the risk for birth defects. Correa et al. (2008) reported an association between pregestational diabetes and longitudinal limb deficiency with odd ratios 6–7. They excluded cases with a known cause. It was a case-control study. They included different birth defects and identified the prevalence of maternal diabetes among the affected subjects and control subjects. In contrast, Nielsen et al. (2004) did not find an association between limb defects and diabetes. It was also case-control study from the Hungarian Register and included all kinds of birth defects. Only 0.3% of the mothers of the cases had diabetes, and thus subgroup analyses consisted of very small numbers of cases in different birth defect groups.

Based on our study and literature data it is very important to control the blood glucose levels of pregnant mothers and to treat high glucose levels properly to reduce the prevalence of congenital limb deficiencies. It is also important to actively search for these anomalies, especially if there is maternal dysglycemia.

We identified an association between limb deficiencies and maternal progesterone (two-fold increase) and antiepileptics (four-fold increase) use just before conception and during the first trimester. Because of a small study population size, we could not further analyze antiepileptics by subgroups. In the literature valproate, phenytoin and phenobarbital have been connected to limb deficiencies (Jentink et al. 2010b, Holmes et al. 2001, Harvey et al. 2003). Klungsoyr et al. (2019), however, did not find any association between epilepsy and limb deficiencies and neither did we find any associations between special medical reimbursements for epilepsy and congenital limb reductions (Study III). This finding will need further studies to expand the patient population so that further analyses on drugs (by ATC code) used by smaller groups of mothers with epilepsy become feasible. Different types of antiepileptic medications have different modes of action and would be expected to affect the developing fetus differently.

In the past decades, maternal sex steroid use has been suspected to cause birth defects (Janerich et al. 1974, Czeizel et al. 1983, Heinonen et al. 1977), but there were also studies against this idea (Källen 1989, Lammer and Cordero 1986, Harlap et al. 1985). Brent (2005) concluded that newer studies imply that progestational drugs do not cause congenital malformations. Despite this, as shown by Study III, we did find an increased risk for limb defects with maternal progesterone use, especially in the multiple anomaly group. This paper and the previous ones cannot be fully compared because of different indications for the use of the hormones and different doses used by the mother. A closer analysis showed that all of the use pertained to natural progesterone, which is used in assisted reproductive technology. Thus, it may not be possible to say if it is the hormone itself, the technology used or maternal and paternal factors related to subfertility that cause the positive association with congenital limb deficiencies. This has been also questioned in papers on infertility treatments and pregnancy outcomes (Hansen et al. 2013, Berntsen et al. 2019)

Infertility treatment increased the risk of limb deficiency six-fold in the multiple anomaly group (multivariable analysis) (Study III). Also, previous studies have reported an increased risk for limb reductions among women who have undergone infertility treatments (Källen et al. 2010, Davies et al. 2012). Our study indicates that the association could be related to a more profound effect of with these treatments, since the association was marked only in the group with multiple defects (Berntsen et al. 2019).

There was no association between BMI and limb deficiencies (Study III), but maternal weight and height information was lacking in many cases. This could influence the results. In previous studies, Oddy et al. (2009) reported a two-fold increase in limb deficiencies in pregnant obese women and in the systematic review and meta-analysis of Stothard et al. (2009) the risk was 1.3-fold. In Oddy's (2009) case-control study the number of limb reduction cases was very small and the results were not statistically significant.

Primiparity (no previous pregnancies or miscarriages) was associated with congenital limb deficiencies (Study III) (OR 1.55 (95%CI 1.29–1.88)). Similar observations have been made by in previous studies (Klungsoyr et al. 2019, Duong et al. 2012). In the study of Duong et al. (2012), which ran from 1997 to 2007, nulliparous women were defined as those with no previous live births and primiparous women were those with one live birth before the index delivery. Multiple pregnancies and subjects with pregestational diabetes were excluded. The odds ratio for congenital limb deficiencies was slightly increased and was 1.2 (95%CI 1.00–1.44) in nulliparous women.

There was also association between beta-blocking agents, muscle relaxants and centrally acting agents and congenital limb deficiencies (Study III). There was a two-

fold to three-fold increase in the risk of limb deficiencies. The finding is new and sufficiently surprising to merit further investigation. None of the subgroup analyses were positive.

Our results show that all risk factors discussed in Study III need to be taken into account when examining pregnant women in maternity health care. When screening ultrasound investigations are made, it is important to actively search for congenital limb deficiencies among pregnant women who use these drugs.

6.3 Prevalence of congenital lower limb deficiencies

The total prevalence of congenital lower limb deficiencies was 2.8 per 10,000 births and the birth prevalence was 2.2 per 10,000 births (Study I). According to previous literature on population-based studies, our figures are higher. In the older data of Aro et al. (1982), Källen et al. (1984) and Calzoari et al. (1990) the birth prevalence of lower limb reductions has varied between 1.4 and 1.7 per 10,000 births. In the study of Froster and Baird (1993) the live birth prevalence was 1.1 per 10,000 live births. They excluded cases with amniotic bands or chromosomal or other syndromes, as well as cases with both upper and lower limb deficiencies. In another study, the same authors report that the birth prevalence of lower limb deficiencies was 1.7 per 10,000 births. The differences between our study and these studies might be explained by differences in study populations, definitions and accuracy of the registry data. Also, after publication of these older studies, the mean maternal age, the proportion of older pregnant women, the use of infertility treatments have increased. (Cleary-Goldman et al. 2005, Sainio et al. 2010, Medical Birth Register, Lahesmaa-Korpinen et al. 2014, Assisted Infertility Treatments) Also, the amount of prenatal screening has increased and the numbers of elective terminations of pregnancy due to fetal anomalies have risen.

There is some more recent data. Klungsoyr et al (2019) reported that the total prevalence of congenital lower limb deficiencies was only 1.3 per 10,000 births. The difference to our study might be partly explained by different classifications. Also, their data begins earlier (1970). In the Norwegian study there was a slight increase in the prevalence in the subsequent years (1970 to 1998 vs 1999 to 2016). In a previous study from Finland (covering the years 1964–1977) the birth prevalence was 1.4. (Aro et al. 1982) On the other hand, Vasluian et al (2013) calculated the total prevalence of lower limb deficiencies to be 4.2 per 10,000 births in northern Netherland. They included recognized conditions in the analysis.

Of the cases in the present study, 71% were live births, 4% stillbirths and 22% elective terminations of pregnancy. In Norway, the proportions were similar (Klungsoyr et al. 2019).

6.4 Mortality of children with congenital lower limb deficiencies

There is not much data on the mortality of children with congenital lower limb deficiencies. In our study (Study I) perinatal mortality was 78 per 1,000 births and infant mortality 46 per 1,000 births. This is more than in Finland on average. Perinatal mortality was 4.7 per 1,000 and infant mortality 2.7 per 1,000 in 2008 (Causes of death 2017). In the study of Klungsoyr et al. (2019) infant mortality was 55 per 1,000 births (upper and lower limb together). In the same study most of the deaths during the first year of life were associated with additional malformations, and the same was observed in Study I. This was also reported by Froster-Iskenius and Baird (1989).

6.5 Gender distribution of subjects with congenital lower limb deficiencies

In previous studies there has been male excess among subjects with congenital lower limb deficiencies (Bedard et a. 2018, Vasluian et al. 2013, Stoll et al. 1996, Forrester and Merz 2003, Rosano et al. 2000, Klungsoyr et al. 2019), for example 55% in the study of Vasluian et al. (2013). This is also true in Study I (53%) and corresponds to the general gender distribution recorded in the Malformation Register (Congenital Malformations 2015)

6.6 Classification of congenital lower limb deficiencies

We used the EUROCAT classification system to classify our cases (EUROCAT 2004). Terminal transverse reductions were the most frequent type of lower limb reductions, followed by postaxial deficiencies (Study I). The same percentage of different subtypes of lower limb deficiencies has been reported in previous studies that have used the EUROCAT classification system (Calzoari et al. 1990, Vasluian et al. 2013, Lin et al. 1993). It is difficult to compare different studies since several different classifications are in use (Bedard 2018, Lowry and Bedard 2016).

6.7 Congenital lower limb deficiencies and associated major anomalies

Almost half of our subjects with lower limb reduction had multiple anomalies. The most common associated anomaly was abdominal followed by urogenital, axial skeleton, central nervous system and cardio-pulmonary anomalies. There are previous reports that subjects with limb reduction have increased relative risks of

non-limb anomalies (Stoll et al. 2010). The proportions of associated anomalies vary between 12% (Calzoari et al. 1990) and 83% (Forrester and Merz 2003). In the study of Froster and Baird (1993), 20% of the live births of lower limb deficiencies had associated anomalies but chromosomal and other syndromes were excluded. A Swedish study group reported a non-limb malformation rate of 27% in unilateral lower limb deficiencies (Källen et al. 1984). Vasluian et al. (2013) reported that 17% of subjects with lower limb cases had additional anomalies, recognized conditions excluded. The comparable figure in Study I is 23% (MCA without known syndrome or chromosomal anomaly). In the studies of Bedard et al (2018) and Klungsoyr et al. (2019), musculoskeletal and other limb anomalies dominated, followed by cardiovascular anomalies. Vasluian et al. (2013) reported digestive tract anomalies as the most frequent site. All studies have reported proportions of the different anomalies that are of similar magnitude as in our study (Bedard et al. 2018, Vasluian et al. 2013, Klungsoyr et al. 2019, Evans et al. 1994).

A third of all our cases had reductions in both the upper and the lower limbs, and one fifth in the isolated group. Vasluian et al. (2013) calculated that 16% of the cases had both upper and lower limb reductions. The percentage was nine in the study of Klungsoyr et al. (2019) but they had 6% of cases in which the affected limbs were unspecified.

Previously it has been reported that around 30% of limb reduction cases associate with some recognized condition (Stoll et al. 2010, McGuirk et al. 2001). In the study of Vasluian et al. (2013), 39% of lower limb reductions were related to a known syndrome or chromosomal anomaly. In our data the percentage was 25%. Chromosomal abnormalities have been reported in 5.6% (Stoll et al. 1992) to 7.2% (Klungsoyr et al. 2019) of the cases; our figure was slightly lower, 4.5%.

Our data show that preaxial lower limb reduction defects are strongly associated with other anomalies. This corroborates previous literature reports (Berard et al. 2018, Rosano et al. 2000, Vasluian et al. 2013).

6.8 Hospital care of congenital lower limb deficiencies

Congenital anomalies pose a significant impact to the healthcare system. Of children with congenital anomalies 80% used healthcare services compared to 54% without birth defects. (Colvin and Bower 2009). Populations-based studies about hospitalizations for congenital heart defects, cleft lips and palates, Down syndrome and neural tube defects have been published (Radcliff et al. 2012, Fitzsimons et al. 2014, Fitzgerald et al. 2013, Robbins et al. 2014, Simeone et al. 2015, Islam et al. 2018, Razzaghi et al. 2015, Peterson et al. 2013). Wu et al. (2013) explored the surgically treated congenital anomalies in Kenya. Spina bifida caused the highest

impact on the healthcare system. In the study of Koskimies-Virta et al. (2019), the impact of congenital upper limb deficiencies on the healthcare system was evaluated. However, hospitalizations of congenital lower limb deficiencies are not known.

We compared hospital admissions and days spent at the hospital of congenital lower limb deficiencies with the corresponding data covering the entire pediatric live-born population. The amount of hospital days was six-fold and the amount of days spent at the hospital were 10-fold compared to the general pediatric population. The need for hospital care is less than for upper limb deficiencies, which amounts to 11-fold compared to whole pediatric population (Koskimies-Virta et al. 2019). Other studies report that the need for hospital care is 2.5-fold in children with birth defect, 11-fold in children with orofacial clefts and five-fold in children with Down syndrome (Colvin and Bauer 2009, Fitzgerald et al. 2013, Fitzsimons et al. 2013)

In a study on limb defects and amputations, the mean number of hospital stays of children with an amputation was 0.3 and without an amputation 0.1. In the same study, the number nights spent at the hospital was seven among patients with an amputation and 2.5 without an amputation. Our figures were higher for the average number of annual hospital admission (0.6, varying from 0.4 for toe deficiencies to 1.5 for terminal amputations) and lower for the days spent in hospital (2.9, varying between 1.9 and 3.9 days). (Weir et al. 2010) Of course, Weir's and our study are not comparable due to different study populations. In both studies the scale of deformities is wide from simple toe reductions to difficult long bone deficiencies and neither our nor Weir's study took into account associated anomalies which would have had an effect on hospital treatment.

Compared to upper limb deficiencies our figures for lower limb deficiencies were lower. Congenital upper limb deficiency patients had mean of one admission per year and five days of hospital care per year. (Koskimies-Virta et al. 2019).

Nowadays, the treatment of congenital limb deficiencies has undergone some changes compared to the techniques used during the study years (Paley and Guardo 2014, Paley 2016, Kulkarni 2019, Laaksonen et al. 2019). In the Finnish study describing the treatment of deformities with motorized lengthening nail (Laaksonen et al. 2019), patients with congenital limb deficiencies spent two to five days in for the surgical procedure. In our study (Study II), long bone deficiencies required a mean of 1.1 hospital admissions and a mean of 3.9 hospital days. Based on these figures the numbers did not differ much between different eras. Our study did not include outpatient visits. We may speculate that the number of outpatient visits may be currently higher because of lengthening nails and external fixators which require control visits during care.

6.9 Surgical treatment of congenital lower limb deficiencies

Considering all patient groups in the present study (terminal amputations, long bone deficiencies, foot and toe deficiencies), the proportion (Kaplan–Meier estimate) of patients requiring at least one operation by age 10 years was over 50% in every group. Except for toe deficiencies, most operations were orthopedic procedures. In all groups, the burden of hospital care was higher than for the whole pediatric population. Long bone deficiencies constituted the biggest need for surgery among the subjects with any lower limb deficiency, while toe deficiencies had the lowest rate of orthopedic operations. To the best of my knowledge, there are no studies to compare these results with.

In this study, long bone deficiencies were classified according to their specific classification systems and the orthopedic procedures in accordance with this. All these procedures were done before 2009. The PFFD patients of Aitken class A (4/5) had limb lengthening procedures and the four Aitken class C and D patients in the study (Study II) did not require orthopedic procedures. Compared to older literature (Gillespie and Torode 1983, Torode and Gillespie 1991) procedures done to our cases followed recommendations done before. Nine patients in Aitken class A and B underwent osteotomy. Because of the nature of register data, it was not possible to pinpoint the level at which these procedures were done, nor was it possible to decide if there were PFFD patients who underwent the Van Nes rotationplasty (Van Nes 1950) in our data. Patients with PFFD may successfully have their hip joint reconstructed with pelvic and femoral osteotomies, especially in mild cases of PFFD. Before lengthening it is necessary to achieve stability of the hip and knee joint. (Westberry and Davids 2009)

In our data (Study II) we had five tibial deficiency patients. Of these, two patients with the Jones type 3 deficiency required amputation. Clinton and Birch (2015) reported of their series of type 3 patients that one patient had knee disarticulation and one Syme amputation and tibiofibular synostosis. Spiegel et al. (2010) reported on the Chopart or Syme amputation in patients with Jones type 2 and 3 defects. According to a review by of Litrenta et al. (2019), the method of choice to treat the rare Jones type 3 deficiencies is the Syme or Chopart amputation. It is assumed that ossification of a proximal tibial cartilaginous anlage will take place and that patients have a functional quadriceps mechanism. According to the same article, the recommended treatment for type 2 patients is tibiofibular synostosis and distal Syme amputation. Schoenecker et al (1989) used distal Syme amputation to treat 12 patients with type 2 deficiency, eight of whom also underwent tibiofibular synostosis. In our small series, type 2 patients had no orthopedic surgery. We also had two cases (2/5, 40%) that we could not classify according to Jones classification system. In the literature the percentage of non-classifiable cases has varied between

11% and 15% (Clinton and Birch 2015, Weber 2008). Non-classifiable cases have undergone osteotomies, amputations and epiphysiodesis procedures. Clinton and Birch (2015) reported that this group of patients was treated with miscellaneous procedures.

Patients with congenital fibular deficiency present with wide range of clinical manifestations. Orthosis, epiphysiodesis, Syme or Boyd amputation, prosthetic rehabilitation, limb lengthening procedures and foot and ankle reconstruction procedures can be used to treat these patients. (Hamdy et al. 2014) The traditional treatment protocol has been that patients with severe foot deformity and big limb length inequality are best treated by amputation and prosthetic rehabilitation (Maffulli and Fixsen 1996, Birch et al. 1999, Changulani et al. 2010). Milder deformities are candidates for limb lengthening procedures. (Hamdy et al. 2014) Birch et al. (2011) have published an algorithm on how to treat these patients. If the foot is not preservable (less than three rays), the treatment method, according to this algorithm, is amputation (if upper limbs are functional). If the foot is preservable, the treatment of choice is conservative treatment or lengthening and epiphysiodesis procedures as dictated by the length of the limb inequality. Further, the patients might need treatment of genu valgum and of knee instability and reconstruction of the foot and ankle (Hamdy et al. 2014). In our series, all AK 2 patients were treated with amputation. These patients have complete absence of the fibula and usually also the foot is poor in most of these cases. In our study (Study II), AK type 1 cases had osteotomies and lengthening procedures according to the guidelines above. Also, seven AK 1A type patients did not have hospital admissions. Maybe their anomaly was so mild that they did not need treatment or maybe the follow-up time was too short to catch data on hospital days.

6.10 Future perspectives

1. In the first study (Study I) we report that the number of major structural anomalies is high among subjects with lower limb deficiencies. Clinically, in the future and now, it is important that this patient group receives a thorough preoperative assessment for identification of these anomalies.
2. The prevalence of congenital limb deficiencies had increased (Study I). In the future, it is important to follow up these figures at least for a decade to check if this trend continues. It is also important to examine the causes for the increasing trend.
3. As new methods to treat these patients have emerged, we need to compare our current results and hospital care figures with newer figures to see how the current treatment protocols affect the lives of patients.

4. It is important that pregnant women who are using medications or have diabetes are followed carefully with regard to the occurrence and risk of limb deficiencies in their offspring. In the future, it is important to expand the study material, after which subgroup analyses become feasible – this is needed for confirmation or exclusion of risk factors that have been identified in this study.

7 Conclusions

1. The total prevalence of congenital lower limb deficiencies was 2.8 per 10,000 births in 1993–2008. Terminal transverse reductions were the most common type of reductions. Perinatal mortality was 78 per 1,000 births.
2. The burden of hospital care was markedly increased in all lower limb deficiency groups compared to the whole pediatric population. The highest burden was in children with terminal amputations and long-bone deficiencies (over ten-fold).
3. Over 50% of patients in all groups were operated on by age 10 years. Most of the operations were orthopedic, except in the group of patients with toe deficiencies. Long bone deficiencies had the highest proportion of operations in the group with lower limb deficiency.
4. Maternal young and high age, primiparity and pregestational diabetes increased the risk of having a child with congenital limb deficiency. Also, maternal medication use (progesterone, antiepileptics, beta blocking agents and muscle relaxants) one month before gestation and during the first trimester increased markedly the risk of limb defects in the offspring.
5. Maternal diabetes, use of the medications mentioned above and risk factors should be followed up carefully throughout the entire pregnancy. Maternal diabetes must be treated as well as possible. Health care actors and the society needs to actively monitor drug safety and search for medication that is not teratogenic.
6. During pregnancy, it is necessary to pay extra attention to limb deficiencies in screening ultrasounds and to look carefully for other anomalies when lower limb deficiency is suspected. A thorough clinical examination and cardiac and abdominal ultrasound are important measures for the newborn child with limb deficiencies, because nearly half of the cases have other, associated major anomalies. This is especially important in the group of patients with longitudinal preaxial reduction.

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References

Abraham E, Waxman B, Shirali S, Durkin M. (1999) Congenital cleft-foot deformity treatment. J Pediatr Orthop 19:404–410.

Achterman C, Kalamchi A. (1979) Congenital deficiency of the fibula. J Bone Joint Surg Br 61-B:133–137.

Ackman J, Altiok H, Flanagan A, Peer M, Graf A, Krzak J, Hassani S, Eastwood D, Harris GF. (2013) Long-term follow-up of Van Nes rotationplasty in patients with congenital proximal focal femoral deficiency. Bone Joint J 95-B:192–198.

Aitken G. (1969) Proximal femoral focal deficiency-definition: a congenital anomaly. Washington: National Academy of Sciences.

Anderson K, Ailes E, Danielson M, Lind J, Farr S, Broussard C, Tinker S. (2018a) Attention-Deficit/Hyperactivity Disorder Medication Prescription Claims Among Privately Insured Women Aged 15–44 Years – United States, 2003–2015. MMWR Morb Mortal Wkly Rep 67:66–70.

Anderson K, Dutton A, Broussard C, Farr S, Lind J, Visser S, Ailes E, Shapira S, Reefhuis J, Tinker S. (2020) ADHD Medication Use During Pregnancy and Risk for Selected Birth Defects: National Birth Defects Prevention Study, 1998–2011. J Atten Disord 24:479–489.

Aro T, Heinonen OP, Saxén L. (1982) Incidence and secular trends of congenital limb defects in Finland. Int J Epidemiol. 11:239–44.

Aro T. (1983) Maternal diseases, alcohol consumption and smoking during pregnancy associated with reduction limb defects. Early Hum Dev 9:49–57.

Aro T, Heinonen OP, Saxén L. (1983) Risk indicators of reduction limb defects. J Epidemiol Community Health 37:50–56.

Assisted Infertility Treatments. Available at: https://thl.fi/en/web/thlfi-en/statistics/statistics-by-topic/sexual-and-reproductive-health. (accessed 22.2.2020)

Bateman B, Huybrechts K, Fischer M, Seely E, Ecker J, Oberg A, Franklin J, Mogun H, Hernandez Diaz S. (2015) Chronic hypertension in pregnancy and the risk of congenital malformations: a cohort study. Am J Obstet Gynecol 212:337.e–337.e14.

Becerra JE, Khoury MJ, Cordero JF, Erickson JD. (1990) Diabetes mellitus during pregnancy and the risks for specific birth defects: a population-based case-control study. Pediatrics 85:1–9.

Bedard T, Lowry RB, Sibbald B, Crawford S, Kiefer G. (2018) Congenital limb deficiencies and major associated anomalies in Alberta for the years 1980–2012. Am J Med Genet A 176:19–28.

Bedard T, Lowry RB, Sibbald B, Kiefer G, Metcalfe A. (2015) Congenital limb deficiencies in Alberta-a review of 33 years (1980–2012) from the Alberta Congenital Anomalies Surveillance System (ACASS). Am J Med Genet A 167A:2599–2609.

Bellizzi S, Ali MM, Abalos E, Betran AP, Kapila J, Pileggi Castro C, Vogel JP, Merialdi M. (2016) Are hypertensive disorders in pregnancy associated with congenital malformations in offspring? Evidence from the WHO Multicountry cross sectional survey on maternal and newborn health. BMC Pregnancy Childbirth 16:198.

Belva F, Henriet S, Van den Abbeel E, Camus M, Devroey P, Van der Elst J, Liebaers I, Haentjens P, Bonduelle M. (2008) Neonatal outcome of 937 children born after transfer of cryopreserved

embryos obtained by ICSI and IVF and comparison with outcome data of fresh ICSI and IVF cycles. Hum Reprod 23:2227–2238.

Bermejo-Sánchez E, Cuevas L, Amar E, Bakker M, Bianca S, Bianchi F, Canfield M, Castilla E, Clementi M, Cocchi G, Feldkamp M, Landau D, Leoncini E, Li Z, Lowry RB, Mastroiacovo P, Mutchinick O, Rissmann A, Ritvanen A, Scarano G, Siffel C, Szabova E, Martínez-Frías M. (2011a) Amelia: a multi-center descriptive epidemiologic study in a large dataset from the International Clearinghouse for Birth Defects Surveillance and Research, and overview of the literature. Am J Med Genet C Semin Med Genet 157C:288–304.

Bermejo-Sánchez E, Cuevas L, Amar E, Bianca S, Bianchi F, Botto L, Canfield M, Castilla E, Clementi M, Cocchi G, Landau D, Leoncini E, Li Z, Lowry RB, Mastroiacovo P, Mutchinick O, Rissmann A, Ritvanen A, Scarano G, Siffel C, Szabova E, Martínez-Frías M. (2011b) Phocomelia: a worldwide descriptive epidemiologic study in a large series of cases from the International Clearinghouse for Birth Defects Surveillance and Research, and overview of the literature. Am J Med Genet C Semin Med Genet 157C:305–320.

Berntsen S, Söderström-Anttila V, Wennerholm U, Laivuori H, Loft A, Oldereid N, Romundstad, Bergh C, Pinborg A. (2019) The health of children conceived by ART: 'the chicken or the egg?'. Hum Reprod Update 25:137–158.

Birch JG, Walsh SJ, Small JM, Morton A, Koch KD, Smith C, Cummings D, Buchanan R. (1999) Syme amputation for the treatment of fibular deficiency. An evaluation of long-term physical and psychological functional status. J Bone Joint Surg Am 81:1511–1518.

Birch J, Lincoln T, Mack P, Birch, C. (2011) Congenital fibular deficiency: a review of thirty years' experience at one institution and a proposed classification system based on clinical deformity. J Bone Joint Surg Am 93:1144–1151.

Blauth W, Borisch NC. (1990) Cleft feet. Proposals for a new classification based on roentgenographic morphology. Clin Orthop Relat Res:41–48.

Boakes JL, Stevens PM, Moseley RF. (1991) Treatment of genu valgus deformity in congenital absence of the fibula. J Pediatr Orthop 11:721–724.

Bokhari A, Connolly S, Coull B, Harvey E, Holmes L. (2002) Effects on toes from prenatal exposure to anticonvulsants. Teratology 66:122–126.

Botto, L, Olney R, Erickson JD. (2004) Vitamin supplements and the risk for congenital anomalies other than neural tube defects. Am J Med Genet C Semin Med Genet 125C:12–21.

Brambati B, Tului L. (2005) Chorionic villus sampling and amniocentesis. Curr Opin Obstet Gynecol 17:197–201.

Brender J, Shinde M, Zhan FB, Gong X, Langlois P. (2014) Maternal residential proximity to chlorinated solvent emissions and birth defects in offspring: a case-control study. Environ Health 13:96.

Brent R. (2005) Nongenital malformations following exposure to progestational drugs: the last chapter of an erroneous allegation. Birth Defects Res Part A Clin Mol Teratol 73:906–918.

Brown F. (1965) Construction of a knee joint in congenital total absence of the tibia (paraxial hemimelia tibia): A preliminary report. J Bone Joint Surg Am 47:695–704.

Brown KL. (2001) Resection, rotationplasty, and femoropelvic arthrodesis in severe congenital femoral deficiency. A report of the surgical technique and three cases. J Bone Joint Surg Am 83-A:78–85.

Buyukkayhan D, Koklu E, Akcakus M, Kurtoglu S. (2007) Femoral hypoplasia in two newborns associated with maternal diabetes mellitus. J Pediatr Endocrinol Metab 20:1151–1154.

Calzolari E, Manservigi D, Garani GP, Cocchi G, Magnani C, Milan M. (1990) Limb reduction defects in Emilia Romagna, Italy: epidemiological and genetic study in 173,109 consecutive births. J Med Genet 27:353–357.

Canfield M, Collins J, Botto L, Williams L, Mai C, Kirby R, Pearson K, Devine O, Mulinare J. (2005) Changes in the birth prevalence of selected birth defects after grain fortification with folic acid in the United States: findings from a multi-state population-based study. Birth Defects Res Part A Clin Mol Teratol 73:679–689.

Carli D, Fairplay T, Ferrari P, Sartini S, Lando M, Garagnani L, Di Gennaro G, Di Pancrazio L, Bianconi G, Elmakky A, Bernasconi S, Landi A, Percesepe A. (2013) Genetic basis of congenital upper limb anomalies: analysis of 487 cases of a specialized clinic. Birth Defects Res Part A Clin Mol Teratol 97:798–805.

Carmichael S, Shaw G, Iovannisci D, Yang W, Finnell R, Cheng S, Lammer E. (2006a) Risks of human limb deficiency anomalies associated with 29 SNPs of genes involved in homocysteine metabolism, coagulation, cell-cell interactions, inflammatory response, and blood pressure regulation. Am J Med Genet A 140:2433–2440.

Carmichael S, Shaw G, Yang W, Iovannisci D, Lammer E. (2006b) Risk of limb deficiency defects associated with NAT1, NAT2, GSTT1, GSTM1, and NOS3 genetic variants, maternal smoking, and vitamin supplement intake. Am J Med Genet A 140:1915–1922.

Carmichael S, Shaw G, Yang W, Lammer E, Zhu H, Finnell R. (2004) Limb deficiency defects, MSX1, and exposure to tobacco smoke. Am J Med Genet A 125A:285–289.

Carter T, Sicko R, Kay D, Browne M, Romitti P, Edmunds Z, Liu A, Fan R, Druschel C, Caggana M, Brody L, Mills J. (2017) Copy-number variants and candidate gene mutations in isolated split hand/foot malformation. J Hum Genet 62:877–884.

Caspers K, Romitti P, Lin S, Olney R, Holmes L, Werler M. (2013) Maternal periconceptional exposure to cigarette smoking and congenital limb deficiencies. Paediatr Perinat Epidemiol 27:509–520.

Caspers-Conway KM, Romitti PA, Holmes L, Olney RS, Richardson SD; National Birth Defects Prevention Study. (2014) Maternal periconceptional alcohol consumption and congenital limb deficiencies. Birth Defects Res A Clin Mol Teratol 100:863–876.

Castori M, Rinaldi R, Cappellacci S, Grammatico P. (2008) Tibial developmental field defect is the most common lower limb malformation pattern in VACTERL association. Am J Med Genet A 146A:1259–1266.

Causes of death 2017. Available at: http://www.stat.fi/til/ksyyt/2017/ksyyt_2017_2018-12-17_tau_005_en.html (accessed 22.2.2020)

Cederholm M, Haglund B, Axelsson O. (2005) Infant morbidity following amniocentesis and chorionic villus sampling for prenatal karyotyping. BJOG 112:394–402.

Changulani M, Ali F, Mulgrew E, Day JB, Zenios M. (2010) Outcome of limb lengthening in fibular hemimelia and a functional foot. J Child Orthop 4:519–524.

Charlton B, Mølgaard-Nielsen D, Svanström H, Wohlfahrt J, Pasternak B, Melbye M. (2016) Maternal use of oral contraceptives and risk of birth defects in Denmark: prospective, nationwide cohort study. BMJ 352:h6712.

Choi IH, Kumar SJ, Bowen JR. (1990) Amputation or limb-lengthening for partial or total absence of the fibula. J Bone Joint Surg Am 72:1391–1399.

Christini D, Levy EJ, Facanha FA, Kumar SJ. (1993) Fibular transfer for congenital absence of the tibia. J Pediatr Orthop 13:378–381.

Cleary-Goldman J, Malone FD, Vidaver J, Ball RH, Nyberg DA, Comstock CH, Saade GR, Eddleman KA, Klugman S, Dugoff L, Timor-Tritsch IE, Craigo SD, Carr SR, Wolfe HM, Bianchi DW, D'Alton M; FASTER Consortium. (2005) Impact of maternal age on obstetric outcome. Obstet Gynecol. 105:983–990.

Cleves M, Hobbs C, Zhao W, Krakowiak P, MacLeod S. (2011) Association between selected folate pathway polymorphisms and nonsyndromic limb reduction defects: a case-parental analysis. Paediatr Perinat Epidemiol 25:124–134.

Clinton R, Birch J. (2015) Congenital tibial deficiency: a 37-year experience at 1 institution. J Pediatr Orthop 35:385–390.

Colvin L, Bower C. (2009) A retrospective population-based study of childhood hospital admissions with record linkage to a birth defects registry. BMC Pediatr 9:32.

Congenital Malformatios 2015. Available at: https://thl.fi/en/web/thlfi-en/statistics/statistics-by-topic/sexual-and-reproductive-health/congenital-anomalies (accessed 22.2.2020)

Correa A, Botto L, Liu Y, Mulinare J, Erickson JD. (2003) Do multivitamin supplements attenuate the risk for diabetes-associated birth defects? Pediatrics 111:1146–1151.
Correa A, Gilboa S, Besser L, Botto L, Moore C, Hobbs C, Cleves M, Riehle-Colarusso T, Waller DK, Reece EA. (2008) Diabetes mellitus and birth defects. Am J Obstet Gynecol 199:237.e–237.e9.
Coventry MB, Johnson EW. (1952) Congenital absence of the fibula. J Bone Joint Surg Am 34 A:941–955.
Crider K, Cleves M, Reefhuis J, Berry R, Hobbs C, Hu D. (2009) Antibacterial medication use during pregnancy and risk of birth defects: National Birth Defects Prevention Study. Arch Pediatr Adolesc Med 163:978–985.
Czeizel AE. (1998) Periconceptional folic acid containing multivitamin supplementation. Eur J Obstet Gynecol Reprod Biol 78:151–161.
Czeizel AE, Dudás I. (1992) Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. N Engl J Med 327:1832–1835.
Czeizel A, Keller S, Bod M. (1983) An aetiological evaluation of increased occurrence of congenital limb reduction abnormalities in Hungary, 1975–1978. Int J Epidemiol. 12:445–449.
Czeizel AE, Kodaj I, Lenz W. (1994) Smoking during pregnancy and congenital limb deficiency. BMJ 308:1473–1476.
Czeizel A, Vitéz M, Kodaj I, Lenz W. (1991) Birth prevalence of different congenital limb deficiency types in a revised, population based Hungarian material, 1975–1984. Acta Morphol Hung 39:229–237.
Davies M, Moore V, Willson K, Van Essen P, Priest K, Scott H, Haan E, Chan A. (2012) Reproductive technologies and the risk of birth defects. N Engl J Med 366:1803–1813.
Dolk H, Armstrong B, Lachowycz K, Vrijheid M, Rankin J, Abramsky L, Boyd PA, Wellesley D. (2010) Ambient air pollution and risk of congenital anomalies in England, 1991–1999. Occup Environ Med 67:223–227.
Dora C, Bühler M, Stover M, Mahomed M, Ganz R. (2004) Morphologic characteristics of acetabular dysplasia in proximal femoral focal deficiency. J Pediatr Orthop B 13:81–87.
Dukhovny S, Van Bennekom C, Gagnon D, Hernandez Diaz S, Parker S, Anderka M, Werler M, Mitchell A. (2018) Metformin in the first trimester and risks for specific birth defects in the National Birth Defects Prevention Study. Birth Defects Res 110:579–586.
Duong H, Hoyt A, Carmichael S, Gilboa S, Canfield M, Case A, McNeese M, Waller D. (2012) Is maternal parity an independent risk factor for birth defects? Birth Defects Res Part A Clin Mol Teratol 94:230–236.
El-Sayed MM, Correll J, Pohlig K. (2010) Limb sparing reconstructive surgery and Ilizarov lengthening in fibular hemimelia of Achterman-Kalamchi type II patients. J Pediatr Orthop B. 19:55–60.
El Tayeby H, Ahmed, Amin Abdel Razek Youssef (2012) Ankle reconstruction in type II fibular hemimelia. Strategies Trauma Limb Reconstr 7:23–26.
Elliott AM, Evans JA, Chudley AE. (2005) Split hand foot malformation (SHFM). Clin Genet 68:501–505.
Erickson JD. (1991) Risk factors for birth defects: data from the Atlanta Birth Defects Case-Control Study. Teratology 43:41–51.
Erkkola M, Karppinen M, Järvinen A, Knip M, Virtanen SM. (1998) Folate, vitamin D, and iron intakes are low among pregnant Finnish women. Eur J Clin Nutr. 52:742–748.
EUROCAT. European network of population-based registries for the epidemiological surveillance of congenital anomalies. Available at: https://eu-rd-platform.jrc.ec.europa.eu/eurocat. (accessed 22.2.2022)
EUROCAT Guide 3 (2nd Ed) For the Description and Classification of Congenital Limb Defects. (2004). Available at: https://eu-rd-platform.jrc.ec.europa.eu/sites/default/files/EUROCAT-Guide-3.pdf. (accessed 22.2.2020)

Evans JA, Vitez M, Czeizel, A. (1994) Congenital abnormalities associated with limb deficiency defects: a population study based on cases from the Hungarian Congenital Malformation Registry (1975–1984). Am J Med Genet 49:52–66.

Fernandez-Palazzi F, Bendahan J, Rivas S. (1998) Congenital deficiency of the tibia: a report on 22 cases. J Pediatr Orthop B 7:298–302.

Finnish Statistics on Medicines. Available at: https://www.kela.fi/web/en/statistical-publications_finnish-statistics-on-medicines. (accessed 22.2.2020)

Firth HV, Boyd PA, Chamberlain PF, MacKenzie IZ, Morriss Kay GM, Huson SM. (1994) Analysis of limb reduction defects in babies exposed to chorionic villus sampling. Lancet 343:1069–1071.

Fisher S, Van Zutphen A, Werler M, Lin A, Romitti P, Druschel C, Browne M. (2017) Maternal Antihypertensive Medication Use and Congenital Heart Defects: Updated Results From the National Birth Defects Prevention Study. Hypertension 69:798–805.

Fitzgerald P, Leonard H, Pikora T, Bourke J, Hammond G. (2013) Hospital admissions in children with down syndrome: experience of a population-based cohort followed from birth. PLoS ONE 8:e70401.

Fitzsimons K, Copley L, Deacon S, van der Meulen Jan H. (2013) Hospital care of children with a cleft in England. Arch Dis Child 98:970–974.

Fixsen JA, Lloyd Roberts GC. (1974) The natural history and early treatment of proximal femoral dysplasia. J Bone Joint Surg Br 56:86–95.

Forrester M, Merz R. (2003) Descriptive epidemiology of limb reduction deformities in Hawaii, 1986–2000. Hawaii Med J 62:242–247.

Franks M, Macpherson G, Figg W. (2004) Thalidomide. Lancet 363:1802–1811.

Frantz CH, Ronan O'Rahilly. (1961) Congenital skeletal limb deficiencies, J. Bone & Joint Surg. 43A:1202–1224.

Froster-Iskenius UG, Baird PA. (1989) Limb reduction defects in over one million consecutive livebirths. Teratology 39:127–135.

Froster UG, Baird PA. (1992) Congenital defects of the limbs and alcohol exposure in pregnancy: data from a population-based study. Am J Med Genet 44:782–785.

Froster UG, Baird PA. (1993a) Congenital defects of lower limbs and associated malformations: a population-based study. Am J Med Genet. 45:60–64.

Froster UG, Baird PA (1993b) Maternal factors, medications, and drug exposure in congenital limb reduction defects. Environ Health Perspect 101:269–274.

Froster UG, Jackson L. (1996) Limb defects and chorionic villus sampling: results from an international registry, 1992–94. Lancet 347:489–494.

Fowler E, Zernicke R, Setoguchi Y, Oppenheim W. (1996) Energy expenditure during walking by children who have proximal femoral focal deficiency. J Bone Joint Surg [Am] 78-A:1857–1862.

Furniss D, Kan S, Taylor IB, Johnson D, Critchley PS, Giele HP, Wilkie AOM. (2009) Genetic screening of 202 individuals with congenital limb malformations and requiring reconstructive surgery. J Med Genet 46:730–735.

Gabos P, El Rassi G, Pahys, J. (2005) Knee reconstruction in syndromes with congenital absence of the anterior cruciate ligament. J Pediatr Orthop 25:210–214.

Galtier Dereure F, Boegner C, Bringer J. (2000) Obesity and pregnancy: complications and cost. Am J Clin Nutr 71:1242–1248S.

Ghanem I. (2008) Epidemiology, etiology, and genetic aspects of reduction deficiencies of the lower limb. J Child Orthop 2:329–332.

Gibbons PJ, Bradish CF. (1996) Fibular hemimelia: a preliminary report on management of the severe abnormality. J Pediatr Orthop B 5:20–26.

Gillespie R, Torode IP. (1983) Classification and management of congenital abnormalities of the femur. J Bone Joint Surg Br 65:557–568.

Gilsanz V. (1983) Distal focal femoral deficiency. Radiology 147:105–107.

Gissler M, Teperi J, Hemminki E, Meriläinen J. (1995) Data quality after restructuring a national medical registry. Scand J Soc Med. 23:75–80.

Gold N, Westgate M, Holmes L. (2011) Anatomic and etiological classification of congenital limb deficiencies. Am J Med Genet A 155A:1225–1235.

Golden C, Ryan L, Holmes L. (2003) Chorionic villus sampling: a distinctive teratogenic effect on fingers? Birth Defects Res Part A Clin Mol Teratol 67:557–562.

Gordon JN, Goggin PM. (2003) Thalidomide and its derivatives: emerging from the wilderness. Postgrad Med J 79:127–132.

Greenlees R, Neville A, Addor MC, Amar E, Arriola L, Bakker M, Barisic I, Boyd PA, Calzolari E, Doray B, Draper E, Vollset SE, Garne E, Gatt M, Haeusler M, Kallen K, Khoshnood B, Latos-Bielenska A, Martinez-Frias ML, Materna-Kiryluk A, Dias CM, McDonnell B, Mullaney C, Nelen V, O'Mahony M, Pierini A, Queisser-Luft A, Randrianaivo-Ranjatoélina H, Rankin J, Rissmann A, Ritvanen A, Salvador J, Sipek A, Tucker D, Verellen-Dumoulin C, Wellesley D, Wertelecki W. (2011) Paper 6: EUROCAT member registries: organization and activities. Birth Defects Res Part A Clin Mol Teratol 91 Suppl 1:S51–S100.

Hamdy R, Makhdom A, Saran N, Birch J. (2014) Congenital fibular deficiency. J Am Acad Orthop Surg 22:246–255.

Hansen J. (2006) Oxidative stress as a mechanism of teratogenesis. Birth Defects Res C Embryo Today 78:293–307.

Hansen M, Kurinczuk J, Milne E, de Klerk N, Bower C. (2013) Assisted reproductive technology and birth defects: a systematic review and meta-analysis. Hum Reprod Update 19:330–353.

Harlap S, Shiono P, Ramcharan S (1985) Congenital abnormalities in the offspring of women who used oral and other contraceptives around the time of conception. Int J Fertil 30:39–47.

Harvey E, Coull B, Holmes L. (2003) Anticonvulsant teratogenesis 5: observer bias in a cohort study. Birth Defects Res Part A Clin Mol Teratol 67:452–456.

Heinonen OP, Slone D, Monson RR, Hook EB, Shapiro S. (1977) Cardiovascular birth defects and antenatal exposure to female sex hormones. N Engl J Med 296:67–70.

Hernandez R, Werler M, Romitti P, Sun L, Anderka M. (2012) Nonsteroidal antiinflammatory drug use among women and the risk of birth defects. Am J Obstet Gynecol 206:228.e–228.e8.

Hitti IF, Glasberg SS, Huggins Jones D, Sabet R. (1994) Bilateral femoral hypoplasia and maternal diabetes mellitus: case report and review of the literature. Pediatr Pathol 14:567–574.

Holmes LB, Harvey EA, Coull BA, Huntington KB, Khoshbin S, Hayes AM, Ryan LM. (2001) The teratogenicity of anticonvulsant drugs. N Engl J Med 344:1132–1138.

Holmes L, Westgate M, Nasri H, Toufaily MH. (2018) Malformations attributed to the process of vascular disruption. Birth Defects Res 110:98–107.

Hosny G. (2005) Treatment of tibial hemimelia without amputation: preliminary report. J Pediatr Orthop B 14:250–255.

Hoyt A, Canfield M, Romitti P, Botto L, Anderka M, Krikov S, Tarpey M, Feldkamp M. (2016) Associations between maternal periconceptional exposure to secondhand tobacco smoke and major birth defects. Am J Obstet Gynecol 215:613.e–613.e11.

Hwang SJ, Beaty TH, McIntosh I, Hefferon T, Panny SR. (1998) Association between homeobox-containing gene MSX1 and the occurrence of limb deficiency. Am J Med Genet 75:419–423.

ICBDSR, International Clearinghouse for Birth Defects Surveillance and Research. Available at: http://www.icbdsr.org. (accessed 22.2.2020)

Islam S, Kaul P, Tran D, Mackie A. (2018) Health Care Resource Utilization Among Children with Congenital Heart Disease: A Population-Based Study. Can J Cardiol 34:1289–1297.

Janerich DT, Piper JM, Glebatis DM. (1974) Oral contraceptives and congenital limb-reduction defects. N Engl J Med 291:697–700.

Jentink J, Dolk H, Loane M, Morris J, Wellesley D, Garne E, de Jong-van den Berg, Lolkje TW. (2010a) Intrauterine exposure to carbamazepine and specific congenital malformations: systematic review and case-control study. BMJ 341:c6581.

Jentink, J, Loane M, Dolk H, Barisic I, Garne E, Morris J, de Jong-van den Berg, Lolkje TW. (2010b) Valproic acid monotherapy in pregnancy and major congenital malformations. N Engl J Med 362:2185–2193.

Jones D, Barnes J, Lloyd Roberts GC. (1978) Congenital aplasia and dysplasia of the tibia with intact fibula. Classification and management. J Bone Joint Surg Br 60:31–39.

Jones LE, Lipson A. (1991) The care of the limb deficient child in Australia. Prosthet Orthot Int 15:140–142.

Kalamchi A, Dawe RV. (1985) Congenital deficiency of the tibia. J Bone Joint Surg Br 67:581–584.

Källén B. (1989) A prospective study of some aetiological factors in limb reduction defects in Sweden. J Epidemiol Community Health 43:86–91.

Källén B, Rahmani TM, Winberg J. (1984) Infants with congenital limb reduction registered in the Swedish Register of Congenital Malformations. Teratology 29:73–85.

Källén B, Finnström O, Lindam A, Nilsson E, Nygren K, Otterblad P. (2010) Congenital malformations in infants born after in vitro fertilization in Sweden. Birth Defects Res Part A Clin Mol Teratol 88:137–143.

Källén K. (1997) Maternal smoking during pregnancy and limb reduction malformations in Sweden. Am J Public Health 87:29–32.

Klaukka T. (2001) The Finnish database of drug utilization. Norwegian J Epidemiol. 11: 19–22.

Klungsøyr K, Nordtveit T, Kaastad T, Solberg S, Sletten I, Vik A. (2019) Epidemiology of limb reduction defects as registered in the Medical Birth Registry of Norway, 1970–2016: Population based study. PLoS ONE 14:e0219930.

Koman LA, Meyer LC, Warren FH. (1982) Proximal femoral focal deficiency: a 50-year experience. Dev Med Child Neurol 24:344–355.

Koskimies E, Lindfors N, Gissler M, Peltonen J, Nietosvaara Y. (2011) Congenital upper limb deficiencies and associated malformations in Finland: a population-based study. J Hand Surg Am 36:1058–1065.

Koskimies-Virta E, Helenius I, Pakkasjärvi N, Nietosvaara Y. (2019) Hospital Care and Surgical Treatment of Children with Congenital Upper Limb Defects. Scand J Surg. 2019 20:1457496919835988. doi: 10.1177/1457496919835988. [Epub ahead of print]

Kuliev A, Jackson L, Froster U, Brambati B, Simpson JL, Verlinsky Y, Ginsberg N, Smidt Jensen S, Zakut H. (1996) Chorionic villus sampling safety. Report of World Health Organization/EURO meeting in association with the Seventh International Conference on Early Prenatal Diagnosis of Genetic Diseases, Tel-Aviv, Israel, May 21, 1994. Am J Obstet Gynecol 174:807–811.

Kulkarni R, Arora N, Saxena S, Kulkarni S, Saini Y, Negandhi R. (2019) Use of Paley Classification and SUPERankle Procedure in the Management of Fibular Hemimelia. J Pediatr Orthop. 39:e708–e717.

Laaksonen T, Haara M, Ahola J-A, Nietosvaara Y. (2019) Alaraajojen pituuseron ja asentovirheiden korjaaminen moottoroidulla ydinnaulalla: Kokemukset kolmestatoista ensimmäisestä potilaasta. Duodecim. 135: 876–881.

Lahesmaa-Korpinen A-M, Artama M, Gissler M, Heino A, Malm H, Ritvanen A. (2014) Raskaudenaikainen lääkkeidenkäyttö, syntyneiden lasten perinataaliterveys sekä epämuodostumat 1996–2010. Available at: https://www.julkari.fi/bitstream/handle/10024/116194/URN_ISBN_978-952-302-199-0.pdf?sequence=1. (accessed 22.2.2020)

Lammer EJ, Cordero JF. (1986) Exogenous sex hormone exposure and the risk for major malformations. JAMA. 255:3128–3132.

Lenz W. (1988) A short history of thalidomide embryopathy. Teratology 38:203–215.

Leoncini E, Botto L, Cocchi G, Annerén G, Bower C, Halliday J, Amar E, Bakker MK, Bianca S, Canessa Tapia MA, Castilla EE, Csáky-Szunyogh M, Dastgiri S, Feldkamp ML, Gatt M, Hirahara F, Landau D, Lowry RB, Marengo L, McDonnell R, Mathew TM, Morgan M, Mutchinick OM, Pierini A, Poetzsch S, Ritvanen A, Scarano G, Siffel C, Sípek A, Szabova E, Tagliabue G, Vollset SE, Wertelecki W, Zhuchenko L, Mastroiacovo P. (2010) How valid are the rates of Down

syndrome internationally? Findings from the International Clearinghouse for Birth Defects Surveillance and Research. Am J Med Genet A. 152A:1670–1680.
Letts M, Vincent N. (1993) Congenital longitudinal deficiency of the fibula (fibular hemimelia). Parental refusal of amputation. Clin Orthop Relat Res:160–166.
Lin S, Marshall EG, Davidson GK, Roth GB, Druschel CM. (1993) Evaluation of congenital limb reduction defects in upstate New York. Teratology 47:127–135.
Lin Y, Lee Y, Jung C, Jaakkola JJK, Hwang B. (2014) Air pollution and limb defects: a matched-pairs case-control study in Taiwan. Environ Res 132:273–280.
Litrenta J, Young M, Birch J, Oetgen M. (2018) Congenital Tibial Deficiency. J Am Acad Orthop Surg. 27:e268–e279.
Liu S, Rouleau J, León JA, Sauve R, Joseph KS, Ray JG. (2015) Impact of pre-pregnancy diabetes mellitus on congenital anomalies, Canada, 2002–2012. Health Promot Chronic Dis Prev Can 35:79–84.
Lowry R, Bedard T. (2016) Congenital limb deficiency classification and nomenclature: The need for a consensus. Am J Med Genet A 170:1400–1404.
Luteijn JM, Brown MJ, Dolk H. (2014) Influenza and congenital anomalies: a systematic review and meta-analysis. Hum Reprod 29:809–823.
Lynch SA, Wright C. (1997) Sirenomelia, limb reduction defects, cardiovascular malformation, renal agenesis in an infant born to a diabetic mother. Clin Dysmorphol 6:75–80.
Maffulli N, Fixsen JA. (1991) Fibular hypoplasia with absent lateral rays of the foot. J Bone Joint Surg Br 73:1002–1004.
Maffulli N, Fixsen JA. (1996) Management of forme fruste fibular hemimelia. J Pediatr Orthop B 5:17–19.
Malm H, Martikainen J, Klaukka T, Neuvonen PJ Prescription drugs during pregnancy and lactation--a Finnish register-based study. (2003) Eur J Clin Pharmacol. 59:127–33.
Manouvrier Hanu S, Holder Espinasse M, Lyonnet S. (1999) Genetics of limb anomalies in humans. Trends Genet 15:409–417.
Martínez-Frías M, Bermejo E, Rodríguez Pinilla E, Frías J. (2004) Risk for congenital anomalies associated with different sporadic and daily doses of alcohol consumption during pregnancy: a case-control study. Birth Defects Res Part A Clin Mol Teratol 70:194–200.
McCandless S, Brunger J, Cassidy S. (2004) The burden of genetic disease on inpatient care in a children's hospital. Am J Hum Genet 74:121–127.
McCarthy JJ, Glancy GL, Chnag FM, Eilert RE. (2000) Fibular hemimelia: comparison of outcome measurments after amputation and lengthening. J Bone Joint Surg Am 82-A:1732–1735.
McGuirk CK, Westgate MN, Holmes LB. (2001) Limb deficiencies in newborn infants. Pediatrics 108:E64.
Medical Birth Register. Available at: https://thl.fi/en/web/thlfi-en/statistics/information-on-statistics/register-descriptions/newborns. (accessed 22.2.2020)
Meinilä J, Koivusalo SB, Valkama A, Rönö K, Erkkola M, Kautiainen H, Stach-Lempinen B, Eriksson JG. (2015) Nutrient intake of pregnant women at high risk of gestational diabetes. Food Nutr Res. 2015 19;59:26676..
Mellin GW, Katzenstein M. (1962) The saga of thalidomide. Neuropathy to embryopathy, with case reports of congenital anomalies. N Engl J Med 267:123–144.
Moore K, Persaud T, Torchia M (2016) In: The developing human. Clinically oriented embryology. Elsevier: Philadelphia. 1–10, 69–89, 337–354, 363–378, 457–486.
Morris J, Springett A, Greenlees R, Loane M, Addor M, Arriola L, Barisic I, Bergman JEH, Csaky-Szunyogh M, Dias C, Draper E., Garne E, Gatt M, Khoshnood B, Klungsoyr K, Lynch C, McDonnell R, Nelen V, Neville A, O'Mahony M, Pierini A, Queisser-Luft A, Randrianaivo H, Rankin J, Rissmann A, Kurinczuk J, Tucker D, Verellen-Dumoulin C, Wellesley D, Dolk H. (2018) Trends in congenital anomalies in Europe from 1980 to 2012. PLoS ONE 13:e0194986.

Mosca V (2014) The foot. In Lovell and Winter's Pediatric Orthopedics. Lippincott Williams and Wilkins: Philadelphia. 1388–1525.

NBDPN Guidelines. Birth Defects Descriptions for NBDPN Core, Recommended, and Extended Conditions. Available at: https://www.nbdpn.org/docs/Appendix_3_1_BirthDefectsDescriptions_2017MAR24.pdf. (accessed 22.2.2020)

Nielsen GL, Nørgard B, Puho E, Rothman KJ, Sørensen HT, Czeizel AE. (2005) Risk of specific congenital abnormalities in offspring of women with diabetes. Diabet Med 22:693–696.

Norio R. (2003a) Finnish Disease Heritage I: characteristics, causes, background. Hum Genet. 2003 112:441–456.

Norio R. (2003b) Finnish Disease Heritage II: population prehistory and genetic roots of Finns. Hum Genet. 2003 112:457–469.

Norio R. (2003c) The Finnish Disease Heritage III: the individual diseases. Hum Genet. 2003 112:470–526.

Oddy W, De Klerk N, Miller M, Payne J, Bower C. (2009) Association of maternal pre-pregnancy weight with birth defects: evidence from a case-control study in Western Australia. Aust N Z J Obstet Gynaecol 49:11–15.

Olesen C, Søndergaard C, Thrane N, Nielsen GL, de Jong-van den Berg L, Olsen J; EuroMAP Group. (2001) Do pregnant women report use of dispensed medications? Epidemiology. 12:497–501.

Olney RS, Khoury MJ, Alo CJ, Costa P, Edmonds LD, Flood TJ, Harris JA, Howe HL, Moore CA, Olsen CL. (1995) Increased risk for transverse digital deficiency after chorionic villus sampling: results of the United States Multistate Case-Control Study, 1988–1992. Teratology 51:20–29.

Orioli I, Amar E, Arteaga Vazquez J, Bakker M, Bianca S, Botto L, Clementi M, Correa A, Csaky Szunyogh M, Leoncini E, Li Z, López-Camelo J, Lowry RB, Marengo L, Martínez-Frías M, Mastroiacovo P, Morgan M, Pierini A, Ritvanen A, Scarano G, Szabova E, Castilla E. (2011) Sirenomelia: an epidemiologic study in a large dataset from the International Clearinghouse of Birth Defects Surveillance and Research, and literature review. Am J Med Genet C Semin Med Genet 157C:358–373.

Pace N, Desrosiers T, Carmichael S, Shaw G, Olshan A, Siega Riz A. (2018) Antioxidant Consumption is Associated with Decreased Odds of Congenital Limb Deficiencies. Paediatr Perinat Epidemiol 32:90–99.

Pakkasjärvi N, Ritvanen A, Herva R, Peltonen L, Kestilä M, Ignatius J. (2006) Lethal congenital contracture syndrome (LCCS) and other lethal arthrogryposes in Finland--an epidemiological study. Am J Med Genet A. 140A:1834–1839.

Paley D. (2016a) Surgical reconstruction for fibular hemimelia. J Child Orthop 10:557–583.

Paley D. (2016b) Tibial hemimelia: new classification and reconstructive options. J Child Orthop 10:529–555.

Paley D, Guardo F (2014). Lenghtening Reconstruction Surgery for Congenital Femoral Deficiency. Available at: https://paleyinstitute.org/wp-content/uploads/Lengthening-Reconstruction-Surgery-for-Congenital-Femoral-Deficiency.pdf (accessed 22.2.2020)

Pappas AM. (1983) Congenital abnormalities of the femur and related lower extremity malformations: classification and treatment. J Pediatr Orthop 3:45–60.

Pereira N, O'Neill C, Lu, V, Rosenwaks Z, Palermo G. (2017) The safety of intracytoplasmic sperm injection and long-term outcomes. Reproduction 154:F61–F70.

Persson M, Cnattingius S, Villamor E, Söderling J, Pasternak B, Stephansson O, Neovius M. (2017) Risk of major congenital malformations in relation to maternal overweight and obesity severity: cohort study of 1.2 million singletons. BMJ 357:j2563.

Peterson C, Dawson A, Grosse S, Riehle Colarusso T, Olney R, Tanner J, Kirby R, Correia J, Watkins S, Cassell C. (2013) Hospitalizations, costs, and mortality among infants with critical congenital heart disease: how important is timely detection? Birth Defects Res Part A Clin Mol Teratol 97:664–672.

Polednak AP, Janerich DT. (1985) Maternal factors in congenital limb-reduction defects. Teratology 32:41–50.

Prentice A, Goldberg G. (1996) Maternal obesity increases congenital malformations. Nutr Rev 54:146–150.

Radcliff E, Cassell C, Tanner J, Kirby R, Watkins S, Correia J, Peterson C, Grosse S. (2012) Hospital use, associated costs, and payer status for infants born with spina bifida. Birth Defects Res Part A Clin Mol Teratol 94:1044–1053.

Rankin J, Chadwick T, Natarajan M, Howel D, Pearce M, Pless Mulloli T. (2009) Maternal exposure to ambient air pollutants and risk of congenital anomalies. Environ Res 109:181–187.

Razzaghi H, Dawson A, Grosse S, Allori A, Kirby R, Olney R, Correia J, Cassell C. (2015) Factors associated with high hospital resource use in a population-based study of children with orofacial clefts. Birth Defects Res Part A Clin Mol Teratol 103:127–143.

Reece EA. (2012) Diabetes-induced birth defects: what do we know? What can we do? Curr Diab Rep 12:24–32.

Register of Congenital Malformations. Available at: https://thl.fi/en/web/thlfi-en/statistics/information-on-statistics/quality-descriptions/congenital-anomalies#Accessibility%20and%20transparency/clarity%20of%20data (accessed 22.2.2020)

Rijnders LJ, Boonstra AM, Groothoff JW, Cornel MC, Eisma WH. (2000) Lower limb deficient children in The Netherlands: epidemiological aspects. Prosthet Orthot Int 24:13–18.

Robbins J, Tilford J, Bird TM, Cleves M, Reading JA, Hobbs C. (2006) Hospitalizations of newborns with folate-sensitive birth defects before and after fortification of foods with folic acid. Pediatrics 118:906–915.

Rogala EJ, Wynne Davies R, Littlejohn A, Gormley J. (1974) Congenital limb anomalies:frequency and aetiological factors. Data from the Edinburgh Register of the Newborn (1964–68). J Med Genet 11:221–233.

Robert E, Harris J, Kallen BA. (1997) The epidemiology of preaxial limb malformations. Reprod Toxicol.11:653–662.

Rosano A, Botto LD, Olney RS, Khoury MJ, Ritvanen A, Goujard J, Stoll C, Cocchi G, Merlob P, Mutchinick O, Cornel MC, Castilla EE, Martínez-Frías ML, Zampino G, Erickson JD, Mastroiacovo P. (2000) Limb defects associated with major congenital anomalies: clinical and epidemiological study from the International Clearinghouse for Birth Defects Monitoring Systems. Am J Med Genet 93:110–116.

Räisänen S, Sankilampi U, Gissler M, Kramer M, Hakulinen-Viitanen T, Saari J, Heinonen S. (2014) Smoking cessation in the first trimester reduces most obstetric risks, but not the risks of major congenital anomalies and admission to neonatal care: a population-based cohort study of 1,164,953 singleton pregnancies in Finland. J Epidemiol Community Health 68:159–164.

Sainio S, Klemetti R, Hemminki E, Gissler M. Yli 40-vuotiaan naisen raskaus (2010). Suomen Lääkärilehti. 65:3129–3134.

Santos J, Nogueira R, Pinto R, Cerveira I, Pereira S. (2013) First Trimester Diagnosis of VACTERL Association. Clin Pract 3:e5.

Schoenecker PL, Capelli AM, Millar EA, Sheen MR, Haher T, Aiona MD, Meyer LC. (1989) Congenital longitudinal deficiency of the tibia. J Bone Joint Surg Am 71:278–287.

Shaw GM, O'Malley CD, Wasserman CR, Tolarova MM, Lammer EJ. (1995) Maternal periconceptional use of multivitamins and reduced risk for conotruncal heart defects and limb deficiencies among offspring. Am J Med Genet 59:536–545.

Shaw G, Nelson V, Carmichael S, Lammer E, Finnell R, Rosenquist T. (2002) Maternal periconceptional vitamins: interactions with selected factors and congenital anomalies? Epidemiology 13:625–630.

Sheffield J, Butler-Koster E, Casey B, McIntire D, Leveno K. (2002) Maternal diabetes mellitus and infant malformations. Obstet Gynecol 100:925–930.

Shi M, Wehby G, Murray J. (2008) Review on genetic variants and maternal smoking in the etiology of oral clefts and other birth defects. Birth Defects Res C Embryo Today 84:16–29.

Simeone R, Oster M, Hobbs C, Robbins J, Collins RT, Honein M. (2015) Population-based study of hospital costs for hospitalizations of infants, children, and adults with a congenital heart defect, Arkansas 2006 to 2011. Birth Defects Res Part A Clin Mol Teratol 103:814–820.

Simmons ED, Ginsburg GM, Hall JE. (1996) Brown's procedure for congenital absence of the tibia revisited. J Pediatr Orthop 16:85–89.

Skjaerven R, Wilcox AJ, Lie RT. (1999) A population-based study of survival and childbearing among female subjects with birth defects and the risk of recurrence in their children. N Engl J Med 340:1057–1062.

Smidt-Jensen S, Permin M, Philip J, Lundsteen C, Zachary JM, Fowler SE, Grüning K. (1992) Randomised comparison of amniocentesis and transabdominal and transcervical chorionic villus sampling. Lancet 340:1237–1244.

Smithells RW, Newman CG. (1992) Recognition of thalidomide defects. J Med Genet 29:716–723.

Spiegel DA, Loder RT, Crandall RC. (2003) Congenital longitudinal deficiency of the tibia. Int Orthop 27:338–342.

Stanitski D, Stanitski C. (2003) Fibular hemimelia: a new classification system. J Pediatr Orthop 23:30–34.

Steel HH, Lin PS, Bets RR, Kalamchi A, Clancy M. (1987) Iliofemoral fusion for proximal femoral focal deficiency. J Bone Joint Surg Am 69:837–843.

Stewart CP, Jain AS. (1995) Congenital limb anomalies and amputees Tayside, Scotland 1965–1994. Prosthet Orthot Int 19:148–154.

Stocker JT, Heifetz SA. (1987) Sirenomelia. A morphological study of 33 cases and review of the literature. Perspect Pediatr Pathol 10:7–50.

Stoll C, Alembik Y, Dott B, Roth MP. (1992) Risk factors in limb reduction defects. Paediatr Perinat Epidemiol 6:323–338.

Stoll C, Calzolari E, Cornel M, Garcia Minaur S, Garne E, Nevin N, Ten Kate L. (1996) A study on limb reduction defects in six European regions. Ann Genet 39:99–104.

Stoll C, Duboule D, Holmes LB, Spranger J. (1998) Classification of limb defects. Am J Med Genet 77:439–441.

Stoll C, Alembik Y, Dott B. Roth M. (2010) Associated malformations in patients with limb reduction deficiencies. Eur J Med Genet 53:286–290.

Stothard K., Tennant PWG, Bell R, Rankin J. (2009) Maternal overweight and obesity and the risk of congenital anomalies: a systematic review and meta-analysis. JAMA 301:636–650.

Sund R. (2012) Quality of the Finnish Hospital Discharge Register: a systematic review. Scand J Public Health 40:505–515.

Swanson AB. (1976) A classification for congenital limb malformations. J Hand Surg Am 1:8–22.

Tabor A, Philip J, Madsen M, Bang J, Obel EB, Nørgaard Pedersen B. (1986) Randomised controlled trial of genetic amniocentesis in 4606 low-risk women. Lancet 1:1287–1293.

Tani Y, Ikuta Y, Ishida O. (2000) Surgical treatment of the cleft foot. Plast Reconstr Surg 105:1997–2002.

Taylor B, Kean J, Paloski M. (2009) Distal focal femoral deficiency. J Pediatr Orthop 29:576–580.

Therapontos C, Erskine L, Gardner E, Figg W, Vargesson N. (2009) Thalidomide induces limb defects by preventing angiogenic outgrowth during early limb formation. Proc Natl Acad Sci U S A 106:8573–8578.

Thomas IH, Williams PF. (1987) The Gruca operation for congenital absence of the fibula. J Bone Joint Surg Br 69:587–592.

Tomson T, Battino D, Bonizzoni E, Craig J, Lindhout D, Perucca E, Sabers A, Thomas S, Vajda F. (2018) Comparative risk of major congenital malformations with eight different antiepileptic drugs: a prospective cohort study of the EURAP registry. Lancet Neurol 17:530–538.

Torode IP, Gillespie R. (1991) The classification and treatment of proximal femoral deficiencies. Prosthet Orthot Int 15:117–126.
Tsou PM. (1982) Congenital distal femoral focal deficiency: report of a unique case. Clin Orthop Relat Res:99–102.
Van Gelder MMHJ, Van Bennekom CM, Louik C, Werler MM, Roeleveld N, Mitchell AA. (2015) Maternal hypertensive disorders, antihypertensive medication use, and the risk of birth defects: a case-control study. BJOG 122:1002–1009.
Van Nes C. (1950) Rotation-plasty for congenital defects of the femur: making use of the ankle for the shortened limb to control the knee joint of the prosthesis. J Bone Joint Surg [Br] 32-B:12–16.
Vargesson N. (2015) Thalidomide-induced teratogenesis: history and mechanisms. Birth Defects Res C Embryo Today 105:140–156.
Vargesson N. (2019) The teratogenic effects of thalidomide on limbs. J Hand Surg Eur Vol 44:88–95.
Vasluian E, van der Sluis Corry K, van Essen A, Bergman JEH, Dijkstra P, Reinders Messelink H, de Walle Hermien EK. (2013) Birth prevalence for congenital limb defects in the northern Netherlands: a 30-year population-based study. BMC Musculoskelet Disord 14:323.
Vergult S, Hoogeboom AJM, Bijlsma E, Sante T, Klopocki E, De Wilde B, Jongmans M, Thiel C, Verheij Joke BGM, Perez Aytes A, Van Esch H, Kuechler A, Barge-Schaapveld Daniela QCM, Sznajer Y, Mortier G, Menten B. (2013) Complex genetics of radial ray deficiencies: screening of a cohort of 54 patients. Genet Med 15:195–202.
Vinikoor Imler L, Davis JA, Meyer R, Luben T. (2013) Early prenatal exposure to air pollution and its associations with birth defects in a state-wide birth cohort from North Carolina. Birth Defects Res Part A Clin Mol Teratol 97:696–701.
Wada A, Nakamura T, Urano N, Kubota H, Oketani Y, Taketa M, Fujii T. (2015) Foot centralization for tibial hemimelia. J Pediatr Orthop B 24:147–153.
Walker J, Knapp D, Minter C, Boakes J, Salazar J, Sanders J, Lubicky J, Drvaric D, Davids J. (2009) Adult outcomes following amputation or lengthening for fibular deficiency. J Bone Joint Surg Am 91:797–804.
Waller DK, Shaw G, Rasmussen S, Hobbs C, Canfield M, Siega Riz A, Gallaway MS, Correa A. (2007) Prepregnancy obesity as a risk factor for structural birth defects. Arch Pediatr Adolesc Med 161:745–750.
Wang Y, Hu J, Druschel C, Kirby R. (2011) Twenty-five-year survival of children with birth defects in New York State: a population-based study. Birth Defects Res Part A Clin Mol Teratol 91:995–1003.
Watkins M, Rasmussen S, Honein M, Botto L, Moore C. (2003) Maternal obesity and risk for birth defects. Pediatrics 111:1152–1158.
Weber M. (2008) New classification and score for tibial hemimelia. J Child Orthop 2:169–175.
Weber M, Siebert C, Goost H, Johannisson R, Wirtz D. (2002) Malleolus externus plasty for joint reconstruction in fibular aplasia: preliminary report of a new technique. J Pediatr Orthop B 11:265–273.
Weir S, Ephraim P, Mackenzie E. (2010) Effects of paediatric limb loss on healthcare utilisation, schooling and parental labour supply. Disabil Rehabil 32:2046–2055.
Werler MM, Bosco JL, Shapira SK; National Birth Defects Prevention Study. (2009) Maternal vasoactive exposures, amniotic bands, and terminal transverse limb defects. Birth Defects Res A Clin Mol Teratol. 85:52–57.
Westberry DE, Davids JR. (2009) Proximal focal femoral deficiency (PFFD): management options and controversies. Hip Int 19 Suppl 6:S1–S25.
Wood VE, Peppers TA, Shook J. (1997) Cleft-foot closure: a simplified technique and review of the literature. J Pediatr Orthop 17:501–504.
Wright MJ, Newell JN, Charlton ME, Hey EN, Donaldson LJ, Burn J. (1995) Limb reduction defects in the northern region of England 1985–92. J Epidemiol Community Health 49:305–308.

Wu V, Poenaru D, Poley M. (2013) Burden of surgical congenital anomalies in Kenya: a population-based study. J Trop Pediatr 59:195–202.

Yang Q, Khoury MJ, Olney RS, Mulinare J. (1997) Does periconceptional multivitamin use reduce the risk for limb deficiency in offspring? Epidemiology 8:157–161.

Zhang R, Marsch F, Kause F, Degenhardt F, Schmiedeke E, Märzheuser S, Hoppe B, Bachour H, Boemers T, Schäfer M, Spychalski N, Neser J, Leonhardt J, Kosch F, Ure B, Gómez B, Lacher M, Deffaa O, Palta M, Wittekindt B, Kleine K, Schmedding A, Grasshoff-Derr S, van der Ven A, Heilmann-Heimbach S, Zwink N, Jenetzky E, Ludwig M, Reutter H. (2017) Array-based molecular karyotyping in 115 VATER/VACTERL and VATER/VACTERL-like patients identifies disease-causing copy number variations. Birth Defects Res 109:1063–1069.

Zhao E, Zhang Y, Zeng X, Liu B. (2015) Association between maternal diabetes mellitus and the risk of congenital malformations: A meta-analysis of cohort studies. Drug Discov Ther 9:274–281.

Åberg A, Westbom L, Källén, B. (2001) Congenital malformations among infants whose mothers had gestational diabetes or preexisting diabetes. Early Hum Dev 61:85–95.

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