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ABSTRACT

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Epidemiology of neurofibromatosis type 1 in Finland: Incidence, mortality, pregnancies and congenital malformations

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Neurofibromatosis type 1 (NF1) is a dominantly inherited cancer syndrome, which is caused by mutations in the *NF1* gene. Because of the high mutation rate of the gene, approximately half of the patients have a new mutation, while none of the parents have the disorder. The incidence of NF1 is estimated to be approximately 1:3,000. The best-known symptoms of NF1 are neurofibromas on the skin, but NF1 is a multisystem disorder associated with a decreased overall survival and increased risk for pathologies such as cancer, learning difficulties, epilepsy and speech defects.

While there are some previous epidemiological studies on NF1-associated pregnancies and mortality of NF1, data is very limited. No epidemiological data is reported on birth size or overall risk for congenital malformations in NF1. We have acquired a nationwide cohort of approximately 1,500 patients with a confirmed diagnosis for NF1, and ten matched controls per NF1 patient were collected. The data was linked with administrative registers to study incidence, mortality, pregnancies, birth size and congenital malformations of NF1.

We observed that the incidence of NF1 in Finland was approximately 1:2,000, which is higher than previously generally accepted. Mortality of NF1 was considerably higher than in the general population. Pregnancy duration was shortened by a fetus with NF1, and the risk for several pregnancy and delivery complications was increased among NF1 mothers. Birth weight was decreased by having a mother with NF1, while having NF1 present in the child increased it. The risk for congenital malformations was almost three-fold among NF1 children compared to matched controls.

Our study highlights a wide spectrum of ailments that NF1 causes, and the results can be utilized when guidelines of treatment and follow-up of NF1 are developed.

Keywords: neurofibromatosis type 1, neurofibromatosis type 2, epidemiology, rasopathy, pregnancy, delivery, congenital malformation, birth size, mortality

TIIVISTELMÄ

LL Jussi Leppävirta

Neurofibromatoosi 1 Suomessa: epidemiologinen tutkimus ilmaantuvuudesta, kuolleisuudesta, raskauksista ja epämuodostumista

Turun yliopisto, lääketieteellinen tiedekunta, kliininen laitos, iho- ja sukupuolitautioppi, biolääketieteen laitos, Turun kliininen tohtoriohjelma; Ihoklinikka, Turun yliopistollinen keskussairaala, Turku, Suomi

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Neurofibromatoosi 1 (NF1) on vallitsevasti periytyvä monille syöpätyypeille altistava oireyhtymä, joka johtuu *NF1*-geenin mutaatioista. *NF1*-geenissä tapahtuu hyvin herkästi mutaatioita, minkä vuoksi noin puolella potilaista on uusi mutaatio ja vain puolella potilaista on vanhemmilta peritty sairaus. NF1:n ilmaantuvuuden on arvioitu olevan noin 1:3000. Parhaiten NF1:n oireista tunnetaan iholta löytyvät hyvänlaatuiset neurofibroomakasvaimet, mutta kyseessä on monen elimen oireyhtymä. NF1:n onkin havaittu lisäävän kuolleisuutta sekä riskiä sairastua mm. syöpään sekä epilepsiaan. Lisäksi esim. oppimisvaikeudet ovat yleisiä NF1-potilailla.

Epidemiologinen tutkimustieto NF1:n vaikutuksista kuolleisuuteen, raskauksiin ja synnytyksiin on niukkaa, eikä epidemiologista tietoa ole lainkaan NF1:n vaikutuksesta lapsen syntymäkokoon tai yleiseen epämuodostumariskiin. Tutkimusta varten muodostettiin koko maan kattava noin 1500 varmistetun NF1-potilaan ryhmä, ja jokaiselle potilaalle kerättiin kymmenen kaltaistettua verrokkihenkilöä. Valtakunnallisten rekisteritietojen avulla tutkittiin NF1:n ilmaantuvuutta sekä oireyhtymän vaikutusta raskauksiin, syntymäkokoon sekä epämuodostumariskiin.

Tutkimuksessa havaittiin, että NF1:n ilmaantuvuus Suomessa on n. 1:2000, eli NF1 on selvästi aikaisempaa arvioitua yleisempi. NF1-potilaiden kuolleisuus oli suurentunut merkitsevästi verrattuna muuhun väestöön. Lapsen NF1 lyhensi raskaudenkestoa ja raskauskomplikaatioiden riski oli suurentunut NF1-äideillä. Äidin NF1 pienensi lapsen syntymäpainoa, mutta lapsen NF1:n vaikutus painoon oli päinvastainen. NF1-lapsen epämuodostumariski oli lähes kolminkertainen kontrolliryhmään verrattuna.

Tuloksemme osoittavat, että NF1:llä on laajoja ja osittain aikaisemmin tuntemattomia vaikutuksia raskauksiin ja synnytyksiin. Tutkimustuloksia voidaan käyttää hyväksi laadittaessa NF1:n seuranta- ja hoitosuosituksia.

Avainsanat: neurofibromatoosi 1, neurofibromatoosi 2, epidemiologia, raskaus, synnytys, epämuodostumat, kuolleisuus, syntymäkoko

TABLE OF CONTENTS

ABBREVIATIONS

AGA	appropriate for gestational age
CALM	café au lait macule
CI	confidence interval
CIM	type 1 Chiari malformation
DNA	deoxyribonucleic acid
GDP	guanosine diphosphate
GTP	guanosine triphosphate
ICD	International Classification of Diseases
IQ	intelligence quotient
IUGR	intrauterine growth restriction
LGA	large for gestational age
MPNST	malign peripheral nerve sheath tumor
MRI	magnetic resonance imaging
mRNA	messenger ribonucleic acid
NF1	neurofibromatosis 1
NF2	neurofibromatosis 2
NIH	National Institutes of Health
NIPT	non-invasive prenatal test
OR	odds ratio
PMR	proportionate mortality ratio
SDS	standard deviation score
SGA	small for gestational age
SMR	standardized mortality ratio
THL	National Institute for Health and Welfare
UBO	unidentified bright object
WHO	World Health Organization

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications, which are referred to in the text by the Roman numerals I-IV.

I. Uusitalo Elina*, Leppävirta Jussi*, Koffert Anna, Suominen Sakari, Vahtera Jussi, Vahlberg Tero, Pöyhönen Minna, Peltonen Juha & Peltonen Sirkku. Incidence and mortality of neurofibromatosis: a total population study in Finland. *The Journal of Investigative Dermatology*, 2015, 135(3), 904.

II. Leppävirta Jussi, Kallionpää Roope A., Uusitalo Elina, Vahlberg Tero, Pöyhönen Minna, Timonen Susanna, Peltonen Juha & Peltonen Sirkku. The pregnancy in neurofibromatosis 1: A retrospective register-based total population study. *American Journal of Medical Genetics Part A*, 2017, 173(10), 2641-2648.

III. Leppävirta Jussi, Kallionpää Roope A., Uusitalo Elina, Vahlberg Tero, Pöyhönen Minna, Peltonen Juha & Peltonen Sirkku. Neurofibromatosis type 1 of the child increases birth weight. *(submitted manuscript)*

IV. Leppävirta Jussi, Kallionpää Roope, A., Uusitalo Elina, Vahlberg Tero, Pöyhönen Minna, Peltonen Juha & Peltonen Sirkku. Congenital anomalies in neurofibromatosis 1: a retrospective register-based total population study. *Orphanet Journal of Rare Diseases, 2018, 13(5).*

*Equal contribution

1 INTRODUCTION

Neurofibromatosis 1 (NF1) is an autosomal dominant cancer predisposition syndrome that is caused by mutations in the *NF1* gene. In other words, half of the children of a parent with NF1 inherits this genetic disorder, which increases the risk for cancer. As the *NF1* gene is prone to mutations, half of the patients with NF1 have a new mutation, while neither of the parents carry a disorder causing mutations in the *NF1* gene. NF1 is classified as a rare disease, but among rare diseases, it can be considered common, as the risk for having NF1 is estimated to be approximately 1:3,000 (Huson et al., 1989; Poyhonen, Kytölä and Leisti, 2000; Lammert et al., 2005; Evans et al., 2010). In Finland, every year approximately 20 infants with NF1 are born, and worldwide 45,000 persons are born with NF1. Best known clinical symptoms of NF1 are found on the skin, including benign nodular lesions called neurofibromas, hyperpigmented café au lait macules (CALM) and freckling of the flexural areas. Neurofibromin, which is a protein product of the *NF1* gene, acts as a tumor suppressor protein inhibiting the Ras signaling transduction pathway. However, it has multiple other functions and interactions, which are currently incompletely known (Jouhilahti et al., 2011). As neurofibromin is expressed during the fetal development (Daston and Ratner, 1992) and throughout the lifetime (Daston et al., 1992; Jouhilahti et al., 2011), it is not surprising that, in addition to an increased risk for cancer, NF1 is associated with numerous conditions including increased mortality (Evans et al., 2011), osteoporosis (Kuorilehto et al., 2005), learning deficits (Krab et al., 2008), speech defects (Alivuotila et al., 2010), pregnancy and delivery complications (Terry et al., 2013) and epilepsy (Ostendorf, Gutmann and Weisenberg, 2013).

Neurofibromatosis 2 (NF2) is a genuinely rare dominantly inherited disease with an incidence of approximately 1:25,000 caused by a mutation in the *NF2* tumor suppressor gene, which codes the merlin protein. The hallmark feature in NF2 is bilateral vestibular schwannomas. Neurological complications are common in NF2 [for review, see Ferner (Ferner, 2010)].

Epidemiological studies on neurofibromatoses are needed to assess the impact of the disorders in the community to guide the allocation of public health resources. In addition, epidemiological studies are crucial for individual patients, because the studies can reveal risks for complications during different stages of life. These studies can be utilized in developing guidelines for treatment and follow-up. Epidemiological studies can also complete the clinical picture of NF1 and find previously unknown associations helping to guide further studies and discover new fields of interest in basic research investigations. However, previous epidemiological studies on NF1 are scarce and cover only limited aspects of NF1. For example, no studies about birth size or congenital malformations in NF1 exist. Finland's extensive administrative registers

together with personal identity codes and electronic hospital registers offer a unique opportunity to execute reliable epidemiological studies.

While an individual rare disease is already by a definition rare at a personal level, it has been estimated that 6-8 % of the population in the European Union are affected by these diseases (European Comission). A rare disease often has a major impact on the quality of life, and rare diseases are causing a significant economic burden (Cohen and Biesecker, 2010; Angelis, Tordrup and Kanavos, 2015). In addition, studies on monogenic diseases can reveal molecular and genetic backgrounds of more common diseases (Peltonen et al., 2006). These aspects highlight the importance of research on rare diseases.

The aim of this study was to determine incidence of NF1 and NF2 in Finland. In addition, mortality, pregnancies, deliveries, birth size and congenital malformations in NF1 were studied. This epidemiological retrospective total population study was executed by acquiring a cohort of 1,410 persons with a confirmed diagnosis of NF1 and ten matched controls per person with NF1. For these study persons, data was gathered from administrative registers, and persons with neurofibromatosis were compared to matched controls.

2 REVIEW OF LITERATURE

2.1 History of NF1

The first possible preserved traces of NF1 date back to 700-200 BC in the form of a discovered female skull found in a cemetery complex in south Siberia (Murphy, Donnelly and Rose, 1998). Another possible historical traces of NF are visible in the photography of a now lost statue from the Hellenistic period (323-31 BC) showing multiple nodular skin lesions (Ragge and Munier, 1994) and Partihan coins representing kings from several generations (123 BC – 58 AD) with a nodule in the face (Todman, 2008). NF1 was possibly for the first time illustrated in the Thirteenth century by a Cistercian monk, Heinricus. Also, a naturalist and philosopher Conrad von Megenberg (1309-1374), possibly illustrated a person with NF1 in the *"Buch de natur"* in approximately 1350. In 1585, French surgeon Ambroise Paré (1510-1590) published *"Les Oeuvres d'Ambroise Paré"* including an illustration accompanied by a written description of a monstrous infant who may have had NF1 (Zanca and Zanca, 1980; Brosius, 2010). However, none of the cases mentioned above can be confirmed to represent persons with NF1.

The first known English report about a patient with NF1 was published in 1768 by a British physician and poet Mark Akenside (1721-1770), who described a patient with multiple tumors on the skin. However, several physicians and over a time period of a century were needed before the nature of the disorder, and the word *"neurofibroma"* were presented. In 1818, Louis Odier (1748-1817) termed tumors originating from the nerves as *"neuroma"* and Irish surgeon Robert William Smith (1807-1873) further developed the classification of neuromas. The latter was also the first person to describe the autopsy reports of patients with NF1. Rudolf Ludwig Virchow (1821-1902) published case reports of multiple neuromas. He also classified neuromas according to the existence of nerves in the neuromas. Finally, Virchows's student Friedrich Daniel von Recklinghausen (1833-1910), after whom the eponym von Recklinghausen's disease was named, recognized the pattern of the disorder including tumors, hyperpigmented macules and freckling of the inguinal and axillary areas. He also was the first person to use a term *"neurofibroma"* to describe a tumor, where fibromas and neuromas are intertwined. Noteworthy in the historical perspective is also that neither John Merrick, better known as the Elephant Man, or Victor Hugo's Quasimodo represented neurofibromatosis (Morse, 1999).

2.2 Pathoetiology of NF1

2.2.1 NF1 gene, mutations and inheritance

NF1 gene is located on the long arm of chromosome 17 (17q11.2) and was identified by cloning in 1990 (Cawthon et al., 1990; Viskochil et al., 1990; Wallace et al., 1990). The gene is very large as it spans approximately 280-290 kb of genomic DNA including 57 constitutive exons and 4 small alternatively spliced exons (Jouhilahti et al., 2011). The mutation rate of the gene is high, but the reasons for this are unknown (Huson et al., 1989; Evans et al., 2010). Only one translation start site and one translational initiation codon are known to exist (Li and Wallace, 2012). Seven partial duplicates called pseudogens, which can interfere mutational analysis, exist in the genome (Legius et al., 1992; Yu et al., 2005). The *NF1* gene is expressed in all tissues and already during fetal development, but the regulation of the expression is incompletely known (Gutmann, Wood and Collins, 1991; Daston et al., 1992; Daston and Ratner, 1992). It has been suggested that the regulation of the *NF1* gene varies among tissues and also between the different stages of development (Daston and Ratner, 1992; Hirvonen et al., 1998; Malminen et al., 2002). It is known that the levels of *NF1* mRNA and the protein product of the *NF1* gene, neurofibromin, can vary considerably in a short period of time, and that the regulation is proceeded at multiple levels (Jouhilahti et al., 2011).

NF1 shows complete penetrance, but new sequencing studies in the near future can change our assumptions about the effects of mutations in the *NF1* gene. Approximately half of the mutations in the *NF1* gene represent new mutations. However, inherited germline mutations are in more than 80 % of the cases inherited from a father, while microdeletions are suggested to be inherited more often from a mother (Jadayel et al., 1990; Upadhyaya et al., 1998). Advanced paternal and maternal age increases risk for sporadic NF1 (Snajderova et al., 2012).

Because of a very large size of the gene, a high mutation rate and the lack of mutational hotspots, there is a huge number of different mutations identified in the *NF1* gene (Baralle and Baralle, 2012; Koczkowska et al., 2018), but in most cases, no genotype-phenotype correlations can be drawn. However, a few described correlations between the mutation and clinical picture exist being specific 3-bp in-frame deletion and a clinical phenotype without cutaneous neurofibromas; individuals harbouring missense mutations and spinal neurofibromatosis; specific missense mutation with café au lait macules and Lisch nodules without cutaneous or plexiform neurofibromas and microdeletions including in the *NF1* gene and neighboring genes leading to a severe phenotype. Especially a phenotype associated with microdeletions is clinically relevant as these mutations account for 5-10 % of pathogenic mutations in the *NF1* gene and

microdeletion-patients have an increased risk for pathologies such as malignant peripheral nerve sheath tumors (MPNST) (De Raedt et al., 2003; Pasmant et al., 2012; Kehrer-Sawatzki, Mautner and Cooper, 2017).

2.2.2 Neurofibromin

The product of the *NF1* gene is a large cytoplasmic protein, neurofibromin, with a molecular mass of 280kDa. It is ubiquitously expressed in every tissues, but higher levels of neurofibromin are found in Schwann cells, oligodendrocytes, neurons, astrocytes and leucocytes (Gutmann et al., 1991; Daston et al., 1992). Neurofibromin has apparently multiple functions and interactions (Peltonen, Kallionpää and Peltonen, 2017). Two functional domains are known in more detail: Sec14 and RasGAP. The function of Sec14 is unknown, but RasGAP has a RAS GTPase activating effect (Ballester et al., 1990; Martin et al., 1990; Xu et al., 1990).

The Ras-pathway plays a central role in cell proliferation, growth and survival. Ras proteins, which are attached to the plasma membrane, transmit signals from the plasma membrane to the nucleus regulating proliferation, growth and survival of the cell. The Ras protein can exist in a guanosine triphosphate (GTP)-bound state or a guanosine diphosphate (GDP)-bound state and cycle between them. However, only the GTP-bound state is an active form of the protein. The RasGAP domain of neurofibromin acts as a GTPase, which accelerates the hydrolysis of the GTP to GDP, thus suppressing the proliferative effect of the Ras pathway. When there are mutations in the *NF1* gene, the amount of neurofibromin is decreased, and the Ras pathway is not normally inhibited, which may lead to accelerated cell proliferation (Trovó-Marqui and Tajara, 2006).

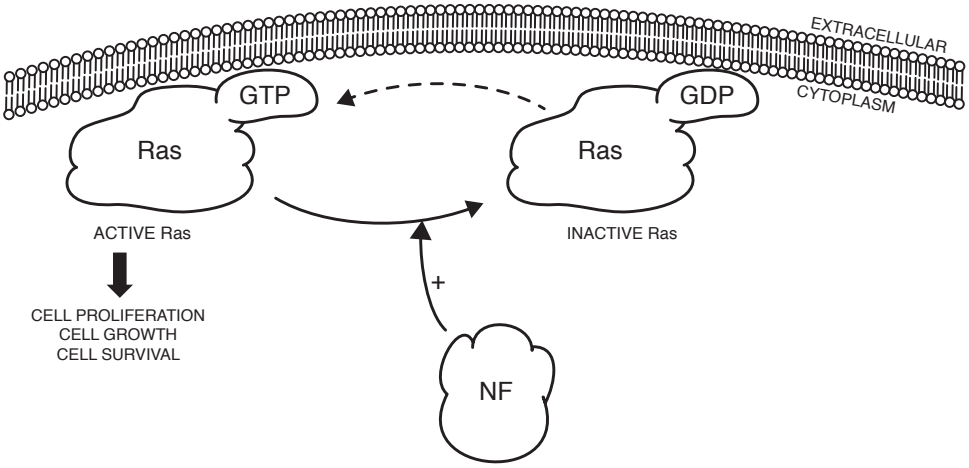

Figure 1. Neurofibromin (NF) inhibits the Ras pathway by accelerating the transformation of the Ras protein from an active guanosine triphosphate (GTP)-bound state to an inactive guanosine diphosphate (GDP)-bound state.

In addition to inhibiting the Ras pathway, neurofibromin has interactions with other pathways including PI3K/Akt/mTOR and Raf/MEK/ERK pathways, which regulate cell proliferation, cell migration, cell survival and cell growth. Also interactions with other proteins are reported, but the effects of these interactions are poorly characterized (Jouhilahti et al., 2011; Peltonen et al., 2017).

2.3 Diagnosis of NF1

2.3.1 Clinical criteria for NF1

Clinical criteria for NF1 are based on the National Institutes of Health (NIH) Consensus Development Conference statement from 1988 (National Institutes of Health Consensus Development Conference., 1988). These clinical criteria have been essential in diagnosing patients and in research, as no official clinical criteria for NF1 existed before 1988. NIH clinical criteria are highly sensitive and specific (Gutmann et al., 1997). Almost all patients with NF1 fulfill NIH clinical criteria already by the age of five, and all patients fulfill the criteria by the age of twenty (DeBella, Szudek and Friedman, 2000). Mutational analysis is not part of official NIH clinical criteria; but this analysis can help diagnosing NF1, when there is a strong suspicion of NF1 and clinical criteria are not (yet) fulfilled.

Diagnosis of NF1 is based on the presence of two of the following (National Institutes of Health Consensus Development Conference., 1988):

- Six or more café-au-lait macules over 5 mm in diameter in prepubertal individuals and over 15 mm in greatest diameter in postpubertal individuals
- Two or more neurofibromas of any type or one plexiform neurofibroma
- Freckling in the axillary or inguinal regions
- Two or more Lisch nodules (iris hamartomas)
- Optic glioma
- A distinctive osseous lesion such as sphenoid dysplasia or thinning of long bone cortex, with or without pseudarthrosis
- First-degree relative (parent, sibling or offspring) with NF1

2.3.1.1 Hyperpigmented macules

The best known clinical findings in NF1 are benign cutaneous neurofibromas, but they usually do not start to grow until puberty. Thus, the most common clinical signs to raise the suspicion of NF1 are hyperpigmented macules (Figure 2) also called cutaneous café au lait macules (CALMs).

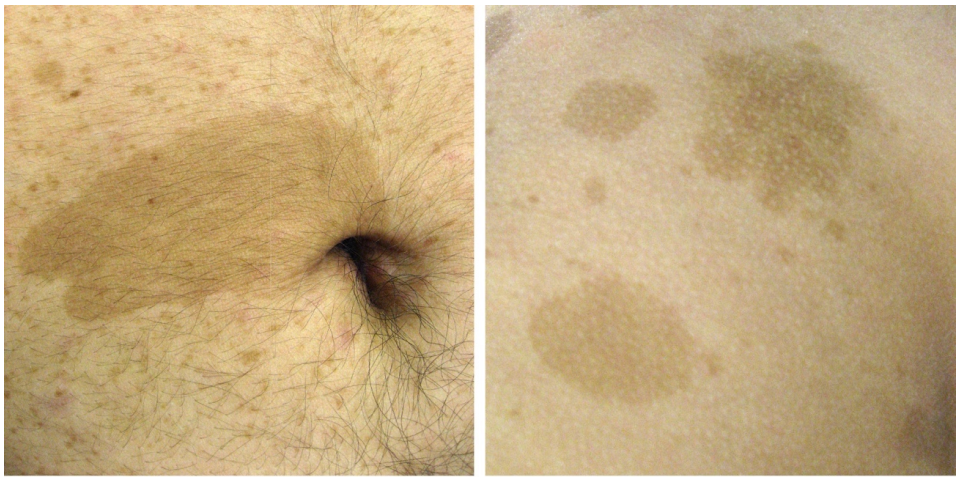

Figure 2. Hyperpigmented macules are often the first clinical finding, which raises the suspicion of NF1. Photo courtesy of Sirkku Peltonen.

They can be found often already on the skin of newborns, and by the age of one year, 95 % of the children with NF1 have them (DeBella et al., 2000). CALMs are darker than the surrounding skin, but the difference is sometimes difficult to recognize. They are macular skin lesions, i.e., CALMs are at the level of the surrounding skin and cannot be felt by palpation. The diameter of a CALM is usually > 5 mm, and the

border is well-defined and smooth. The size of macules increases with age proportionately to body growth, and the diameter can exceed 20 cm (De Schepper et al., 2006). Multiple CALMs should raise the suspicion of NF1. However, up to 28 % of infants without NF1 have a solitary CALM, so a clinician should be careful when evaluating the possibility of NF1 (McLean and Gallagher, 1995). Among white non-NF1 newborns, more than a single CALM is rare, but among black newborns, two or more CALMs can be found in ~6.5 % of newborns (Alper and Holmes, 1983). Differential diagnosis of CALM includes melanocytic nevus, lentigo, Becker nevus, nevus spilus, postinflammatory hyperpigmentation, pityriasis versicolor, urticaria pigmentosa and hypermelanotic macules found in tuberous sclerosis.

2.3.1.2 Neurofibromas

Cutaneous neurofibromas (Figure 3) are benign tumors, which consist of components of peripheral nerves and grow within the dermis. The number of cutaneous neurofibromas varies significantly among individuals from a few to thousands; and they commonly begin to appear during puberty as a bulging of the skin (nodular lesions) localized on the back and abdomen. They can also be purplish macules resembling bruises and palpate soft. For an unknown reason, cutaneous neurofibromas do not undergo malignant transformation, but they can significantly reduce the quality of life.

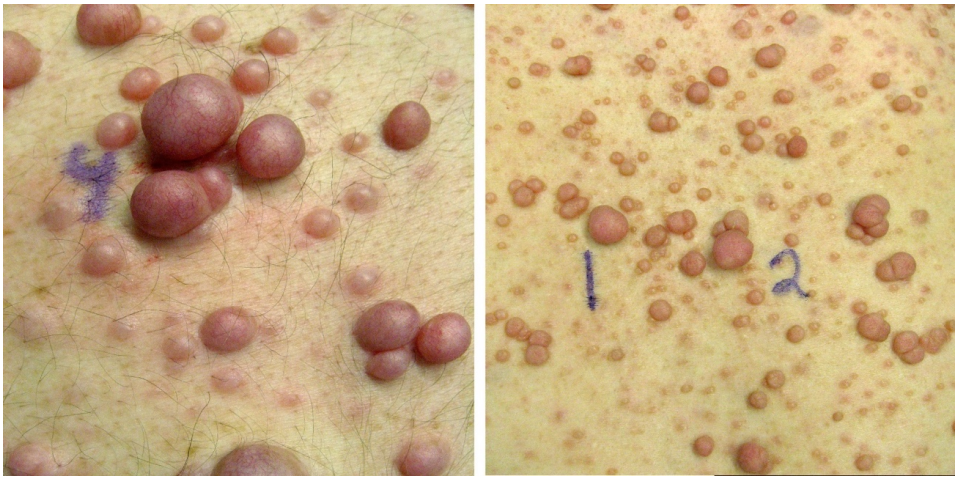

Figure 3. Cutaneous neurofibromas are benign tumors, which most commonly appear during puberty. Photo courtesy of Sirkku Peltonen.

Subcutaneous neurofibromas are palpated as moving firm nodules under the skin. Palpation can cause pain and a tingling sensation for the patient, but the possible operative removal of the subcutaneous neurofibroma must only be made after careful

consultation by a specialist, because the operation has risk for damaging the nerve (Peltonen and Pöyhönen, 2012a).

Plexiform neurofibromas can be confined to the nerves as nodular tumors, or they can be diffuse spreading to neighboring tissues and associate with pigmentation of the overlying skin, hair growth, bone hypertrophy and vascular changes (Ferner, 2007). In a clinical examination, 27 % of the patients with NF1 were found to have at least one plexiform neurofibroma (Huson, Harper and Compston, 1988). However, inspection and palpation alone cannot find even the majority of the plexiform neurofibromas, as in the MRI study by Nguyen et. al. (2011), 37 of 65 children (57 %), aged between 1.7 and 17.6 years, were found to have at least one plexiform neurofibroma.

2.3.1.3 Freckling of the axillary and inguinal regions

Another typical skin lesion in NF1 is freckling of the axillary and inguinal regions (Figure 4). Freckling is often missed, and it should be particularly looked for when evaluating patients suspected to have NF1. Freckling often appears at the age of 3-5 years, and the majority of the patients have freckling by the age of seven. Clinically freckling consist of numerous small macules that are the same color as CALMs (Peltonen and Pöyhönen, 2012b).

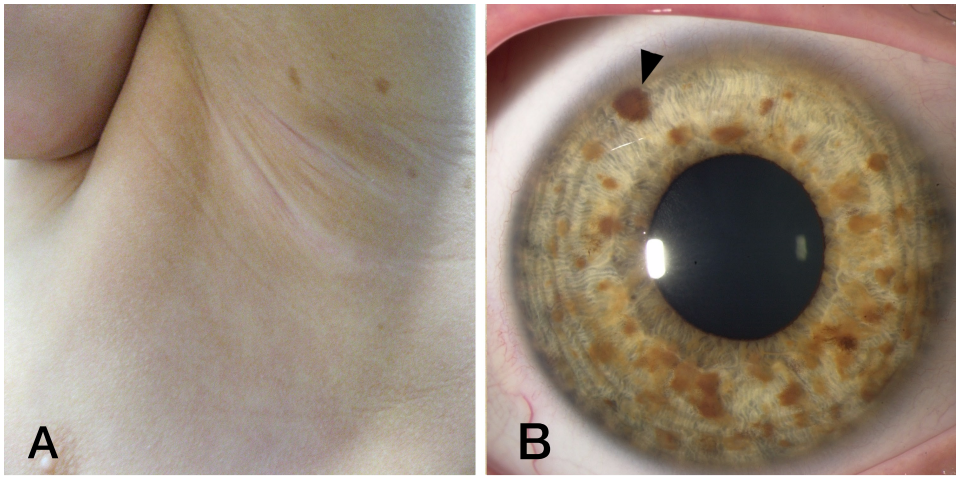

Figure 4. A) Freckling of the axillar region; B) Lisch nodules (arrowhead). Photo courtesy of: A) Sirkku Peltonen, B) Vesa Aaltonen

2.3.1.4 Lisch nodules

Lisch nodules (Figure 4) are found on the slit lamp examination as brown or yellow dome-shaped, well-defined nodules on the surface of the iris. They are composed of pigmented cells, mast cells and fibroblasts (Richetta et al., 2004). Lisch nodules can be visible without magnification, but the differential diagnosis from nevi cannot be made without slit lamp examination by an ophthalmologist (Abdolrahimzadeh et al., 2016). They are rare before the age of two, but already at the age between five and six years, more than half of the children with NF1 have Lisch nodules, and among adults, the majority (>90 %) of the patients have these lesions (Lewis and Riccardi, 1981; Lubs et al., 1991). Lisch nodules are not known to cause any ophthalmological complications. Because practically every adult patient with NF1 has Lisch nodules, they provide a valuable diagnostic tool.

2.3.1.5 Optic gliomas

Optic gliomas in NF1 are pilocytic astrocytomas. Optic nerve gliomas are rare among general population, and 50 % of children with optic glioma have NF1. Thus, optic glioma should always raise the suspicion of NF1 and lead to further examination. Optic gliomas can be asymptomatic and be found in MRI studies performed for other reasons. Symptoms related to optic glioma are decreased visual acuity, proptosis, headache and vomiting. Typically, optic gliomas appear before the age of six years, but they can develop also for older patients. They affect about 15 % of NF1 patients. Routine MRI studies, performed under anesthesia in children, are not indicated to diagnose possible optic glioma. However, visual assessment by an ophthalmologist is recommended beginning in early childhood. Optic gliomas in NF1 are often indolent, but they can cause precocious puberty, poor visual acuity and hydrocephalus (Ferner, 2007; Peltonen and Pöyhönen, 2012c).

2.3.1.6 Skeletal lesions

NF1 is associated with long bone dysplasia, scoliosis, sphenoid wing dysplasia, bone cysts, hand anomalies, bony overgrowth, anterior chest wall anomalies, osteopenia, osteoporosis, increased risk for fractures and short stature (Stevenson and Yang, 2011). Traditionally sphenoid dysplasia, dystrophic scoliosis and pseudarthoris of long bones are listed as diagnostic criteria of NF1.

Sphenoid wing dysplasia can occur among persons without NF1, but over half of the cases are associated with NF1. Again, diagnosis of sphenoid wing dysplasia should

always lead to further examination. The frequency of sphenoid wing dysplasia is 1.0-11.3 % in NF1 (White et al., 1986; Friedman and Birch, 1997; Ferner, 2007). Usually sphenoid wing dysplasia is asymptomatic and is found when imaging studies are performed for other reasons (Alwan, Tredwell and Friedman, 2005).

Typical long bone dysplasia in NF1 is pseudarthrosis, which is described as a thinning of the long bone cortex, bowing of the bone and development of false joint. Usually pseudarthrosis occurs in the tibia, but also the fibula is often affected. In addition, there are reports of pseudarthrosis in the radius and ulna. Pseudarthrosis may not be diagnosed until the child learns to stand up and walk. 1-4 % of NF1 patients have pseudarthrosis, but it is rare in the general population (Alwan et al., 2005; Peltonen and Pöyhönen, 2012d; Kjell et al., 2016).

Scoliosis is apparent in 10 % of patients with NF1 (Akbarnia et al., 1992). It can be divided into the dystrophic form, with bony abnormalities evident in radiographic imaging and the non-dystrophic form without visible bony abnormalities. Dystrophic scoliosis is typical for NF1 and is characterized by severe angulation. It most commonly develops during the first decade and can progress rapidly requiring prompt operational management (Alwan et al., 2005).

2.3.2 Mutation analysis of the NF1 gene

Diagnosis of NF1 is based on clinical criteria set by the NIH. However, mutational analysis can be helpful when evaluating a patient with a suspicion of NF1, e.g., an infant with multiple CALMs, when the clinical criteria are not fulfilled yet. Mutational analysis of the *NF1* gene has been challenging due to the large size of the gene, lack of mutational hotspots, wide mutational spectrum, large number of exons, vast number of different mutations and pseudogenes. Currently modern multistep mutation detection protocols find ~95 % of the mutations in patients with NF1 (Messiaen et al., 2000; Evans et al., 2016; Friedman, 2017). Also, prenatal and preimplantation genetic diagnosis are available, but the extent of their usage in Finland is unknown.

2.3.3 Atypical Forms of NF1

2.3.3.1 Spinal NF

Spinal NF is a rare form of NF1, which is characterized by CALMs and multiple symmetrically distributed neurofibromas in the spinal nerve roots. However, patients

with spinal NF lack other findings of NF1. No genotype-phenotype correlation has been found explaining this form of NF1 (Messiaen, 2003).

2.3.3.2 Watson syndrome

Watson syndrome is a syndrome with multiple CALMs, pulmonic stenosis and learning difficulties. Nowadays, it is regarded as a variant of NF1. It is extremely rare, and a *NF1* mutation has been found in most of the families (Watson, 1967; Allanson et al., 1991; Friedman, 2017).

2.3.3.3 Mosaic NF1

When a somatic mutation in the *NF1* gene has occurred during the embryonic development, patients may have NF1 symptoms, like pigmentary lesions and neurofibromas, only on a limited area of the skin. An affected area can cover only a small part of the skin or even half of the body. If a somatic mutation has occurred in the beginning of the embryonic development, NF1 symptoms can cover even the whole body. Lesions can be distributed symmetrically or asymmetrically. Even when lesions are widespread, the phenotype is not usually as severe as patients with complete NF1. The prevalence of mosaic NF1 is estimated to be 1:36,000-40,000, but this frequency is probably underestimated as the phenotype is often milder, and patients may not seek to medical investigations as easily as patients with complete NF1. The existence of germ line mutations cannot be proven by traditional sequencing technologies, but the mutation can be transmitted to the next generation. Thus, genetic counseling should be provided to persons with a suspected mosaic NF1 (Poyhonen, 2000; García-Romero, Parkin and Lara-Corrales, 2016).

2.3.4 Differential diagnosis

Differential diagnoses for CALMs are presented above. In addition, several syndromes with CALMs exist including Legius syndrome (Brems et al., 2007), NF2, McCune-Albright syndrome, LEOPARD syndrome, DNA mismatch repair cancer syndromes, neurocutaneous melanosis, Peutz-Jeghers syndrome and Carney complex. Single neurofibromas are occasionally removed from persons without NF1, and histologically confirmed neurofibroma alone should never lead to diagnosis of NF1 without further symptoms. Several syndromes with multiple cutaneous tumors, such as schwannomas and lipomas, exist, but none of these are characterized by multiple neurofibromas (Peltonen and Pöyhönen, 2012e).

2.4 Pathoetiology of NF2

2.4.1 NF2 gene, mutations and inheritance

The *NF2* gene is located on the long arm of chromosome 22 and consists of 17 exons and one alternatively spliced exon. The *NF2* gene is expressed throughout embryologic development. During adulthood, the gene is especially expressed in meningeal cells, neurons, lens and Schwann cells (Pećina-Šlaus, 2013). An individual with NF2 has a 50 % probability to transmit the disease. However, mosaicism among patients with NF2 is more common than in NF1, and there are estimations that 20-30 % of the sporadic cases of NF2 represent mosaicism. In mosaicism, usually only part of the germ cells carries mutations, and therefore the risk for transmitting the disease is less than 50 %. If the offspring inherits the mutation, then the disease is usually more severe than in the parent (Ferner, 2007; Evans, 2009; Kresak and Walsh, 2016). Also the mutation rate of the *NF2* gene is high and approximately 50 % of the new patients represent *de novo* mutations (Pećina-Šlaus, 2013). Differently from NF1, several genotype-phenotype correlations are known in NF2. Nonsense mutations and frameshift mutations seem to result in a severe phenotype and reduced survival. Missense mutations, which lead to a complete protein product, and large deletions resulting complete lack of protein, are associated with milder phenotypes. Genotype-phenotype correlation in splice-site mutations is more variable depending at least partly on the exon where the mutation is located (Evans, 2015).

2.4.2 Merlin

The product of the *NF2* gene is a 65 to 70 kDa protein named merlin or schwannomin. Merlin is classified as a tumor suppressor protein, and it has interactions with a large range of molecules. However, no interactions between merlin and neurofibromin are known. Merlin is needed during the embryologic development. Subcellular localization of merlin is complex, but the protein has functions at the cell cortex, plasma membrane, cytoskeleton and in the nucleus (Cooper and Giancotti, 2014). The main function of merlin is supposed to be promoting contact dependent growth inhibition of the cells. It also acts as a link between the plasma membrane and the actin cytoskeleton and affects the organization of the cytoskeleton. Merlin interacts with multiple pathways including inhibition of the Ras/MEK/ERK and Hippo pathway, which promotes apoptosis and restricts cell proliferation (Pećina-Šlaus, 2013).

2.5 Diagnosis and manifestations of NF2

2.5.1 Clinical diagnosis and symptoms

NF2 is diagnosed often before any subjective clinical symptom is present, because the disorder is suspected due to NF2 of the parent (Evans et al., 1992a). However, as mentioned above, approximately 50 % of the patients represent new mutations, in which case, the disorder is not found until clinical symptoms are present. Alternatively, NF2 can become diagnosed when the disorder is found incidentally in imaging studies proceeded for other reasons. NF2 can be diagnosed according to Manchester clinical criteria when one of the following criteria is fulfilled (Evans, 2009):

- Bilateral vestibular schwannomas
- A first-degree relative with NF2 and at least one of the following:
    - Unilateral vestibular schwannoma
    - At least two of: meningioma, schwannoma, glioma, neurofibroma, posterior subcapsular lenticular opacities
- Unilateral vestibular schwannoma and
    - At least two of: meningioma, schwannoma, glioma, neurofibroma, posterior subcapsular lenticular opacities
- Multiple meningiomas and at least one of the following:
    - Unilateral vestibular schwannoma
    - At least two of: schwannoma, glioma, neurofibroma, cataract

Also other clinical criteria are published, of which, the Baser criteria includes molecular testing, a scoring system weighing separate symptoms differently and taking also into account the age of the patient when the symptom is occurring (Baser et al., 2011).

The most common presenting symptoms include hearing loss, tinnitus and problems with balance, which are associated with vestibular schwannomas (Evans, 2015). Also, visual deficits, cranial nerve palsies, nausea, vomiting and motor or sensory changes can lead to a diagnosis of NF2.

The mean age at onset of the clinical findings has been reported to be approximately 20-25 years and, by the age of 50, nearly all patients have symptoms. Among adults, the presenting symptoms are most often those related with vestibular schwannomas. On the contrary, the first symptoms among children are often caused by non-vestibular schwannomas, and only in 15-30 % of the cases, the presenting symptoms are caused by eighth cranial nerve tumors.

Opposite to NF1, the suspicion of NF2 is only seldom raised because of findings on the skin, but the skin can serve sometimes as a useful aid in the diagnosis of NF2. CALMs can be found sometimes in NF2 patients, but the number of the lesions is lower than among NF1 patients. NF2 patients can also have different types of cutaneous tumors on the skin. The most frequently found tumors in the skin of NF2 patients are plaque lesions, which are slightly elevated and more pigmented than the surrounding skin. These lesions have also often excessive hair growth. Other types of tumors are subcutaneous nodular and intracutaneous lesions, which usually are histologically found as schwannomas. However, occasionally also histologically defined neurofibromas are found (Evans, 2015).

In familial cases, genetic testing is sensitive and specific (Evans, 2009). However, genetic testing of NF2 is complicated, because mosaicism among *de novo* cases is common. This is illustrated by the fact that only 60 % of classically affected isolated patients with NF2 have an identifiable mutation in the blood. Thus, genetic testing is not sufficient to rule out NF2, and clinical screening should cover patients with NF2 and individuals who are at risk for NF2 (Evans et al., 2012).

Cutaneous tumors in NF2 can occasionally represent histologically neurofibromas raising a suspicion of NF1. However, in NF2, most of the cutaneous lesions are schwannomas, and another lesion should be biopsied if no other clinical findings supporting diagnosis for NF1 are found. Following currently used clinical criteria, differential diagnosis between NF2 and NF1 is not problematic. More problematic is differential diagnosis between NF2 and schwannomatosis when multiple noncranial schwannomas are present, but the eighth nerve is spared, and the mutation in *NF2* is not found. These patients can represent mosaic NF2 or schwannomatosis (Evans, 2015).

2.6 Prevalence and incidence of NF1 and NF2

2.6.1 Definition of prevalence and incidence

Prevalence is defined as the proportion of persons possessing the clinical condition at a given prevalence date and can be expressed as:

$$prevalence = \frac{number\ of\ persons\ with\ the\ clinical\ condition\ in\ the\ defined\ population}{number\ of\ persons\ in\ the\ defined\ population}$$

Incidence is defined as the proportion of the persons initially free of the clinical condition but developing it during the defined time period. In the case of an inherited disorder, such as NF1, incidence can be defined as the proportion of persons with the disorder born between a defined time period of all persons born between the same defined time period, and terms *birth incidence* and *birth prevalence* are often used. The birth incidence can be expressed as:

$$incidence = \frac{number\ of\ persons\ with\ the\ clinical\ condition\ born\ during\ the\ defined\ time\ period}{a\ total\ number\ of\ persons\ born\ during\ the\ defined\ time\ period}$$

2.6.2 Prevalence and incidence of NF1

The prevalence estimations of NF1 have varied between 1:960 and 1:7,813 (Sergeyev, 1975; Garty, Laor and Danon, 1994). However, the prevalence of ~1:3,000 has been regarded as generally accepted. Prevalence studies of NF1 are summarized in Table 1, and detailed information is given in the chapter.

The first clinical study involving a relatively large number of patients with NF1 was performed by Borberg (1951) in Denmark. The cohort consisted of 82 patients with NF1, who were hospitalized between 1924 and 1944. Based on Borberg's cohort, Littler and Morton (1990) estimated that the prevalence of NF1 was 1:3,704 (Borberg, 1951; Mulvihill, 1986; Littler and Morton, 1990). The seminal *"Multiple neurofibromatosis"* was published by Crowe et al. in 1956. In that study, over 250,000 hospitalized patients with epilepsy or mental retardation were screened in Michigan, USA. The synthesis of three different methods to approximate the prevalence of the disorder concluded that the prevalence of NF1 is between 1:2,500 and 1:3,300 (Crowe, Schull and Neel, 1957).

In Russia, a total of 94,000, 16-year-old persons were examined during the premilitary examination, and persons with suspected CALMs were referred for further examination. The estimated prevalence in the study was 1:7,813, which is the lowest reported up-to-date (Sergeyev, 1975).

Table 1. Prevalence of NF1. Modified from original publication I.

Author(s)	Country	Number of NF1 patients	Prevalence
Littler and Morton (1990)[1]	Denmark	82	1:3,704
Crowe et. al. (1956)	USA	NA[2]	1:2,500-1/3,300
Sergeyev et. al. (1975)	Russia	9	1:7,813
Samuelsson and Axelsson (1981)	Sweden	96	1:4,600
Fuller et. al. (1989)	New Zealand	52	1:2,190
Huson et. al. (1989)	United Kingdom (Wales)	135	1:4,510
Clementi et. al. (1990)	Italy	202	1:4,292
Garty et. al. (1994)	Israel	390	1:960
Ingordo et. al. (1995)	Italy	11	1:5,000
Fazii et. al. (1998)	Italy	14	1:1,009
Pöyhönen et. al. (2000)	Finland	197	1:4,436
Lammert et. al. (2005)	Germany	51	1:2,996
McKeever et. al. (2008)	United Kingdom (Northern Ireland)	75	1:5,681
Evans et. al. (2010)	United Kingdom (England)	979	1:2,712
Orraca et. al. (2014)	Cuba	17	1:1,141
Kallionpää et. al. (2017)[3]	Finland	1,476	1:2,052

[1]Based on Borberg's (1951) cohort.
[2]Prevalence estimation is based on synthesis of three different approaches, involving different number of cases
[3]Prevalence estimation is based on the combination of incidence numbers and survival rates.

In 1981, a total of 96 patients with NF1 were found by searching patients from medical institutions, municipal health services and private practices in Gothenburg, Sweden. The prevalence of neurofibromatosis was estimated to be 1:4,600 representing the minimum prevalence of the disorder (Samuelsson and Axelsson, 1981).

The NIH clinical criteria were published in 1987, which should have led to a uniform classification of patients in epidemiological studies. Yet, the criteria have not been completely followed in all studies published after 1987. In 1989, Fuller et al. conducted a study, where 113,700 persons were screened by reviewing medical records and then, physicians and family members of patients with neurofibromatosis in Dunedin, New Zealand were contacted. A total of 52 persons with NF1 were identified for a crude prevalence of 1:2,190. A high prevalence was discussed to illustrate probably a small population effect (Fuller, Cox and Gardner, 1989). In the study performed in Southeast Wales between 1983 and 1986, patients with NF1 were identified by examining hospitalized patients with a diagnosis for NF1, reviewing the medical records of the patients with treatment for scoliosis or pseudarthrosis, reviewing the medical records of the patients receiving genetic consultation and searching the histopathology register for NF1-related tumors. Finally, first-degree relatives of the

index cases were examined when possible. A total of 135 persons with NF1 were found giving an estimated prevalence of 1:4,510 (Huson et al., 1989).

In Italy, databases of hospitals and genetics were searched for neurofibromatosis, and the incidence of NF1 was estimated to be 1:4,292 (Clementi et al., 1990). The highest prevalence up-to-date was found in a study among 374,440 seventeen-year-old recruits for military service, including 220,215 males and 154,225 females in Israel. A total of 390 persons with NF1 were identified resulting in a prevalence of 1:960. The diagnoses were confirmed by the Israel Defense Force physicians, and only unclear cases were examined by experts in dermatology or genetics (Garty et al., 1994). In Italy, 56,183 young men were examined between 1991 and 1993 during the premilitary examination for the Italian Navy. 11 cases of NF1 were identified giving a prevalence of 1:5,000. However, no criteria for NF1 diagnosis was reported (Ingordo et al., 1995). A few years later a total of 21,181 young males were examined in Italy during the premilitary examination, and 14 persons with NF1 were identified. Thus, the prevalence was 1:1,009 being one of the highest reported (Fazii et al., 1998).

In Northern Finland with a population of 733,037, patients having NF1 were traced by searching medical records of the hospitals, contacting physicians, reviewing the histological specimens and examining the relatives of the index person whenever possible. 197 persons with NF1 were identified giving an overall prevalence of 1:4,436. A peak prevalence of 1:2,983 was observed in the age group of 10-19-year-old people (Poyhonen et al., 2000). In Germany, 152,819 six-year-old children were examined in a medical examination prior to enrollment in an elementary school between 2000 and 2001, and the observed prevalence for neurofibromatosis was 1:2,996 (Lammert et al., 2005). In Northern Ireland, with a total population of 425,250, medical records of patients under sixteen years old and visiting clinics of genetics were reviewed. 75 persons with NF1 were found giving a prevalence of 1:5,681 (McKeever et al., 2008).

An epidemiological study reviewing genetic and cancer registers in Manchester region, Northwest England with total population of 4.1 million people was published in 2010 (Evans et al., 2010). Persons with NF1 were confirmed according to NIH clinical criteria. In that study, 979 cases of NF1 were found yielding a prevalence of 1:4,560. Birth incidence was calculated to be 1:2,712. A recent epidemiological study was carried out in Cuba where 19,392 children aged 9-11 years old were screened. Among these children, 17 were diagnosed for having NF1. The overall prevalence among 9-11-year-old children was 1:1,141 (Orraca et al., 2014). Utilizing our cohort of patients with NF1, the observed prevalence of NF1 was 1:4,088 in December 2005. Combining incidence numbers and mortality rates, the estimated prevalence was calculated to be 1:2,052 (Kallionpää et al., 2017).

2.6.3 Prevalence and incidence of NF2

Prevalence of NF2 has been estimated to be approximately 1:56,000-1:116,000 (Evans et al., 1992a, 2010) and incidence 1:25,000-1:87,000 (Antinheimo et al., 2000; Evans et al., 2005). The studies on the prevalence and incidence of NF2 are presented in Table 2.

Table 2. Prevalence and incidence of NF2.

Author(s)	Country	Number of NF2 patients	Prevalence/ incidence
Evans et. al. (1992a)	United Kingdom (England)	150	1:116,000/1:33,000
Antinheimo et. al. (2000)	Finland	17	NA/1:87,410
Evans et. al. (2005)	United Kingdom (England)	48	1:68,250-100,750/ 1:24,844
Evans et. al. (2010)	United Kingdom (England)	113	1:56,161/1:33.206

NA, not available

In the study carried out in the UK between 1989 and 1991, patients with NF2 were searched by contacting clinicians and reviewing the records of the cancer register for schwannomas and meningiomas. In addition, the majority of the patients and first-degree relatives were examined. A total of 150 cases were identified including 110 persons alive. The prevalence of NF2 was estimated to be approximately 1:116,000. The birth incidence was estimated to be 1:33,000 (Evans et al., 1992a).

In Southern Finland, patients diagnosed with schwannoma or meningioma were acquired from the hospital registers of Helsinki University Central Hospital and the Finnish Cancer Registry. Then, information about neoplasms among relatives of these patients were searched from the Finnish Cancer Registry. The birth incidence of NF2 was estimated to be 1:87,410 (Antinheimo et al., 2000). In Northwest England, patients with vestibular schwannomas were searched, and the data was crosslinked with the regional NF2-register. The prevalence of NF2 was calculated to be 1:68,250-1:100,750. Combining data of vestibular schwannomas and the NF2 register, the birth incidence was estimated to be 1:24,844 (Evans et al., 2005).

The observed incidence and prevalence of NF2 in Northwest England during the last decades were reported in 2010. The highest birth incidence was observed between 1974 and 1983 being 1:33,206. The prevalence was calculated to be 1:56,161 (Evans et al., 2010).

2.7 Mortality of NF1 and NF2

Mortality can be analyzed in multiple different ways. It can be evaluated straightforwardly by calculating the difference in the life expectancy compared to the matched control population. Survival analysis, which analyzes time to death, is also often used. Proportionate mortality ratio (PMR) is used especially when different causes of death are analyzed, and it is defined as:

$$PMR = \frac{the\ number\ of\ deaths\ from\ a\ specific\ cause}{the\ number\ of\ deaths\ expected\ from\ a\ spesific\ cause}$$

2.7.1 Mortality and causes of death in NF1

Mortality and causes of death among NF1 patients have been analyzed in studies based on administrative death certificate registers and clinical patient cohorts. Two studies relying on death certificates have been published: one conducted in the United States and one in Italy. In the United States, a total of 3,770 persons with NF1 were identified among over 32 million deaths between 1983 and 1997. The mean age at death of NF1 patients was reduced over 15 years compared to the general population, and the difference was especially high among females with NF1 being almost 18 years. The differences remained significant when including only persons over the age of 40 years. Malignant tumors, vascular disease, scoliosis, epilepsy and mental retardation were mentioned more often than expected on death certificates. Of the malignancies, the frequency of the malignant tumors of connective and other soft tissue were significantly increased. Also, malignant tumors of the brain were more common among patients with NF1 than in the general population. Vascular disease was more common only in the subgroup of patients who died at under 30 years of age (Rasmussen, Yang and Friedman, 2001).

In Italy, the mean age at death was even more reduced among patients with NF1 compared to the general population (55.5 vs. 76.2 years), when deaths of 632 NF1 patients identified from the death certificates were analyzed. The proportionate mortality ratios for connective and soft tissue tumors was 22.3, for brain malignancies 4.2 and for respiratory disorders 1.5 in the NF1 group (Masocco et al., 2011).

The first study reporting increased mortality associated with neurofibromatosis was published in 1986 by Sørensen et al. However, as the original patient cohort was acquired over 40 years earlier, NF1 was not distinguished from NF2 (Sørensen, Mulvihill and Nielsen, 1986).

In Sweden, 70 adult NF1 patients recruited from outpatient clinics and hospitals were followed retrospectively during the years 1978-1990. To evaluate mortality, death certificates, medical records and histology specimens were reviewed. The mean age at death was 61.2 years among women and 52.8 among men being approximately 15 years less than in the general population. The overall estimated risk ratio of death was 4.3. Seven of 22 deaths were for cancer, and the additional six deaths for NF1-related (non-cancer) causes were identified (Zöller et al., 1995).

In France, a total of 1,895 patients referred to the national specialty center of neurofibromatosis were followed between 1980 and 2006. The vital status at the end of the follow-up period was available for 1,226 patients of whom 67 died. The median age at death was only 31.7 years, and death was related to NF1 in 96.6 % of the cases where the cause of death was known. The most common cause of death was MPNST (33/56 known causes of death). Overall PMR was 2.02. Among patients aged 10-20 years, PMR was 5.2; and among patients aged 20-40, the PMR was 4.1 (Duong et al., 2011).

Mortality among 1,186 patients with NF1, identified in the genetics register services in Manchester, was analyzed in a retrospective study in 2011. During the study period between years 1957 and 2009, a total of 131 patients died, and the mean age at death was 43.6 years, while there was a loss of 8 years of life expectancy. The median survival of NF1 patients was 71.5 years. The most common cause of death was MPNST followed by glioma, which was the most common cause of death among persons aged less than 20 years. Cardiovascular diseases were overrepresented among men, while among females, breast cancer caused death more often than in the general population (Evans et al., 2011). The same register was analyzed by Wilding et al. (2012), and a median life expectancy of 71.5 years was observed. 55 % of the deaths were caused by NF1 (Wilding et al., 2012).

In a special clinic in Cincinnati, 520 children with NF1 were followed between 1997 and 2007. Also in this study, the most common cause of death was MPNST. In addition, the authors observed that mortality was significantly increased among patients with plexiform neurofibromas being 3.2 % compared to 0.5 % in the group of NF1 children without plexiform neurofibromas (Prada et al., 2012), which supports the prior findings by Khosrotehrani et al. (2005) who found that plexiform neurofibromas on the face of the children were associated with increased mortality (Khosrotehrani et al., 2005).

2.7.2 Mortality and causes of death in NF2

The epidemiological studies about mortality of NF2 are uncommon. In the study by Evans et al. (1992), 40 deaths occurred among 150 patients with NF2, and the mean age at death was 36.3 years. All but one death was considered to be caused by NF2. The actual survival was estimated to be 62 years (Evans et al., 1992b). In Japan, the mortality of 75 NF2 patients, who were identified during 1986-1987, was analyzed. Overall 5-, 10- and 20-year survival rates were 85 %, 67 % and 38 %, respectively. However, the causes of death were not available (Otsuka et al., 2003). A total of 113 cases of NF2, including 35 deaths, were identified in the genetic register service in Manchester. The median life expectancy was 69.0 years, and 66 % of the deaths were related to NF2 (Wilding et al., 2012). In France, 80 patients with NF2 were retrospectively followed between 1987 and 2011. During the study period, seven patients died, and the mean age at death was 38.9 years (Aboukais et al., 2014). Hexter et al. (2015) identified 1,192 NF2 patients in the UK National NF2 Register. A total of 241 deaths occurred during ~11,000 patient-years, and in 103 cases, the death certificate was available. NF2 was definite cause of death in 44 deaths, probable in 31 deaths and possible in 9 deaths. Median age at death was 45.9 years among patients with truncating mutations, 74.2 years among patients with splice-site mutation and 79.1 among patients with missense mutations. An improved mortality rate since 1980 was observed in the study (Hexter et al., 2015).

In the United Kingdom, the mortality of 368 NF2 patients from 261 families was analyzed. A total of 74 patients died during the follow-up period and the causes of death included tumor burden (n=51), postoperative complications (14), NF2-associated malignancies (3), traffic accidents (2), suicides (2), fall for problems with balance (1) and myocardial infarction (1). Early onset of symptoms, meningiomas and non-missense mutations were associated with increased mortality among NF2 patients (Baser et al., 2002). Also, Otsuka et al. (2003) observed that the early onset of symptoms was associated with increased mortality. 5-, 10- and 20-year survival rates among NF2 patients with the onset of symptoms before the age of 25 years were 80, 60 and 28 %, respectively. Among patients with onset of symptoms at age of 25 years or later, the figures were 100, 87 and 62 %, respectively. The difference between mortalities was statistically significant (Otsuka et al., 2003). The increased mortality associated with early onset of symptoms, meningiomas and truncating mutations was also reported in the study involving 241 deaths among 1,192 NF2 patients in the United Kingdom (Hexter et al., 2015).

2.8 Duration of the pregnancy

The duration of the pregnancy is commonly reported in terms of gestational age, which is the length of the pregnancy after the first day of the last menstrual period. Gestational age is expressed as weeks and days. Preterm birth, defined as gestational age less than 37 weeks, causes 75 % of the perinatal morbidity and has lifelong effects on morbidity and mortality (Goldenberg et al., 2008). In the US, the annual cost of preterm births is several billion dollars.

Morbidity and mortality are increased most among births that occur before week 34 but are increased also among those who are born later but are not full term (Frey and Klebanoff, 2016). Impaired fetal growth is associated with a vast spectrum of complications including atherosclerosis, hypertension, dyslipidemia (Skilton et al., 2011), diminished long term survival and reproduction (Swamy, 2008), respiratory morbidity (Narang, 2010), epilepsy (Crump et al., 2011) and alcoholism (Manzardo et al., 2011).

Also post-term births are associated with complications during the perinatal period and later in childhood (El Marroun et al., 2012; Schierding et al., 2014). While the effects of preterm births are studied extensively, post-term birth, defined as gestational age more than 42 weeks, has attracted much less attention, although being relatively common with the rate of 4.2-4.8 % (*WHO Recommendations for Induction of Labour*, 2011). There are also observations that the risks for complications increase already beyond 39 weeks of gestation (Caughey, Washington and Laros, 2005).

2.8.1 Classification of gestational age

The evaluation of gestational age can be based on menstrual history, clinical investigation and ultrasound dating of which the ultrasound dating during the first-trimester is recommended (Butt et al., 2014). In Finland, general early pregnancy ultrasound is offered comprehensively ("Screenings in Finland 2014. The present state of health care screenings and future prospects," 2015).

The WHO classifies births into three main categories (Blencowe et al., 2012):

- preterm (< 37 weeks gestation),
    - moderate or late preterm (32 - < 37 weeks gestation)
    - very preterm (28 - < 32 weeks gestation)
    - extremely preterm (< 28 weeks gestation)
- term (37 - 41 weeks gestation)

- post-term (≥ 42 weeks gestation)

2.8.2 Background mechanisms of the timing of the delivery

Timing of the birth has four etiological factors: fetal genetic factors, maternal genetic factors, familial environmental factors and pregnancy-specific environmental factors. Of these, pregnancy-specific factors are considered to contribute 45-61 % of the gestational age. For fetal-genetic factors, maternal-genetic factors and familial environmental factors, the figures are 5-35 %, 13-25 % and 2-13 %, respectively. While maternal genetic and environmental factors have been studied extensively, interest in fetal genetic factors that influence the timing of the birth has arisen only lately (York et al., 2014).

Familial environmental factors include those that remain unchanged between the pregnancies, e.g., socioeconomic or immigration status. On the other hand, pregnancy-specific environmental factors include those that vary between pregnancies, e.g., medication. Environmental factors that are associated with preterm birth include low socioeconomic and educational status, single marital status, low and high maternal age, close temporal proximity of previous delivery, a low prepregnancy BMI, nutritional deficiencies, previous preterm delivery, multiple gestation, placental abruption, extremes in the volume of amniotic fluid, abdominal surgery, multiple medical disorders (e.g., diabetes, thyroid disease, asthma), history of cervical cone biopsy sample and other procedures in the cervical area, social and psychological stress, depression, tobacco using, intrauterine and intra-amniotic infection and heavy consumption of alcohol (Goldenberg et al., 2008).

To analyze the genetic factors determining the timing of the delivery classical twin design studies, models including extended family structures, candidate gene association studies and genome-wide association studies have been used. These studies have provided evidence that both maternal and fetal genomes likely contain alleles that take part in the determination of timing of the delivery (York et al., 2014). Only a little is known about the molecular mechanism behind the fetal genetic contribution to the timing of the birth. It is hypothesized that the fetal genome could affect the production and response to corticotropin-releasing hormone, placental development and the integrity of fetal tissues (York et al., 2013).

In a recent genome-wide association study, four loci (*EBF1, EEFSEC, AGTR2* and *WNT4)* were associated with pregnancy duration. These genes are known to play roles in uterine development, maternal nutrition, and vascular control support (Zhang et al., 2017).

2.8.3 Gestational age in NF1

To my knowledge there are no previous reports that study specifically the effects of NF1 on gestational age. Observations based on the case reports, case series and limited epidemiological studies suggest that NF1-related pregnancies may be shorter than those of the general population. However, it is unknown if this effect is caused by the NF1 of the mother or the fetus as the studies do not separate the cases based on the NF1-status of the mother and the child.

Blickstein and Lancet (1987) reported five infants with intrauterine growth restriction and of these infants, two were born preterm (Blickstein and Lancet, 1987). Sharma et al. (1991) followed ten pregnancies of mothers with NF1, and the mean gestational age was 33 weeks with only four full term pregnancies and five pregnancies with a gestational age of less than 32 weeks (Sharma, Gulati and Malik, 1991). In 1996, 14 pregnancies of 8 women with neurofibromatosis were evaluated. Of seven live births, five were born before 37 weeks gestation (Hadi, 1995). On the contrary, among 247 pregnancies of 105 women with NF1 in Denver, US, the incidence of preterm delivery was only 6 %, which was less than in the general population. No definition of preterm delivery was provided in the study (Dugoff and Sujansky, 1996). Segal et al. (1999) compared 13 deliveries of eight NF1 patients to deliveries of 65 healthy parturients during 1994-1996. The mean gestational age among mothers with NF1 was 36.8 weeks, which was 2.4 weeks less than among controls (p=.029) (Segal et al., 1999).

In the large retrospective register-based epidemiological study in the United States, 19 million pregnancy-related admissions were identified and of those, 1,552 were associated also with NF1. No information about the NF1-status of the born child was available. Neither was the mean and/or median gestational age reported. However, preterm labor was more common in the pregnancies of NF1 females compared to controls (13.5 % vs. 9.1 %, p<.001) (Terry et al., 2013). In Denmark, the diagnoses of 1,348,106 persons, born between 1974-1996, were acquired for the time period of 1994-2007. Diagnoses were then linked to the information of gestational age and birth size. Children born appropriate for gestational age (AGA) at 32 weeks gestation had a 2.47-fold risk for having NF1 compared to children born AGA on 40 weeks gestation. However, no information about the NF1 status of the mother was available (Á Rogvi, Forman and Greisen, 2015).

2.9 Pregnancy and delivery complications

Pregnancy and delivery complications are not uncommon and cause significant morbidity for mothers and infants. Pregnancy-related complications also cause substantial

health care costs (Law et al., 2015). During the first trimester, approximately 10 % of clinically observed pregnancies end in miscarriage, and the figure is considerably higher if also biochemical pregnancies are included (Bottomley and Bourne, 2009; Committee on Practice Bulletins—Gynecology, 2015). Later in the pregnancy, the most common maternal complications are gestational diabetes mellitus (10-11 %) (Lamberg et al., 2012), pregnancy-related hypertension (6-7 %) and preeclampsia (4-5 %) (Ekholm and Laivuori, 2011). However, multiple other complications exist, e.g., placental ablation (1.4 %) and placenta previa (0.5 %) (Heinonen, 2011). Also, preterm and post-term deliveries can be classified as pregnancy complications. The delivery can be complicated, e.g., by prolonged labor, malpresentation of the fetus, cephalopelvic disproportion, fetopelvic disproportion and lacerations. The most common major operation proceeded during the delivery is cesarean section. Cesarean sections can be classified as elective sections and non-elective sections. Elective cesarean sections are planned before the labor, while non-elective cesarean sections are unplanned.

2.9.1 Pregnancy and delivery complications in NF1

NF1-related pregnancies have been associated with an increased risk for multiple complications. Some authors have even suggested in the past, when similar suggestions were common also with many other chronic diseases, that females with severe NF1 should not get pregnant (Blickstein, Lancet and Shoham, 1988). However, most of the presented associations are based on case reports and case series. To my knowledge, only one large-scale epidemiological study has been carried out of NF1 and pregnancy.

2.9.1.1 Benign and malignant tumors during the pregnancy

Case reports have been published reporting growth of previous neurofibromas or the appearance of new neurofibromas during pregnancy resulting in complications, e.g., paraparesis (Ansari and Nagamani, 1976; Isikoglu et al., 2002; Dham, Kwa and Campellone, 2012). The increase of neurofibromas in size or in numbers has been supported by studies that have involved a relative large number of patients. In a study of 105 NF1 women, a total of 64 (61 %) women reported new neurofibromas during pregnancy. Four of the women observed their first cutaneous neurofibromas during the pregnancy. 55 (52 %) of the women observed an enlargement of existing neurofibromas, and of these women, 33 % observed that neurofibromas decreased in size after delivery (Dugoff and Sujansky, 1996). In a recent study by Cesaretti et al. (2013), an increased number and/or size of neurofibromas during pregnancy was reported in 31 (47.7 %) of 65 women. Two women noted neurofibromas for the first

time during the pregnancy. Of these 33 women, seven (21.2 %) reported that neurofibromas decreased in size after pregnancy (Cesaretti et al., 2013).

Large-scale studies about cancer risk or the risk for MPNST during pregnancy do not exist, but several case reports describe patients whose MPNST was diagnosed during pregnancy (Ginsburg, Hernandez and Johnson, 1981; Puls and Chandler, 1991; Posma, 2003; Kellogg and Watson, 2010; Nelson, Greer and Wendel, 2010). Posma et al. (2003) reported a patient who was diagnosed with MPNST during her second pregnancy. The tumor was resected, and the patient had postoperative radiotherapy. After a three-year long remission, she became pregnant again, and during the postpartum period, MPNST relapsed. The patient died only three months after the delivery (Posma, 2003). In addition to recurrence of MPNST, a fatal recurrence of glioblastoma located in the basal ganglia during pregnancy has been reported (Hadi, 1995).

2.9.1.2 Gestational hypertension and cerebrovascular disease

It has been suggested that NF1 is associated with hypertension. However, no epidemiological data about hypertension among adult patients with NF1 exist. Case series have suggested that gestational hypertension could be more common among NF1 females than among controls (Swapp and Main, 1973; Edwards, Fooks and Davey, 1983; Sharma et al., 1991). In addition to common pregnancy-induced hypertension, case reports of more uncommon etiologies behind the hypertension during the pregnancy have been reported including renovascular hypertension (Pilmore, Na Nagara and Walker, 1997; Hagymásy et al., 1998), and hypertension caused by pheochromocytomas (Geisler and Lloyd, 1963). On the other hand, in a study of 27 pregnancies among ten women with NF1 in the United Kingdom, only two pregnancies were complicated by gestational hypertension (Jarvis and Crompton, 1978). Dugoff and Sujansky (1996) reported that the incidence of gestational hypertension among 247 pregnancies of 105 females with NF1 was only 2 %, which does not differ from the general population (Dugoff and Sujansky, 1996).

In the United States, Terry et al. acquired diagnoses of almost 20 million pregnancy-related hospital admissions using the US Nationwide Inpatient Sample between 1988-2009. 1,553 pregnancy-related hospital admissions were associated with diagnosis of NF1, and of those, 1,248 were related to delivery. The rate of gestational hypertension among NF1 patients was 3.5 %, which was significantly higher than the 2.3 % observed in the general population. The odds ratio for gestational hypertension in NF1-related pregnancies was 1.6 [95 % confidence interval (CI) 1.2-2.0]. When excluding NF1 patients with prior chronic hypertension or renal artery stenosis, the odds ratio for gestational hypertension remained significant being 1.6 (95 % CI 1.3-2.2). Cerebrovascular disease was more frequent among NF1 mothers than in the control group

(unadjusted OR 10.0, 95 % CI 5.2-19.3), and the difference remained significant after excluding patients with NF1 and preexisting chronic hypertension or renal artery stenosis (unadjusted OR 11.9, 95 % CI 6.2-22.9) (Terry et al., 2013). However, as less than 10 cases of cerebrovascular diseases during pregnancy were observed among 1,553 hospitalizations of patients with NF1, these complications were very uncommon even among NF1 mothers.

2.9.1.3 Preeclampsia

Preeclampsia is one of the most common pregnancy complications, and it is defined as hypertension and proteinuria during pregnancy in a previously normotensive patient (Ekholm and Laivuori, 2011). Hence, it is not surprising that there are case reports of NF1 and preeclampsia or eclampsia (Agarwal, Dahiya and Sangwan, 2003; Lee et al., 2013). The association between preeclampsia and NF1 is strongly supported by an extensive epidemiological study by Terry et al. (2013), who observed a significantly increased rate of preeclampsia among women with NF1 (OR 2.8, 95 % CI 2.3-3.4). On the contrary, Jarvis and Crompton (1978) or Dugoff and Sujansky (1996) did not find an increased rate of preeclampsia or eclampsia in their case series (Jarvis and Crompton, 1978; Dugoff and Sujansky, 1996), but the number of patients with NF1 was considerably smaller in these studies than in the epidemiological study mentioned before.

2.9.1.4 Intrauterine growth restriction

Intrauterine growth restriction (IUGR) is a clinical definition referring to a condition where a fetus does not achieve its genetically determined potential size. It has long been associated with the pregnancies of NF1 women. Multiple case reports have been published of NF1 and IUGR (Edwards et al., 1983; Belton, Ferguson and Catanzarite, 1984; Blickstein and Lancet, 1987; Hadi, 1995), and larger studies have afterwards confirmed this association. Weissman et al. (1993) observed that the rate of IUGR among 34 pregnancies of NF1 women was 13.0 % (Weissman et al., 1993). In Israel, among 13 NF1-related deliveries, the prevalence of IUGR was 46.2 %, which was significantly higher than the 8.95 % observed in the control group (p=.0005) (Segal et al., 1999). In the study by Cesaretti et al. (2013), 20.9 % of the NF1-related pregnancies were associated with IUGR, which is more than observed in the general population (Cesaretti et al., 2013). In the US, the frequency of IUGR among pregnancies of NF1 women was 7.9 %, and the OR was 4.6 (95% CI 3.7-5.6) (Terry et al., 2013). The only study, to my knowledge, that did not observe increased rate of IUGR in NF1-related pregnancies was by Dugoff and Sujansky (1996) in the

US. They reported that only 4 % of pregnancies of NF1 women were complicated by IUGR, while the number in the general population was 4-8 % (Dugoff and Sujansky, 1996).

2.9.1.5 Cesarean sections

Several studies have reported that the rate of cesarean sections is increased in NF1-related pregnancies. Weissman et. al. (1993) observed that among 34 pregnancies of nine women with NF1, the rate of cesarean section was 26 %, which is higher than expected (Weissman et al., 1993). Also, in a study by Dugoff and Sujansky (1996), the rate of cesarean sections was increased. 36 % of the deliveries were carried out by cesarean section, while in the general population, the figure was 9.1-23.5 %. The most common indication for cesarean section in the NF1 group was cephalopelvic disproportion (37 %) followed by fetal distress (18 %), malpresentation (18 %), elective repeat cesarean section (18 %), placental abruption (2 %), pheochromocytoma (2 %) and spinal neurofibroma (2 %) (Dugoff and Sujansky, 1996). Cesaretti et al. (2013) reported pregnancy outcomes of 43 women with 79 pregnancies. Approximately 20 % of the women were clinically followed through the pregnancy and for the rest, information was obtained from medical records and personal interviews. Of the 65 full-term pregnancies, 20 (30.8 %) needed cesarean sections. Five cesarean sections were proceeded for insufficient dilatation, 3 for NF1-related maternal complication, 3 for fetopelvic disproportion, 2 for breech presentation, 2 for a wrapped umbilical cord, 1 for maternal choice, 1 for oligohydramnios, 1 for elective repeat cesarean and 1 for twin delivery (Cesaretti et al., 2013). Also in the epidemiological study by Terry et al. (2013), the rate of cesarean sections was increased compared to the general population (43.4 % vs. 25.7 %, OR 2.0, 95 % CI 1.8-2.3). However, the reasons leading to higher rate of cesarean sections could not be further analyzed (Terry et al., 2013).

2.9.1.6 Perinatal mortality

Perinatal mortality is commonly defined as the number of stillbirths and deaths during the first seven days after birth (WHO). Previous case series have suggested that perinatal mortality in NF1 is drastically increased. Sharma et al. (1991) followed a total of ten pregnant NF1 women, and the perinatal mortality was as high as 60 % (Sharma et al., 1991). The rate of stillbirths (8.7 %), and first-trimester spontaneous abortions (20.7%) were higher than expected in a study of 34 pregnancies of nine NF1 women in Israel (Weissman et al., 1993). In a study of 14 pregnancies of 8 women with NF1, the incidence of live birth, therapeutic abortion and spontaneous

abortion were 50.0 %, 42.8 % and 7.1 %, respectively (Hadi, 1995). On the other hand, a case series published already in 1978 reported only one stillbirth among 27 pregnancies (Jarvis and Crompton, 1978).

In the study by Pöyhönen et. al. (2000), perinatal mortality was not analyzed, but the frequency of miscarriages among women with NF1 was 7.5 %, which is not a higher number than in the general population in Finland (Hemminki and Forssas, 1999; Poyhonen et al., 2000). Dugoff and Sujansky (1996) did not find an increased perinatal mortality among 247 pregnancies of 105 women with NF1 (Dugoff and Sujansky, 1996). Among 79 pregnancies of 43 women with NF1 studied by Cesaretti et al. (2013), 65 (82.3 %), pregnancies were carried to term. The remaining 14 pregnancies included five first trimester spontaneous abortions (6.3 %), seven elective abortions not related to NF1 (8.9 %) and two therapeutic abortions after prenatal diagnosis (2.5 %). No stillbirths were observed (Cesaretti et al., 2013). Also a recent epidemiologic study conducted in the US supports the finding that the prognosis of pregnancies of NF1 women is not as poor as previously thought, and the rate of stillbirths or maternal mortality was not significantly increased compared to the general population (Terry et al., 2013).

2.9.1.7 Other pregnancy complications

Placental abruption is an obstetric emergency, where part or all of the placenta prematurely separates from the uterus. NF1-related pregnancies with placental abruption have only rarely been reported, and the rate of placental abruption was not increased in a study conducted in Denver, US (Dugoff and Sujansky, 1996). In the same study, 5.8 % of 172 newborns delivered at term were in breech presentation. This is a slightly higher rate than in the general population (Cammu et al., 2014), but this study did not have control group, and the significance of the finding cannot be evaluated. Other even less frequently reported complications include obstructed labor because of pelvic neurofibroma (Griffiths and Theron, 1978), hemothorax of mother (Brady and Bolan, 1984), epidural hematoma caused by dural puncture (Esler, Durbridge and Kirby, 2001) and oligohydramnios (Belton et al., 1984; Kosec and Márton, 2006; Cesaretti et al., 2013).

2.10 Birth size

Birth size is extensively studied, because the measures are relatively easily available, it is a strong predictor for an infant's survival and it is associated with several diseases in the later life. However, birth weight alone is insufficient to evaluate birth size, and

therefore it is nowadays expressed in relation to gestational age. In other words, a born child can be low in absolute weight, but appropriate when adjusted for gestational age (Wilcox, 2001). Birth weight is classified as small for gestational age (SGA, less than the 10th percentile or less than 2 SDs for gestational age), appropriate for gestational age (AGA, birth weight 10th to 90th percentile or birth weight -2 SD ≥ and ≤ 2 SD for gestational age) and large for gestational age (LGA, more than 90th percentile or more than 2 SDs for gestational age). In addition, some studies include height in the classification of birth size. Mortality of newborns who are SGA is increased. Also, the risk for stillbirth, seizures, sepsis, intraventricular hemorrhage, necrotizing enterocolitis and hypoxic-ischemic encephalopathy is increased. Newborns who are LGA are at an increased risk of neonatal mortality, brachial plexus palsy, mechanical ventilation, traumatic delivery and stillbirth (Chauhan et al., 2017).

Birth size is not only associated with complications in the neonatal period, but it predicts also risk of mortality and diseases later in life. In 2011, Risnes et al. (2011) conducted a meta-analysis of an association between birth size and all-cause mortality, cancer mortality and cardiovascular mortality. Birth size was inversely associated with all-cause mortality and cardiovascular mortality in both sexes, while a positive association between birth size and cancer mortality was observed in males. However, gestational age was not provided in all studies that included two large studies. It is thus unclear how much of the observed difference was due to variation in gestational age (Risnes et al., 2011).

In a large umbrella review including 39 articles published between 2005 and 2015, the association between birth weight and morbidity in later life was studied. Convincing (criteria: >1000 cases, $p<10^{-6}$, 95 % prediction interval excluding the null value, no small-study effects and excess significance bias) associations were found between low birth weight and all-cause mortality, and an inverse association existed between an increase in weight and cardiovascular mortality. The level of evidence was classified as convincing also for an increased risk for childhood stunting among newborns with SGA. Highly suggestive associations (>1000 cases, $p<10^{-6}$, largest study with a statistically significant effect) were found between low birth weight and perinatal mortality in developing countries, wheezing disorders in childhood, coronary heart disease, asthma in childhood, RSV-related lower respiratory infections in childhood and chronic kidney disease. Inverse associations were highly suggestive or suggestive (<1000 cases, $p<10^{-3}$) for being overweight in adulthood and overall intelligence during adolescence. The associations between high birth size and leukemia, being overweight, and type 1 diabetes mellitus were highly suggestive or suggestive. An increase in weight was correlated with mortality from cancer, and inverse correlations were found for coronary heart disease, maternal cardiovascular mortality, paternal cardiovascular mortality and type 2 diabetes mellitus. Also other associations were found, but evidence for these was weak (Belbasis et al., 2016).

Birth size is explained by maternal genetic factors, fetal genetic factors and environmental factors. Approximately 50 % of birth weight and birth length are explained by genetic factors (Lunde et al., 2007). Environmental factors include fetal diseases, parity, maternal age, smoking, drug abuse, maternal diseases such as gestational diabetes mellitus, infectious diseases, undernutrition and maternal body composition (Voigt et al., 2010).

2.10.1 Birth size in NF1

No epidemiological studies exist about birth size and NF1, and the only study reporting a relatively large number of birth size measurements, including weight and length, was carried out in Brazil. The study involved 85 males and 61 females. Among males, the mean birth weight was 3,180 grams, and the birth length was 50.7 cm. For females, the numbers were 2,920 grams and 47.1 cm, respectively. The study did not include a control group, and standard WHO growth charts were used to classify measurements: below 3rd percentile weight/height was considered small and above 97th percentile as large. 5.8 % of males and 1.6 % of females were considered underweight, but the frequency of low birth length was not reported (Ribeiro and Coutinho, 2015).

In the case series by Sharma et al. (1991), ten NF1 patients were followed during their pregnancy. Only four children were born alive and among these, mean birth weight was 1,924 grams, and the mean gestational age was only 33 weeks (Sharma et al., 1991). Dugoff and Sujansky (1996) reported pregnancy outcomes of 105 females with NF1. The mean birthweight was 3,374 grams, and intrauterine growth restriction was observed in 3.8 % of the pregnancies (Dugoff and Sujansky, 1996). In Israel, among 13 children of 8 mothers with NF1, the mean birth weight was 2,379 grams, while in the control group, it was 3,186 grams. Also pregnancy duration was shortened in the NF1 group being 36.8 weeks (39.2 weeks in the control group) (Segal et al., 1999). Indirect conclusions about birth size can also be made by the frequency of IUGR, which is summarized in Chapter 2.9.1.4. The frequency of IUGR among children of NF1 mothers has been 4.0-46.2 % (Weissman et al., 1993; Dugoff and Sujansky, 1996; Segal et al., 1999; Cesaretti et al., 2013; Terry et al., 2013). However, the frequency of IUGR describes only the prevalence of exceptionally small children.

2.10.2 Height in NF1

Most of the information about the anthropometrics of NF1 patients is provided by studies involving adults and adolescents. In most studies, patients have been shorter

than controls or persons in the general population, and this has been especially apparent among adults. These studies are summarized in Table 3.

Table 3. Frequency of short stature of NF1 patients.

Author(s)	NF1 patients, (n)	Age of the patients, years	Frequency of short stature, (%)	Definition of short stature
Riccardi (1981)	134	NA	16	≤ 3rd percentile
Huson et. al. (1988)	102	0-> 70	34	≤ 3rd percentile
Garty et. al. (1994)	390	17	NA	NA
Clementi et. al. (1999)	317	2-24	~10	≤ 3rd percentile
Carmi et. al. (1999)	89	>8	26/43[1]	< 10th percentile
Szudek et. al. (2000)	385	NA	13	≤ -2 SD
Vassilopoulou-Sellin et. al. (2000)	251	<18	12	< 5th percentile
Boulanger and Larbrisseau (2005)	279	<18	18	< 10th percentile
Soucy et. al. (2013)	2013	>3	7	≤ -2 SD
Karvonen et. al. (2013)	80	0-7	12/18[2]	≤ -2 SD
Koga et. al. (2014)	96	>20	NA	NA

NA, not available. SD, standard deviation
[1]Frequency of short stature during prepubertal period/adulthood
[2]Frequency of short stature among boys/girls

In the study by Riccardi (1981), the median height of 137 patients with neurofibromatosis was at the 24th percentile and the mean at the 34th percentile, while the relative frequency of height at or below the 3rd percentile was 16 % (Riccardi, 1981). Huson et al. (1988) excluded patients with skeletal complications or other known causes for short stature in their study. 35 (34 %) of 102 patients were at or below the 3rd percentile for height. The difference in height between patients with NF1 and unaffected siblings was highly significant (p=.001) (Huson et al., 1988).

Garty et al. (1994) identified 390 persons with NF1 during the recruiting for military service. Height and weight were significantly decreased among both sexes (p<.001) (Garty et al., 1994). In Italy, measurements for height, weight and head circumference were available for 159 girls and 158 boys with NF1 aged between 2 and 24 years. Growth curves were constructed for NF1 patients, and curves were compared to the growth curves of the general population. No significant difference in height was found in boys up to 12 years and in girls up to 7 years. After that, the children with NF1 were shorter compared to the general population (Clementi et al., 1999). Carmi et al. (1999) followed prospectively 89 children with NF1. Short stature was common already in the prepubertal period, but the frequency increased towards adulthood. Short stature was more common among patients with familial NF1 (Carmi et al., 1999).

In a study by Szudek et al. (2000), stature was measured for 385 patients with NF1 that were identified in the National Neurofibromatosis Foundation International Database. Measurements were taken from the first visit to the clinic, and no information on measurements at birth were provided. Data on age distribution of the patients was not provided. Patients with pseudarthrosis, delayed/early puberty, glioma or scoliosis were excluded from the analysis. The frequency of short stature, defined as at least 2 SDs below the reference mean, was significantly increased at 13 % (Szudek, 2000). In a NF1 clinic in Texas, 42 (17%) of 251 children with NF1 were at or above the 75th percentile for age and gender adjusted height, while 112 (45 %) children were at or below the 25th percentile. Again, no information about birth height or a detailed distribution of age at the time of measurement was provided (Vassilopoulou-Sellin, Klein and Slopis, 2000). In France, information about 279 children with NF1 was retrospectively analyzed. The frequency of short stature, defined as height below the 10th percentile for reference mean, was 17.9 %, but information about birth size was not provided (Boulanger and Larbrisseau, 2005).

The height of 170 children with NF1 was studied in the United States. 116 (68 %) children were shorter than the age- and gender-adjusted population mean (p<.001). When height was adjusted for parents' height, children of non-NF1 parents were shorter than predicted (p<.001), while children of a NF1 parent were not significantly shorter than expected. NF1 children were shorter than their unaffected siblings (p=.001). Authors also compared height before and after puberty, but the timing of puberty did not seem to affect the height (Soucy et al., 2013). In a study of bone phenotype in NF1, the comparison of baseline characteristics between 18 patients with NF1 and their siblings were included in the analysis, and NF1 patients were significantly shorter (p=.006) (Armstrong et al., 2013). In Finland, height was measured for 80 children with NF1, aged between 0 and 7 years, and they were shorter compared to the general population. For males, the mean height was -0.62 SD from the population mean, and for females, the number was -0.98. 12.0 % of males and 17.7 % of females had height at or below 2 SD from the population mean (Karvonen et al., 2013). In a Japanese study, the height and weight of 96 adults with NF1 were analyzed. Mean height was significantly shorter between both sexes in the NF1 group compared to the control group (p<.001 for males, p=.002 for females) (Koga et al., 2014).

Patients in the studies referenced above were not classified by the type of the mutation, i.e., NF1 patients with microdeletions and non-microdeletions were studied as a single group. However, NF1 patients with microdeletions seem to be taller than patients with other types of mutations. Spiegel et al. (2005) analyzed the growth of ten children with a NF1 microdeletion, aged between two and six years, and the mean height was 1.7 SD above the mean in the general population (p<.001). The difference was

significant both among males and females. Overgrowth remained statistically significant when height was adjusted for parental height (Spiegel et al., 2005). Another study by Ning et al. (2016) confirmed these results. Authors compared growth of 56 NF1 patients with a microdeletion to 226 non-microdeletion NF1 patients. The mean height of patients with a microdeletion, aged 2-18 years was 0.38 standard deviations above the age and sex-adjusted reference measurements, while the number among non-microdeletion NF1 patients was -0.58 (p<.001). However, in early infancy (<2 years), height did not differ between patients with microdeletions and patients with other type of mutations. Head circumference was similar in both study groups (Ning et al., 2016).

2.10.3 Weight in NF1

Information about the weight of patients with NF1 is limited, and the results are somewhat conflicting. In the study by Garty et. al. (1994) absolute weight was decreased among 17-year-old NF1 persons compared to the general population, but there was no significant difference in BMI between study groups (Garty et al., 1994). Clementi et. al. (1999) observed that weight was indifferent from the general population during the whole growth period, but after that, a slightly overweight value was presented (Clementi et al., 1999). On the contrary, in a study of 96 adults with NF1 in Japan, BMI was significantly lower among males with NF1 than in the control group (p=.024), but no significant difference was found among females (Koga et al., 2014). In a study of bone phenotype in NF1, standardized weight did not differ between patients with NF1 and their siblings (Armstrong et al., 2013).

2.10.4 Head circumference in NF1

Multiple studies confirm that head circumference is increased among NF1 patients. While most of the studies involve patients in their adolescence and adulthood, studies of head circumference during early childhood are scarce. In 1981, head circumference of 133 patients with NF1 was analyzed, and also patients under two years old were included. The mean head circumference was at the 70th percentile and 37 (27 %) had a head circumference at or above the 97th percentile. Among children who were 2 years old or less, the average head circumference was at the 48th percentile. Thus, the author suggested that macrocephaly could have a postnatal onset (Riccardi, 1981). In a Finnish study of 80 NF1 children aged between 0 and 7 years, the mean head circumference of boys was 0.41 SD above the population mean. For girls, head circumference was 0.03 below the population mean. While the head circumference was not increased among girls, head circumference-to-height ratio was increased already in

early childhood, and the median age when the ratio was at least 2 SDs above the population mean was only 0.3 years (Karvonen et al., 2013).

In addition to the results reviewed above, a few studies have observed a head circumference of older children, adolescents and adults. To my knowledge, the first study about the head circumference involving a relatively large number of patients was published already in 1973 by Weichert et al. (1973). Head circumference of 27 children with neurofibromatosis was measured. In addition, radiographic measures of the skull were available for 20 patients. Head circumference was at least 2 SDs above the reference mean in 8 (30%) of 27 patients with neurofibromatosis. When analyzing radiographic measures, 18 (75 %) had an enlarged skull (Weichert et al., 1973). Huson et al. (1988) excluded patients with brain tumors and large plexiform neurofibromas and compared the head circumference of NF1 patients to unaffected siblings. 52 out of 115 children with NF1 had a head circumference at or above the 97th percentile, while the number among unaffected siblings was 6 out of 40. The difference between these groups was statistically significant (p=.001) (Huson et al., 1988). In a study by Clementi et al. (1999), among 317 NF1 patients, head circumference was increased during childhood, adolescence and adulthood (Clementi et al., 1999). Szudek et al. (2000) analyzed head circumference of 436 patients with NF1 and 24 % had a head circumference of 2 SDs or more above the reference population mean (Szudek, 2000).

2.11 Congenital malformations

Congenital malformations are observed, on average, in 4.8 %, of newborns in Finland, so birth defects affect approximately 2,800 newborns annually. In 2011, malformations of the cardiovascular system were the most common (43 % of all malformations), followed by malformations in the musculoskeletal system (15 %), urinary system (10 %) and malformations of eye, ear, face and neck (7 %) ("EUROCAT Website Database"). The prevalence of prenatally diagnosed congenital malformations is approximately 9.5:1,000 births, but the rate varies between countries in Europe ("EUROCAT Annual Surveillance Report"). In Finland, a first-trimester ultrasound screening is offered for all pregnant women. The main aim of this ultrasound is checking plurality, the place of the placenta and gestational age, but occasionally anomalies can already be found in the first trimester ultrasound. In addition, combined screening in early pregnancy, including measurement of the nuchal translucency and a serum test, is offered ("Prenatal Screening Policies in Europe"). In some municipalities, the Non-Invasive Prenatal Test (NIPT) is offered for women who belong to the risk group for chromosomal aberrations. A second trimester morphological ultrasound is performed primarily in gestational weeks 18-21 ("Prenatal Screening Policies in Europe"). In addition to the national screening program, anomalies can be

found in the investigations carried out in the private sector. The investigations include, for example, 4D ultrasound studies and NIPTs. In 2012-2013, on average, 334 pregnancies were terminated for fetal anomaly following prenatal diagnosis, and in 2013, the prevalence of the terminations for fetal anomaly was 55:10,000 births in Finland ("EUROCAT Website Database").

In Europe, perinatal mortality associated with congenital malformations is approximately 0.9:1,000 newborns ("EUROCAT Annual Surveillance Report"). Congenital malformations have also long-term consequences. In a study of almost 14,000 patients with a congenital malformation, the 20-year survival was 85.5 %, which is less than in the general population (Tennant et al., 2010). In addition to mortality and morbidity caused by congenital malformations, they cause an extensive economic burden. No updated data on the cost of birth defects in Finland exist, but in the United States, the estimated annual cost of hospitalization associated with birth defects was 22.9 billion dollars in 2013, which is 3.0 % of the cost of all hospitalizations and 5.2 % of total hospital costs. Cardiovascular defects accounted for the largest percentage of the costs followed by defects in the central neural system, chromosomal defects and defects of genitourinary system (Arth et al., 2017).

2.11.1 Congenital malformations in NF1

In the literature, NF1 is connected with many congenital malformations, but most of the associations are based on case reports or limited case series. However, some NF1-related malformations, e.g., pseudarthoris and sphenoid dysplasia, are widely known and even included in the NIH clinical criteria for NF1. The largest studies of congenital malformations are on cardiovascular abnormalities (Lin et al., 2000) and bone malformations (Ruggieri et al., 1999), but there are no epidemiological studies on overall risk for malformations among patients with NF1.

2.11.1.1 Cardiovascular malformations

NF1 and cardiovascular malformations are commonly reported in case reports. Most of the published malformations involve heart, major arteries or intracranial vessels, while malformations of the peripheral vascular system are reported less frequently. Best characterized abnormalities associated with NF1 are Moyamoya syndrome and pulmonal stenosis.

To my knowledge, the first systemic study about cardiovascular malformations among patients with neurofibromatosis was published in 1974 by Neiman et al. (1974). Au-

thors reviewed medical records of all patients with neurofibromatosis seen at the University of Michigan Medical Center between 1951-1971 that included 78 patients. Six (7.7 %) of the patients had also congenital heart disease, which is significantly more than expected. Congenital malformations included ventricular septal defect (1), pulmonic stenosis (2), a patient with aortic coarctation and bicuspid aortic valve (1), atrial septal defect (1) and complete heart block (1) (Neiman et al., 1974).

The largest study about cardiovascular malformations in NF1 was published in 2000. 97 of 2,322 patients fulfilling NIH clinical criteria for NF1 in the National Neurofibromatosis Foundation International Database were found to have a cardiovascular disease, and of these, 54 (2.3 %) had a cardiovascular malformation. The most significant finding was the high frequency of pulmonic stenosis, which covered 46 % of all cardiovascular malformations, while the proportion was only 11 % in the population that was used as a reference. Another significant finding was the small number of complex cardiovascular malformations and the lack of hypertrophic cardiomyopathy. In addition to patients with intracardiac malformation, 16 patients had a peripheral vascular abnormality including two patients with Moyamoya syndrome. The number of cardiovascular malformations of 2,322 patients with NF1 are presented in Table 4 (Lin et al., 2000).

Table 4. The frequency of cardiovascular malformations among 2,322 patients with NF1 (Lin et. al. 2000).

	Number of patients, n (%)	Proportion of all malformations (%)
NF1 patients	2322 (100)	
Cardiovascular malformation	54 (2.3)	100
Tetralogy of Fallot	2 (0.1)	3.7
Pulmonic stenosis	25 (1.1)	46.3
Aortic stenosis	2 (0.1)	3.7
Aortic coarctation	5 (0.2)	9.3
Atrial septal defect (ASD)	4 (0.2)	7.4
Ventral septal defect (VSD)	6 (0.3)	11.1
Patent ductus arteriosus (PDA)	1 (<0.1)	1.9
Mitral valve prolapse	1 (<0.1)	1.9
Possible, not specified or inadequately specified	8 (0.3)	14.8

Tedesco et al. (2002) studied 48 NF1 patients and 30 control subjects by echocardiography including Doppler scans. The mean age of the patients was ten years at the time of the study. Cardiac abnormalities were found in 13 (27 %) patients with NF1 but none among control subjects. The abnormalities included atrial septal defect secundum (2), pulmonary artery stenosis (1), aortic coarctation (1), aortic regurgitation (2), mitral valve prolapse (1), mitral regurgitation (2), atrial septal aneurysm (2) and suspected hypertrophic cardiomyopathy (2) (Tedesco et al., 2002).

Moyamoya disease is described as an occlusive disease of the cerebral vasculature. Specifically, the internal carotid arteries and the circle of Willis are involved. As a consequence of the progressive occlusion, an abnormal collateral network develops and is seen as a "puff of smoke" in the angiography, and this disease is named after the Japanese name *Moyamoya*, which means "puff of smoke". The most common symptoms at presenting are ischemic stroke, transient ischemic attack and hemorrhage (Scott and Smith, 2009). When the abnormality is secondary to an underlying disease, such as NF1, the term Moyamoya syndrome is used. Case reports and case series have reported an association between NF1 and Moyamoya syndrome. In a retrospective study of 353 NF1 children in the United States, a total of 316 had undergone MRI of the brain. Eight children were found to have cerebrovascular abnormalities, and two (0.6%) were diagnosed with Moyamoya (Rosser, Vezina and Packer, 2005). In Spain, medical records of 197 children with NF1 were retrospectively reviewed. 168 children had undergone brain MRI, and four (2.4 %) were found to have Moyamoya (Duat-Rodríguez et al., 2014). The prevalence of Moyamoya in the general population varies geographically, and the highest prevalence is found in Japan being approximately 3 cases per 100,000 (0.003 %) children. In Europe, the prevalence is approximately one-tenth of the prevalence in Japan (Scott and Smith, 2009). In any case, the prevalence of Moyamoya syndrome found among patients with NF1 is significantly higher than in the general population.

Microdeletion of the *NF1* gene is associated with a more severe disease, and this seems to be true also with congenital cardiovascular complications. 16 NF1 patients with a *NF1* gene deletion, and 16 NF1 patients without such a deletion were studied clinically and by echocardiography. Six (37.5 %) of the NF1 patients with *NF1* gene deletion had at least one congenital heart defect. Heart defects included two cases of mitral insufficiency and one case of ventricular septal defect, aortic stenosis and aortic insufficiency each. In addition, three patients had hypertrophic cardiomyopathy, and two patients had an intracardiac tumor. Congenital heart defects were not found among NF1 patients without deletion of the *NF1* gene (Nguyen et al., 2013).

2.11.1.2 Musculoskeletal malformations

The relationship between congenital bowed tibia and neurofibromatosis has been well known for decades, and it is also included in the NIH clinical criteria for NF1. The prevalence of bowed tibia in NF1 children has been reported to be 3.8-5 % (Friedman and Birch, 1997; Crawford and Schorry, 2006). As congenital bowed tibia is very rare in the general population, approximately 75 % of the children with bowed tibia can be diagnosed for NF1 (Feldman, Jordan and Fonseca, 2010). While the tibia is the most common location for bowing and pseudarthrosis, it can rarely be located in the

other bones including the ulna, radius and fibula (Mathoulin, Gilbert and Azze, 1993; Hisaoka et al., 2004; Durga Nagaraju et al., 2007). Another established skeletal anomaly in NF1 is sphenoid wing dysplasia. It is commonly found incidentally when imaging studies are processed, but it can cause ophthalmic complications, cosmetic issues related to facial asymmetry and herniation of the temporal lobe in the orbit (Ferner, 2007). Approximations of the prevalence of sphenoid wing dysplasia vary from 1.0 % up to 11.3 % (White et al., 1986; Friedman and Birch, 1997; Ferner, 2007). Occurrence of sphenoid wing dysplasia correlates with long bone dysplasia (Alwan et al., 2007).

In Italy, 135 children with NF1 were followed between 1990 and 1996 by Ruggieri et al. (1999). They were clinically investigated, but imaging studies were performed only if there was a suggestion of orthopedic complications. A total of 12 (8.9 %) patients with NF1 had congenital bone malformations. Polydactyly was found in four (3.0 %) patients, while in the general population, the prevalence of polydactyly is approximated to be 0.014-0.12 %. Also, the number of segmentation anomalies of the vertebras was higher than expected. Six patients had a segmentation anomaly of vertebrae, and one patient had a segmentation anomaly of vertebrae and ribs. However, there was not a control group in the study (Ruggieri et al., 1999).

In addition, there are multiple case reports of rare anomalies among patients with NF1 including dysplasia of the skull (Rangarajan et al., 2015; Solanki et al., 2015), but there are no epidemiological studies or even case series confirming these associations.

2.11.1.3 Malformations of the central nervous system

Multiple malformations and abnormalities of the central nervous system have been published in case reports and case series. However, no epidemiological data on malformations exist, and most of the case series involve only a few patients. Thus, the overall prevalence of the malformations in the central nervous systems is poorly known.

The most frequent brain abnormalities of NF1 patients are so called unidentified bright objects (UBO). These are lesions that are observed as bright areas with ill-defined borders on T2-weighted MRI images. They are observed in 43-93 % of patients with NF1 (Bognanno et al., 1988; Griffiths et al., 1999). While the prevalence of UBOs among children at age between 8-9 years is 84 %, the prevalence of the lesions decreases as the age increases being only 20 % in the age group of 20-25 years (Gill et al., 2006). In the general population, the prevalence of UBOs is approximately 0.5 % (Katzman, 1999). UBOs are benign lesions, but otherwise their clinical importance is

unknown. In a study of 37 NF1 children, thalamo-striatal UBOs were associated with decreased IQ and visuospatial performance (Chabernaud et al., 2009), while the study of 24 NF1 patients did not find an association between UBOs and emotional or behavioral problems (Cohen et al., 2015). Among 630 NF1 patients in Taiwan, UBOs were not associated with epilepsy (Hsieh et al., 2011). Previously, UBOs were sometimes described as hamartomas, but imaging studies have shown that they probably represent intramyelinic vacuolization, which is supported also by histopathologic findings (DiPaolo et al., 1995; Billiet et al., 2014). As the etiology of the lesions is not known, it is unclear if UBOs represent truly congenital malformations of the brain.

Another suggested brain abnormality associated with NF1 is a type 1 Chiari malformation (CIM). In CIM, cerebellar tonsils are displaced caudally at least 3 mm into the upper cervical spinal canal. As the brainstem is often involved in herniation, symptoms can vary from a mild headache to severe myelopathy (Tubbs et al., 2007). Miraglia et al. (2016) reviewed MRI scans of 428 NF1 patients between 1994 and 2014. MRI scans of the brain and spinal cord are routinely processed for every patient in Italy, and among these patients, 9 (2.1 %) patients were found to have CIM. They did not have a control group in the study, but prevalence was higher than expected (Miraglia et al., 2016). Tubbs et al. (2011) reported that 25 (5.0 %) of the 500 patients with symptomatic and surgically operated CIM had also NF1, which is more than expected when considering that the prevalence of NF1 is far less than 5.0 % (Tubbs et al., 2011). MRI scans of 604 patients with NF1 were reviewed retrospectively in the United States by Acosta et al. (2012). 14 (2.3 %) patients were found to have CIM. However, the authors did not provide detailed information about the indications for the MRI imaging (Acosta et al., 2012).

Malformations of cortical development are associated with several neurocutaneous syndromes, and the mTOR signaling system is essential in cortical development (Crino, 2015). Thus, it is not surprising that case reports of NF1 and malformations of cortical development have also been reported. Polymicrogyria is one of the most often reported malformation (Balestri et al., 2003; Clark and Neville, 2008; Ruggieri et al., 2011; Barba et al., 2013), but also other malformations are reported including, for example, grey matter heterotopia and agenesis of corpus callosum (Voudris, Skardoutsou and Vagiakou, 2003; Balestri et al., 2003; Acosta et al., 2012; Runke and Salanova, 2013). However, in a study of 604 NF1 patients, only five patients had abnormalities of the cortical development including two patients with hemihypertrophy, two patients with grey matter heterotopia and one patient with double cortex. As the number of the cases is limited, and the study did not have a control group, no conclusions about the prevalence of these malformations compared to general population can be made (Acosta et al., 2012). In the United States, 72 consecutive brain MRIs of children with NF1 were studied retrospectively, but no malformations of cortical development were found (Toelle et al., 2015).

2.11.1.4 Malformations of the eye, head, ear and neck

A couple dozen case reports or small case series have been published on congenital glaucoma in NF1. In many occasions, plexiform neurofibroma around the eye explains the glaucoma, but congenital glaucoma can also occur without plexiform neurofibroma. These other etiologies include angle closure due to thickening of the ciliary body and choroid, fibrovascularization, developmental angle abnormalities and congenital ectropion uvea (Payne et al., 2003; Edward et al., 2012). However, there is no information about the actual prevalence of congenital glaucoma among NF1 children. In addition to congenital glaucoma, there are case reports of other congenital malformations of eye, e.g., chorioretinal coloboma (Duman et al., 2016), but information about many of these malformations are limited to single reports. Malformations of the head, ear and neck are only rarely reported in the literature, and these are often associated with the existing plexiform neurofibroma in the area.

2.11.1.5 Other malformations

In addition to organ systems mentioned in the previous sections, malformations in other organ systems, i.e., urinary, genital, digestive and respiratory systems, have also been reported (Oktenli et al., 2004; Jorge and Jorge, 2006; Yayli et al., 2008; Jat et al., 2008; Gorospe Sarasúa, Saldaña Garrido and Ayala Carbonero, 2015). Information about malformations in these organ systems is scarce, and thus any conclusions cannot be drawn about the prevalence of these malformations among patients with NF1.

3 AIMS OF THE STUDY

The objectives of this study were:

1. To evaluate the incidence of NF1 and NF2
2. To analyze the mortality of patients with NF1
3. To evaluate NF1-associated pregnancies and deliveries
4. To analyze the birth size of children with NF1 and children of NF1 mothers
5. To determine the frequency of congenital malformations associated with NF1

4 MATERIALS AND METHODS

4.1 Study permits and ethical considerations

The study compiles with the Declaration of Helsinki, and the study protocol was approved by the Ethics Committee of the Hospital District of Southwest Finland. Permits for the study were obtained from the National Institute of Health and Welfare (THL) and all secondary and tertiary referral centers in mainland Finland. In addition, permits were acquired from each register separately. Favorable opinion was also received from the Office of the Data Protection Ombudsman, which is an independent authority controlling and guiding the processing of personal data. The study was fully based on the information found in the administrative registers and hospital electronic databases. Study persons were not contacted personally, and thus written consent from the study person was not needed. Personal identity codes were replaced by randomly generated study person numbers before statistical analyses to protect the privacy of the study persons.

4.2 Study population

4.2.1 Formation of the cohorts of patients with neurofibromatosis

The International Classification of Diseases (ICD) is a classification system, which provides codes to classify diseases. The ICD is used in a clinical setting, and for research purposes. The classification system can be used in, for example, incidence, prevalence and mortality studies. ICD is maintained by the WHO and is revised periodically (WHO). In Finland, ICD-9 was used between 1987 and 1995, and since then, ICD-10 has been used (Sund, 2012).

Every resident in Finland has a unique personal identity code, which includes gender and birth date. It remains immutable for their lifetime and enables following, also retrospectively, persons over time (Population Register Centre). As a personal identity code is used in hospitals and administrative registers, it can be used to crosslink data among different databases.

The Finnish health care system is based on the primary health care provided by public municipal health care centers. Specialist care is provided by 20 hospital districts, which are funded by the municipalities. Each of these hospital districts has one or several hospitals, which are divided to primary referral centers (the district hospital),

secondary referral centers (the central hospital) and tertiary referral centers (university hospitals) (Teperi et al., 2009). In addition, health care services are provided by a few non-governmental organizations, of which, Folkhälsan and the Family Federation of Finland have provided also genetic counseling (Väestöliitto). In Finland, the private sector has only a minor role in treating multiorgan disorders or otherwise complicated diseases.

The Care Register for Health Care is a continuation of the Hospital Discharge Register and administrated by THL. The first information was collected into the register in 1959, and since 1969, collected information nationwide has included personal identity codes and ICD-8 codes of the inpatient hospital visits. In 1987, ICD-9-classification was introduced, and since then, the information has been collected into electronic databases. The ICD-10 classification has been used since 1996. Since 1998 the register has covered also outpatient hospital visits (Sund, 2012).

To find Finnish patients with neurofibromatosis as completely as possible, medical records of all secondary and tertiary level hospitals of mainland Finland, the Care Register for Health Care, the Family Federation of Finland and Folkhälsan were included in the search. Only a secondary referral center in the Åland Islands, with a population of ~29,000, was excluded. However, the tertiary referral center of the hospital district of the Åland Islands was included. As the inpatient hospital visits were electronically archived since 1987, the persons were searched between January 1987 and December 2011. Information was acquired for all patients, who had visited the above-mentioned institutions with any of the following diagnoses:

ICD-9 classification

- 237.7, Neurofibromatosis

ICD-10 classification

- Q85.00, Neurofibromatosis, unspecified
- Q85.0, Neurofibromatosis
- Q85.09, Other neurofibromatosis
- Q85, Phakomatoses
- Q85.01, Neurofibromatosis type 1

In addition to diagnosis for neurofibromatosis, we included in the search also more general diagnoses, e.g., Q85 (phakomatoses), to find the patients who may have had an unclear clinical presentation at the time of hospitalization or were mistakenly registered with the wrong diagnosis. The initial database search resulted in 2,335 persons, who had been hospitalized with any of the diagnoses listed above. Then, the medical records of these patients were reviewed to confirm or reject the diagnosis of NF1 or

NF2 according to clinical criteria listed in the previous Chapters 2.3.1 and 2.5.1. The Cohort entry date was described as the date when the patient was for the first time hospitalized with the diagnosis of neurofibromatosis. The formation of the study cohort of patients with neurofibromatosis is illustrated in Figure 5. In addition, information about the first-degree relatives of suspected patients with neurofibromatosis was acquired.

4.2.2 Formation of the control population

Control persons were sampled from the Population Register Centre of Finland, which maintains the Population Information System. This register contains information about all Finnish residents, including, but not limited to, date of birth, municipality, sex, date of emigration and date of birth (Population Register Centre). This data is accessible and linkable to other data sources by the personal identity code. For all confirmed NF1 patients, ten control persons were sampled from the register. These controls were adjusted to municipality (at the time of cohort entry date), sex and year of birth. First-degree relatives of NF1 patients were excluded. To ensure the privacy of control persons, the Population Register Centre generated random study person codes for controls. This code included a reference to matched NF1 patient, so that NF1-control sets could be formed.

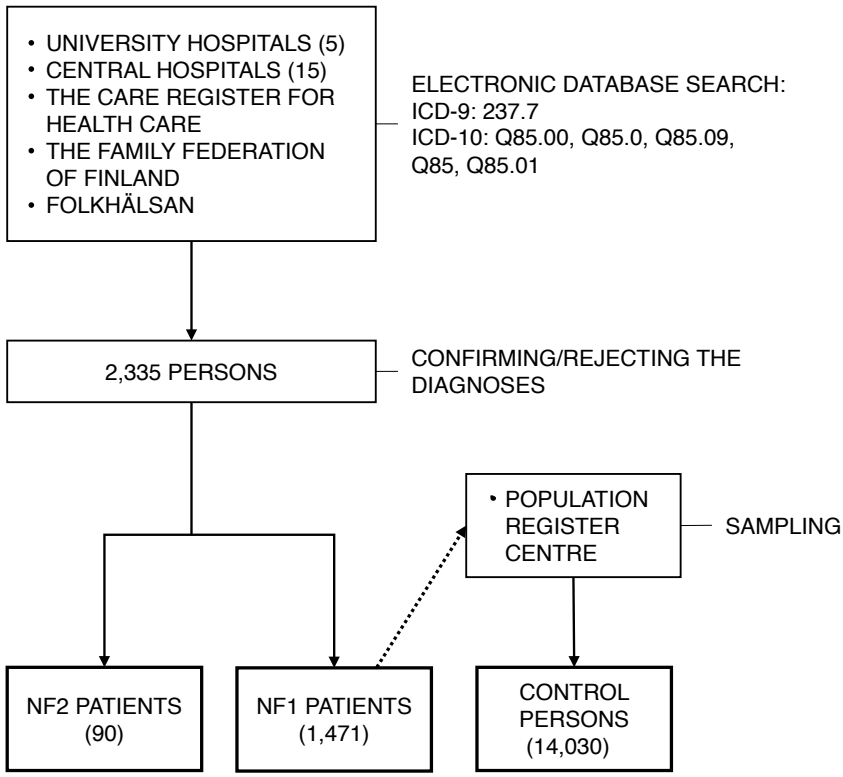

Figure 5. Formation of the study cohorts.

4.2.3 Archiving of data

Data of patients with neurofibromatosis and their first-degree relatives were stored into register program, which was designed by the research group specifically for archiving neurofibromatosis data. Stored data including information about fulfilling the diagnostic criteria, study person number, family relations, date of birth and date of death. The program code was written by Genosyst Ltd in 2010. The database program is web-based and running on the server of University of Turku with double-layer password protection. It is also hidden from search engines.

4.3 Data sources

The personal identity code was used to acquire information from the nationwide registers. The registers serving as data sources in this study included: statistics of causes of death (maintained by Statistics Finland), the Care Register for Health Care (THL),

the Medical Birth Register (THL) and the Register of Congenital Malformations (THL). The availability of the data during the different time periods is presented in Figure 6.

Figure 6. The availability of the data in the registers over the years.

4.3.1 The Statistics of Causes of Death

Statistics Finland is a public authority, which is specifically established for collecting, analyzing and publishing statistics. The statistics of causes of death has been operated since 1936. The death certificate is delivered to THL by the physician who has established the death or by forensic pathologist if autopsy is required. Subsequently, a forensic pathologist in THL checks the correctness of the death certificate, and then the death certificate is delivered to Statistics Finland. Between 2000 and 2015 only 0.1-0.9 % of the death certificates has been missing.

Statistics of death include ICD-codes of underlying, immediate, intermediate and contributing causes of death. In the current study, the underlying cause of death was used to classify the deaths when analyzing the mortality due to different disease groups. It is described as disease that initials the series of events causing the death (Statistics Finland).

4.3.2 The Care Register for Health Care (the Hospital Discharge Register)

The Care Register for Health Care is briefly reviewed in Section 4.2.1. In 2012, a systematic review of the quality of the Care Register for Health Care was published by Sund (2012). The completeness of the register was found to be very good, and the accuracy was good or satisfactory. The main limitations of the register concern outpatient visits to hospitals (Sund, 2012). In our study, the register was used to find persons with neurofibromatosis to form the study cohort and complete the information on morbidity during pregnancy and delivery. All ICD-10 diagnosing codes of hospital visits during pregnancy until 42 days after the delivery were acquired from the register to evaluate morbidity during the pregnancy.

4.3.3 The Medical Birth Register

The Medical Birth Register is maintained by THL and was established in 1987. Information in the register is provided by maternity hospitals, Population Information System and Statistics Finland (causes of death). Data provided by hospitals is sent to THL, correctness of the data is checked, and apparently incorrect data, including systematical or single errors, is returned to the hospital for correction. Because the collection of the data is compulsory, the register covers extensively births in Finland (Gissler and Shelley, 2002).

The register covers data on all live births and stillbirths with a gestational age of at least 22 weeks or birth weight of at least 500 grams. Maternal data in the register includes personal data of the mother (e.g., age, marital status, cohabiting), data on previous pregnancies and deliveries, data on present pregnancy (e.g., diseases during pregnancy, risk factors relating to pregnancy and hospital care during pregnancy), data on delivery (e.g., gestational age at the time of the delivery, mode of delivery, pain relief during labor and diagnoses relating pregnancy and delivery). The data on infant include the sex, weight at birth, length at birth, head circumference at birth, Apgar score, pH of the umbilical cord, diagnoses and data on care interventions. The data is collected until the age of seven days or discharge. The full data content of the Medical

Birth Register can be found online at https://www.thl.fi/en/web/thlfi-en/statistics/information-on-statistics/register-descriptions/newborns.

Because reforms were introduced to the Medical Birth Register in 1990, 1996 and 2004, available data varies among individual pregnancies. For all pregnancies in the study, the best estimate of gestational age at the time of delivery, the type of delivery (vaginal/cesarean delivery), marital status, cohabiting status and smoking during pregnancy were available. Information about hypertension during pregnancy, the occurrence of placental abruption, induction of labor, methods of pain relief during delivery, socioeconomic position and more detailed information on the type of delivery were available since 1991 covering the majority of the pregnancies in the study. Data on gestational diabetes, maternal weight and maternal height were available since the last reform in 2004.

4.3.4 The Register of Congenital Malformations

The Register of Congenital Malformations was established in 1962 by THL and contains data on congenital structural and congenital chromosomal anomalies. It is obligatory to report observed congenital malformations in Finland, and the register covers all live births and stillbirths in Finland. It also contains information about inducted abortions. Data sources of the register are from physicians' records, hospitals, prenatal clinics, child-welfare clinics, cytogenetic laboratories, the Medical Birth Register, the Care Register for Health Care, the Register of Induced Abortions, the Register of Visual Impairment, Statistics Finland and National Supervisory Authority for Welfare and Health (Valvira).

The congenital anomalies are described in the register both as verbal diagnoses and as ICD-codes. Also, additional data, e.g., pattern of the anomaly, examinations and etiology of the anomaly, are collected into the register. It also contains data on maternal background, current pregnancy and anomalies in family members (National Institute for Health and Welfare). The full list of variables in the register can be accessed online: https://www.thl.fi/en/web/thlfi-en/statistics/information-on-statistics/register-descriptions/register-of-congenital-malformations.

4.4 Statistical analysis

Analyses of incidence included all patients with NF1 or NF2. Other study outcomes were analyzed only for NF1 patients. However, as mentioned in Chapter 2.3.1, NF1 may not be diagnosed at birth, while at the age of five, most patients have findings required to fulfill clinical criteria for NF1 (Lammert et al., 2005). Therefore, only

children who were born before year 2007 were included in the analyses, where the effect of the infant's NF1 was analyzed. Cases with missing data for confounding or outcome variables were excluded from the analysis of corresponding outcome variable. Throughout the study, 95 % CIs were calculated, and two-tailed p values <.05 were considered statistically significant.

4.4.1 Incidence

Birth incidence was calculated as the number of new patients with neurofibromatosis born during the time period divided by the total number of the live births in Finland during the corresponding time period. The number of total live births was provided by Statistics Finland (Official Statistics of Finland), and incidence was calculated for each year between 1905 and 2011. In addition, mean incidence was calculated for three- and ten-year long intervals. Information about place of birth was used to exclude persons, who were born abroad.

4.4.2 Standardized mortality ratio

Mortality compared to general population was analyzed by calculating the standardized mortality ratio (SMR), which is defined as (Everitt, 2002):

$$SMR = \frac{observed\ deaths\ in\ the\ study\ group}{expected\ deaths\ in\ the\ study\ group}$$

To calculate expected deaths among patients with NF1, person years were calculated for both sexes separately grouped by calendar year and the age of the person. Then, these sex data, calendar year and age-specific person years were multiplied by the corresponding death rates in the general population provided by Statistic Finland. Thus, mortality was standardized for age, sex and calendar year. The statistical significance was analyzed with Mid-P test. In the current study, SMRs were calculated separately for overall mortality, both sexes, age groups over and under 50 years and selected medical conditions.

4.4.3 Control groups and subgroup analyses

In the analyses of pregnancy and delivery outcomes, frequency of congenital malformations and birth size, patients with NF1 were compared to control persons, who were matched for age, sex and municipality. In addition, subgroup analyses were carried out by stratifying the cohort by NF1 diagnosis of the mother and child, e.g., the

effect of infant's NF1 was studied by analyzing subgroup, where the mother did not have NF1, but the infant had the disorder. Also, other subgroup analyses were proceeded in cases, where the data was not available for the whole study period.

4.4.4 Congenital malformations

ICD-9 diagnoses in the Register of Congenital Malformations were converted to ICD-10 diagnoses, as this revised classification system is currently used in clinical setting in Finland. All the diagnosis codes and verbal diagnoses were manually reviewed to ensure that the converting was performed as accurately as possible. Only major congenital malformations, as described by the EUROCAT (European surveillance of congenital malformations), were included in the analysis (EUROCAT Association, 2014). Malformations were classified by organ systems according to ICD-10 classification of diseases. In the cases, if malformation was part of a syndrome, then only the syndrome was accounted as a case in the analyses. For example, for a patient with trisomy 21 (Down syndrome), cardiac anomalies would not be accounted as malformations, because they are part of the syndrome. When a patient had multiple malformations in different organ systems, they all were considered as cases in the corresponding organ system. However, multiple malformations in the same organ system were accounted only once. Also, when analyzing the overall frequency of congenital malformations, a patient was accounted as a single case irrespective of whether a patient had a single or multiple malformation.

4.4.5 Birth size measurements

An infant's height, weight and head circumference measurements were converted to standard deviation scores (SDS) to analyze birth size. SDS is defined as the difference in standard deviations from the population mean, and recently updated Finnish growth charts were used as reference (Sankilampi et al., 2013). These charts include gestational age- and sex-specific means and standard deviations separately for parous and nulliparous women. Reference growth charts were available for infants born from 23 to 43 weeks gestational age, and deliveries carried out before 23 weeks or after 43 weeks gestational age were excluded from birth size analyses. Also, patients with birth size measurements less than -6 SDS or above 6 SDS were excluded, as they probably represent measurement/typing errors. If information on parity was missing, then gestational age- and sex-specific references were used. To analyze possible disproportion of weight and length, ponderal index (birth weight/birth length^3) at birth was calculated.

4.4.6 Statistical models

Pregnancy and delivery outcomes, birth size measurements and the frequency of congenital malformations were analyzed by mixed model analysis. A linear mixed model was used to analyze continuous variables and a generalized mixed model with binomial distribution for categorical variables. To take into account case-control matching and consecutive pregnancies, the study person code and number of the NF1-control set were used as random intercepts. When the statistical models did not estimate with two random variables, only the study person code was used as a random variable, because it has more effect on the models than case-control matching (number of NF1-control set). 95 % CIs and two-tailed *p* values were calculated. In addition, odds ratios (OR) were calculated for categorical variables.

Unadjusted and adjusted analyses were proceeded. Unadjusted models included only random variables in the model. Adjusted analyses included smoking status, age of the mother and year of the pregnancy and delivery as confounding variables. In addition, the models for analyzing the frequency of congenital malformations were adjusted for parity and birth size analyses for anthropometrics and gestational diabetes of mother. However, as data on gestational diabetes, maternal weight and maternal height were not available before year 2004, only a subgroup of all newborns could be adjusted for these factors.

4.4.7 Statistical and graphic software

Person years were calculated with Microsoft Excel and R statistical software using RStudio interface and *pyears* function from 'survival' package. Mixed model analyses were performed with the SAS System for Windows version 9.4. Figures were drawn with Adobe Illustrator CC version 18.0.0.

5 RESULTS

5.1 Incidence of NF1 (I)

The highest incidence of 1:1,786 was observed for persons born in 1996. As one-year incidence is sensitive to year-to-year variation, mean incidences for three-year periods were calculated and are illustrated in Figure 7. This calculated incidence increased until reaching the maximum of 1:1,871 among persons born between 1994 and 1996.

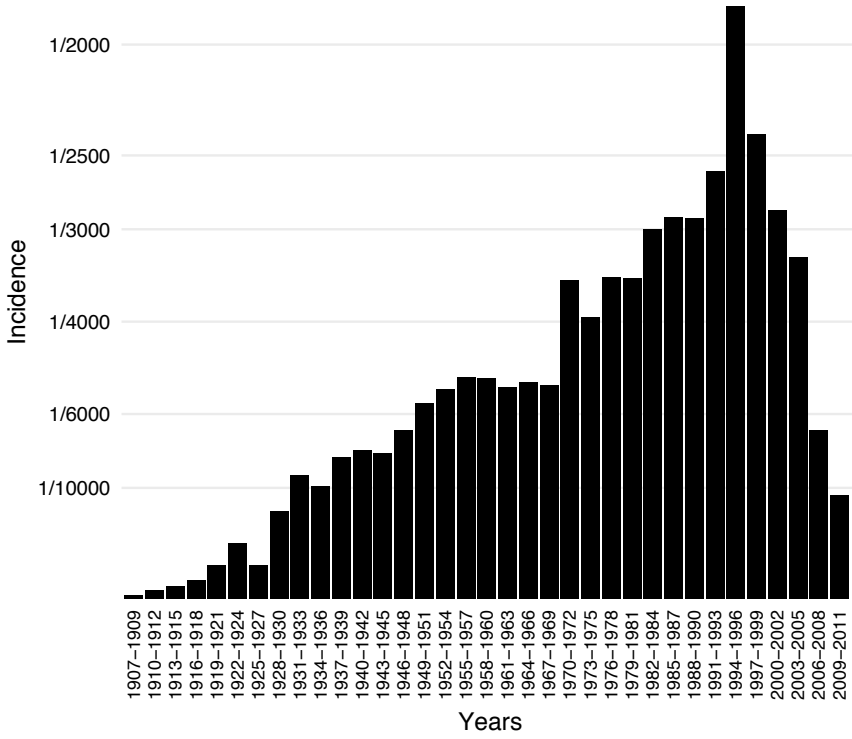

Figure 7. The mean incidence of NF1 in three-year long periods. Modified from original publication I.

5.2 Incidence of NF2 (I)

Because NF2 is known to be considerably less common than NF1, also year-to-year variation is larger. Thus, in addition to one-year incidences, incidences for ten-year periods were calculated. The peak one-year incidence was 1:12,911 observed in 1970. The peak ten-year incidence of 1:39,337 was observed between 1970 and 1979.

5.3 Mortality in NF1 (I)

A total of 214 deaths were identified in the NF1 cohort between 1987 and 2012. For males, the mean age at death was 52.3 years, which was 16.5 years less than the mean age at death among their counterparts in the general population. For females, the numbers were 51.9 years and 26.1 years, respectively. Standardized mortality ratios classified by gender, age and cause of death are shown in Figure 8. Total mortality was increased in males and females, but it was more significant in females. The highest mortality was observed among females aged less than 50 years. Mortalities for respiratory diseases, cancer and cardiovascular disease were increased among both genders, while mortality for dementia was increased only in males.

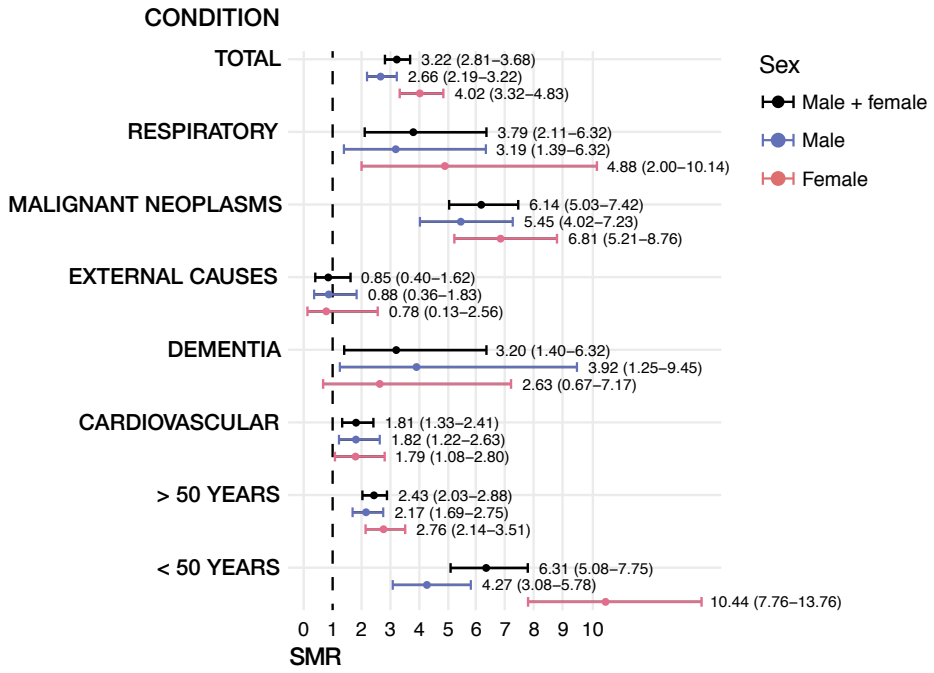

Figure 8. Standardized mortality ratios classified by gender, age and cause of death.

5.4 Pregnancy and delivery (II)

During 1987-2006, a total of 313 singletons with NF1 were born. A total of 375 deliveries, including nine twin deliveries, of 176 women with NF1 were identified. The baseline characteristics of NF1 mothers and matched mothers in the control group are presented in Table 5. NF1 mothers were significantly shorter and less often married than control mothers. Otherwise no statistically significant differences were observed between the groups.

Table 5. Baseline characteristics of NF1 mothers and matched control mothers. Modified from original publication II.

Characteristic	NF1 mothers (n = 357)	Control mothers (n = 4,399)	*p* value for difference
Age (y)	28.5 ± 5.2	28.8 ± 5.3	.346
Height (cm)	159.9 ± 5.6	165.9 ± 5.9	<.001
BMI (kg/m^2)	24.2 ± 4.4	24.4 ± 4.8	.860
Married	196 (54.9)	2,717 (61.8)	.012
Cohabiting	316 (88.5)	3,946 (89.7)	.991
Smoking during pregnancy	46 (12.9)	699 (15.9)	.397

Data are mean ± standard deviation or number (proportion in %).
BMI, body mass index

5.4.1 Duration of the pregnancy

Pregnancy durations grouped by NF1 status of mother and child are shown in Table 6. Gestational age at birth was statistically significantly decreased in the group consisting of NF1 children and non-NF1 children of NF1 mothers. In addition, pregnancy duration was shortened in the subgroups of NF1 children of NF1 mothers and NF1 children of non-NF1 mothers. Thus, the only subgroup where pregnancy duration was not decreased significantly was non-NF1 children of NF1 mothers. Deliveries were also classified by the mode of delivery (vaginal/cesarean section, detailed data reported in the original publication). When including only vaginal deliveries, results in the above-mentioned groups remained statistically significant. Analyzing only cesarean deliveries pregnancy duration was shortened compared to matched control in all groups but among NF1 children of non-NF1 mothers.

Table 6. Duration of NF1-related pregnancies. Modified from original publication II.

Mother / child	Mean pregnancy duration ± SD (weeks, n)	Adjusted mean difference (weeks, 95% CI)[1]	*p* value for difference[1]
Control/control[2]	39.82 ± 1.69 (4,377)	Ref.	Ref.
NF1/NF1 or non-NF1	39.17 ± 2.36 (355)	0.65 (0.42-0.88)	<.001
Control/control[3]	39.80 ± 2.11 (3,175)	Ref.	Ref.
NF1/NF1	38.92 ± 1.70 (118)	0.86 (0.52-1.21)	<.001
NF1/non-NF1	39.51 ± 2.17 (144)	0.31 (-0.03-0.64)	.072
Control/control[4]	39.82 ± 1.64 (4,521)	Ref.	Ref.
Non-NF1/NF1	39.37 ± 1.73 (310)	0.43 (0.24-0.62)	<.001

[1]Adjusted for maternal age, year of the delivery and smoking during pregnancy.
[2]Controls matched to NF1 mothers between 1987-2013.
[3]Controls matched to NF1 mothers between 1987-2006.
[4]Controls matched to NF1 children of non-NF1 mothers between 1987-2006.

The proportion of preterm deliveries classified by NF1 status of mother and child are presented in Table 7. Preterm births were significantly more common in the group consisting of NF1 and non-NF1 children of NF1 mothers and in the subgroup of non-NF1 children of NF1 mothers compared to controls. The proportions of very preterm and extremely preterm births are reported in the original publication. The rate of very preterm births was higher than in controls among all children (including non-NF1 and NF1 children) of NF1 mothers and NF1 children of NF1 mothers. Extremely preterm births occurred more often than expected in the group consisting of all children of NF1 mothers.

Table 7. Proportion of preterm births. Modified from original publication II.

Mother / child	Adjusted OR for preterm birth (95% CI)[1]	Proportion of preterm births of all births in the group, %
NF1/NF1 or non-NF1[2]	1.96 (1.26-3.06)	9.0
NF1/NF1[3]	1.65 (0.80-3.43)	7.6
NF1/non-NF1[3]	2.26 (1.24-4.12)	9.7
Non-NF1/NF1[4]	1.29 (0.77-2.17)	5.5

OR, odds ratio.
[1]Adjusted for maternal age, year of the delivery and smoking during pregnancy.
[2]Controls matched to NF1 mothers between 1987-2013.
[3]Controls matched to NF1 mothers between 1987-2006.
[4]Controls matched to NF1 children of non-NF1 mothers between 1987-2006.

5.4.2 Pregnancy and delivery complications

ORs and 95 % CIs for pregnancy and delivery complications of NF1 mothers compared to matched controls are shown in Figure 9. Hypertension before pregnancy, hypertension during pregnancy/preeclampsia, poor fetal growth, oligohydramnios, maternal care for disproportion, placental abruptions and cesarean sections, were more common among NF1 mothers than in controls. We did not observe any deaths among NF1 mothers during the pregnancy or within 42 days after delivery. One death occurred in the control group.

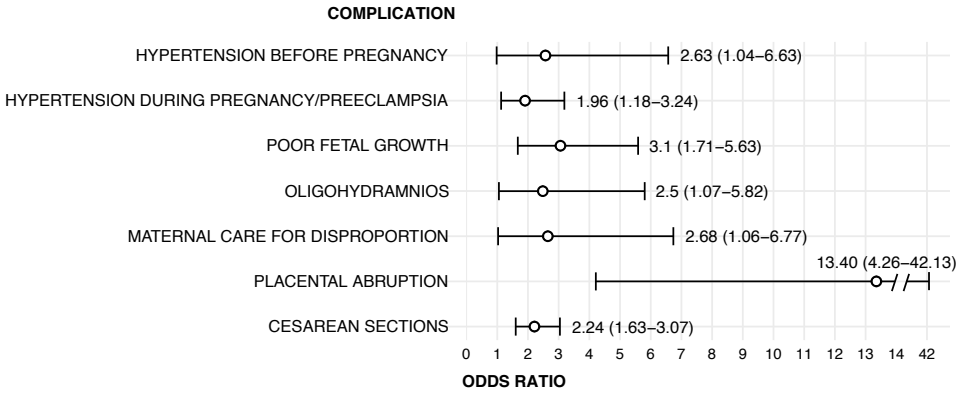

Figure 9. ORs and 95 % CIs for pregnancy and delivery complications of mothers with NF1, adjusted for maternal age, year of the delivery and smoking during pregnancy.

The rate of cesarean sections was further analyzed. The OR for cesarean section among NF1 mothers remained significant, when the analysis was additionally adjusted for hypertension during pregnancy/preeclampsia (OR 1.96, 95 % CI 1.30-2.94). Including only non-elective cesarean sections, no statistically significant difference in the rate of cesarean-section was observed (OR 1.13, 95 % CI 0.67-1.93). Cesarean sections were also further classified by NF1 status of mother and child. The rate of cesarean sections was increased, in addition to above-mentioned group of NF1 mothers, in subgroups consisting of NF1 mothers giving birth to a NF1 child and a NF1 mother giving birth to a non-NF1 child. The rate for cesarean sections was not increased among non-NF1 mothers of a NF1 child. Thus, NF1 in the mother increased the likelihood for a cesarean section, but the child's NF1 did not have the same effect. Non-elective cesarean sections were more common than expected only in the group of NF1 mothers giving birth to non-NF1 child (OR 2.53, 95 % CI 1.04-6.18).

5.4.3 Pain relief during delivery

An epidural block was used as pain relief during delivery less often among NF1 mothers than controls (OR 0.70, 95 % CI 0.51-0.96), and the difference remained significant when only vaginal deliveries were included in the analysis (OR 0.68, 95 % CI 0.48-0.97). Otherwise there were no differences in the usage of pain relief during delivery.

5.5 Birth size (III)

Baseline characteristics for mothers of NF1 children, children with NF1 and matched controls are presented in Table 8. Mothers of NF1 children were slightly older, but otherwise differences in maternal background characteristics were not found. NF1 children were born earlier, were shorter and had increased ponderal index. However, these are unadjusted numbers, and as such, conclusions on birth size cannot be drawn.

Table 8. Baseline characteristics for mothers of NF1 children, NF1 children and matched controls. Modified from original manuscript III.

Characteristic	Infants with NF1 (n=442)	Controls (n=4,548)	*p*
Maternal characteristics			
Age (y)	29.5±5.4	29.0±5.1	.025
Height (cm)[1]	163.9±6.5	165.6±6.2	.081
Weight (km)[1]	69.0±15.8	67.3±15.1	.590
BMI (kg/m^2)[1]	25.4±5.4	24.5±5.3	.259
Smoking during pregnancy	73 (16.5)	671 (14.8)	.291
Married or cohabiting	391 (88.5)	4139 (91.0)	.116
Parity (1+)	277 (62.7)	2740 (60.3)	.687
Gestational diabetes[1]	4 (7.5)	37 (7.0)	.877
Infant characteristics			
Sex, male	241 (54.5)	2475 (54.4)	.809
Gestational age (weeks)	39.2 ± 1.8	39.8 ± 1.6	<.001
Birth weight (g)	3600 ± 667.0	3573 ± 537.0	.113
Birth length (cm)	49.9 ± 2.8	50.3 ± 2.3	<.001
Head circumference at birth[1]	35.1 ± 2.1	34.8 ± 1.5	.242
Ponderal index (kg/m^3)	28.7 ± 2.9	27.9 ± 2.5	<.001

BMI, body mass index. Data are mean ± standard deviation or number (proportion in %), when the unit is not defined. [1]Available for the 53 infants with NF1 and 532 controls born since 2004.

Table 9 shows baseline characteristics for NF1 mothers, their children and matched controls. NF1 mothers were shorter and weighed less than their counterparts in the control group. Their pregnancy duration was shortened, and born children were shorter, weighed less and had decreased ponderal index compared to controls.

Table 9. Baseline characteristics for mothers with NF1, their children and matched controls. Modified from original manuscript III.

Characteristic	Mothers with NF1 (n=357)	Controls (n=4,396)	*p*
Maternal characteristics			
Age (y)	28.5±5.2	28.8±5.3	.360
Height (cm)[1]	159.9±5.6	165.9±5.9	<.001
Weight (km)[1]	62.0 ± 12.2	67.3 ± 14.4	<.001
BMI (kg/m^2)[1]	24.2 ± 4.4	24.4 ± 4.8	.860
Smoking during pregnancy	46 (12.9)	697 (15.9)	.398
Married or cohabiting	318 (89.1)	3964 (90.2)	.200
Parity (1+)	218 (61.1)	2516 (57.2)	.057
Gestational diabetes	17 (12.2)	174 (10.2)	.725
Infant characteristics			
Sex, male	177 (49.6)	2222 (50.6)	.737
Gestational age (weeks)	39.2 ± 2.4	39.8 ± 1.7	<.001
Birth weight (g)	3268 ± 695.5	3552 ± 532.6	<.001
Birth length (cm)	48.9 ± 3.3	50.3 ± 2.3	<.001
Head circumference at birth[1]	34.8 ± 2.1	35.0 ± 1.6	.095
Ponderal index (kg/m^3)	27.4 ± 3.0	27.8 ± 2.5	.013

BMI, body mass index. Data are mean ± standard deviation or number (proportion in %), when the unit is not defined. [1]Available for the 139 infants with NF1 and 1704 controls born since 2004

Birth size measurements in SDSs accompanied with means and 95 % CIs are presented in Table 10. Only births since 2004 are included, because data on gestational diabetes, maternal weight and maternal height were not available before year 2004. Birth weight was increased among NF1 children. On the contrary, the birth weight was decreased among children of NF1 mothers. The number of children born LGA for weight was increased in the group including NF1 children of non-NF1 and NF1 mothers (OR: 7.30, 95 % CI: 1.60-33.36), and in the group of NF1 children of non-NF1 mothers (OR: 9.79, 95 % CI: 1.70-56.35). The frequency of children born SGA for weight was increased among non-NF1 children of NF1 mothers, but the difference was not statistically significant (OR: 3.75, 95% CI: 0.88-16.09). When including births since 1987, the frequency of children born SGA for weight was increased among all children of NF1 mothers (OR: 4.48, 95% CI: 2.92-6.89) and in the subgroup of non-NF1 children of NF1 mothers (OR: 6.95, 95% CI: 4.06-11.91). However, these analyses were not adjusted for gestational diabetes, maternal height and maternal weight.

Head circumference at birth was increased among NF1 children, and OR for head circumference above 2 SDS was increased in the group including NF1 children of NF1 and non-NF1 mothers (OR 5.72, 95 % CI: 1.70-19.60), and in the group including NF1 children of non-NF1 mothers (OR: 5.95, 95 % CI: 1.09-32.41). Among children of mothers with NF1, significant differences in head circumference were not found.

In birth length measurements, birth length was decreased among children of NF1 mothers.

Table 10. Birth size measurements in SDS classified by NF1 status of mother and child. Modified from original manuscript III.

Mother/ child	Birth weight SDS, adjusted mean (n)	p^1	Birth head circumference SDS, adjusted mean (n)	p^1	Birth length SDS, adjusted mean (n)	p^1
Control/control	-0.09 (455)	Ref.	-0.03 (448)	Ref.	-0.07 (453)	Ref.
NF1 or non-NF1/NF1	0.44 (43)	.002	0.55 (42)	<.001	0.03 (43)	.575
Control/control	-0.07 (455)	Ref.	-0.02 (448)	Ref.	-0.04 (453)	
Non-NF1/NF1	0.83 (24)	<.001	0.52 (24)	.011	-0.12 (24)	.479
Control/control	-0.18 (1581)	Ref.	-0.04 (1554)	Ref.	-0.20 (1574)	Ref.
NF1/NF1 or non-NF1	-0.46 (126)	.012	0.10 (120)	.243	-0.42 (1125)	.049
Control/control	-0.25 (425)	Ref.	-0.19 (417)	Ref.	-0.33 (425)	Ref.
NF1/non-NF1	-1.08 (24)	<.001	-0.55 (23)	.127	-0.81 (24)	.044

SDS, standard deviation score (difference from gestational age, sex and parity adjusted population-based birth size references)

[1] *p*-value adjusted for age of the mother, smoking during pregnancy, year of the delivery, gestational diabetes, weight of the mother and height of the mother

5.6 Congenital malformations (IV)

Baseline characteristics for mothers of NF1 children, NF1 children and their controls matched by children are presented in Table 11. Mothers of NF1 children were significantly less often in upper white-collar and more frequently in blue-collar socioeconomic positions. In addition, pregnancy duration was decreased among the NF1 group, and children with NF1 were more often born LGA than their counterparts in the control group.

Table 11. Baseline characteristics for mothers of NF1 children, NF1 children and their matched controls. Modified from original publication IV.

Characteristic	Mothers of offspring with NF1 (n=443)	Mothers of control children (n=4550)	*p*
Age (y)	29.5±5.4	29.0±5.1	.071
Smoking during pregnancy	73 (16.5)	671 (14.7)	.300
Married or cohabiting	392 (88.5)	4141 (91.0)	.123
Socioeconomic position			
Upper white	40 (11.2)	572 (15.5)	.048
Lower white	151 (42.2)	1603 (43.5)	.857
Blue-collar	84 (23.5)	706 (19.1)	.035
Other	55 (15.4)	589 (16.0)	.874
Parity (1+)	278 (62.8)	2742 (60.3)	.669
Sex, offspring			.784
Male	241 (54.4)	2476 (54.4)	
Female	202 (45.6)	2074 (45.6)	
Gestational age, offspring (weeks)	39.2 ± 1.9	39.8 ± 1.6	<.001
Birth size, offspring			
SGA	29 (6.5)	230 (5.1)	.241
AGA	363 (81.9)	4042 (88.8)	<.001
LGA	46 (10.4)	239 (5.3)	<.001

Data are mean ± standard deviation or number (proportion in %). SGA, small for gestational age. AGA, appropriate for gestational age. LGA, large for gestational age.

Characteristics of mothers and children classified in relation to existence of congenital malformations are presented in Table 12. The only difference in the baseline characteristics was found in birth size. Children born SGA had an increased risk for congenital malformations.

Table 12. Baseline characteristics for mothers and children in relation to congenital malformations. Modified from original publication IV.

Characteristic	No major congenital anomaly (n=4886)	Major congenital anomaly (n = 107)	*p*
Age (y)	29.1±5.2	29.1±5.7	.946
Smoking during pregnancy	722 (14.8)	22 (20.6)	.127
Married or cohabiting	4439 (90.9)	94 (87.9)	.255
Socioeconomic position			
Upper white	596 (15.1)	16 (16.7)	.771
Lower white	1717 (43.5)	37 (38.5)	.225
Blue-collar	764 (19.3)	26 (27.1)	.098
Other	630 (15.9)	14 (14.6)	.634
Parity (1+)	2965 (60.7)	55 (51.4)	.171
Sex, offspring			.993
Male	2659 (54.4)	58 (54.2)	
Female	2227 (45.6)	49 (45.8)	
Gestational age, offspring (weeks)	39.8 ± 1.7	39.4 ± 2.1	.072
Birth size, offspring			
SGA	245 (5.0)	14 (13.1)	<.001
AGA	4320 (88.4)	85 (79.4)	.002
LGA	277 (5.7)	8 (7.5)	.521

Data are mean ± standard deviation or number (proportion in %). SGA, small for gestational age. AGA, appropriate for gestational age. LGA, large for gestational age.

The frequencies and ORs for congenital malformations of NF1 children compared to matched controls are shown in Table 13 and numbers of individual malformations in Table 14. The overall risk for malformations was significantly increased. In addition, increased ORs for malformations in circulatory, urinary and musculoskeletal systems, and in the combined group of eye, ear, face and neck were observed. The overall risk for congenital malformation was analyzed also for subgroups classified by NF1 status of mother and child. The frequency of congenital malformations was increased among NF1 children of NF1 mothers (OR 3.27, 95 % CI 1.42-7.52) and NF1 children of non-NF1 mothers (OR 2.66, 95 % CI 1.48-4.78). However, a significantly increased risk was not observed in the groups including non-NF1 children.

Table 13. ORs of congenital anomalies among NF1 children compared to matched controls. Modified from original publication IV.

Organ group	NF1 (1/1,000)	OR, adjusted (95 % CI)[1]	*p*[1]
All	49.7	2.78 (1.71-4.54)	<.001
Nervous system	0	NA	NA
Eye, ear, face and	6.8	4.66 (1.42-15.31)	.011
Circulatory system	20.3	3.35 (1.64-6.83)	<.001
Respiratory system	0	NA	NA
Cleft lip and cleft palate	0	NA	NA
Other digestive system	2.3	NA	NA
Genital organs	0	NA	NA
Urinary system	9.0	4.26 (1.36-13.35)	.013
Musculoskeletal system	13.5	2.77 (1.09-7.02)	.032
Other	2.3	3.88 (0.41-36.90)	.238
Chromosomal	0	NA	NA

OR, odds ratio; NA, not enough events for statistical analysis
[1]Adjusted for smoking during pregnancy, maternal age, year of the pregnancy and parity.

Table 14. The number of individual congenital malformations of children with NF1. From original publication IV.

Congenital anomaly	n
Eye, ear, face and neck	
Coloboma of iris	1
Ptosis	2
Circulatory system	
Ventricular septal defect	1
Patent ductus arteriosus (gestational age ≥ 37 weeks)	2
Subvalvular aortic stenosis	1
Aortic valve insufficiency	1
Ostium secundum atrial septal defect	1
Pulmonary valve stenosis (gestational age ≥ 37 weeks)	2
Arteriovenous malformation of brain	1
Other digestive system	
Anorectal atresia with fistula	1
Urinary system	
Hydronephrosis (dilatation > 10mm)	3
Double ureter	1
Musculoskeletal system	
Polydactyly	5
Syndactyly	2
Craniosynostosis (middle sagittal suture)	1
Other	
Fetal alcohol syndrome	1

6 DISCUSSION

The results of previous studies on the epidemiology of NF1 and NF2 have varied considerably and evaluating the total burden of these disorders on the health care system has been difficult. Thus, there has been a need for more accurate estimations of prevalence and mortality of the disorders. The assumptions about NF1 and pregnancy have largely been based on case reports and case series, in which pregnancy outcomes have often been severely affected by NF1. The same has been true with congenital malformations. Thus, there has been a need for a high quality epidemiological study to evaluate NF1-related risk at a personal level. Results may also be used to develop recommendations for follow-up and screening. In addition, as all the functions and interactions of neurofibromin are not completely known, an epidemiological study may reveal novel effects of the disorder and direct basic research.

6.1 Incidence of NF1 (I)

There are no previous nationwide epidemiological studies, including all age groups, about the incidence of NF1. Noteworthy, when reviewing the studies published before 1987, is the fact that no common clinical criteria was announced, and the definition of NF1 has varied considerably among studies: e.g., in the study by Sergeyev, NF1 clinical criteria included only pigmentary changes on the skin (Sergeyev, 1975), Crowe et al. had a rather wide range of inclusion criteria (Crowe et al., 1957), whereas Samuelsson and Axelsson had criteria consisting of CALMs, neurofibromas and axillary freckling (Samuelsson and Axelsson, 1981).

Our study shows that the incidence of NF1 is closer to 1:2,000 than previously generally accepted at 1:3,000. While we conducted an extensive database search including also diagnosis codes closely related to NF1, it is not possible to identify all patients with the disorder. There are still undiagnosed cases, probably in all age groups, and due to mortality, all children with NF1 do not reach the age when the diagnosis could be made after the clinical criteria. Thus, the incidence of ~1:2,000 represents minimum incidence, and the actual incidence is probably higher. The relatively high incidence of NF1 in our study highlights the strength of electronic hospital registers in Finland, which allow to acquire extensively patients with a certain disorder. Especially this is true with diseases, e.g., NF1 and NF2, whose treatments are centralized to secondary and tertiary referral centers.

The peak incidence of NF1 was observed in the 1990's, which is probably explained by the enhanced general awareness of the disorder and the timing of the data collecting. We included hospital visits since 1987 in our data search, and as NIH diagnostic

criteria were established also in 1987, these criteria should have been used systematically to diagnose patients with NF1 born since 1980. Persons born before this may not have been diagnosed as extensively due to a lack of clear clinical criteria for the disorder. In addition, the patients born in the mid-1990's were old enough to fulfill the NIH criteria by the time of our patient search. They also have been diagnosed and followed up by specialists and are thus better covered in this study.

In our study, the observed incidence had an increasing tendency from 1905 to 2000. However, this probably reflects immortality bias, as patients included in our study had to be alive in 1987. Also, some patients born before 1970's, who have got their diagnosis before 1987, may not have needed treatment for NF1 in secondary or tertiary referral centers since 1987, and these patients are not included in our cohort. On the other hand, the youngest age groups born in 2000's may not have yet fulfilled the diagnostic criteria at the time of database search and review of the medical records.

6.2 Incidence of NF2 (I)

Prior information about the incidence of NF2 is scarce, and no nationwide studies exist. In the previous studies, the incidence estimates of NF2 have been between 1:25,000 and 1:87,000 (Antinheimo et al., 2000; Evans et al., 2005). The results of the current study, with the incidence of 1:39,000 during the highest ten-year period, are in line with the previous reports. As with NF1, this number represents a minimum incidence, and real incidence is probably somewhat higher, because of undiagnosed patients, mortality before year 1987 and the lack of hospital visits associated with NF2 diagnosis after year 1987, when collection of our study cohort started.

6.3 Mortality in NF1 (I)

In our study, the lifespan of NF1 patients was significantly shortened compared to the general population. In males and females, the mean age at the time of death was 16.5 and 26.1 years less, respectively, than in the general population. In the studies, which relied on death certificates, by Rasmussen et al. (2001) and Masocco et al. (2011), the age at death was shortened 13.7-19.5 years in males and 17.9-21.8 years in females (Rasmussen et al., 2001; Masocco et al., 2011). Also Evans et al. (2011) observed in their study, which was based on the patients identified in genetics services, that survival of NF1 patients was significantly reduced. Males with NF1 lived 7.2 years less than males in the general population, and females lived 8.0 years less than their counterparts in the general population (Evans et al., 2011). The mean age at death in our study was decreased slightly more than in some other studies, which may

be at least partly explained by the fact, that in our study, the median age of the patients in the cohort was only 33.0 at the end of follow-up period. In our study, overall SMR was 3.2, which is in line with previous studies, which have observed SMR between 2.0 and 4.3 (Zöller et al., 1995; Duong et al., 2011). Our study also implies that the effect of NF1 on survival is stronger among females than males. Especially, in females under 50 years, the mortality was considerably higher than in males. Also in other studies, the effect of NF1 on mortality has been stronger among females than among males (Imaizumi, 1995; Rasmussen et al., 2001; Duong et al., 2011; Masocco et al., 2011). However, Evans et al. (2011) did not report a significant difference in mortality between sexes (Evans et al., 2011). We also observed that mortality was especially high in the age group < 50 years, which was also observed in other studies (Duong et al., 2011; Masocco et al., 2011). In conclusion, studies clearly show that patients with NF1 die at a younger age than persons in the general population. However, there is considerable variation among the studies in the amount of the reduced life expectancy, and prospective follow-up studies with a control group are needed to assess the exact effect of NF1 on life expectancy and the differences between sexes.

Cancer-related deaths and cardiovascular deaths accounted for 58 % and 13 % of the excess mortality in the current study, respectively. Especially MPNSTs and brain tumors have been associated with NF1 for decades, and mortality for these malignancies has been observed to be increased in multiple mortality studies (Rasmussen et al., 2001; Duong et al., 2011; Masocco et al., 2011; Evans et al., 2011). In addition, the recent study by Uusitalo et al. (2016) reported that also the overall cancer risk, excluding NF1 specific tumors, was significantly increased and cancer survival decreased (Uusitalo et al., 2016). Death from cardiovascular diseases has previously been reported to be up to four times more common in NF1 males than in the general population (Evans et al., 2011), but there are also opposite observations (Rasmussen et al., 2001; Masocco et al., 2011). In our study, SMR for cardiovascular mortality was slightly but significantly increased. A novel finding in our study was that the mortality for dementia was increased among patients with NF1.

The observed proportion of deaths associated with diagnosis for NF1 of all deaths in the studies relying solely on death certificates has been as low as 1:8,700-1:10,685 (Rasmussen et al., 2001; Masocco et al., 2011), while the incidence of NF1 is known to be considerably higher. In the study carried out in the UK, only 36 % of death certificates of confirmed NF1 patients had NF1 as a contributing cause of death on the death certificate (Evans et al., 2011). In our study, 31 % of death certificates of NF1 patients included a NF1 diagnosis. These factors suggest that studies utilizing death certificates as the only data source are of limited value, because the majority of NF1 patients are not identified, and the cohort of cases with NF1 on the death certificate may be heavily biased.

6.4 Pregnancy and delivery (II)

There are no previous studies on pregnancy duration in NF1. Our results suggest that the mean duration of NF1-related pregnancies is shortened compared to controls. However, in the subgroup analysis, only NF1 of the fetus reduced significantly the mean pregnancy duration, while the effect of mother's NF1 did not reach statistical significance. In the epidemiological study carried out in the United States, risk for preterm labor was increased among NF1 mothers, and in the Danish register, the study frequency of preterm births was increased among children with NF1. However, as these studies reported data only on the frequency of preterm pregnancies, it is not possible to evaluate if NF1-related pregnancies were generally shortened, or if only a subgroup of pregnancies was affected. In our study, the proportion of preterm births among NF1 children of non-NF1 mothers was not statistically increased, which indicates that NF1 of the children affects systematically but rather mildly on pregnancy duration. At the same time, mothers' NF1 seems to affect fewer cases, but the effect is more profound. This is illustrated by the fact that the risk for preterm deliveries was significantly increased in the subgroup of NF1 mothers giving birth to a non-NF1 infant.

While our study reports a novel finding that NF1 in the fetus decreases pregnancy duration, it is not totally unexpected, as recent studies have concluded that fetal genetic factors contribute 5-35 % of the timing of the birth (York et al., 2014). It should also be noted that the placenta is mostly of fetal origin. The precise etiology of the shortened pregnancy duration can only be hypothesized, but as NF1 affects endothelial cells (Gitler et al., 2002; Bajaj et al., 2012), our results may reflect the NF1-related vasculopathy in placenta. The mean pregnancy duration for births of infants with NF1 was 0.43 weeks shorter than that in the control group, which may not be clinically significant. However, our results are scientifically remarkable, because at the moment, only little is known about the effect of fetus' disorders on the timing of birth.

The frequency of cesarean sections among NF1 mothers was increased compared to the control group in all subgroups except for a non-NF1 mothers giving birth to a NF1 infant. Thus, NF1 of the mother is suggested to increase the risk for cesarean sections more than NF1 in the fetus. The frequency of non-elective sections was increased only in the subgroup of NF1 mothers giving birth to a non-NF1 child, and also in this subgroup, the lower bound of the 95 % CI was only minimally above one, and the overall 95 % CI was wide (1.04-6.18). Our observations on increased frequency of cesarean sections are in line with a previous study carried out in the United States (Terry et al., 2013). In our study non-elective cesarean sections were only slightly overrepresented in the group of NF1 mothers giving birth to a non-NF1 child, and the frequency of diagnosis for prolonged delivery was not increased in NF1-re-

lated groups and the diagnosis for fetal distress, potentially reflecting impaired placental function was not more common among NF1-related pregnancies than in the control groups. These results do not support the hypothesis by Terry et. al. that a higher rate of failed induction could explain the higher frequency of cesarean sections. Thus, the etiology of increased frequency of cesareans sections remains unexplained.

Multiple other complications, including hypertension/preeclampsia, placental abruption, maternal care for disproportion, poor fetal growth (IUGR) and oligohydramnios were more common among NF1 mothers than controls. Of these, the risk for hypertension, preeclampsia and poor fetal growth was also reported to be increased in the previous epidemiological study by Terry et al. (Terry et al., 2013). However, while placental abruptions and oligohydramnios are reported previously in case reports (Belton et al., 1984; Dugoff and Sujansky, 1996; Cesaretti et al., 2013), it is a new finding that the frequencies of these complications are increased in the epidemiological study setting. The etiology of these complications is unclear, but the higher than expected prevalence of hypertension during the pregnancy among NF1 mothers may partly explain the higher rate of placental abruptions.

We observed that epidural block was used significantly less often among NF1 females than among controls. No difference was found in usage of other pain relief methods during delivery. While the reasons behind the decreased use of epidural blockage cannot be explained by the current study, this result may reflect an anesthesiologists' concern about possible spinal neurofibromas, which could increase the risk of complications during the procedure. In any case, it is essential to ensure that NF1 patients get sufficient pain relief during delivery, and that an MRI of the spinal area has to be performed if there is any concern on possible spinal neurofibromas.

While we found an increased frequency of many pregnancy complications and decreased pregnancy duration in NF1-related pregnancies, it is noteworthy that we could not identify any deaths among women with NF1 during pregnancy or within 42 days after the delivery. Because of the increased rate of pregnancy complications, pregnant females with NF1 need a close follow-up during the pregnancy, and especially hypertension should be treated effectively. However, most of the NF1-related pregnancies are not associated with complications, and possible complications can usually be treated. Thus, potential pregnancy complications are not a reason to recommend patients, with NF1 but in otherwise good health, to avoid pregnancy.

6.5 Birth size (III)

NF1 of the mother reduced gestational age standardized birth weight, which is in line with the previous assumptions based on case reports and the observed increased frequency of IUGR in the epidemiological study of pregnancies in NF1 (Terry et al., 2013). Women with NF1 are significantly shorter and weigh less than their counterparts in the general population, but after adjusting the infant's birthweight to the mother's height and weight, the results remained significant. These findings suggest that NF1 of the mother is an independent factor reducing birth weight of the infant. The difference to controls was greater among non-NF1 infants of NF1 mothers than in the group including all infants of NF1 mothers. This further supports the conclusion that mothers' NF1 has an independent effect on birth weight. Most of the studies analyzing long-term morbidity associated with low birth weight have compared infants born SGA to infants born AGA (see Chapter 2.10). We observed that the risk for being born SGA for weight among children of NF1 mothers was significantly increased, which suggests that NF1 mothers' effect on birth weight is also clinically important. Further studies are needed to assess the long-term effects of small birth weight on morbidity and mortality. In future studies, birth weight should also be considered as a confounding factor when analyzing incidence of diseases, which are known to be associated with small birth weight.

A completely novel and somewhat unexpected finding was that the birth weight of infants with NF1 was increased compared to controls, and also the frequency of infants with birth weight above 2 SD for the gestational age-adjusted population mean was increased. This finding was supported also by subgroup analyses, as the difference to controls was accentuated in the group of NF1 children of non-NF1 mothers. While the large birth size is not associated with as high morbidity as the small birth size, it is known to increase risk for diabetes mellitus, for example, and a higher than expected proportion of large birth size is clinically significant.

Large head circumference has been associated with NF1 for decades (Weichert et al., 1973). However, no information about the measurements at birth exist. In the current study, NF1 of the infant increased significantly the head circumference at birth, but the mother's NF1 did not have a significant effect. It is not known if a large head circumference has some long-term effects, but it increases the risk for prolonged labor, fetal distress and maternal distress during delivery (Elvander, Högberg and Ekéus, 2012).

Birth length was not affected by NF1 of the child, when it was adjusted for the height and weight of the mother. While the adult height of patients with NF1 is shortened, it does not seem to be noticeable at birth. However, the infants of mothers with NF1

were shorter than controls. In the study by Carmi et al. (1999), adult height of patients with sporadic NF1 without a CNS pathology was not significantly shortened. On the other hand, among familial cases without CNS pathology height was shortened significantly. While the number of cases reaching adult height during the study period in their study was low (n = 28), the results suggest that parent's NF1 affects significantly the stature of the child. As also mid-parental height of children was shortened in the study, parents' height is not sufficient enough alone to explain shortened adult height (Carmi et al., 1999).

The background mechanisms, which cause increased birth weight among NF1 children, are unclear. While head circumference at birth is increased, the possible increase in the volume of the head cannot alone explain the increase in birth weight. Increased birth weight could also be caused by retention of fluids and altered glucose or fat metabolism. One could also speculate that because neurofibromin acts as a tumor suppressor protein, a decreased inhibitive effect of neurofibromin in NF1 may also cause uncontrolled growth during the prenatal period. However, no previous studies supporting these explanations are known to exist. Morphometric analyses and studies of body composition of newborns are needed to further evaluate the etiology of the results of the current study. Also, the etiology of decreased birth weight among children of NF1 mothers is unknown, because the effect of mothers' NF1 on birth weight is not explained solely by the decreased maternal weight or height. Altered expression of *NF1* in the uterus could play a role in the infant's decreased birth weight and increased rate of pregnancy complications, but there are no studies, to my knowledge, analyzing the expression of the *NF1* gene in the uterus. Studies have suggested that there is dysregulation of the PI3K/Akt/mTor signaling pathway in endometriosis (McKinnon et al., 2016), and endometriosis is associated with fetal growth restriction, preterm birth and placental abruption (Leone Roberti Maggiore et al., 2017). Further studies should be conducted before any conclusions about the effect of NF1 on the uterus can be made.

Our results on head circumference are in line with the previous studies. While there are reports of increased head circumference during early childhood (Karvonen et al., 2013), this is the first study reporting that the head circumference among NF1 children is increased already at birth. Increased head circumference is thought to be caused by an increase in brain volume. Studies suggest that in the corpus callosum and frontal regions volume of white matter increases, and in posterior regions, grey matter drives the increased brain volume (Greenwood et al., 2005). Skeletal abnormalities are common in NF1 patients (Elefteriou et al., 2009), and these could be caused by defects in bone cells (Cung et al., 2015). This intrinsic bone cell defect may contribute also to an increased head circumference.

6.6 Congenital malformations (IV)

Our study clearly shows that the overall risk for congenital malformations is increased among infants with NF1, but NF1 of the mother does not seem to increase the frequency of the congenital malformations. While there is a large study on cardiovascular malformations in NF1, and a relatively large study on skeletal malformations, no previous information about the overall risk for malformations in NF1 exist. Information about malformations in other organ systems have relied completely on case reports and case series.

We identified malformations of the cardiovascular system in nine children with NF1, which is significantly more than expected. Two of these children had pulmonary valve stenosis. These findings are supported by the previous study by Lin et al. (Lin et al., 2000). They found that both the overall number of cardiovascular malformations and number of pulmonary valve stenosis were significantly increased. Pulmonic valve stenosis is associated with Watson syndrome and patients with microdeletions (Friedman et al., 2002), but we could not confirm if persons with pulmonic valve stenosis in our cohort had one of these subtypes of NF1. Neurofibromin is known to be essential in the development of heart, and *NF1*$^{-/-}$ mice die *in utero* with a double outlet right ventricle. *NF1* is expressed during embryonic development in myocardial and mesenchymal cells of the endocardial cushions, which refer to subset of cells essential for formation of heart septa. It is observed that in *NF1* knockout mice endocardial cushion tissue can be enlarged obstructing blood flow causing venous congestion. This may reflect a series of events causing pulmonary valve stenosis in humans. However, endocardial cushion formation and interactions among different cell types are not completely understood, and the precise molecular mechanism behind heart malformations is unknown (Friedman et al., 2002).

In our study, malformations of the musculoskeletal system were more common among NF1 children than in controls. Malformations included polydactylies, syndactylies and craniosynostosis, but no muscular malformations were found. An increased rate of skeletal malformations have been previously observed in Italy, where polydactylies were also overrepresented (Ruggieri et al., 1999). Neurofibromin is expressed throughout the embryologic development in osteoprogenitor cells and mature osteoblasts, osteoclasts and chondrocytes. The lack of neurofibromin causes disturbed differentiation of osteoblasts and problems of adhesion and migration of osteoclasts, which can explain the musculoskeletal anomalies (Stevenson and Yang, 2011; He et al., 2011). In addition, Shinawi and Patel (2007) speculated about the role that upregulation of SHH plays in neurofibromin-deficient cells in polydactyly, because SHH and its target GLI1 are known to participate in polarization of the limbs during development (Shinawi and Patel, 2007). Craniosynostosis is only rarely reported in

association with NF1, but there is evidence that the frequency of craniosynostosis may be increased in other Rasopathies (Ueda et al., 2017).

In addition to an increased rate of malformations in cardiovascular and musculoskeletal systems, we observed a significantly increased risk for malformations in the urinary system and in the area of the head, face, ear and neck. The medical records of these patients were reviewed to ensure that the patients did not have plexiform neurofibromas located near the affected area. However, MRI was not performed for every patient, and thus the existence of plexiform neurofibromas could not be fully excluded. The number of cases in these groups is rather low, and the results have to be intercepted with some caution.

Only major congenital malformations were included in the analysis, but we could not evaluate the morbidity caused by the malformations. Taking into account the increased rate of overall risk for malformations, it is plausible that also severe anomalies are more common, and a close follow-up is needed when a child is suspected to have NF1. Especially cardiac manifestations should be excluded, and it could be beneficial to perform echocardiography for these persons.

6.7 Strengths

The current study is the first involving a nationwide cohort of patients with a confirmed diagnosis for neurofibromatosis. Many previous studies include only patients who are identified in special clinics, e.g., genetic clinics, which can cause bias towards more complicated cases. We acquired patients from all secondary and tertiary referral centers in mainland Finland, which minimizes this bias. As all patients with neurofibromatosis should be referred to special health care at least for diagnosis and treatment of NF1 complications, our approach should cover patients extensively. Patients in our study were collected independently from the studied outcome variables, i.e., different registers were used to acquire the study cohort and the data used in analyses. When the same registers are used to identify the patients, and collecting the analyzed data, the study cohort is easily biased towards more complicated cases. For example, problems with studies relying only on death registers are highlighted in Chapter 6.3. In addition to the total-population approach of the study, confirming all diagnoses of NF1 is an absolute strength of the study. This was illustrated during the formation of the study cohort, as approximately one-third of patients in the initial cohort were excluded, because they did not fulfill the clinical criteria for NF1. Thus, any study relying only on diagnoses found in the registers should be taken with caution, as a considerable part of the cohort may not fulfill strict clinical criteria for NF1. Only rarely NF1 is diagnosed at birth, which causes difficulties in studying birth-related outcomes of NF1 patients. However, the personal identity code, which is described

in Chapter 4.2.1, provides an opportunity to acquire data retrospectively for patients who are diagnosed for NF1 possibly a decade or more after birth. In addition, the personal identity code allows linking the data between the mother and the child, and subgroups can be formed to separate the effect of mother's and child's NF1 on outcome variables. Administrative registers in Finland are exceptionally extensive and cover information about practically every pregnancy and delivery in Finland. Thus, Finland may be one of few countries where this kind of study would be possible to perform.

6.8 Limitations

As always, the current study has some important limitations. First, the relatively small population in Finland (5,451,270 on December 31st, 2013) limits the number of patients in the study cohort. This is significant especially in subgroup analyses, which includes a rather small number of cases leading to a low statistical power. Second, despite our approach to acquiring patients, cases with more complications may be overrepresented in the study, because they usually have more hospital visits. Third, because patients had to have a neurofibromatosis-associated hospital visit after 1987, younger age groups are overrepresented in our study. Fourth, according to the recent study by Kallionpää et al. (2017), the estimated prevalence of NF1 in Finland is approximately 1:2000 (Kallionpää et al., 2017), which would mean over one thousand missing patients in our cohort. Fifth, due to reforms in the administrative registers, available data varies between study periods. This is especially significant in birth size analyses, as the anthropometrics and gestational diabetes of mother were available only since 2004.

6.9 Future directions

A natural continuation for the study would be to extend the study cohort to increase the power of the study. This could also allow analysis of the frequency of individual congenital malformations. In addition to Finland, other Nordic countries have extensive administrative registers, and these would provide a platform for extending the study cohort. Another direction is to study the long-term morbidity caused by observed complications and malformations. This requires following the study cohort, while analysis of perinatal morbidity is possible to analyze utilizing the current data. Thirdly, analyzing placentas of mothers with NF1, and mothers of NF1 children could reveal background mechanisms for the complications and other alterations dur-

ing pregnancy. Also, analyzing the microenvironment of uterus could provide information about the molecular mechanisms and deepen our understanding about NF1 and pregnancy.

7 CONCLUSIONS

The following main conclusions can be drawn on the basis of this study:

- The incidence of NF1 is approximately 1:2,000, which is higher than previously generally accepted. The incidence of NF2 was in line with previous studies being approximately 1:39,000 in our study. (I)

- NF1 increases mortality significantly. Especially, mortality among NF1 females aged less than 50 is considerable higher than in the general population. (I)

- NF1 of the fetus slightly shortens pregnancy duration. While the shortening is clinically insignificant, it is noteworthy because only little is known about the role that the fetus plays on the timing of the delivery. (II)

- Cesarean section, hypertension during pregnancy/preeclampsia, oligohydramnios, placental abruption, poor fetal growth and maternal care for disproportion were significantly more common among mothers with NF1 than in controls. (II)

- NF1 of the mother decreases birth weight, while NF1 of the fetus increases it. Head circumference at birth is increased by NF1 of the fetus but is not significantly affected by NF1 of the mother. (III)

- The frequency of congenital malformations is increased among NF1 children. Congenital malformations are increased in cardiovascular, musculoskeletal and urinary systems. Also risk for malformations in the group of eye, ear, head and neck was increased. (IV)

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