TURUN YLIOPISTO
UNIVERSITY OF TURKU

MORBIDITY IN NEUROFIBROMATOSIS 1

Epidemiological Perspectives on Breast Cancer and Diabetes

Roope Kallionpää

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
SARJA – SER. D OSA – TOM. 1630 | MEDICA – ODONTOLOGICA | TURKU 2022

TURUN
YLIOPISTO
UNIVERSITY
OF TURKU

MORBIDITY IN NEUROFIBROMATOSIS 1

Epidemiological Perspectives on Breast Cancer and Diabetes

Roope Kallionpää

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
SARJA – SER. D OSA – TOM. 1630 | MEDICA – ODONTOLOGICA | TURKU 2022

University of Turku

Faculty of Medicine
Institute of Biomedicine, Cell Biology and Anatomy, and
Department of Clinical Medicine, Dermatology and Venereal Diseases
Turku Doctoral Programme of Molecular Medicine (TuDMM)
Cancer Research Unit

Supervised by

Professor Juha Peltonen, MD, PhD
Cancer Research Unit
Institute of Biomedicine
University of Turku, Finland

Docent Sirkku Peltonen, MD, PhD
Department of Dermatology
University of Turku, Finland
Professor, Department of Dermatology and Venereology
University of Gothenburg, Sweden

Reviewed by

Professor Arto Mannermaa, PhD
Institute of Clinical Medicine, Pathology and Forensic Medicine
Head, Multidisciplinary Cancer Research Community RC Cancer
University of Eastern Finland
Research Manager, Vice-Head, Biobank of Eastern Finland
Kuopio, Finland

Professor of Surgery (emeritus), Research Professor
Jukka-Pekka Mecklin, MD, PhD
Sport and Health Sciences
University of Jyväskylä and
Central Finland Health Care District
Jyväskylä, Finland

Opponent

Docent Jussi Koivunen, MD, PhD
FICAN North
Department of Oncology and Radiotherapy
Oulu University Hospital
Oulu, Finland

The originality of this publication has been checked in accordance with the University of Turku quality assurance system using the Turnitin OriginalityCheck service.

Cover: Kristian Krokfors, “Trio”, 1997, fragment

ISBN 978-951-29-8854-9 (PRINT)
ISBN 978-951-29-8853-2 (PDF)
ISSN 0355-9483 (Print)
ISSN 2343-3213 (Online)
Painosalama, Turku, Finland 2022

UNIVERSITY OF TURKU
Faculty of Medicine
Institute of Biomedicine, Cell Biology and Anatomy, and Department of Clinical Medicine, Dermatology and Venereal Diseases
ROOPE KALLIONPÄÄ: Morbidity in Neurofibromatosis 1: Epidemiological Perspectives on Breast Cancer and Diabetes
Doctoral Dissertation, 163 pp.
Turku Doctoral Programme of Molecular Medicine (TuDMM)
May 2022

ABSTRACT

Neurofibromatosis type 1 (NF1) is a dominantly inherited rare disorder caused by pathogenic variants of the *NF1* tumor suppressor gene. The syndrome can be diagnosed based on its clinical manifestations, such as café-au-lait macules, skinfold freckling, and benign cutaneous neurofibroma tumors. NF1 affects several organ systems, yet it is best known as a tumor predisposition syndrome.

In this thesis, a Finnish cohort of 1,476 individuals with NF1 was used to study the prevalence of NF1 and the risks for breast cancer and diabetes among individuals with NF1. For a more precise assessment of the risk for contralateral breast cancer in NF1, the Finnish NF1 cohort was analyzed together with four other European NF1 cohorts. Moreover, the effect of NF1 on the concentration of circulating free plasma DNA (cfDNA) was assessed in a small-scale clinical study. Breast cancer diagnoses of individuals with NF1 were obtained from the Finnish Cancer Registry. Diagnoses of diabetes were inferred from drug purchases and hospital visits and hospital stays. The characteristics of NF1-related breast cancer were also analyzed.

The results demonstrate that the overall prevalence of NF1 may be as high as 1/2,052. NF1 is associated with increased mortality throughout the lifetime, and the age-specific prevalence of NF1 declines in older age groups. Women with NF1 face a marked risk for breast cancer, and the risk for being diagnosed with breast cancer is 7.8% by 50 years of age. The survival after NF1-related breast cancer is worse compared to breast cancer in the general population. Breast cancers diagnosed in individuals with NF1 also exhibit poor prognostic factors, such as hormone receptor negativity. Moreover, women with NF1 and breast cancer have a 16% risk for contralateral breast cancer within 20 years. In contrast, the risk for diabetes and type 2 diabetes, in particular, is decreased among individuals with NF1. The NF1 syndrome, as such, may not significantly alter plasma cfDNA concentration.

The results highlight the need for identifying all individuals with NF1 in order to provide them surveillance for NF1-related complications, such as breast cancer. The findings demonstrate the role of the *NF1* gene in the pathogenesis of two common diseases, namely breast cancer and diabetes.

KEYWORDS: breast cancer, circulating free DNA, contralateral breast cancer, diabetes, neurofibromatosis type 1, NF1, prevalence

TURUN YLIOPISTO
Lääketieteellinen tiedekunta
Biolääketieteen laitos, Solubiologia ja anatomia ja
Kliininen laitos, Iho- ja sukupuolitautioppi
ROOPE KALLIONPÄÄ: Sairastavuus tyypin 1 neurofibromatoosissa: epidemiologisia näkökulmia rintasyöpään ja diabetekseen
Väitöskirja, 163 s.
Molekyylilääketieteen tohtoriohjelma (TuDMM)
Toukokuu 2022

TIIVISTELMÄ

Tyypin 1 neurofibromatoosi (NF1) on vallitsevasti periytyvä harvinaissairaus, joka aiheutuu muutoksista *NF1*-kasvunrajoitegeenissä. Sairaus voidaan diagnosoida kliinisten oireiden, kuten ihon maitokahviläiskien, taivealueiden kesakoiden ja hyvänlaatuisten neurofibrooma-ihokasvainten perusteella. NF1 vaikuttaa moniin elinjärjestelmiin, mutta parhaiten se tunnetaan kasvainalttiusoireyhtymänä.

Tässä tutkimuksessa tarkasteltiin NF1:n yleisyyttä sekä potilaiden alttiutta sairastua rintasyöpään ja diabetekseen. Tutkimuksessa käytettiin 1476 henkilön suomalaista NF1-kohorttia. Vastakkaisen rinnan rintasyövän riskiä tutkittaessa hyödynnettiin tietoja myös neljästä muusta eurooppalaisesta NF1-kohortista. Lisäksi tarkasteltiin NF1:n vaikutusta plasman vapaiden nukleiinihappojen pitoisuuteen. NF1-potilaiden rintasyöpädiagnoosit haettiin Suomen Syöpärekisteristä. Diabetes-diagnoosit pääteltiin lääkeostotiedoista sekä sairaalakäynneistä ja -jaksoista. Lisäksi tarkasteltiin NF1:een liittyvän rintasyövän ominaisuuksia.

Tulokset osoittavat, että NF1:n vallitsevuus on jopa 1/2052. NF1 aiheuttaa lisääntynyttä kuolleisuutta kaikissa ikäryhmissä, minkä vuoksi NF1:n vallitsevuus laskee vanhemmissa ikäryhmissä. NF1:een liittyy merkittävä rintasyöpäriski, ja NF1:tä sairastavilla naisilla on 7,8 %:n todennäköisyys sairastua rintasyöpään ennen 50 vuoden ikää. NF1 huonontaa rintasyövän ennustetta, ja NF1-naisten rintasyövät ovat usein hormonireseptorinegatiivisia. Lisäksi rintasyöpään sairastuneilla NF1-naisilla on 16 %:n riski sairastua vastakkaisen rinnan rintasyöpään 20 vuoden kuluessa. Sen sijaan erityisesti tyypin 2 diabeteksen riski on NF1:ssä pienempi kuin vertailuväestössä. NF1-sairaus itsessään ei vaikuta merkittävästi muuttavan plasman vapaiden nukleiinihappojen pitoisuutta.

Tulokset korostavat tarvetta tunnistaa kaikki NF1:tä sairastavat henkilöt, jotta he pääsevät taudin edellyttämän seurannan piiriin. Tulosten perusteella *NF1*-geeni vaikuttaa kahden yleisen sairauden, rintasyövän ja diabeteksen, kehitykseen.

AVAINSANAT: diabetes, NF1, plasman vapaa DNA, prevalenssi, rintasyöpä, tyypin 1 neurofibromatoosi, vastakkaisen rinnan syöpä

Table of Contents

Abbreviations

Akt	Protein kinase B
ATC	Anatomical Therapeutic Chemical classification of drugs
cfDNA	Circulating free DNA
CI	Confidence interval
CMML	Chronic myelomonocytic leukemia
CNS	Central nervous system
CSRD	Cysteine-serine-rich domain
ctDNA	Circulating tumor DNA
ERK	Extracellular signal-regulated kinase
GAP	GTPase activating protein
GEF	Guanine nucleotide exchange factor
GFR	Growth factor receptor
GIST	Gastrointestinal stromal tumor
GRB	Growth factor receptor-bound protein
GRD	GAP-related domain
HER2	Human epidermal growth factor receptor 2
HLA	Human leukocyte antigen
HR	Hazard ratio
ICD-9	International Classification of Diseases, 9th edition
ICD-10	International Classification of Diseases, 10th edition
ICD-O-3	International Classification of Diseases for Oncology, 3rd edition
JMML	Juvenile myelomonocytic leukemia
LADA	Latent autoimmune diabetes of adults
MAPK	Mitogen-activated protein kinase
MEK	Mitogen-activated protein kinase kinase
MPNST	Malignant peripheral nerve sheath tumor
MRI	Magnetic resonance imaging
mTOR	Mammalian target of rapamycin
NF1	Neurofibromatosis type 1
NF2	Neurofibromatosis type 2
NIH	National Institutes of Health

OR	Odds ratio
PDK1	3-Phosphoinositide-dependent protein kinase 1
PET	Positron emission tomography
PH	Pleckstrin homology-like domain
PI3K	Phosphoinositide 3-kinase
PIP_2	Phosphatidylinositol (4,5)-bisphosphate
PIP_3	Phosphatidylinositol (3,4,5)-trisphosphate
PMR	Proportionate mortality ratio
PTEN	Phosphatase and tensin homolog
RaR	Rate ratio
Ras	“Rat sarcoma virus,” a family of small GTPases
RiR	Risk ratio
Rit1	Ras-like without CAAX protein 1
SD	Standard deviation
SIR	Standardized incidence ratio
SMR	Standardized mortality ratio
SHOC-2	Leucine-rich repeat protein SHOC-2
SHP2	Src homology region 2 domain-containing phosphatase-2
SOS1	Son of sevenless 1
SPRED1	Sprouty-related, EVH1 domain-containing protein 1
TBD	Tubulin-binding domain
TCGA	The Cancer Genome Atlas

List of Original Publications

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

I Roope A. Kallionpää, Elina Uusitalo, Jussi Leppävirta, Minna Pöyhönen, Sirkku Peltonen, Juha Peltonen. Prevalence of neurofibromatosis type 1 in the Finnish population. *Genetics in Medicine*, 2018; 20(9): 1082–1086.

II Elina Uusitalo, Roope A. Kallionpää, Samu Kurki, Matti Rantanen, Janne Pitkäniemi, Pauliina Kronqvist, Pirkko Härkönen, Riikka Huovinen, Olli Carpen, Minna Pöyhönen, Sirkku Peltonen, Juha Peltonen. Breast cancer in neurofibromatosis type 1: overrepresentation of unfavourable prognostic factors. *British Journal of Cancer*, 2017; 116: 211–217.

III D. Gareth R. Evans*, Roope A. Kallionpää*, Maurizio Clementi, Eva Trevisson, Victor-Felix Mautner, Sacha J. Howell, Lauren Lewis, Ouidad Zehou, Sirkku Peltonen, Antonella Brunello, Elaine F. Harkness, Pierre Wolkenstein, Juha Peltonen. Breast cancer in neurofibromatosis 1: survival and risk of contralateral breast cancer in a five country cohort study. *Genetics in Medicine*, 2020; 22(2): 398–406.

IV Roope A. Kallionpää, Sirkku Peltonen, Jussi Leppävirta, Minna Pöyhönen, Kari Auranen, Hannu Järveläinen, Juha Peltonen. Haploinsufficiency of the *NF1* gene is associated with protection against diabetes. *Journal of Medical Genetics*, 2021; 58(6): 378–384.

V Roope A. Kallionpää, Kaisa Ahramo, Marianna Aaltonen, Paula Pennanen, Juha Peltonen, Sirkku Peltonen. Circulating free DNA in the plasma of individuals with neurofibromatosis type 1. *American Journal of Medical Genetics Part A*, 2021; 185(4): 1098–1104.

* Equal contribution

1 Introduction

Neurofibromatosis type 1 (NF1) is a hereditary syndrome caused by pathogenic variants in the *NF1* gene encoding neurofibromin protein (Wallace et al., 1990; Xu et al., 1990; Gutmann et al., 2017; Koczkowska et al., 2020). The NF1 syndrome can be diagnosed based on its clinical characteristics (Legius et al., 2021), and a clinically diagnosed NF1 syndrome is a specific sign of an individual harboring a pathogenic variant in the *NF1* gene. The NF1 syndrome thus serves as a model of the consequences of a deficiency in functional neurofibromin. Since NF1 is one of the most common rare diseases, it affects a high number of patients worldwide. Given a birth incidence of 1/3,000–1/2,000 (Uusitalo et al., 2015), the annual number of children born with NF1 is 1,400–2,100 in the European Union and 42,000–63,000 globally. Research on NF1 can therefore potentially influence the treatment of a large number of patients. From a research point of view, the high frequency of NF1 means that it is possible to study relatively large cohorts of individuals with NF1.

The earliest signs of the NF1 syndrome are pigmentary findings on the skin, such as café-au-lait macules that may be present already at birth (DeBella et al., 2000; Morris et al., 2021). Family history or multiple café-au-lait macules may prompt a clinical suspicion of NF1 before the fulfillment of the diagnostic criteria, and sequencing of the *NF1* gene may confirm the diagnosis in such cases. Disease manifestations frequently seen during childhood include plexiform neurofibromas, optic pathway tumors, skeletal abnormalities, and cognitive deficits (Ferner et al., 2007; Gutmann et al., 2017). The hallmark tumors of NF1, cutaneous neurofibromas, typically start to grow during the puberty (DeBella et al., 2000). Puberty is also associated with an increase in the risk for malignancies. Malignant peripheral nerve sheath tumors (MPNSTs) are particularly frequent among individuals with NF1 after 20 years of age, and individuals with NF1 are also predisposed to various other tumors throughout their life (Uusitalo et al., 2016). Health issues associated with NF1 also include, for example, cardiovascular disease, osteoporosis, and dementia (Uusitalo et al., 2015; Kenborg et al., 2020; Ottenhoff et al., 2020; Kallionpää et al., 2021a). NF1 syndrome is associated with marked excess mortality (Rasmussen et al., 2001; Masocco et al., 2011; Duong et al., 2011; Wilding et al., 2012; Uusitalo et al., 2015). Because of the risk for multiple life-threatening co-morbidities,

individuals with NF1 need regular surveillance for the timely detection of any new complications. For instance, cancer can, in general, only be cured if diagnosed at a non-metastatic stage.

This thesis consists of five original studies, where the prevalence of NF1, the risks for diabetes, breast cancer and contralateral breast cancer in NF1, and circulating free plasma DNA among individuals with NF1 are examined. Four of the studies are epidemiological and based on information linked from various nationwide registers, and one is a clinical study examining blood samples drawn from volunteers with and without NF1. Diabetes and breast cancer are common also in the general population, and lessons learned by studying NF1 can contribute to the development of better therapies. On the other hand, if NF1 alters the risk of a common disease, the absolute change is greater than in the case of rare health issues.

In this thesis, epidemiological research serves two purposes. First, it can reveal disease associations and characteristics pertinent to the treatment of patients. Thus, the research can lead to the monitoring of relevant symptoms and improved patient care, health, and quality of life. Second, epidemiological research can reveal disease mechanisms and biological phenomena that are behind the disease phenotype. Epidemiological research can therefore help to reveal concepts relevant to basic science and point out questions that should be addressed using biochemical and cell biological experiments. Importantly, breast cancer and diabetes have shared cell signaling pathways involved in their pathogenesis. Studies on the prevalence of NF1 and the associated risks for breast cancer and diabetes advise surveillance, yet they also show that the *NF1* gene is an important driver of breast cancer development and can affect the pathogenesis of diabetes.

2 Review of the Literature

2.1 Neurofibromatosis type 1 (NF1)

Neurofibromatosis type 1 (NF1) is a monogenic, dominantly inherited multi-organ syndrome (Gutmann et al., 2017). The NF1 syndrome is caused by pathogenic variants in the *NF1* gene, which is located in chromosome 17 (Wallace et al., 1990; Xu et al., 1990; Koczkowska et al., 2020). The classical NF1 is caused by a germline pathogenic *NF1* variant. The variant originates from either a maternal or a paternal gamete, and the variant is therefore present in all diploid cells of an individual and can be passed to the offspring. While many of the individuals with NF1 have inherited the disorder, at least half have a *de novo* pathogenic variant and no parent with the NF1 syndrome (Huson et al., 1989; Poyhonen et al., 2000; Evans et al., 2010).

Mutations of the *NF1* gene can also occur after fertilization, during gestation, or at any age during the lifespan. Mosaic NF1 is due to an *NF1* mutation occurring during embryonic or fetal development, which leads to *NF1* haploinsufficiency in a subset of cells and causes segmental disease manifestations. The mutation may or may not be present in the germ cells, and the germline involvement determines the risk of passing the condition to children. Mutations that are not present in the germ cells are termed somatic and, when occurring in adults, they typically only affect a single cell population. The accumulation of somatic mutations is an inherent part of cancer development.

2.1.1 Diagnosis of NF1

The diagnosis of NF1 is based on the clinical diagnostic criteria first established in the National Institutes of Health (NIH) consensus conference in 1987 (National Institutes of Health Consensus Development Conference, 1988) and subsequently updated in 2021 (Legius et al., 2021). The diagnostic criteria aim at sensitivity and specificity, which requires that the manifestations included in the criteria are common among individuals with NF1, yet the manifestations also need to be rare or absent among individuals without NF1. Thus, even if a disease manifestation is common among individuals with NF1 and strongly associated with NF1, it cannot

Table 1. The diagnostic criteria of neurofibromatosis type 1 (NF1). The diagnosis of NF1 can be set if two of the criteria are met.

1987 CRITERIA[a]	2021 CRITERIA[b]
Six or more café-au-lait macules >5 mm in greatest diameter in prepubertal individuals and >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 region	
Optic pathway glioma	
Two or more Lisch nodules	Two or more iris Lisch nodules, or two or more choroidal abnormalities
A distinctive osseous lesion, such as sphenoid dysplasia or thinning of long bone cortex, with or without pseudarthrosis	A distinctive osseous lesion such as sphenoid dysplasia, anterolateral bowing of the tibia, or pseudarthrosis of a long bone
	A heterozygous pathogenic *NF1* variant with a variant allele fraction of 50% in apparently normal tissue
A first-degree relative with NF1 by the above criteria	A parent with NF1 by the above criteria

[a] National Institutes of Health Consensus Development Conference (1988); [b] Legius et al. (2021)

be included as a diagnostic criterion if it is frequent also in the general population. This is the case of, for example, cardiovascular disease, which seems to be associated with NF1 (Uusitalo et al., 2015; Kenborg et al., 2020) but is not specific to NF1.

The 1987 diagnostic criteria of NF1 list seven criteria, two of which are required for the diagnosis of NF1 (Table 1). The diagnosis can often be reached based on cutaneous or ophthalmological findings, and approximately 95% of individuals with NF1 fulfill the 1987 diagnostic criteria by the age of six years (DeBella et al., 2000). After the establishment of the 1987 diagnostic criteria, gene sequencing has become commonly available. Consequently, it is often possible to detect a pathogenic variant of the *NF1* gene to confirm the diagnosis. Moreover, sequencing has revealed that some of the individuals with only pigmentary features actually have another hereditary condition, the Legius syndrome, caused by pathogenic germline variants of the *SPRED1* gene (Brems et al., 2007). Therefore, the diagnostic criteria for NF1 were revised in 2021 and the detection of a pathogenic heterozygous *NF1* variant present in all cells was included as a new criterion (Table 1; Legius et al., 2021). Moreover, at least two choroidal abnormalities are included in the ophthalmological criterion in addition to the Lisch nodules. The osseous lesions, which are taken into account in the diagnosis, were revised as sphenoid dysplasia, anterolateral bowing of the tibia or pseudarthrosis of a long bone. According to the new criteria, any first-

degree relative with NF1 is not adequate for establishing the NF1 diagnosis, but only the parents' NF1 status is considered to facilitate the differentiation of NF1 from the congenital mismatch repair deficiency syndrome and mosaic NF1.

The pigmentary cutaneous findings associated with NF1 include the hyperpigmented café-au-lait macules and axillary or inguinal freckling (Legius et al., 2021). Café-au-lait macules are present in the vast majority of individuals with NF1 (Easton et al., 1993; DeBella et al., 2000; Ferner et al., 2007; Duong et al., 2011; Morris et al., 2021). Since café-au-lait macules are typically visible by the age of one year, they are often the first disease manifestation to occur (DeBella et al., 2000; Morris et al., 2021). The presence of at least six café-au-lait macules in an otherwise asymptomatic small child is a strong predictor of having the NF1 syndrome even in the absence of a family history of NF1 (Ben-Shachar et al., 2017). The café-au-lait macules associated with NF1 arise from the somatic inactivation of the *NF1* gene in melanocytes typically due to a second-hit mutation (De Schepper et al., 2008).

Neurofibromas are the hallmark tumor of NF1. They arise in close association with peripheral nerve tributaries (Jouhilahti et al., 2011). All neurofibromas harbor a clonal Schwann cell population with a somatic second-hit mutation in the *NF1* gene (Maertens et al., 2006a). Neurofibromas also contain multiple other cell types, such as fibroblasts, mast cells, and perineurial cells, and an abundant collagen matrix (Peltonen et al., 1988; Jouhilahti et al., 2011; Kallionpää et al., 2021b). Cutaneous neurofibromas usually start to grow during puberty and occur in most adults with NF1 (Easton et al., 1993; DeBella et al., 2000; Szudek et al., 2000; Ferner et al., 2007; Duong et al., 2011; Morris et al., 2021). Cutaneous neurofibromas may grow up to a diameter of a few centimeters, after which they cease their growth, and they never turn malignant (Jouhilahti et al., 2011). Despite their benign nature, cutaneous neurofibromas cause disfigurement and thereby are a major psychological burden to the affected individuals (Kodra et al., 2009; Granström et al., 2012). Moreover, the tumors may be itchy or painful (Riccardi, 1993; Ortonne et al., 2018; Stewart et al., 2018). In addition to cutaneous neurofibromas, individuals with NF1 may develop subcutaneous or plexiform neurofibromas. Plexiform neurofibromas have been estimated to occur in 20–50% of individuals with NF1 (Easton et al., 1993; Szudek et al., 2000; Ferner et al., 2007; Duong et al., 2011; Morris et al., 2021). A plexiform neurofibroma can undergo a transformation into an MPNST (Ferner et al., 2007; Stewart et al., 2018). Nerve compression caused by a benign plexiform neurofibroma may cause disability, and the tumor may be difficult to biopsy and impossible to excise due to intratumoral heterogeneity and the risk for neural damage (Gutmann et al., 2017).

Lisch nodules and choroidal abnormalities are the typical ophthalmologic findings in NF1 (Ferner et al., 2007; Duong et al., 2011). Lisch nodules can be identified by a slit-lamp examination. Moreover, optic pathway gliomas are present

in 12–20% of individuals with NF1 (Listernick et al., 1994; Ferner et al., 2007; Rosenfeld et al., 2010; Cecen et al., 2011; Friedrich and Nuding, 2016; Morris et al., 2021). These tumors typically develop during early childhood and may interfere with the development of vision or cause precocious puberty, yet many of them are asymptomatic and have a good prognosis (Listernick et al., 1994; Guillamo et al., 2003; Ferner et al., 2007; Listernick et al., 2007; Friedrich and Nuding, 2016). Optic pathway gliomas in NF1 are reviewed in more detail below along with other central nervous system (CNS) tumors (Section 2.2.6).

NF1 also affects bone health. Individuals with NF1 are at an increased risk for osteoporosis (Kuorilehto et al., 2005; Elefteriou et al., 2009; Heervä et al., 2012, 2013), and *NF1*-haploinsufficient osteoclasts show increased resorption capacity (Heervä et al., 2010). Individuals with NF1 also show an increased frequency of scoliosis (Elefteriou et al., 2009; Kenborg et al., 2020). Particularly typical of NF1 is dystrophic scoliosis that manifests early, progresses rapidly, and requires surgical intervention (Elefteriou et al., 2009; Gutmann et al., 2017). The diagnostic criteria of NF1 specifically mention two bone manifestations being sphenoid wing dysplasia not related to a plexiform neurofibroma and anterolateral bowing of the tibia (National Institutes of Health Consensus Development Conference, 1988; Legius et al., 2021). The anterolateral bowing of the tibia may lead to pseudarthrosis, yet pseudarthrosis is not required for fulfilling the criterion for a NF1 diagnosis (Legius et al., 2021).

Sequencing of the *NF1* gene has proven difficult due to its large size and the several pseudogenes of *NF1* (Messiaen et al., 2000; Uusitalo et al., 2014). Moreover, the pathogenic variant underlying the disease phenotype may be located outside the coding regions of the *NF1* gene. Methods based on the analysis of mRNA have yielded the highest detection rates (95–96%) of pathogenic variants of the *NF1* gene (Messiaen et al., 2000; Evans et al., 2016). Nevertheless, the failure to identify a pathogenic variant in the *NF1* gene is not sufficient to exclude NF1 when the clinical criteria of NF1 are met (Miller et al., 2019). Genetic testing for NF1 is particularly useful in the case of small children, because sequencing may confirm the diagnosis in children with café-au-lait macules only (Evans et al., 2016; Ben-Shachar et al., 2017; Miller et al., 2019). In familial cases, the inheritance of NF1 can be confirmed or excluded by the targeted sequencing of the parental variant locus. Genetic testing may also aid genetic counseling in the case of variants with a known correlation with phenotype (Section 2.1.3), although identifying the causative variant is usually unnecessary for establishing the NF1 diagnosis in adults (Bergqvist et al., 2020).

2.1.2 Incidence and prevalence of NF1

Highly varying estimates of the incidence and prevalence of NF1 have been published over the decades. However, it is generally accepted that NF1 is a rare disease, that is, has an overall prevalence less than 1/2,000, and that NF1 is one of the most common rare diseases. Since NF1 is a genetic condition with full penetrance (Huson et al., 1989; McGaughran et al., 1999; DeBella et al., 2000), the incidence of NF1 always reflects the number of children born with NF1. The incidence may be modulated by societal factors, such as the social consequences of disfigurement caused by NF1, and the availability and use of prenatal diagnostics in families with NF1. While it has been estimated that approximately half of the individuals with NF1 have inherited the disorder (Huson et al., 1989; Poyhonen et al., 2000; Evans et al., 2010), prenatal screening may decrease the proportion of familial variants. The incidence of NF1 sets the highest possible prevalence of NF1, that is, the birth incidence equals prevalence among newborns. Given the increased mortality associated with NF1 (Rasmussen et al., 2001; Duong et al., 2011; Masocco et al., 2011; Wilding et al., 2012; Uusitalo et al., 2015), the age-specific prevalence of NF1 likely declines in older age groups.

Many studies on the incidence and prevalence of NF1 are based on searching the affected individuals from hospital registries covering a defined population. Such settings allow studying a large population, yet they necessarily rely on medical records and may be biased to identify those with the most severe disease manifestations or those with some kind of need for medical attention. Studies based on medical genetics clinics have often information including first-degree relatives, and in some studies, the authors have also contacted and examined the relatives of the affected individuals identified using medical records (Huson et al., 1989; Clementi et al., 1990; Poyhonen et al., 2000). Extending the cohorts to family members and relatives reduces the risk for bias, yet such approaches may still lead to the under-representation of variants associated with a mild disease phenotype.

Evans and co-workers (2010) used data from a genetic register service and found an overall prevalence of 1/4,560 and incidence of 1/2,712 for NF1 in North West England. Both Huson and co-workers (1989) and Poyhonen and colleagues (2000) extended their cohorts by contacting and examining the relatives of the affected individuals. They estimated the overall prevalence of NF1 as 1/4,150 and 1/4,436, respectively. The incidence estimates obtained in the two studies were 1/2,558 and 1/2,703. These studies also confirmed the Mendelian dominant mode of inheritance of NF1 by showing that approximately half of the children of affected parents had NF1 (Huson et al., 1989; Poyhonen et al., 2000; Evans et al., 2010). Clementi and co-workers (1990) also attempted to contact the first-degree relatives of probands seen in a genetic counselling service, yet they found a markedly lower overall prevalence of 1/6,711, and a prevalence of 1/4,292 among children aged 0–9 years.

Another study focused on individuals younger than 16 years and found a prevalence of 1/5,681 (McKeever et al., 2008).

In contrast to the studies using hospital registers to identify families affected by NF1, a German study gathered information from preschool examinations covering all six-year-old children in certain federal states in Germany (Lammert et al., 2005). The authors did not find a markedly higher prevalence of NF1 than those reported in the hospital-based studies, as they ended up with an estimated prevalence of 1/2,996. However, only cutaneous pigmentary manifestations, osseous lesions, and family history were considered as signs of NF1, because the study focused on small children. It is therefore possible that not all affected individuals had developed sufficiently marked disease manifestations, even though most children aged six are known to fulfill the diagnostic criteria for NF1 (DeBella et al., 2000). Another school-based, cross-sectional study found a prevalence of 1/1,141 among children aged 9–11 in a Cuban province (Orraca et al., 2014). In this study, the children were older than in the German study with a similar setting, which may partly explain the higher prevalence estimate.

Two cross-sectional studies of defined age-cohorts have been based on pre-military medical examinations. An Italian study encompassing 21,181 18-year-old males found an age-specific prevalence of 1/1,513 for NF1 (Fazii et al., 1998). In a cohort of 374,440 17-year-old military recruits in Israel, 390 individuals with NF1 were found corresponding to a prevalence of 1/960 (Garty et al., 1994). The study settings allowed identification of individuals with even mild disease manifestations as compared with hospital-based ascertainment. All individuals with NF1 fulfill the diagnostic criteria by the age of 20 years (DeBella et al., 2000), so the study populations of Fazii and co-workers (1998) and Garty and co-workers (1994) were old enough to have visible manifestations of NF1. Moreover, the cohorts were young enough to avoid a significant level of an excess in mortality because of NF1.

2.1.3 The molecular biology underlying NF1

Individuals with NF1 always have a pathogenic germline variant of one allele of the causative gene, *NF1*, while the other allele is intact (Wallace et al., 1990; Xu et al., 1990; Koczkowska et al., 2020). A murine model has demonstrated that the biallelic loss of *NF1* in all cells is lethal (Jacks et al., 1994). While most pathogenic alterations of *NF1* are intragenic, NF1 is caused by microdeletions encompassing the whole *NF1* gene in approximately 5% of the affected individuals (Cnossen et al., 1997). The *NF1* gene is large with 280 kilobases and 57 constitutive exons and so is the neurofibromin protein of 2,818 amino acids (Wallace et al., 1990; Marchuk et al., 1991). Neurofibromin dimerizes within cells, and inactivation of one monomer has

been suggested to impair the activity of the whole dimer (Carnes et al., 2019; Sherekar et al., 2020).

Neurofibromin is a large protein that has multiple protein-protein interactions (Scheffzek and Shivalingaiah, 2019). The best known and characterized function of neurofibromin is its role as a Ras-GTPase activating protein (GAP) (Martin et al., 1990). The Ras proteins, named after the "Rat sarcoma virus" and encoded by *NRAS*, *HRAS*, and *KRAS* genes, are active when bound to GTP and inactive when bound to GDP (Simanshu et al., 2017). The conversion of the inactive Ras-GDP to active Ras-GTP is mediated by guanine nucleotide exchange factor (GEF) proteins, such as Son of sevenless 1 (SOS1) in response to an activating signal from a receptor tyrosine kinase (Figure 1). Ras proteins have intrinsic GTPase activity converting the active Ras-GTP back into Ras-GDP, yet the conversion rate is slow in the absence of GAPs (Simanshu et al., 2017). Neurofibromin is a GAP that accelerates the conversion of Ras-GTP to Ras-GDP and thus downregulates Ras function (Martin et al., 1990). Activated Ras further activates downstream signaling molecules, such as the mitogen-activated protein kinase (MAPK) cascade consisting of Raf, mitogen-activated protein kinase kinase (MEK), and extracellular signal-regulated kinase (ERK) proteins; and also the phosphoinositide 3-kinase (PI3K) – protein kinase B (Akt) – mammalian target of rapamycin (mTOR) pathway (Figure 1). These signaling pathways lead to cell proliferation, migration, and survival and are often hyperactivated in cancer (Krauthammer et al., 2015; Simanshu et al., 2017).

In addition to its GAP-related domain (GRD), neurofibromin also has a tubulin-binding domain (TBD); a cysteine-serine-rich domain (CSRD); a Sec14 domain; a pleckstrin homology-like (PH) domain; and a carboxy-terminal domain interacting with proteins, such as focal adhesion kinase and syndecan (Ratner and Miller, 2015). The GRD interacts not only with Ras-GTPase but also with the Sprouty-related, EVH1 domain-containing protein 1 (SPRED1), whose pathogenic variants underlie the Legius syndrome (Dunzendorfer-Matt et al., 2016). The SPRED1 protein facilitates the localization of neurofibromin on the cell membrane, which is required for interaction with Ras (Dunzendorfer-Matt et al., 2016).

The *NF1* gene is large, and the deficiency in functional neurofibromin may be caused by variants affecting different parts of the gene. Consequently, over 3,000 different pathogenic germline *NF1* variants have been observed and only few correlations between the *NF1* genotype and disease manifestations have been established (Koczkowska et al., 2020). Individuals with pathogenic missense variants at codons 844–848, arginine 1276, or lysine 1423 generally have more serious disease manifestations than individuals with NF1 on average (Koczkowska et al., 2018, 2020). Especially plexiform and spinal neurofibromas are frequent among individuals harboring these germline variants, and the risk for malignancies may also be increased. Codons 844–848 are located within the CSRD, while residues

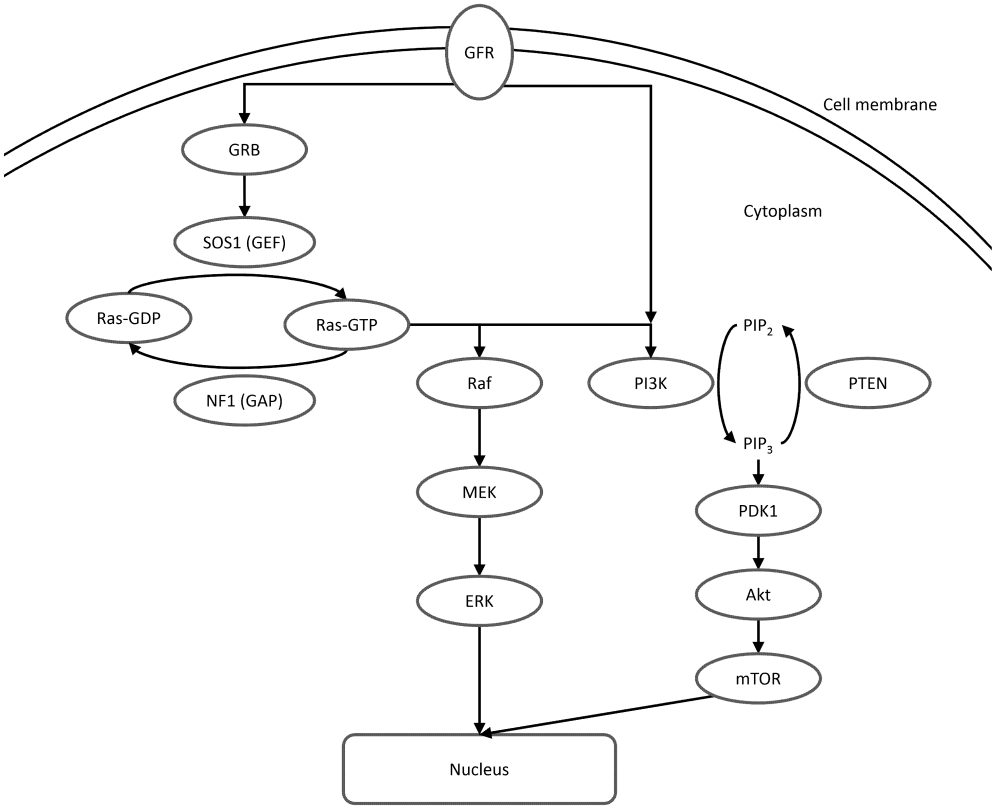

Figure 1. A schematic view of the classical Ras signaling pathway. Activation of a growth factor receptor (GFR) by an extracellular ligand transmits a signal through the cell membrane. Growth factor receptor-bound protein (GRB) and Son of sevenless 1 (SOS1) facilitate the conversion of inactive Ras-GDP to active Ras-GTP. Neurofibromin (NF1) activates the intrinsic GTPase activity of the Ras proteins thus driving the conversion of Ras-GTP back into inactive Ras-GDP. Thus, SOS1 is a guanine nucleotide exchange factor (GEF) and NF1 is a GTPase activating protein (GAP). Activated Ras can activate the mitogen-activated protein kinase (MAPK) pathway including Raf, mitogen-activated protein kinase kinase (MEK), and the extracellular signal-regulated kinase (ERK) proteins as well as the phosphoinositide 3-kinase (PI3K). PI3K converts phosphatidylinositol (4,5)-bisphosphate (PIP_2) to phosphatidylinositol (3,4,5)-trisphosphate (PIP_3), which leads to activation of 3-phosphoinositide-dependent protein kinase 1 (PDK1), protein kinase B (Akt), and the mammalian target of rapamycin (mTOR). The effects of PI3K activation are downregulated by the phosphatase and tensin homolog (PTEN). Activation of the Ras downstream pathways ultimately results in altered transcription and typically drives cell survival, proliferation, and migration. The figure is simplified and, for example, the feedback loops regulating pathway activation (Klinger et al., 2013) and membrane-anchoring of Ras have been omitted. The figure is based on Oda et al. (2005), Aoki et al. (2016), and Peltonen et al. (2017).

1276 and 1423 are part of the GRD. Microdeletions of *NF1* encompassing the whole gene and its flanking regions also cause severe disease manifestations, such as an increased risk for MPNSTs (De Raedt et al., 2003). On the other hand, the in-frame

deletion of methionine 992 in the CSRD and the missense variants of methionine 1149 in the TBD and arginine 1809 in the PH domain are associated with a phenotype characterized by the lack of externally visible plexiform neurofibromas and few to no cutaneous neurofibromas (Upadhyaya et al., 2007; Rojnueangnit et al., 2015; Koczkowska et al., 2020). Studies exploring the correlation of disease manifestations between family members with NF1 have also identified a genetic component unlinked to the *NF1* gene affecting at least some disease manifestations such as the number of café-au-lait macules (Easton et al., 1993; Szudek et al., 2002). Despite the apparent contribution of modifier genes on NF1 phenotype, only few such genes have been identified (Sung et al., 2020; Harder, 2022).

2.1.4 RASopathies

NF1 belongs to a group of disorders called RASopathies. These disorders have shared etiology of germline variants leading to Ras/MAPK pathway hyperactivation (Rauen, 2013; Tajan et al., 2018). The Ras/MAPK activation may either be caused by a loss of inhibition as in NF1 and the Legius syndrome or by activating variants like in the Noonan syndrome (Tajan et al., 2018). RASopathies have shared clinical manifestations, such as craniofacial dysmorphology; neurocognitive impairment; tumor predisposition; and cardiovascular, musculoskeletal, ocular, and cutaneous abnormalities (Rauen, 2013; Aoki et al., 2016; Tajan et al., 2018).

In analogy to NF1, the Legius syndrome is caused by a single gene, *SPRED1* (Brems et al., 2007). Legius syndrome is characterized by the pigmentary findings also seen in NF1, that is, café-au-lait macules and skinfold freckling (Brems et al., 2007). However, the Legius syndrome is not associated with the tumors seen in NF1 (Brems et al., 2007; Messiaen et al., 2009). In contrast, many of the RASopathies can be caused by variants occurring in several different genes, and different variants of a gene may be associated with different phenotypes (Table 2). For example, the Noonan syndrome is caused by pathogenic variants of the *PTPN11* gene in approximately half of the affected individuals, yet similar clinical manifestations may also be due to variants of *SOS1*, *RAF1*, *BRAF*, *KRAS*, *NRAS*, *RRAS*, *RIT1*, *SHOC2*, and *CBL* (Rauen, 2013; Tajan et al., 2018). Given the variation in the underlying genes, Noonan syndrome associated with the *SHOC2* gene may also be considered as a separate condition called the Noonan syndrome-like disorder with loose anagen hair, and *CBL* variants may cause the Noonan syndrome-like disorder (Aoki et al., 2016).

In addition to the actual RASopathies, neurofibromatosis type 2 (NF2) is often mentioned together with NF1. This is mostly due to historical reasons and confusion regarding the genetic etiology of the two conditions before the identification of the disease-causing genes. NF2 is caused by pathogenic variants of the *NF2* gene,

Table 2. A selection of RASopathies and their causative genes and proteins. The table is based on the reviews of Rauen (2013), Aoki et al. (2016), and Tajan et al. (2018).

DISORDER	GENE / PROTEIN	ETIOLOGY
Capillary malformation–arteriovenous malformation syndrome	*RASA1* / p120-RasGAP	Impairment of Ras-GAP function
Cardio-facio-cutaneous syndrome	*BRAF* / B-Raf	Increased kinase activity
	MAP2K1 / MEK1	Increased kinase activity
	MAPK2K2 / MEK2	Increased kinase activity
	KRAS / K-Ras	Impairment of Ras-GTPase activity or binding of guanine nucleotides
Costello syndrome	*HRAS* / H-Ras	Impairment of Ras-GTPase activity or binding of guanine nucleotides
Legius syndrome	*SPRED1* / SPRED1	Impaired downregulation of Raf phosphorylation by Ras; impaired interaction of neurofibromin and Ras
Neurofibromatosis type 1	*NF1* / neurofibromin	Impairment of Ras-GAP and other functions
Noonan syndrome	*PTPN11* / SHP2	Dysregulated tyrosine phosphatase activity
	SOS1 / SOS1	Extensive Ras-GEF function
	RAF1 / C-Raf	Increased activity or increased dimerization
	KRAS / K-Ras	Impairment of Ras-GTPase activity or binding of guanine nucleotides
	NRAS / N-Ras	Impairment of Ras-GTPase activity
	RIT1 / Rit1	Activation of a Ras-like protein
	BRAF / B-Raf	Increased kinase activity
	RRAS / R-Ras	Increased Ras signaling
	SHOC2 / SHOC-2	Impaired dephosphorylation of C-Raf
	CBL / E3 ubiquitin-protein ligase CBL	Impaired receptor tyrosine kinase degradation
Noonan syndrome with multiple lentigines, i.e., LEOPARD syndrome	*PTPN11* / SHP2	Reduced activity of protein tyrosine phosphatase
	RAF1 / C-Raf	Increased activity

GAP: GTPase activating protein; GEF: guanine nucleotide exchange factor; MEK: mitogen-activated protein kinase kinase; Rit1: Ras-like without CAAX protein 1; SHOC-2: leucine-rich repeat protein SHOC-2; SHP2: Src homology region 2 domain-containing phosphatase-2; SOS1: Son of sevenless 1; SPRED1: Sprouty-related, EVH1 domain-containing protein

encoding protein Merlin (Rouleau et al., 1993). While Merlin has several functions, it primarily acts as a part of the Hippo signaling pathway (Zheng and Pan, 2019), and NF2 is therefore usually not considered as a RASopathy (Rauen, 2013; Aoki et al., 2016; Tajan et al., 2018). Like NF1, NF2 predisposes the affected individuals to various tumors, i.e., vestibular and other schwannomas, meningiomas, and ependymomas (Asthagiri et al., 2009). The NF2 syndrome is also associated with, for example, cataracts and peripheral neuropathy, and the syndrome causes increased mortality (Asthagiri et al., 2009; Forde et al., 2021).

2.2 Cancer in NF1

2.2.1 Cancer in general

Cancer is characterized by uncontrolled cell proliferation. The growth, invasion, and metastasis of neoplastic cells eventually interferes with the normal function of vital organs and, when untreated or incurable, leads to death. Based on the data from the Finnish Cancer Registry, a total of 35,327 new cancers were diagnosed in Finland in 2019, and the mean 5-year survival after cancer diagnosis was 68–70% during 2017–2019 (Pitkäniemi et al., 2021). In the 5-year period of 2015–2019, the lifetime risk for cancer was 36–38%, and the lifetime risk for cancer-related death was 18–21% in Finland. The most common cancer type was breast cancer among women and prostate cancer among men. Breast cancer represents approximately 13% of all cancers and 28% of female cancers in Europe (Dyba et al., 2021).

From a cell biology perspective, cancer cells are self-sufficient in growth-inducing signals, insensitive to growth-inhibiting and apoptotic signals, can replicate indefinitely and evade immune eradication, are able to drive angiogenesis, can reprogram their energy metabolism to support growth, and can invade neighboring tissues and metastasize to distant sites (Hanahan and Weinberg, 2000, 2011). The mechanisms enabling these capabilities include, for example, production of pro-angiogenic agents and growth factors for autocrine signaling, receptor overexpression, loss of tumor suppressor proteins, and activating mutations in signaling pathways leading to cell growth and proliferation. The development of cancer is an evolutionary process where cells capable to prosper are enriched. Random mutations may or may not be beneficial for the cells, yet those supportive of cell growth become prevalent in the cell population (Hanahan and Weinberg, 2011). The selective pressure induced by treatment may favor new traits, such as a resistance to chemotherapeutic agents or independence from a certain signaling pathway.

2.2.2 Breast cancer in general

Despite the hallmark features of cancer described above, the detailed clinical and molecular characteristics of cancers are highly heterogeneous. Not only do the cancers at different sites differ, but also the tumors occurring in similar locations may show variable characteristics. For example, breast cancer can be classified in several ways. Histologically, breast cancers have long been grouped based on the structures-of-origin with the most common type of breast cancer being ductal carcinoma, followed by luminal tumors, and other special types (Azzopardi et al., 1982). The luminal type is associated with a more favorable prognosis and a higher proportion of hormone receptor-positive cancers than ductal carcinomas (Cristofanilli et al., 2005). The division of breast cancers to hormone receptor (estrogen/progesterone receptor)-positive, human epidermal growth factor receptor 2 (HER2)-positive, and triple-negative tumors is therapeutically important (Allison et al., 2020; Burstein et al., 2021). Breast cancers expressing estrogen receptor are treated with anti-estrogen therapy, such as tamoxifen that is a selective estrogen receptor modulator. The amplification of the *ERBB2* gene encoding the HER2 protein can be targeted with, for example, trastuzumab, an anti-HER2 antibody (Wang and Xu, 2019).

Studies of gene expression patterns have further led to the classification of breast cancers into luminal A, luminal B, HER2-amplified, and basal cancers (Perou et al., 2000; Sørlie et al., 2001). The luminal A subtype is associated with the most favorable prognosis, while the basal subtype results in poorer survival (Sørlie et al., 2001; Spitale et al., 2009). Although the subtypes have been established based on a large-scale gene expression analysis, the subtypes can also be differentiated based on immunohistochemical markers such as the estrogen and progesterone receptors, *ERBB2* amplification, cytokeratins, and the proliferation biomarker Ki-67 (Perou et al., 2000; Sørlie et al., 2001; Spitale et al., 2009; Burstein et al., 2021).

The genetic background of a breast cancer can mold its phenotype. For example, breast cancers occurring in carriers of the pathogenic germline variants of *BRCA1* are more likely to be negative for hormone receptors and show more often basal-like features than sporadic breast cancers (Foulkes et al., 2004; Lakhani et al., 2005). The pathogenic variants of *BRCA1* are associated with a 50–80% lifetime risk for breast cancer (King et al., 2003; Fackenthal and Olopade, 2007). Pathogenic germline variants of the *TP53* tumor suppressor gene causing the Li-Fraumeni cancer predisposition syndrome are, in turn, associated with an increased likelihood of *ERBB2* amplifications (Wilson et al., 2010; Masciari et al., 2012). In addition to the genetic contributors, also lifestyle and environmental factors, such as obesity, lack of physical activity, and parity, can affect breast cancer risk and cancer subtype (Barnard et al., 2015; Rojas and Stuckey, 2016). Diabetes is also a risk factor of breast cancer, yet the association is partly linked to obesity (Boyle et al., 2012).

2.2.3 Overall cancer risk in NF1

NF1 is notorious for being a tumor-predisposition syndrome. In addition to the benign hallmark tumors, cutaneous and plexiform neurofibromas, NF1 is associated with a high risk for malignancies. The overall cancer risk among individuals with NF1 has been estimated to be 2.5–9.5-fold compared to the general population (Table 3). The risk estimates vary from study to study, which may be related to the methods of ascertainment of individuals with NF1, tracking of cancer diagnoses, and cohort age structure. Importantly, studies where diagnoses of cancer, such as MPNST, are used to search for individuals with NF1 are likely to overestimate the cancer risk (Landry et al., 2021). Incomplete cancer registration and follow-up may, on the other hand, lead to an underestimation of the cancer risk (Airewele et al., 2001). In addition to the longitudinal cancer risk studies, some studies have aimed at estimating the cancer-related mortality in NF1 based on death certificates (Table 4). However, when also the NF1 status is established based on death certificate information only, studies are more likely to find individuals with NF1 and a cancer typical for NF1 than individuals with NF1 with other types of cancer or without cancer (Rasmussen et al., 2001; Masocco et al., 2011).

A Finnish population-based study estimated a 60% lifetime risk of cancer in NF1, which is approximately twice the risk in the general population (Uusitalo et al., 2016). For comparison, the cancer risk by 70 years of age is 90–100% and 58–70% in the well-known cancer predisposition syndromes Li-Fraumeni syndrome and Lynch syndrome, respectively (Chompret et al., 2000; Mai et al., 2016; Møller et al., 2017; Bucksch et al., 2020).

Sex and age have been identified as major modifiers of cancer risk in NF1. Females with NF1 generally have a higher standardized incidence ratio (SIR) for cancer than males with NF1 (Sørensen et al., 1986; Airewele et al., 2001; Walker et al., 2006; Uusitalo et al., 2016). This is mostly due to a higher frequency of optic pathway gliomas and breast cancers among women than among men (Uusitalo et al., 2016; Peltonen et al., 2019). The sex difference is also reflected in mortality, as NF1 is associated with a slightly greater increase in mortality among women than among men, and NF1 causes a greater reduction in the average age at death among women than among men (Rasmussen et al., 2001; Duong et al., 2011; Masocco et al., 2011; Uusitalo et al., 2015). It is obvious that the risk for breast cancer is largely sex-specific, but also the risk for optic pathway glioma may be related to hormonal factors. For example, women with NF1 frequently report growth of cutaneous neurofibromas during pregnancy suggesting a hormonal influence on these tumors (Well et al., 2020).

Table 3. Studies reporting overall cancer risk in neurofibromatosis type 1 (NF1).

STUDY	AREA OF ORIGIN	N AT RISK	RELATIVE INCIDENCE (95% CI)	ABSOLUTE RISK FOR CANCER (95% CI)	TUMOR TYPES CONSIDERED	STUDY SETTING
Sørensen et al. (1986)	Denmark	212	SIR 2.5 (1.9–3.3)		All malignant neoplasms and benign CNS neoplasms but not benign neoplasms of the peripheral nervous system (neurofibromas)	Register-based follow-up of an NF1 cohort established based on cutaneous findings
Zöller et al. (1995)	Gothenburg, Sweden	70	RiR 4.0 (2.0–7.1)		All malignant neoplasms, all CNS tumors; neurofibromas excluded	Register-based follow-up of previously identified individuals with NF1
McGaughran et al. (1999)	North West England, UK	523		15–20%	All malignant tumors and optic gliomas	Individuals with NF1 seen in a population-based clinic, cancers from interviews, medical records and centralized registries
Airewele et al. (2001)	Texas, US	138	SIR 3.0 (1.5–5.4)		All invasive malignancies and all brain tumors, excluding carcinoma *in situ* and non-melanoma skin cancers	Individuals with confirmed NF1 visiting a single, specialized clinic, cancer history by questionnaire
Walker et al. (2006)	UK	448	SIR 2.7 (1.9–3.7)	20% (14–29%) by 50, 36% (27–46%) by 70	All sites	Families recruited *via* a patient support group, NF1 diagnoses confirmed by a doctor, questionnaire to identify affected family members, linkage to cancer registries

(Table 3 continued)

STUDY	AREA OF ORIGIN	N AT RISK	RELATIVE INCIDENCE (95% CI)	ABSOLUTE RISK FOR CANCER (95% CI)	TUMOR TYPES CONSIDERED	STUDY SETTING
Cheuk et al. (2013)	China	123		12% by 20, 16% by 30	All malignant neoplasms and brain tumors	Diagnosis-based search for individuals with NF1 in general and oncology-specific registries
Seminog and Goldacre (2013)	England, UK	6,739	RaR 4.0 (3.7–4.3)		All cancers	Individuals with NF1 ascertained by diagnosis-based search from hospital admission registry
Uusitalo et al. (2016)	Finland	1,404	SIR 5.0 (4.4–5.7)	25.1% (20.2–29.7%) by 30, 38.8% (33.3–43.8%) by 50, 59.6% (53.7–64.7%) by 85	All sites, including benign intracranial tumors	Total population-based ascertainment of individuals with NF1, register-based follow-up
Landry et al. (2021)	Texas, US	1,607	OR 9.5 (8.5–10.5)	22.5% by 30, 34.0% by 50	Non-neurofibroma neoplasms	Individuals with NF1 searched using related diagnoses, such as MPNSTs

CI: confidence interval; CNS: central nervous system; MPNST: malignant peripheral nerve sheath tumor; OR: odds ratio; RaR: rate ratio; RiR: risk ratio; SIR: standardized incidence ratio

N at risk refers to the number of individuals with NF1 followed up for cancer diagnoses.

Table 4. Studies reporting mortality due to malignant neoplasms in neurofibromatosis type 1 (NF1).

STUDY	AREA OF ORIGIN	NUMBER OF INDIVIDUALS AT RISK	MORTALITY (95% CI)	STUDY SETTING
Rasmussen et al. (2001)	US	3,770	PMR 1.21 (1.14–1.28)	Based on death certificates only
Masocco et al. (2011)	Italy	632	PMR 0.9 (0.79–1.09)	Based on death certificates only
Uusitalo et al. (2015)	Finland	1,471	SMR 6.14 (5.03–7.42)	Total population-based ascertainment of individuals with NF1, neoplasms from death certificates

CI: confidence interval; PMR: proportionate mortality ratio; SMR: standardized mortality ratio

The age profile of NF1-associated cancer is very different from the general population. While cancer in the general population is largely a disease of the elderly, individuals with NF1 have a marked risk for cancer throughout their lives (Uusitalo et al., 2016). As a result, the excess risk associated with NF1 peaks at childhood and early adulthood, and the cancer incidence in NF1 matches the general population level after 70 years of age (Uusitalo et al., 2016).

While only few genotype–phenotype associations linking specific germline variants of the *NF1* gene to clinical disease manifestations are known, individuals with microdeletions of the *NF1* gene have an approximately two-fold risk for MPNST (De Raedt et al., 2003). On the other hand, *NF1* microdeletions are rarely seen among women with NF1 and breast cancer suggesting that the increased tumor-predisposition associated with the microdeletion genotype does not extend to all cancer types equally (Wang et al., 2018a; Yap et al., 2018; Frayling et al., 2019).

In addition to the high cancer incidence in NF1, cancer in the context of NF1 seems to be associated with poor survival. While the evidence is strongest for MPNST, as described below, poor survival of individuals with NF1 and cancer compared to matched cancer patients without NF1 also holds for many other tumor types (Uusitalo et al., 2016).

2.2.4 Malignant peripheral nerve sheath tumor

MPNSTs are rare sarcomas. With an estimated incidence of 1.46 per million person-years (Bates et al., 2014), approximately eight new MPNSTs are expected to be diagnosed in Finland each year. MPNSTs are highly characteristic to NF1 especially when diagnosed at a young age. MPNST may arise from a preexisting plexiform neurofibroma or develop without a known prior tumor. Detecting the malignant

transformation of a plexiform neurofibroma may be challenging and require multiple biopsies or positron emission tomography (PET) (Ferner et al., 2008; Dare et al., 2020). The SIR of MPNST and other peripheral nervous system and connective tissue malignancies in NF1 has been estimated as 122–2,056 (Walker et al., 2006; Seminog and Goldacre, 2013; Uusitalo et al., 2016) indicating an extremely high incidence in NF1 compared to the general population. In addition, one study reported an odds ratio (OR) of 9,043 for MPNST in NF1 (Landry et al., 2021). The estimates of the lifetime risk of individuals with NF1 for MPNST have varied from 7.5% to 16% (McGaughran et al., 1999; Evans et al., 2002; Ingham et al., 2011; Uusitalo et al., 2016). In addition to NF1, MPNSTs are associated with the Li-Fraumeni syndrome and may occur sporadically especially after radiotherapy of another tumor (Evans et al., 2012; Miao et al., 2019). Notably, prior radiotherapy is associated with a particularly high risk for a secondary MPNST in the context of NF1 (Evans et al., 2002; Sharif et al., 2006; Evans et al., 2011).

The average age at the diagnosis of MPNST is lower among individuals with NF1 compared to sporadic MPNST cases (Doorn et al., 1995; Evans et al., 2002; McCaughan et al., 2007; Watson et al., 2017; Miao et al., 2019; Landry et al., 2021). The median age of diagnosis for NF1-associated MPNST has been reported as 25–26 years (Doorn et al., 1995; Evans et al., 2002), while a median age of 60 years has been reported for sporadic MPNSTs (Doorn et al., 1995). The peak frequency in a large, unselected cohort of MPNST patients was observed among those aged 75–79 years (Bates et al., 2014). Therefore, a patient with MPNST of less than 50 years of age should always be examined for signs of the NF1 syndrome.

The mainstay of MPNST treatment is surgery. Negative surgical margins are a crucial prognostic factor (Bates et al., 2014; Watson et al., 2017). Because obtaining negative margins is more likely to be possible in tumors located in the extremities than in the torso, the peripheral location is associated with better outcomes (Shearer et al., 1994; Stucky et al., 2012; Miao et al., 2019).

Many studies have reported a worse survival after MPNST in NF1 compared to the general population (Evans et al., 2002; Carli et al., 2005; McCaughan et al., 2007; Stucky et al., 2012; Kolberg et al., 2013; Watson et al., 2017; Miao et al., 2019; Martin et al., 2020). The median survival after NF1-associated MPNST is only 2.4 years (Ingham et al., 2011). The 5-year survival after NF1-associated MPNST has been reported as 21–33.5% and the 10-year survival as 15–23.5% (Evans et al., 2002; Carli et al., 2005; Ingham et al., 2011; Landry et al., 2021). NF1-associated MPNSTs have been reported to be larger (Stucky et al., 2012; Miao et al., 2019; Dare et al., 2020) and more often symptomatic at diagnosis (Watson et al., 2017) than sporadic MPNSTs, which may indicate that the diagnosis is made at a later disease stage. A delay of the diagnosis could be due to, for example, benign neurofibromas (Stucky et al., 2012). Carli and co-workers (2005) reported a reduced response rate to

chemotherapy in NF1-associated pediatric MPNSTs compared to sporadic MPNSTs. An improved prognosis of NF1-associated MPNST has been reported in the more recent years, which may be due to an earlier diagnosis or utilization of novel therapeutic combinations (Ingham et al., 2011; Kolberg et al., 2013; Martin et al., 2020). The temporal change in the prognosis of NF1-associated MPNST suggests that these cancers are not inherently different than their sporadic counterparts (Kolberg et al., 2013). As a result of the high incidence and poor prognosis of MPNST in NF1, MPNSTs are a major cause of death among individuals with NF1 accounting for 26–60% of deaths (Duong et al., 2011; Evans et al., 2011).

2.2.5 Gastrointestinal stromal tumor

Another stromal tumor frequently occurring among individuals with NF1 is gastrointestinal stromal tumor (GIST). Landry and co-workers (2021) reported an OR of 272 for GIST in NF1, while Miettinen et al. (2006) estimated a 45-fold incidence in NF1. Uusitalo et al. (2016) reported a SIR of 34.2 for GIST in NF1. NF1-associated GIST may be misdiagnosed as neurofibroma or schwannoma (Miettinen et al., 2006). The interstitial cells of Cajal are considered the cells of origin of GIST, and analogous to Schwann cells in neurofibromas, they may harbor a somatic *NF1* second-hit mutation in NF1-associated GIST (Maertens et al., 2006b).

NF1-associated GIST is often located in the small intestine (Miettinen et al., 2006; Mussi et al., 2008; Dare et al., 2020; Landry et al., 2021), while the stomach is a more frequent site in the general population (von Mehren and Joensuu, 2018). A study based on hospital discharge diagnoses found a hazard ratio (HR) of 15.6 for tumors of the small intestine in NF1 compared to the control population, while the risk for tumors of the stomach did not significantly differ between the NF1 and control groups (Ylä-Outinen et al., 2019). Approximately half of the individuals with NF1 and a clinically diagnosed GIST have multiple lesions (Miettinen et al., 2006; Maertens et al., 2006b; Mussi et al., 2008; Dare et al., 2020). Individuals with NF1 and multiple GISTs often also have Cajal cell hyperplasia (Mussi et al., 2008).

NF1-associated GISTs may be asymptomatic and only detected during an autopsy (Zöller et al., 1997). Also, they have been suggested to be less likely to metastasize than sporadic ones (Mussi et al., 2008). Nevertheless, NF1-associated GIST can also metastasize and lead to death (Miettinen et al., 2006; Mussi et al., 2008; Ylä-Outinen et al., 2019). Unlike GISTs in the general population, GISTs in individuals with NF1 do not usually harbor *KIT* or *PDGFRA* mutations, which yields NF1-associated GISTs unresponsive to imatinib treatment (Miettinen et al., 2006; Maertens et al., 2006b; Mussi et al., 2008; Dare et al., 2020). Imatinib is the standard first-line treatment of sporadic, advanced GIST (von Mehren and Joensuu, 2018).

2.2.6 Tumors of the central nervous system

NF1 is associated with a marked predisposition to tumors of the brain and CNS. The estimates of the relative CNS tumor risk in NF1 compared to the general population have ranged from 22.6 to 42.7 (Walker et al., 2006; Seminog and Goldacre, 2013; Uusitalo et al., 2016). Landry and co-workers (2021) even reported ORs of 5,473 and 82.2 for low- and high-grade gliomas, respectively, in NF1 compared to the general population, yet the study might be biased towards individuals affected by tumors. The high incidence of CNS tumors among individuals with NF1 also translates to increased mortality related to tumors of the CNS (Rasmussen et al., 2001; Masocco et al., 2011; Uusitalo et al., 2016; Peltonen et al., 2019).

The most common type of CNS tumors in individuals with NF1 is optic pathway glioma accounting for at least half of the CNS tumors diagnosed in children with NF1 (Guillamo et al., 2003; Peltonen et al., 2019). The estimates of the prevalence of optic pathway gliomas among individuals with NF1 vary depending on the study setting. Some studies have reported optic pathway gliomas in 5% of individuals with NF1 (McGaughran et al., 1999; Singhal et al., 2002), while patient series with systematic neuroimaging or material from highly specialized clinics have yielded prevalence estimates in the range of 12 to 20% (Listernick et al., 1994; Rosenfeld et al., 2010; Cecen et al., 2011; Friedrich and Nuding, 2016). Interestingly, some studies have suggested a higher risk for CNS tumors among females than males with NF1 (Listernick et al., 1994; Evans et al., 2011; Uusitalo et al., 2016; Peltonen et al., 2019), while others have not observed such a sex difference (Singhal et al., 2002; Guillamo et al., 2003; Friedrich and Nuding, 2016).

NF1-associated optic pathway tumors are typically grade I pilocytic astrocytomas (Guillamo et al., 2003; Peltonen et al., 2019). They are often asymptomatic and have a more benign course than their sporadic counterparts (Listernick et al., 1994; Singhal et al., 2002; Guillamo et al., 2003; Friedrich and Nuding, 2016; Listernick et al., 2007; Miller et al., 2019; Landry et al., 2021). Symptoms of optic pathway gliomas include visual loss, strabismus, exophthalmia, and precocious puberty (Listernick et al., 1994; Guillamo et al., 2003; Friedrich and Nuding, 2016). Optic pathway gliomas in NF1 typically develop early and are unlikely to become symptomatic after seven years of age (Listernick et al., 1994; Friedrich and Nuding, 2016). Moreover, especially radiotherapy has yielded poor outcomes (Singhal et al., 2002; Guillamo et al., 2003) and causes a marked risk for secondary malignancies (Singhal et al., 2002; Evans et al., 2006; Sharif et al., 2006) and other morbidities (Guillamo et al., 2003). Consequently, only an optic pathway glioma that causes clinically significant symptoms should be treated, and it is generally recommended that neuroimaging should not be used to screen for these tumors in asymptomatic individuals with NF1 (Listernick et al., 1994, 1997, 2007;

Miller et al., 2019). However, ophthalmological surveillance is warranted for early detection of vision loss (Listernick et al., 1997, 2007; Miller et al., 2019).

Another common CNS tumor in children with NF1 is brainstem glioma (Guillamo et al., 2003; Peltonen et al., 2019). Like the optic pathway gliomas, also brainstem gliomas are often asymptomatic in individuals with NF1 (Mahdi et al., 2017). Despite the typical benign course of optic pathway gliomas and brainstem gliomas among individuals with NF1, NF1 is also associated with high-grade brain tumors, such as glioblastoma multiforme (Shearer et al., 1994; Guillamo et al., 2003; Peltonen et al., 2019; Landry et al., 2021). High-grade CNS tumors may occur both in children and adults with NF1 (Guillamo et al., 2003; Rosenfeld et al., 2010; Peltonen et al., 2019).

2.2.7 Breast cancer

Several studies have reported an increased risk for breast cancer among women with NF1 (Table 5). The relative risk seems to be particularly high among women younger than 50 years (Walker et al., 2006; Sharif et al., 2007; Madanikia et al., 2012; Wang et al., 2012). The risk after 50 years of age has been suggested to be at the general population level (Madanikia et al., 2012), yet a meta-analysis documented an increased risk also among older women (Suarez-Kelly et al., 2019). Some population-based studies and case reports have reported males with NF1 and breast cancer, yet no formal analysis of breast cancer risk among males with NF1 has been conducted (Wilson et al., 2004; Seminog and Goldacre, 2015).

The role of the *NF1* gene in breast cancer development has been highlighted in a mouse model with deficient DNA replication and a high rate of spontaneous mutations causing a high incidence of mammary tumors resembling luminal breast cancer (Wallace et al., 2012). *Nf1* mutations causing Ras hyperactivation were observed in 59 out of 60 murine mammary tumors. Another rodent study reported that *Nf1* heterozygous rats with a defective GAP-related domain developed multiple aggressive mammary tumors at a young age (Dischinger et al., 2018). Interestingly, mammary tumors also occurred in some male rats. The role of the genetic background was also highlighted in the study, as the same *Nf1* alteration was associated with a different risk for mammary tumors in various lines of *Nf1* heterozygous rats (Dischinger et al., 2018).

Table 5. Studies reporting the relative incidence of female breast cancer in neurofibromatosis type 1.

STUDY	AREA OF ORIGIN	FEMALES AT RISK	FEMALES WITH BREAST CANCER	RELATIVE INCIDENCE (95% CI)
Walker et al. (2006)	UK	227	5	SIR 1.87 (0.61–4.37)
Sharif et al. (2007)	North West England, UK	405	14	SIR 3.5 (1.9–5.9)
Madanikia et al. (2012)	Maryland, US	126	4	SIR 1.71 (0.54–4.12)
Wang et al. (2012)	Michigan, US	76	9	SIR 5.2 (2.4–9.8)
Seminog and Goldacre (2013)	England, UK	~3,370	58	SIR 2.3 (1.7–2.9)
Landry et al. (2021)	Texas, US	840	47	OR 3.8 (2.9–5.1)

CI: confidence interval; OR: odds ratio; SIR: standardized incidence ratio

2.2.8 Other cancers

The risk for malignant melanoma in NF1 has been of interest, since somatic mutations of the *NF1* gene are frequent in sporadic melanomas and especially in desmoplastic melanomas (Hodis et al., 2012; Krauthammer et al., 2015; Shain et al., 2015; Peltonen et al., 2017). *NF1* deficiency is also mechanistically linked to melanoma development (Maertens et al., 2013; Whittaker et al., 2013; Nissan et al., 2014; Shalem et al., 2014). While some studies have reported an increased risk for melanoma in NF1 (Seminog and Goldacre, 2013; Landry et al., 2021), others have not detected a melanoma risk significantly higher than in the general population (Uusitalo et al., 2016; Zhang et al., 2019). Geographical differences may be accentuated in the case of melanoma, since the highest risk for melanoma in NF1 has been reported in Texas (Landry et al., 2021). It has been suggested that the cutaneous manifestations of the NF1 syndrome may lead to covering the skin with clothes even in warm temperatures, and social isolation and physical restrictions could reduce the time spent outdoors (Zhang et al., 2019). Such behavioral mechanisms could compensate for a minor additional risk caused by the germline *NF1* variant. The high frequency of somatic *NF1* mutations in sporadic melanoma naturally does not have to mean that pathogenic germline *NF1* variants would be associated with an excess risk for melanoma.

Leukemia, and particularly juvenile myelomonocytic leukemia (JMML), is often mentioned in association with NF1 (Miller et al., 2019). JMML has shared

characteristics with chronic myelomonocytic leukemia (CMML) (Emanuel, 2008), and JMML was long recorded as CMML in the absence of a specific registration code. JMML is an extremely rare childhood leukemia with an overall incidence rate of 0.7–1.3 per million (Passmore et al., 2003). The association between NF1 and JMML was first suggested in a 1978 report of seven individuals with NF1 and chronic myelogenous leukemia (Bader and Miller, 1978). Later on, a relative risk of 221 for CMML in NF1 was estimated (Stiller et al., 1994). The risk for leukemia in NF1 has been documented in large cohort studies with estimates of relative risk ranging from 2.5 to 28.2 (Cheuk et al., 2013; Seminog and Goldacre, 2013; Landry et al., 2021). Moreover, two deaths due to JMML were reported in an English cohort of 1,186 individuals with NF1 (Evans et al., 2011). On the other hand, some studies have found no cases of JMML in association with NF1 (Walker et al., 2006; Uusitalo et al., 2016; Peltonen et al., 2019). It thus seems that the absolute risk for JMML is rather low in NF1.

NF1 has also been observed to predispose the affected individuals to, for example, thyroid, lung, and ovarian carcinomas (Seminog and Goldacre, 2013; Uusitalo et al., 2016; Landry et al., 2021). A highly characteristic endocrine tumor is pheochromocytoma that arises in the adrenal medulla and may, for example, cause hypertension (Seminog and Goldacre, 2013; Uusitalo et al., 2016; Landry et al., 2021).

2.2.9 Subsequent primary cancers

Multiple cancers may arise because of shared environmental risk factors such as tobacco smoke (Teppo et al., 1985; Coyte et al., 2014), mutagenicity of the treatment for a prior tumor (de Vathaire et al., 1999; Eulo et al., 2020), or as a result of genetic predisposition (Eulo et al., 2020). A history of a prior cancer is generally associated with a slightly increased risk for a subsequent malignancy (Neglia et al., 2001; Coyte et al., 2014; Jégu et al., 2014; Molina-Montes et al., 2015).

Studies on cancer risk in NF1 often report patients with multiple primary tumors (Schneider et al., 1986; Sørensen et al., 1986; Doorn et al., 1995; Zöller et al., 1997; McGaughran et al., 1999; Singhal et al., 2002; Barbaric et al., 2003; Walker et al., 2006; Cecen et al., 2011; Kim et al., 2012; Landry et al., 2021). While individuals with NF1 harbor a pathogenic germline variant of a tumor suppressor gene and may therefore be prone to develop several cancers, the *NF1* deficiency may also predispose them to the adverse effects of oncological treatments. Mouse models have found an increased rate of tumors after radiation in *Nf1*-haploinsufficient mice compared to wild-type mice (Chao et al., 2005; Nakamura et al., 2011; Choi et al., 2012).

Studies systematically examining the risk for subsequent tumors in NF1 are scarce. An analysis of individuals diagnosed with MPNST before the age of 30 years found a 946-fold increase in the incidence of second malignant neoplasms compared to individuals with any prior cancer (Williams et al., 2020). Especially the risk for a second MPNST was high. Individuals with MPNST at an age of less than 30 years likely, although not necessarily, have NF1. Subsequent malignant neoplasms were found in 11% of children with NF1 treated for cancer at a single institution from 1960 to 1995 (Maris et al., 1997). Especially monosomy 7 myelodysplastic syndrome was commonly seen, and the authors suggested an association with alkylating chemotherapy used to treat the first malignancy (Maris et al., 1997). The largest cohort of subsequent cancers in individuals with NF1 published to date exhibited a 7.3% cumulative risk for second neoplasms within 20 years of a childhood cancer, and a HR of 2.6 compared to non-NF1 survivors of childhood cancer (Bhatia et al., 2019; de Blank et al., 2020). The risk for second primary malignancies was higher among patients whose first tumor had been treated with radiotherapy, yet no association between alkylating chemotherapy and subsequent neoplasms was observed (Bhatia et al., 2019).

The risk posed by radiotherapy to individuals with NF1 was initially shown by a study reporting a risk ratio of 3.0 for individuals whose optic pathway glioma had been treated with radiotherapy, as compared to individuals with an optic pathway glioma but without a history of radiotherapy (Sharif et al., 2006). Because of the risk for subsequent malignancies, radiotherapy of optic pathway glioma in individuals with NF1 is to be avoided (Evans et al., 2006).

Multiple breast cancers among individuals with NF1 have also been reported (Sharif et al., 2007; Wang et al., 2012, 2016; Yap et al., 2018). A meta-analysis reported bilateral breast cancer in 12.4% of 286 women with NF1 and breast cancer (Suarez-Kelly et al., 2019).

2.3 Cancer screening and surveillance

Cancer is a gradually progressing disease where the loss of cellular growth control first leads to extensive proliferation eventually followed by dissemination of the malignant cells outside the primary tumor. Metastatic cancer is generally incurable. Therefore, an important approach for reducing cancer-related mortality is diagnosing malignant tumors at an early stage, before metastasis, which can be achieved *via* different screening approaches. However, not all pre-malignant tumors progress to clinically detectable disease during a lifetime, yet screening may also lead to the detection of such indolent tumors (Haber and Velculescu, 2014; Niell et al., 2017). The rate of tumors detected is therefore not a relevant outcome measure when assessing the efficacy of cancer screening, yet an effective screening protocol should

lead to a lower average tumor stage at diagnosis and ultimately reduce cancer mortality (Lousdal et al., 2016; Niell et al., 2017).

Currently, population-based screening programs for breast cancer (Sarkeala et al., 2008a), cervical cancer (Lönnberg et al., 2012), and colorectal cancer (Koskenvuo et al., 2019; Sarkeala et al., 2021) have been established in Finland. The breast cancer screening is reviewed in detail below. The cervical cytological screening every five years reduces the risk for cervical cancer after 30 years of age (Lönnberg et al., 2012). Population-based colorectal cancer screening programs are often based on the detection of fecal blood and follow-up colonoscopy among those who initially test positive (Lauby-Secretan et al., 2018), yet the sensitivity of such tests may vary depending on, for example, tumor location within the colon (Koskenvuo et al., 2019). Nevertheless, the biennial screening reduces the mortality caused by colorectal cancer (Lauby-Secretan et al., 2018). Individuals at an increased risk for colorectal cancer, such as those with the Lynch syndrome, may be invited directly to colonoscopy screening (Seppälä et al., 2021).

In the following section, screening and surveillance approaches particularly relevant for the NF1-associated cancer risks are reviewed. In addition to considering magnetic resonance imaging (MRI) and PET used for the monitoring of NF1-associated tumor burden and mammography screening for the detection of breast cancer, analysis of circulating free DNA (cfDNA) is discussed. In contrast to the well-established imaging-based screening and surveillance approaches, the analysis of cfDNA is an emerging technology, whose role in the clinical care has not been fully established (Ignatiadis et al., 2021). While imaging and the analysis of cfDNA can be used for the detection of cancer, they also have value in the diagnostic work-up, follow-up, and therapeutic targeting of an already detected tumor (Schwarzenbach et al., 2011; Heitzer et al., 2015; Mann et al., 2019).

2.3.1 Monitoring of NF1-associated tumor burden

Plexiform neurofibromas may be externally visible or they may lie deeper and be only detectable by imaging. In either case, the presence of plexiform neurofibromas in 20–50% of individuals with NF1 (Easton et al., 1993; Szudek et al., 2000; Ferner et al., 2007; Duong et al., 2011; Morris et al., 2021) and their potential for transformation into an MPNST emphasize the need for surveillance. The cornerstone for the surveillance of NF1-associated plexiform neurofibromas is monitoring for clinical symptoms by physicians and the patients themselves (Stewart et al., 2018; Miller et al., 2019). Persistent pain, changes in tumor consistency, and a rapid growth may indicate malignant degeneration. Clinically detected changes can be further examined by MRI and PET (Stewart et al., 2018; Miller et al., 2019), as they can aid the differentiation of a plexiform neurofibroma from an MPNST when malignancy

is suspected (Ferner et al., 2008; Evans et al., 2017; Ahlawat et al., 2020). Comparison with previous imaging may be helpful in detecting changes in the tumor. While ^{18}F-fluorodeoxyglucose-PET is useful for detecting malignant transformation, it may not allow determining the MPNST grade (Ferner et al., 2008).

Whole-body MRI can be used to quantify the tumor burden to obtain a baseline for comparison with future imaging at the time of transition from pediatric to adult care (Ahlawat et al., 2020). Moreover, information on the existing tumor burden and the detection of deep-seated, clinically undetected plexiform neurofibromas may guide the planning of surveillance (Mautner et al., 2008; Ahlawat et al., 2020). While the evidence is still scarce, repeated whole-body MRI for the screening of asymptomatic individuals with NF1 is not currently recommended, yet it may be useful among individuals with NF1 and a particularly high risk for MPNST due to, for example, *NF1* microdeletion (Ahlawat et al., 2020).

2.3.2 Breast cancer screening: breast self-examination and mammography

Breast self-examination has been suggested to allow for the early detection of breast cancers. Its benefits include the lack of ionizing radiation and an ease of access allowing monthly examination, which would ideally lead to an earlier detection of tumors. While randomized clinical trials are scarce, there is no evidence showing that breast self-examination would reduce breast cancer mortality (Kösters and Gøtzsche, 2003; Nelson et al., 2009). Moreover, breast self-examination may even increase the rates of unnecessary diagnostic procedures and therefore cause harm. In a study of a clinical breast examination by a radiographer nurse in conjunction with a mammography screening visit, symptoms, such as lumps, were associated with the risk for breast cancer, yet the sensitivity of clinical breast examination alone was poor (Singh et al., 2015).

Mammography screening of the general population often starts at the age of 50 years, which is also the case in Finland (Sarkeala et al., 2008a; Roman et al., 2014). The screening may either be invitation-based or opportunistic (Hofvind et al., 2008). In, for example, Finland and Norway, women aged 50–69 are invited to mammography screening every second year (Sarkeala et al., 2008a; Roman et al., 2014). Especially, the first round of screening increases breast cancer incidence, as prevalent cancers are detected prior to symptoms (Nyström et al., 2002). Also, overall, mammography screening leads to the detection of more early-stage tumors than would be detected without screening (Lousdal et al., 2016). In addition to malignant and low-risk tumors, the screening is associated with false-positive findings that may lead to further imaging or biopsies. The risk for false-positive findings is 1.8–4.1% per screening round, and the cumulative risk over ten screening

rounds is 18–23% (Roman et al., 2014; Singh et al., 2016). In addition to the direct medical harms related to the further examinations, false-positive findings may cause psychological distress and affect future participation in screening (Salz et al., 2011). Despite the potential downsides of mammography screening, the screening programs have been found beneficial in decreasing breast cancer-related mortality (Nyström et al., 2002; Swedish Organised Service Screening Evaluation Group, 2006; Sarkeala et al., 2008b; Niell et al., 2017). In Finland, the risk ratio for breast cancer death is 0.72 among those attending the mammography screening (Sarkeala et al., 2008b), and a highly concordant risk ratio of 0.73–0.79 has also been observed in Sweden (Nyström et al., 2002; Swedish Organised Service Screening Evaluation Group, 2006).

It has been suggested that mammography screening of genetically predisposed individuals should start at the age when the 5-year risk for breast cancer exceeds the 5-year risk observed in the general population undergoing screening (Tung et al., 2016). Moreover, MRI should be used when the 5-year risk for breast cancer exceeds the peak 5-year risk seen in the general population (Tung et al., 2016). Breast MRI has a greater sensitivity than mammography alone or in combination with ultrasonography, yet also false-positive findings are frequent (Berg et al., 2012; Raikhlin et al., 2015; Niell et al., 2017). However, many factors need to be considered when extending the screening of individuals who are at an increased risk for breast cancer. Young women generally have denser breasts than postmenopausal women, which reduces the sensitivity of mammography (Burton et al., 2017). Moreover, mammography exposes the women to ionizing radiation, and the earlier start of screening increases the cumulative exposure (Hendrick, 2010; Miglioretti et al., 2016). This is particularly a concern in the case of NF1, where the patients already harbor a defective tumor suppressor gene and are at an increased risk for many tumors (Sharif et al., 2006; Evans et al., 2006; Choi et al., 2012; Evans, 2012). Furthermore, the cutaneous neurofibromas associated with NF1 may interfere with the reading of mammograms and compromise sensitivity (Zhou et al., 2012; Da Silva et al., 2015; Howell et al., 2017). Due to these considerations, it has been highlighted that more studies on the utility of mammography screening and breast MRI in NF1 are urgently needed (Stewart et al., 2018).

Maani and co-workers (2019) conducted a retrospective review of medical records of 61 women with NF1 undergoing mammography, ultrasonography, or MRI for breast cancer screening. They found an excellent attendance and a high rate of 49% of women who were referred to further investigation at least once. Out of the 27 women with positive findings in the initial screening, four were diagnosed with breast cancer (Maani et al., 2019). Therefore, the rate of false-positive findings was rather high. Importantly, another study found no distress or anxiety associated with

breast cancer screening or even recall after a screening visit among women with NF1 (Crook et al., 2022).

2.3.3 Circulating free DNA

Analysis of cfDNA isolated from plasma or serum represents an emerging approach for cancer screening and surveillance. Cells from a primary or metastatic tumor or circulating tumor cells may release DNA into the blood plasma (Schwarzenbach et al., 2011; Crowley et al., 2013; Heitzer et al., 2015). The tumor-derived cfDNA is termed "circulating tumor DNA" (ctDNA). Analyses based on cfDNA are applied also in fields outside oncology, since non-invasive prenatal testing can be used to detect chromosomal aberrations during pregnancy (Wang et al., 2013; Amant et al., 2015).

The analysis of cfDNA and ctDNA can be applied for cancer detection, prognostic evaluation, monitoring of treatment response and recurrence, and for the targeting of therapy (Schwarzenbach et al., 2011; Crowley et al., 2013; Haber and Velculescu, 2014; Heitzer et al., 2015; Ignatiadis et al., 2021). Changes in cfDNA concentration may be associated with tumor burden and malignant transformation, and somatic variants associated with cancer may imply the development of a clinically undetected tumor. For example, ctDNA may reveal disease relapse up to 11 months before clinical relapse in early breast cancer (Ignatiadis et al., 2021). Especially among patients who are at an increased risk for cancer, cfDNA-based screening could allow for the diagnosis of malignant tumors at a curable stage (Haber and Velculescu, 2014; Jones et al., 2021). Since ctDNA can be derived from the whole tumor and its metastases, it is not spatially restricted like traditional tissue biopsies and can therefore provide a more comprehensive molecular portrait of the disease, which is essential especially when targeted therapies are used (Crowley et al., 2013; Heitzer et al., 2015; Ossandon et al., 2018; Siena et al., 2018; Ignatiadis et al., 2021). Moreover, cancer may acquire resistance to therapy by different mechanisms at various metastatic sites, which can be feasibly detected using ctDNA analysis (Razavi et al., 2018). The cfDNA-based "liquid biopsy," i.e., drawing a blood sample, is also less invasive than traditional biopsies and can be done repeatedly over the disease course (Schwarzenbach et al., 2011; Crowley et al., 2013; Haber and Velculescu, 2014; Heitzer et al., 2015; Bonner et al., 2018; Ignatiadis et al., 2021). Moreover, early detection of recurrence may allow proactive treatment and therefore better treatment results (Crowley et al., 2013).

The cfDNA primarily originates from apoptotic and necrotic cells, yet also other mechanisms such as autophagy, phagocytosis, and the release of extracellular vesicles contribute to the cfDNA (Jahr et al., 2001; Grabuschnig et al., 2020). Most of the cfDNA may be contained in exosomes that protect DNA from cleavage by

DNAses (Fernando et al., 2017), and cfDNA has been estimated to have a half-time of at least 15 minutes (Thierry et al., 2010; Schwarzenbach et al., 2011; Heitzer et al., 2015). Also, tissue damage, inflammation, smoking, physical exertion, or cardiac insufficiency may increase the concentration of plasma cfDNA (Schwarzenbach et al., 2011; Heitzer et al., 2015). However, T cells and endothelial cells, which are the cells most intimately in contact with the circulation, rarely release cfDNA (Jahr et al., 2001).

Despite the other sources of cfDNA, persons with cancer often show higher cfDNA concentrations than healthy individuals, yet there is marked variation between individuals and between different types of cancer. The cfDNA concentration in healthy individuals typically ranges from 0 to 100 ng/ml, while those with cancer may have cfDNA levels up to 1,000 ng/ml (Leon et al., 1977; Jahr et al., 2001; Wu et al., 2002; Boddy et al., 2005; Umetani et al., 2006a; Chun et al., 2006; Schwarzenbach et al., 2008; Kamat et al., 2010; Schwarzenbach et al., 2011; Kim et al., 2014; Yu et al., 2019). Tumor progression is known to increase the cfDNA concentration (Leon et al., 1977; Wu et al., 2002; Sunami et al., 2008; Kim et al., 2014), and surgery often causes at least transient reduction in cfDNA levels (Szpechcinski et al., 2008; Kim et al., 2014; Namløs et al., 2017). A high cfDNA concentration has also been associated with poor survival in various cancer types, such as breast cancer, non-small cell lung cancer, ovarian cancer, and colorectal cancer (Schwarzenbach et al., 2008; Kamat et al., 2010; Dawson et al., 2013; Tissot et al., 2015; Cheng et al., 2018). The association of cfDNA concentration and prognosis may be because of a correlation between the tumor stage and cfDNA concentration (Lamminaho et al., 2021). Individuals with and without cancer may show overlapping cfDNA concentrations, and therefore the concentration alone is not a specific cancer biomarker (Leon et al., 1977; Jahr et al., 2001; Wu et al., 2002; Schwarzenbach et al., 2011; Heitzer et al., 2015).

The size of cfDNA ranges from 70–200 bp up to 21 kb (Jahr et al., 2001; Schwarzenbach et al., 2011). The size distribution of ctDNA has been reported to differ from cfDNA from other sources, and the association may depend on tumor type (Wu et al., 2002; Wang et al., 2003; Umetani et al., 2006a; Mouliere et al., 2018; Ossandon et al., 2018; Cristiano et al., 2019). Both increased fragmentation of ctDNA (Wu et al., 2002; Thierry et al., 2010; Mouliere et al., 2018; Ossandon et al., 2018) as well as a higher integrity of ctDNA compared to cfDNA (Wang et al., 2003; Umetani et al., 2006a, 2006b) have been reported. Nevertheless, cfDNA size can provide a way for estimating the proportion of ctDNA out of all cfDNA (Crowley et al., 2013). Moreover, tumor progression may affect DNA integrity (Umetani et al., 2006a; Thierry et al., 2010; Cheng et al., 2018), and the integrity of cfDNA may have prognostic value (Lamminaho et al., 2021). The length of cfDNA fragments depends at least on the mechanism of release, as apoptosis yields multiples of

nucleosomal fragments of 180 bp, while necrosis may result in the release of DNA fragments longer than 10 kb (Jahr et al., 2001; Grabuschnig et al., 2020). DNA has also been suggested to leak out from cells during proliferation (Mouliere et al., 2018).

The highest specificity of cfDNA-based analyses can be achieved if a tumor-specific variant is known and its presence in the cfDNA can be monitored (Dawson et al., 2013; Thierry et al., 2014; Heitzer et al., 2015; Bonner et al., 2018; Ignatiadis et al., 2021). Another option is to search for mutations typical in a given type of cancer (Kammesheidt et al., 2018; Ossandon et al., 2018; Jones et al., 2021) or to sequence the cfDNA for the detection of any variants (Heitzer et al., 2015; Bonner et al., 2018; Ignatiadis et al., 2021). In such approaches, it is essential to discriminate tumor-associated somatic variants from germline variants (Ignatiadis et al., 2021). The sensitivity of ctDNA analysis depends on the concentration of ctDNA and also the proportion of ctDNA out of total cfDNA (Risberg et al., 2018; Ignatiadis et al., 2021). For example, in the case of non-invasive prenatal testing, a high maternal cfDNA concentration decreases the sensitivity of the assay to detect alterations in fetal DNA (Wang et al., 2013). Therefore, any conditions affecting the baseline cfDNA concentration may affect the sensitivity of cfDNA-based screening. While cfDNA alone is not completely specific, it can be used to supplement other biomarkers or imaging. For example, the risk for malignancy in a neural tumor that is detected by imaging, but inaccessible for biopsy, can be estimated based on the cfDNA (Bonner et al., 2018; Jones et al., 2021). Moreover, cfDNA findings can be supplemented with protein-based assays, such as prostate-specific antigen levels in the case of prostate cancer (Wu et al., 2002; Chun et al., 2006; Bonner et al., 2018; Ignatiadis et al., 2021).

2.4 Diabetes and NF1

Diabetes is a common disease associated with excess mortality (DiMeglio et al., 2018; Zheng et al., 2018). Diabetes is characterized by impaired insulin production or action that leads to an excessive blood glucose concentration. The two main categories of diabetes are types 1 and 2, yet also less frequent disease forms exist, such as the maturity-onset diabetes of the young that is a monogenic form of diabetes (Redondo et al., 2020).

2.4.1 Type 1 diabetes in general

Type 1 diabetes is an autoimmune disease where the body's own immune system attacks the insulin-producing pancreatic cells leading to insufficient insulin production. Clinical type 1 diabetes is preceded by autoantibodies indicating the

body's autoimmune reaction towards itself (Krischer et al., 2017; DiMeglio et al., 2018), and the disease most often has onset already during childhood (Diaz-Valencia et al., 2015). Latent autoimmune diabetes of adults (LADA) has also been described (Tuomi, 2005; Redondo et al., 2020). While LADA is an autoimmune disease, onset in adulthood and lack of insulin-dependency in the early phase may lead to an initial diagnosis of type 2 diabetes.

Type 1 diabetes has a strong genetic component with type 1 diabetes of a parent, sibling, or identical twin conferring risks of 1–9%, 6–7%, and 30–70% for type 1 diabetes, respectively (DiMeglio et al., 2018). Approximately 50% of the heredity of type 1 diabetes is associated with the human leukocyte antigen (HLA) genes (Noble and Valdes, 2011; Pociot and Lernmark, 2016; DiMeglio et al., 2018). Especially class II HLAs modify the risk for type 1 diabetes, and particularly HLA-DR3-DQ2 and HLA-DR4-DQ8 confer an increased risk for diabetes (Noble and Valdes, 2011; Pociot and Lernmark, 2016; Jerram and Leslie, 2017; Pociot, 2017; Inshaw et al., 2020). In addition, multiple non-HLA loci associated with type 1 diabetes have been identified affecting, for example, the immune system and the insulin gene (Pociot and Lernmark, 2016; Jerram and Leslie, 2017; Pociot, 2017; DiMeglio et al., 2018; Inshaw et al., 2020). Genetic factors not only affect the predisposition to type 1 diabetes, but they may also modify the age of onset (Inshaw et al., 2020). Some variants in, for example, HLA genes and the *INS* gene encoding insulin also protect against type 1 diabetes (Nejentsev et al., 2009; Pociot, 2017). In addition to genetics, environmental factors, including the prenatal environment, affect the onset of type 1 diabetes (Jerram and Leslie, 2017; DiMeglio et al., 2018). The incidence of type 1 diabetes is particularly high in Finland (Patterson et al., 2019).

2.4.2 Type 2 diabetes in general

In contrast to type 1 diabetes, type 2 diabetes is typically diagnosed later in life and is associated with various environmental factors, such as a sedentary lifestyle, excessive energy intake, and obesity (Zheng et al., 2018). Type 2 diabetes is the predominant type of diabetes, and its global prevalence is increasing along with the rising standard of living (Zheng et al., 2018). Type 2 diabetes is primarily a disease of insulin action, and it is characterized by insulin resistance. The insulin receptor is a tyrosine kinase receptor whose downstream signaling is mediated by PI3K and Ras (Figure 1). The receptor activates PI3K both directly and *via* Ras, and the PI3K–Akt–mTOR pathway is considered essential in mediating the cellular effects of insulin (Saltiel, 2021). Pharmacological inhibition of PI3K or mTOR is used in the treatment of, for example, breast cancer. The inhibition of PI3K or mTOR frequently causes hyperglycemia because inhibition of the pathway decreases the activation

induced by insulin stimulation and therefore leads to insulin resistance (Sivendran et al., 2014; André et al., 2019). The *PTEN* gene encodes a negative regulator of PI3K–Akt activity (Figure 1), and haploinsufficiency of *PTEN* leads to a decrease in the negative regulation of Akt activation. Individuals with pathogenic germline variants of *PTEN* display increased insulin sensitivity, i.e., lower levels of insulin are sufficient to produce a similar response as seen in healthy controls (Pal et al., 2012). Taken together, a higher level of PI3K–Akt–mTOR activation increases insulin sensitivity, while reduced activation causes insulin resistance and hyperglycemia.

Type 2 diabetes is linked to various lifestyle factors and, consequently, it often coincides with other diseases, such as the metabolic syndrome, gestational diabetes, dyslipidemia, and hypertension (Fletcher et al., 2002; Zheng et al., 2018). Many of the risk factors of type 2 diabetes are also shared with, for example, breast cancer (Barnard et al., 2015; Rojas and Stuckey, 2016; Zheng et al., 2018). While type 2 diabetes is more strongly associated with environmental factors than type 1 diabetes, the family history is also an indicator of the risk for type 2 diabetes (Fletcher et al., 2002; Lyssenko et al., 2008; Zheng et al., 2018). It is naturally difficult to discern genetics from inherited lifestyle and environmental factors. However, certain genes have been linked to the risk for type 2 diabetes (Xue et al., 2018). For example, variants of the *WSF1*, *HNF1A*, *PAM*, *AKNRD55*, and *PPIP5K2* genes increase the risk for type 2 diabetes (Lyssenko et al., 2008; Morris et al., 2012; Steinthorsdottir et al., 2014). Some genes associated with the risk for type 2 diabetes, such as *FTO1*, *TCF7L2*, and *PRC1*, are also linked with breast cancer risk (Zhao et al., 2016). Variants of the *SLC30A8*, *PPARG*, and *TCF2* genes are associated with a reduced risk for type 2 diabetes (Deeb et al., 1998; Gudmundsson et al., 2007; Flannick et al., 2014, 2019). Loss-of-function variants of the G-protein coupled receptor gene *MC4R* confer an increased risk for type 2 diabetes, yet the gain-of-function variants of the same gene reduce the risk for type 2 diabetes (Lotta et al., 2019). In *CCND2*, both predisposing and protective variants have been described (Steinthorsdottir et al., 2014; Mahajan et al., 2018).

2.4.3 The risk for diabetes in NF1

Little is known about the risk for diabetes in NF1. NF1 is associated with an increased risk for death due to cardiovascular disease (Zöller et al., 1995; Evans et al., 2011; Uusitalo et al., 2015), which would suggest a potential increased risk for diabetes as well. However, diabetes-related deaths seem to be rare in NF1, as proportionate mortality ratios of 0.2–0.27 have been reported in death certificate-based studies (Rasmussen et al., 2001; Masocco et al., 2011). The low rate of diabetes-related deaths may, however, be because of the excess mortality associated with NF1 at ages younger than the typical onset of type 2 diabetes. It is plausible that

so many individuals with NF1 die due to other causes before the onset of type 2 diabetes that the risk for diabetes seems artificially low in death certificates. A study based on insurance claims reported an odds ratio of 0.4 for diabetes in NF1 (Madubata et al., 2015). A recent analysis of hospitalizations of Danish patients with NF1 estimated a risk ratio of 0.4 (95% confidence interval [CI] 0.2–0.97) for type 1 diabetes and 0.8 (95% CI 0.4–1.5) for type 2 diabetes in NF1 (Kenborg et al., 2020). However, the study did not encompass outpatient care, which is the typical treatment setting of diabetes. The rates of preexisting diabetes in pregnant women (Terry et al., 2013) and gestational diabetes (Leppävirta et al., 2019) are similar among individuals with NF1 and among controls.

Individuals with NF1 have been found to show lower fasting blood glucose and leptin levels, increased insulin sensitivity, and a higher resting energy expenditure compared to controls (de Souza et al., 2015, 2019; Martins et al., 2016, 2018). While it is difficult to perform mechanistic studies of energy metabolism in humans, animal models allow examining even the long-term effects of high-glucose diet with a controlled energy intake. A recent study characterized the metabolic features of *Nf1* heterozygous mice (Tritz et al., 2021). Interestingly, the results were highly concordant with the observations in individuals with NF1, as *Nf1* heterozygous mice showed increased insulin sensitivity and did not develop insulin resistance even after a prolonged administration of dietary glucose. The *Nf1*-haploinsufficient mice also exhibited decreased fat mass and altered fat distribution compared to wild-type mice and showed lower blood glucose and leptin levels (Tritz et al., 2021). The *Nf1* heterozygous mice were able to process glucose more efficiently than their wild-type littermates. After a 16-week administration of glucose in drinking water, the *Nf1*-haploinsufficient mice had gained less weight than controls and did not develop hyperglycemia like wild-type mice (Tritz et al., 2021).

3 Aims

The overall aim of this thesis was to estimate the prevalence of NF1 and to gain new information on the comorbidities associated with NF1. Specifically, two diseases that are relatively common in the general population, breast cancer and diabetes, were studied in the context of NF1. The information on the prevalence is essential for assessing how many individuals with NF1 should be undergoing surveillance and for identifying any biases affecting cohort studies. Knowledge about the comorbidities of NF1 allows targeting of surveillance efforts and may thus improve the health of individuals with NF1. The marked cancer risk associated with NF1 highlights the need for better surveillance and screening of individuals with NF1. Since the neurofibromin protein encoded by the *NF1* gene is part of the signaling networks involved in the pathogenesis of both cancer and diabetes (Figure 1), the epidemiology of NF1 also elucidates the molecular biology underlying these diseases.

The specific aims of the thesis were to:

1. estimate the prevalence of NF1 by age group (Study I),
2. study the effect of NF1 and the underlying *NF1* haploinsufficiency on breast cancer risk and the prognosis of breast cancer to improve surveillance and treatment (Studies II and III),
3. study the effect of germline deficiency of functional neurofibromin on the risk for diabetes (Study IV),
4. examine whether NF1 affects the baseline concentration of circulating free plasma DNA and could therefore interfere with the sensitivity of cfDNA-based analyses (Study V).

4 Materials and Methods

4.1 Study populations

Studies I–IV are based on the Finnish NF1 cohort. The cohort has been previously collected (Uusitalo et al., 2015). The Finnish NF1 cohort is based on searching all hospital visits related to neurofibromatosis in the 15 central and five university hospitals in mainland Finland from 1987 to 2011 and is therefore representative of the whole population of mainland Finland. Hospital visits of interest were identified with the International Classification of Diseases, 9th edition (ICD-9) diagnosis code: 2377A and the ICD-10 codes: Q85.00, Q85.0, Q85.09, Q85, and Q85.01. ICD-9 has been used to record hospital visits in 1987–1995 and ICD-10 since 1996. The diagnosis codes also included NF2 and unspecified neurofibromatosis because the initial search was aimed at comprehensively identifying all potential individuals with NF1 rather than being specific for NF1. The medical records of all individuals identified in the initial search were then examined to confirm NF1 diagnosis according to the NIH diagnostic criteria for NF1 (National Institutes of Health Consensus Development Conference, 1988). The procedure yielded 1,476 individuals with confirmed NF1. However, the number of individuals included in Studies I–IV varied because of different follow-up periods and availability of necessary information (Figure 2). For the same reason, the cohort size also slightly differed from previous publications (Uusitalo et al., 2015, 2016). For each individual with NF1, the cohort entry date was the date of the first NF1-related hospital visit that led to the inclusion of the individual in the cohort.

In Study III focusing on the risk for contralateral breast cancer, the Finnish NF1 cohort was analyzed together with data from four other NF1 cohorts being the Manchester regional NF1 registry of 2,148 patients from the United Kingdom, the Paris NF1 registry of 1,895 patients from France, the Hamburg neurofibromatosis clinic cohort of 2,019 patients from Germany, and 811 patients from the Padua NF1 clinic in Italy. The Manchester cohort of individuals with NF1 was ascertained based on referrals from physicians and clinical examination of family members of known patients with NF1, and the cohort is representative of the population in the

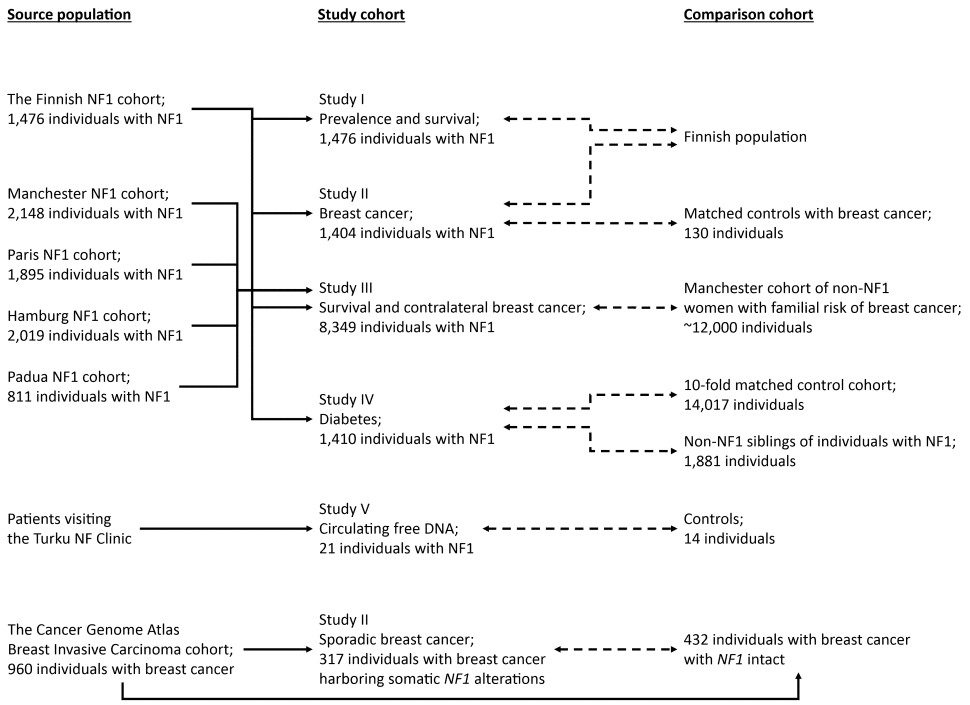

Figure 2. Source populations, study cohorts and comparison cohorts in each study. NF1: neurofibromatosis type 1

Manchester area in North West England. The Paris, Hamburg, and Padua cohorts are based on individuals visiting specialized NF1 clinics. The individuals with NF1 from the Finnish, Manchester, Paris, and Hamburg cohorts had not received additional breast cancer screening because of their NF1, yet they may have participated in the general population screening programmes established from 1989 to 2005 for women older than 49 years of age. The individuals with NF1 in the Padua cohort had been screened for breast cancer using annual mammography and/or ultrasonography starting at the age of 45 years beginning in 2009 and 2010.

The NF1 patients for the assays of cfDNA concentrations (Study V) were recruited from the NF clinic operative in Turku University Hospital. The participants were adults, fulfilled the NIH diagnostic criteria for NF1, and came in for a regular follow-up visit related to their NF1. The 21 individuals with NF1 enrolled in the study were aged 18–64 years. They were all included in the Finnish NF1 cohort used in Studies I–IV.

In Studies I and II, individuals with NF1 were compared with the Finnish general population. When breast cancer characteristics were examined in Study II, five controls matched for the age at diagnosis and sex were obtained for each individual

with NF1 from the Auria Biobank (Turku, Finland). When survival after breast cancer was assessed, five control breast cancers matched for the age at diagnosis, year of diagnosis, estrogen receptor status, and sex were retrieved for each individual with NF1. In Study III on breast cancer characteristics and survival and the risk for contralateral breast cancer, a Manchester-based cohort of non-NF1 women with breast cancer and enhanced screening was used as a reference. These women had a 17% or greater risk for breast cancer based on family history. They had been screened annually with mammography at ages of 30–49 and every 18 months at ages of 50–59.

When the risk for diabetes was examined in Study IV, individuals with NF1 were compared with two cohorts being their 1,881 non-NF1 siblings and a ten-fold control cohort of 14,017 individuals matched to individuals with NF1. The siblings were defined by at least one shared parent, and those with known or suspected NF1 were excluded from the non-NF1 sibling cohort. Comparison of individuals with NF1 with their non-NF1 siblings allowed controlling for the effects of genes other than *NF1* and environmental factors such as family socioeconomic status. For each individual with NF1, a maximum of ten control individuals matched for sex, area of residence, and age were also retrieved from the Finnish Population Register Centre. The controls were required to live in the same municipality as the respective individual with NF1 on the cohort entry date. The date of birth of the controls had to be within a maximum of 12 months from the birth of the respective individual with NF1. Due to the small population of some municipalities, the full number of controls could not be retrieved for all individuals with NF1. First-degree relatives of individuals with NF1 were excluded from the control cohort.

For Study V, the controls were recruited from the personnel of the University of Turku and Turku University Hospital. The controls were healthy by their own announcement, but no medical records were reviewed. The controls were not individually matched to individuals with NF1, yet the controls were selected to obtain an approximately similar age and sex distribution as in the NF1 group.

In Study II, data on primary invasive breast cancers were retrieved from The Cancer Genome Atlas (TCGA) project (Cancer Genome Atlas Network, 2012). These data were used to examine somatic *NF1* alterations in breast cancers of the general population. The cancers were grouped according to their *NF1* gene status: breast cancers with *NF1* intact were compared with breast cancers harboring somatic *NF1* mutations or shallow or deep *NF1* deletions. Information on deletions has been algorithmically produced in the TCGA data (Cancer Genome Atlas Network, 2012), and shallow deletions likely represent heterozygous loss and deep deletions likely represent homozygous loss of a gene.

Studies I–IV were register-based and retrospective and therefore the use of the Finnish NF1 cohort was exempt from obtaining informed consent from the

participants. The studies were approved by the Ethics Committee of the Hospital District of Southwest Finland and had research permissions from the Finnish Institute for Health and Welfare, The Social Insurance Institution of Finland, The National Supervisory Authority for Welfare and Health (Valvira), Statistics Finland, the Finnish Population Register Centre, and all participating hospitals. The international cohorts included in Study III also had local approvals. Study V was approved by the Ethics Committee of the Hospital District of Southwest Finland, it had a research permission from Turku University Hospital, and all participants provided written informed consent. The study was preregistered in ClinicalTrials.gov with identifier NCT02680431. All studies adhered to the principles of the Declaration of Helsinki.

4.2 Data sources and outcomes of interest

For the Finnish NF1 cohort, the Finnish personal identity code was used as the key for retrieving data from various registers. Dates of birth, death, and emigration were retrieved from the Finnish Population Register Centre in order to define the follow-up time of each participant in Studies I–IV. The use of data from the Finnish Population Register Centre allowed a complete follow-up in the sense that no missing data were present.

Study I focused on estimating the prevalence and survival in NF1. The age-, sex- and calendar year-specific mortality rates of the general population and the population sizes in each age, sex, and year category were obtained from Statistics Finland. Prevalence was defined as the ratio of the numbers of individuals with NF1 and individuals in the general population alive in each age category on the last day of each year. In the survival analysis, time from birth to death was of interest.

Breast cancer diagnoses of the Finnish patients, and the cancer incidence rates of the general population were obtained from the Finnish Cancer Registry for Studies II and III. The causes of death were obtained from Statistics Finland. The Finnish Cancer Registry has collected information on cancers diagnosed in Finland since 1953, and health care providers are required to report cancers in the registry. As a result, the Finnish Cancer Registry has a very high coverage of most cancer types diagnosed in Finland (Leinonen et al., 2017). The information on the international cohorts in Study III was based on medical records of the patients and on local cancer registry information. Breast cancer was defined with the ICD-10 code: C50 or the International Classification of Diseases for Oncology, third edition (ICD-O-3) topology code: C50.

Data for Study II were also collected from the medical records of each individual with NF1 and breast cancer. Archived formalin-fixed, paraffin-embedded breast cancer samples of the individuals with NF1 were obtained from the pathology units

of the participating hospitals. Only invasive breast cancers and only one sample per individual with NF1 were retrieved. Breast cancer tissue samples were immunolabeled as described below, and samples showing nuclear immunoreactivity for estrogen and progesterone receptor in more than 10% of tumor cells were considered positive for the receptors. Breast cancer subtyping was based on the estrogen and progesterone receptors, *ERBB2* amplification, Ki-67, and cytokeratin 5. Auria Biobank provided information collected from the medical records for the control breast cancers. The information collected on the Finnish NF1-related breast cancers in Study II was also used in the Study III examining survival after breast cancer and the risk for contralateral breast cancer.

Hospital visits and hospital stays for Study IV were retrieved from the Care Register for Health Care maintained by the Finnish Institute for Health and Welfare, and the drug purchases were obtained from the Drug Reimbursement Register maintained by the Social Insurance Institution of Finland. The Care Register for Health Care has recorded inpatient care using ICD-9 coding since 1987 and ICD-10 coding since 1996. In 1998, also specialized outpatient care was incorporated in the register. The Care Register for Health Care contains a total of six diagnosis codes associated with each hospital visit or hospital stay. The Drug Reimbursement Register contains information on reimbursed outpatient purchases of prescription drugs since 1995 and is comprehensive since 1996. The drug purchases are recorded using the Anatomical Therapeutic Chemical (ATC) classification.

Diagnoses of diabetes were retrieved using ICD-10 codes: E10–E14 from the Care Register for Health Care. Purchases of insulin were collected using ATC code: A10A and blood glucose-lowering drugs other than insulins with the ATC code: A10B from the Drug Reimbursement Register. When type 1 and type 2 diabetes were analyzed separately, the ICD-10 code: E10 was used for type 1, and the ICD-10 code: E11 and the ICD-9 code: 250xA were used for type 2. The letter "x" denotes any digit. Since insulins can be used to treat either type 1 or type 2 diabetes, type 1 diabetes was defined solely by its ICD-10 diagnosis code. However, since type 1 diabetes always leads to insulin dependency, only individuals with an insulin purchase (ATC A10A) at any time were included. This allowed exclusion of coding errors where other types of diabetes would have been recorded with an ICD-10 code for type 1 diabetes. Patients with type 2 diabetes are often treated in a primary care setting and therefore cannot be comprehensively identified using the Care Register for Health Care. Consequently, both purchases of anti-diabetic drugs and the specific ICD-9 and ICD-10 diagnosis codes were considered as evidence of type 2 diabetes. However, the individuals identified as having type 1 diabetes were excluded from the analyses related to type 2 diabetes. To study the comorbidities and predisposing conditions of diabetes, the ICD-10 codes: E66 and E78 were used to search for diagnoses of obesity and disorders of lipoprotein metabolism, respectively. Both the

primary and secondary diagnoses included in the Care Register for Health Care were taken into account.

In Study V, the primary outcome was the concentration of plasma cfDNA that was measured as described below and normalized relative to the plasma protein concentration. As a sensitivity analysis, the plain concentration of cfDNA without normalization was also studied. The doctor treating the patients extracted the history of cancer, optic pathway glioma, and plexiform neurofibroma from medical records. In addition, the clinician estimated the numbers of cutaneous and subcutaneous neurofibromas at the time of blood sampling.

4.3 Histology and immunohistochemistry of breast cancers (II)

The formalin-fixed, paraffin-embedded breast cancer samples retrieved from pathology archives were cut into 3-µm sections. Samples stained with hematoxylin and eosin were examined for histological type and grade by a specialist in pathology according to the World Health Organization classification of tumors of the breast (Lakhani et al., 2012). The tumors were also immunolabeled for the estrogen and progesterone receptors, Ki-67, HER2, cytokeratins 5/6, and cytokeratin 14 using the BenchMark XT automated immunostaining instrument (Roche/Ventana, Tucson, AZ, USA) and ultraView Universal DAB Detection Kit (Roche/Ventana). Sections positive for HER2 immunostaining were further examined for *ERBB2* amplification using the BenchMark XT instrument. *In situ* hybridization for *ERBB2* was performed using the Ventana HER2 DNA probe and the ultraView SISH Detection Kit and for chromosome 17 using the Inform Chromosome 17 probe and ultraView Alkaline Phosphatase Red ISH Detection Kit (all from Roche/Ventana). A pretreatment with ISH Protease 3 (Roche/Ventana) for 8 minutes was followed by *ERBB2* hybridization at 52°C for 6 hours and chromosome 17 hybridization at 44°C for 2 hours. A specialist in breast pathology scored the breast cancers for estrogen and progesterone receptor and cytokeratin positivity, for *ERBB2* amplification and for Ki-67 labeling (<14% or ≥14%) according to clinical practices.

4.4 Analysis of circulating free DNA (V)

Peripheral blood was drawn into lithium-heparin tubes. The samples were processed within four hours of sampling to avoid cell lysis and release of intracellular DNA into plasma. It has been previously reported that the plasma cfDNA concentration is stable for at least four hours after sampling (Jung et al., 2003; Lam et al., 2004; Crowley et al., 2013). The samples were processed to isolate a mononuclear cell fraction for another study (Pennanen et al., 2021) and blood plasma for the present

study. Blood was diluted 1:1 with phosphate-buffered saline and fractionated using Ficoll-Paque PLUS (GE Healthcare Bio-Sciences, Uppsala, Sweden) gradient centrifugation at 2,000 g for 30 min. Plasma was aspirated without disturbing the underlying mononuclear cell fraction and stored at −80°C until analysis.

After thawing the samples for analysis, 3 ml of each plasma sample was centrifuged at 1,000 g for 10 min and the supernatant was carefully aspirated for use in the analysis. The aim was to remove any potentially remaining cells whose lysis could release intracellular DNA. Plasma protein concentration was measured using the bicinchoninic acid assay (Pierce BCA Protein Assay Kit, Thermo Fisher Scientific, Rockford, IL, USA) at dilutions 1:20 and 1:30, and the computed original concentrations were averaged. The cfDNA was isolated from 2 ml of plasma using the QIAamp Circulating Nucleic Acid Kit (Qiagen, Hilden, Germany) according to the kit instructions, and the extracted DNA was eluted in a volume of 50 µl. The concentration of DNA in the eluate was measured in duplicate using the Qubit 2.0 Fluorometer (Invitrogen, Eugene, OR, USA) and Qubit HS Assay Kit (Invitrogen) that utilizes a dye specific for double-stranded DNA.

All plasma samples were visually evaluated for hemolysis. Cell degradation indicated by hemolysis could be caused by, for example, aberrant blood drawing or defective sample processing and would likely imply contamination of the plasma cfDNA with intracellular DNA. Two samples from individuals with NF1 and two samples from controls were excluded because of visually detected hemolysis. Hemolysis can also be detected based on the spectrophotometric measurement of absorbance at the hemoglobin peak absorbance of 414 nm (Shah et al., 2016). After excluding the four samples based on visually detected hemolysis, all remaining samples demonstrated a low absorbance at 414 nm as measured using the NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

4.5 Statistical methods

In all studies, two-tailed *P* values <0.05 were considered statistically significant. The proportional hazards assumption of the Cox proportional hazards model was assessed using scaled Schoenfeld residuals. The R software (versions 3.2.2–3.3.0, 3.6.1) was used for the analyses.

4.5.1 Study periods

The study periods for Studies I–IV are shown in Table 6. In the cfDNA study (V), no longitudinal follow-up data were available. In the epidemiological studies based on the Finnish NF1 cohort (I, II, and IV), the follow-up started at the cohort entry of each individual with NF1 from 1987 to 2011 or the beginning of study period,

Table 6. Study periods in Studies I–IV.

STUDY	BEGINNING OF STUDY PERIOD	END OF STUDY PERIOD
I		
PREVALENCE	31.12.1990	31.12.2011
SURVIVAL	1.1.1987	31.12.2014
II		
BREAST CANCER INCIDENCE	1.1.1987	31.12.2013
III		
FINNISH NF1 COHORT	1.1.1987 / first breast cancer	31.12.2014
INTERNATIONAL COHORTS	cohort entry / first breast cancer	last clinical contact
IV		
PRIMARY STUDY PERIOD	1.1.1998	31.12.2014
SENSITIVITY ANALYSIS	1.1.1987	31.12.2014

whichever was later. In Study III on breast cancer survival and risk for contralateral breast cancer, the follow-up started at the cohort entry or the first breast cancer depending on the analysis. Starting the follow-up before the cohort entry would have led to an immortality bias, since those who died before their prospective cohort entry could not be detected as having NF1. Many diseases, especially cancer, increase the risk for death. The risk for such diseases would be underestimated in the period preceding the cohort entry, because there would be bias towards those without these diseases.

In the study on diabetes in NF1 (IV), the cohort entry of the respective individual with NF1 was used for controls, as all control individuals were required to be alive on the date of the cohort entry of the respective individual with NF1. The follow-up of the non-NF1 siblings started at the latter of birth or the cohort entry of the first sibling with NF1, because the inclusion of the non-NF1 siblings in the cohort was contingent on having a sibling with NF1.

In Study IV, the primary study period started on January 1st, 1998, since information on both drug purchases and specialized outpatient care were available since 1998 (Table 6). As a sensitivity analysis, type 2 diabetes was also studied starting from 1987. However, since drug purchases were only available since 1995, they were omitted from this analysis.

In Studies I–IV, the follow-up always ended at death or emigration, since register-based follow-up is only possible for individuals living within a national

registration system. Moreover, the availability of register data ended on December 31st, 2013 in Study II and on December 31st, 2014 in Studies I and IV. Also, in Study III, the follow-up of the Finnish NF1 cohort ended on December 31st, 2014, and the follow-up of the international cohorts was based on the last clinical contact with each individual with NF1 or register-based information.

4.5.2 Estimation of NF1 prevalence (I)

In Study I, the observed prevalence of NF1 was calculated in five-year age categories during years 1990–2011 and more specifically on December 31st, 2005. It has been reported that the great majority of individuals with NF1 fulfill the diagnostic criteria by the age of 6 years (DeBella et al., 2000) implicating that those born before year 2006 were likely to have been diagnosed by the end of the ascertainment period of the NF1 cohort in 2011. The number of live individuals with NF1 in each stratum was divided by the general population in the corresponding stratum to obtain prevalence estimates. The 95% CIs were estimated with the Wilson score interval with continuity correction.

However, the observed prevalence underestimates the true prevalence of NF1, since it is highly unlikely that all individuals living with NF1 at a given moment would have been diagnosed and included in the NF1 cohort. Therefore, two approaches to estimate the true prevalence of NF1 were undertaken. Firstly, the highest prevalence observed in each age category over the years 1990–2011 was taken to represent the prevalence in this age group, similar to the work of Evans and colleagues (2010). The approach assumed that the incidence of NF1 was constant, and the prevalence only changed due to differential mortality compared to the general population. However, since the study period was limited and some age groups showed higher ascertainment than others, the method was prone to artifacts.

The second approach was also based on assuming a constant incidence of NF1, but the estimation was not based on the directly observed age-specific prevalence. Instead, the highest prevalence observed among boys aged 0–4 years was assumed to represent the incidence of NF1. In this age group, the effect of mortality associated with NF1 only plays a small role. The analysis was focused on boys since they may be diagnosed earlier than girls, and there is no evidence to suggest that the incidence of NF1 would differ by sex. The incidence was then multiplied with the ratio of survival probabilities calculated for individuals with NF1 and the general population in each age group to obtain age-specific prevalence estimates, similar to the approach applied by others (Crowell et al., 2021). The 95% CIs were estimated using the asymptotic normal distribution method (Tofighi and MacKinnon, 2011).

4.5.3 Breast cancer incidence, survival, and characteristics in the Finnish NF1 cohort and in the TCGA dataset (II)

The SIR was computed to compare breast cancer incidence in the NF1 cohort and the Finnish general population in Study II. Age group, calendar period, and sex-specific cancer incidence rates in the general population were multiplied with the person-years observed in the NF1 cohort in each stratum to compute the number of expected breast cancers. The SIR was then obtained as the ratio of observed and expected breast cancers, and the 95% CIs were based on the Poisson distribution. The competing risk for death was taken into account when the cumulative risks for breast cancer by the age of 85 years or within a specific age range were estimated.

When survival after breast cancer was estimated in Study II using the NF1 cohort and matched controls or data from TCGA, the follow-up started from the surgery of the cancer, and deaths due to any cause were considered events. The cumulative survival proportion was estimated using the Kaplan-Meier method, and the groups were compared using the Cox proportional hazards model with stratification to take into account the matching of individuals with NF1 and controls.

Breast cancer characteristics were compared between individuals with NF1 and matched controls using generalized linear mixed effects models to account for the matching. The characteristics of the sporadic TCGA breast cancers were compared using the Chi squared test and Fisher's exact test.

4.5.4 Survival and risk of contralateral breast cancer in five international cohorts (III)

In Study III, individuals with NF1 and no enhanced breast cancer screening, that is, the cohorts from Finland, Manchester, Paris, and Hamburg, were compared with individuals with NF1 and enhanced screening from Padua and the non-NF1 women at increased risk for breast cancer. Three outcomes were examined: 1) overall survival after breast cancer, 2) breast cancer-specific survival after breast cancer, and 3) cumulative risk for contralateral breast cancer.

Kaplan-Meier estimates were computed for each outcome, and the groups were compared using the Cox proportional hazards model. The follow-up started at the first breast cancer or, in another analysis, the latter of cohort entry and the first breast cancer. The two approaches were included to demonstrate the effect of an immortality bias in the time preceding the cohort entry. In the survival analyses, deaths due to any cause or deaths due to breast cancer were considered as events. Censoring was caused by emigration, last information on patient status, or, in the breast cancer-specific analysis, death due to other causes than breast cancer. When the incidence of contralateral breast cancer was studied, contralateral breast cancer was considered as an event, and death, emigration, and the last information on patient

status led to censoring. The incidence of contralateral breast cancer was computed with and without the competing risk of death. The characteristics of the breast cancers were compared using the Chi-squared test and Fisher's exact test.

4.5.5 The risk for diabetes (IV)

The rates of diabetes were estimated based on the Poisson distribution in Study IV. Cox proportional hazards models were used for comparisons of individuals with NF1 and the control and non-NF1 sibling cohorts. Since the controls were individually matched to patients with NF1, the groups were dependent, and a frailty term was included to allow heterogeneity between the subgroups of each individual with NF1 and the respective controls. A frailty term was also included for each family when individuals with NF1 and their non-NF1 siblings were compared. The occurrence of a diagnosis or drug purchase of interest was defined as an event, while death, emigration, or the end of follow-up was handled as censoring.

4.5.6 Concentration of plasma cfDNA (V)

In Study V, cfDNA levels were compared between individuals with NF1 and controls and within the NF1 group by the presence of various clinical characteristics. The variation in plasma dilution during sample processing was accounted for by normalizing cfDNA concentration with plasma protein concentration. Measured cfDNA concentration values were divided with a normalization coefficient that was obtained by dividing the plasma protein concentration of each sample with the average of protein concentrations observed in all samples. Natural logarithm transformation led to a Gaussian distribution of the normalized cfDNA values and allowed construction of linear regression models for comparisons. Separate age-adjusted and sex-adjusted models were studied in addition to an unadjusted model, yet the low number of participants did not allow incorporation of both age and sex in the same model.

5 Results

5.1 Prevalence of NF1 (I)

A total of 1,279 individuals from the Finnish NF1 cohort were alive on December 31st, 2005. This corresponds an observed prevalence of 1/4,088 (95% CI 1/4,320–1/3,869), which is of similar magnitude as previously reported (Section 2.1.2). The year 2005 marked the highest prevalence observed in the material because of the high rate of NF1 diagnoses among those born in the mid-1990s. Studying the maxima of prevalences observed in each age group over the period 1990–2011 confirmed that the youngest patients had been diagnosed at a much higher rate than those born before 1990, since the prevalence appeared to steeply decrease around 15 years of age. Interestingly, boys seemed to be diagnosed with NF1 at a younger age than girls.

Consequently, the observed prevalence is likely not an accurate estimate of the prevalence of NF1, since it is unlikely that there would have been a sudden and substantial change in the true incidence of NF1. The mortality of individuals with NF1 was higher than in the general population throughout the lifetime, and the age-specific prevalence of NF1 was therefore expected to decline along with increasing age. When the age-specific survival probabilities of individuals with NF1 and the general population were used to estimate the prevalence of NF1, a decline from 1/1,706 (95% CI 1/2,158–1/1,410) among children aged 0–4 years to 1/3,380 (95% CI 1/4,545–1/2,690) among adults aged 70–74 years was seen. The estimated overall prevalence of NF1 among individuals aged 0–74 years was 1/2,052 (95% CI 1/2,176–1/1,941) in Finland. This would correspond to 2,370 individuals with NF1 living in Finland in 2005.

5.2 Risk for breast cancer and contralateral breast cancer (II, III)

The risks for breast cancer and second contralateral breast cancer were significantly increased in NF1, and the highest relative risk for breast cancer was observed among young women with NF1. Thirty-one female breast cancers were diagnosed in the Finnish NF1 cohort during the follow-up in 1987–2013 yielding a SIR of 2.82 (95%

CI 1.92–4.00) for breast cancer in NF1 compared to the general population. When stratified by age group, the SIR was 5.1 (95% CI 2.9–8.1) among women younger than 50 years of age and 2.0 (95% CI 1.2–3.1) among women older than 49 years of age. The cumulative risk for breast cancer by 50 years of age was 7.8% (95% CI 3.9–11.5%) among women with NF1 and 2.1% in the general population. The respective numbers by the age of 85 years were 18.0% (95% CI 11.4–24.1%) and 9.7%. Women with NF1 had a risk of 4.7% (95% CI 1.5–7.9%) at ages 30–39, 3.9% (95% CI 0.77–7.0%) at ages 40–49, and 5.9% (95% CI 1.9–9.8%) at ages 50–59 for being diagnosed with breast cancer. The corresponding numbers in the Finnish general population were 0.34%, 1.5%, and 2.6%, respectively. Regarding the risk factors of breast cancer, the proportions of nulliparous women or those with the first birth before 30 years of age did not differ between individuals with NF1 with or without breast cancer. Three women with NF1 were tested for pathogenic variants of *BRCA1/2*, yet none were detected. No ovarian cancers were seen among the individuals with NF1 and breast cancer.

The risk for contralateral breast cancer was studied by following up 142 women with NF1 and breast cancer from the Finnish, Manchester, Paris, Hamburg, and Padua cohorts. There were 12 contralateral breast cancers diagnosed at a median age of 53 years. The cumulative risk for being diagnosed with a contralateral breast cancer within 20 years of the first breast cancer was 27% (95% CI 12–57%). When competing mortality was taken into account, the risk for contralateral breast cancer was 16% (95% CI 8.1–30%). Half of the contralateral breast cancers were detected in screening, and half were detected because of symptoms.

5.3 Survival after breast cancer diagnosis (II, III)

Survival after breast cancer diagnosis was worse among women with NF1 compared to matched controls. Tissue samples were available from the breast cancers of 26 women with NF1 in the Finnish NF1 cohort. These women were compared with 130 controls who had cancers matched with the age and year of diagnosis and estrogen receptor status. The Kaplan-Meier estimate for 5-year all-cause survival was 68% (95% CI 52–89%) among women with NF1 and 82% (95% CI 76–89%) among the matched controls (HR 2.34, 95% CI 0.99–5.6).

When the female breast cancers observed in the Finnish NF1 cohort were combined with the data from the Manchester, Hamburg, and Paris cohorts, women with NF1 and breast cancer had lower survival compared to non-NF1 women undergoing enhanced screening irrespective of cancer grade. Out of the 115 women with NF1 and breast cancer, 53 had died during the follow-up with 26 of them due to breast cancer. The 5- and 10-year all-cause survival estimates of women with NF1 and breast cancer were 65% (95% CI 55–77%) and 50% (95% CI 39–63%),

respectively, when the follow-up started from the latter of breast cancer and cohort entry. The breast cancer-specific 10-year survival was 64% (95% CI 54–77%). As expected, the survival probabilities were overestimated if the follow-up was allowed to start before cohort entry. The non-NF1 women undergoing enhanced screening because of familial risk for breast cancer displayed 93% (95% CI 89–96%) and 90% (95% CI 86–94%) 5- and 10-year all-cause survival, respectively. Women from the Padua cohort had been screened for breast cancer since 45 years of age, and their all-cause and breast cancer-specific 10-year survival estimates were 93% (80–100%) and 100%, respectively. The women from Padua had significantly lower risk for death after breast cancer than women from the four other NF1 cohorts (HR 0.18, 95% CI 0.04–0.72). Among women with NF1, estrogen receptor negativity was associated with reduced survival (HR 2.8, 95% CI 1.2–6.8).

5.4 Breast cancer characteristics (II, III)

The histopathological characteristics of breast cancers from women with NF1 differed from control breast cancers. Twenty-six breast cancers from the Finnish NF1 cohort were compared with 130 female control breast cancers matched with the age at diagnosis. Breast cancers of women with NF1 were more often estrogen and progesterone receptor-negative (54% and 65%) than breast cancers of the controls (21% and 22%; P=0.001 and P<0.001, respectively). Moreover, *ERBB2* amplifications were more frequent in the breast cancers of individuals with NF1, as *ERBB2* amplification was observed in 31% of NF1-related cancers and 10% of control cancers (P=0.006). The breast cancers of women with NF1 were of a higher grade (P=0.050) and a larger size (P=0.019), yet lymph node involvement was not significantly higher in NF1 than among controls (P=0.332). Out of the 26 NF1-related breast cancers, 89% were ductal carcinomas and 7.7% were lobular carcinomas. The luminal B subtype represented 35% of the cancers, while 15% of the NF1-related breast cancers were luminal A, 31% were the HER2 subtype, and 19% were triple-negative. Despite the association of NF1 with hormone receptor negativity, the proportion of triple-negative breast cancers did not significantly differ between the NF1 (19%) and control (13%) groups (P=0.399).

In the international cohorts of women with NF1 and breast cancer, the cancer was estrogen receptor-negative in 5/16 (31%) women from Manchester, 3/12 (25%) women from Hamburg, 1/6 (17%) women from Paris, and 1/12 (8.3%) women from Padua. With all five cohorts combined, the rate of estrogen receptor negativity was 32%. HER2 positivity ranged from 0.0% in the Hamburg and Paris cohorts to 22% in Padua, 31% in Manchester, and 32% in Finland. The combined rate of HER2 positivity of 24% was significantly higher than the 11% seen in the non-NF1 cohort with enhanced screening (P=0.03). NF1-related breast cancers were also less

frequently *in situ* and, when invasive, less often stage 1 than tumors seen among the non-NF1 women with enhanced screening.

5.5 Somatic *NF1* alterations in breast cancers of the general population (II)

Analysis of the primary breast cancers included in the TCGA dataset revealed similar features in sporadic breast cancers with somatic *NF1* alterations as observed in the breast cancers of women with NF1. Mutations, shallow deletions, or deep deletions of the *NF1* gene were present in 33% of the TCGA breast cancers. The 5-year survival after breast cancer harboring somatic *NF1* alteration was worse than after breast cancer with *NF1* intact (77%, 95% CI 71–84% and 87%, 95% CI 82–92%, respectively; HR 1.9, 95% CI 1.14–3.00). The breast cancers with somatic alterations in the *NF1* gene were more often estrogen- and progesterone receptor-negative and harbored *ERBB2* amplification more frequently than cancers without *NF1* alterations (P<0.001 for all).

5.6 Risk for diabetes (IV)

A comparison of 1,349 individuals with NF1 to 13,870 controls and 1,871 non-NF1 siblings of individuals with NF1 in 1998–2014 yielded a relative rate of diabetes of 0.34 (95% CI 0.23–0.49) compared to controls and 0.35 (95% CI 0.23–0.55) compared to the non-NF1 siblings indicating a clearly decreased risk. The rate of diabetes diagnoses was 1.7 (95% CI 1.1–2.4) per 1,000 person-years in the NF1 cohort, 5.1 (95% CI 4.8–5.4) in the control cohort, and 4.3 (95% CI 3.5–5.2) in the cohort of the non-NF1 siblings.

The risk for type 1 diabetes was decreased among individuals with NF1, yet the association was not statistically significant with HRs of 0.58 (95% CI 0.27–1.25) and 0.55 (95% CI 0.23–1.33) compared to controls and the siblings without NF1, respectively. There were seven individuals with NF1, 129 controls and 19 siblings without NF1 who had a diagnosis of type 1 diabetes during 1998–2014. Only two males with NF1 (29%) had type 1 diabetes, while male predominance was observed in the control (56%) and the non-NF1 sibling (63%) cohorts. The differences in the sex distribution were reflected in the HR estimates, as the HR for type 1 diabetes in NF1 was 0.95 (95% CI 0.38–2.36) among females and 0.29 (95% CI 0.07–1.2) among males compared to controls. However, the small number of individuals with NF1 and type 1 diabetes limits any conclusions regarding the effect of sex.

The HR for type 2 diabetes showed a markedly and statistically significantly lower relative rate in NF1 compared with controls (0.27, 95% CI 0.17–0.43) or the non-NF1 siblings (0.28, 95% CI 0.16–0.47). The numbers of individuals with type 2

diabetes were 19, 779, and 83 among those with NF1, controls, and the non-NF1 siblings, respectively. Individuals with NF1 had 1.1 (95% CI 0.68–1.8) new diagnoses of type 2 diabetes per 1,000 person-years, while the respective rates were 4.4 (95% CI 4.1–4.7) among controls and 3.4 (95% CI 2.7–4.2) among the non-NF1 siblings of individuals with NF1. As in the case of type 1 diabetes, the effect of NF1 on the risk for type 2 diabetes was more pronounced among males than among females, yet the difference to controls or to the non-NF1 siblings was significant irrespective of sex. The great majority of individuals with NF1 and type 2 diabetes had purchased anti-diabetic medication during the follow-up time, while only a minority (37%) had hospital encounters related to type 2 diabetes.

The results regarding type 2 diabetes remained essentially similar when the analysis was limited to individuals younger than 50 years of age. This was also the case when only hospital visits and hospital stays were considered and years 1987–2014 were studied among 1,410 individuals with NF1, 14,017 matched controls, and 1,881 non-NF1 siblings. The decreased risk for type 2 diabetes in NF1 was not related to a lower risk for obesity or dyslipidemias, since the HR for a diagnosis of obesity or dyslipidemia was 1.04 (95% CI 0.76–1.43) in NF1 versus controls and 1.20 (95% CI 0.77–1.87) in NF1 versus the non-NF1 siblings.

5.7 The effect of NF1 on circulating free plasma DNA (V)

Only a minor effect of NF1 on plasma cfDNA concentration was observed. Two samples suggestive of hemolysis were excluded from the NF1 group, and two samples were excluded from the control group. The mean age of individuals with NF1 was 36.0 years (standard deviation [SD] 12.9), and the mean age of the controls was 38.7 years (SD 12.0). Analysis of the 19 plasma samples from individuals with NF1 and 12 controls revealed medians of normalized cfDNA concentrations of 19.3 ng/ml (range 6.6–78.6) and 15.9 ng/ml (range 4.8–47.0), respectively. The difference between individuals with NF1 and controls was not statistically significant (P=0.369). A negative correlation was observed between age and cfDNA among individuals with NF1 (Spearman's rho −0.55). Consequently, when the comparison between individuals with NF1 and controls was adjusted for age, NF1 was marginally significantly associated with a higher normalized cfDNA concentration (P=0.023 for NF1, P=0.032 for the interaction between NF1 and age). When the effects of NF1 and sex on normalized cfDNA were studied, no significant associations were found. The results remained essentially unchanged when a plain cfDNA concentration was used instead of the normalized cfDNA concentration.

Among individuals with NF1, those with at least six subcutaneous neurofibromas or a plexiform neurofibroma had a slightly higher normalized cfDNA concentration

than those with less than six subcutaneous neurofibromas or without a known plexiform neurofibroma, yet the differences were not statistically significant (P=0.514 and P=0.122, respectively). No association between the number of cutaneous neurofibromas and normalized cfDNA concentration was observed.

6 Discussion

The results of the present study elucidate the prevalence of NF1 and the associated risks for breast cancer and diabetes. In addition, a small-scale trial on the effect of NF1 on the plasma cfDNA concentration serves as a starting point for further studies. Although the epidemiologic Studies I–IV were register-based, they can also elucidate the molecular biology of the *NF1* gene. The Finnish NF1 cohort is well suited for the epidemiological study of NF1, since the cohort is relatively large, and the NF1 diagnoses of all the included individuals have been confirmed according to the NIH diagnostic criteria for NF1, which increases the reliability and statistical power of the analyses. If individuals with potentially misregistered diagnoses, such as NF2 and tuberous sclerosis, were included, a false association between NF1 and, for example, vestibular schwannoma could be observed. Moreover, individuals with an unaltered risk for a disease, such as diabetes, would contribute person-time thus diluting the estimated effect of NF1. However, the Finnish NF1 cohort alone provides insufficient statistical power for studying very uncommon events, such as contralateral breast cancer. The risk for contralateral breast cancer is nevertheless clinically relevant, and an international collaboration in Study III was necessary to allow a quantitative study. The Finnish nationwide registries allow a long-term follow-up with practically no missing information. The Finnish registers can also provide pertinent control data, such as the population level statistics for estimating the prevalence of NF1 in Study I, and general population breast cancer incidence rates in Study II. When diabetes in NF1 was studied, comparisons with matched controls and the non-NF1 siblings of individuals with NF1 yielded highly concordant results, which increases the confidence that the decreased risk for diabetes is indeed related to NF1.

The excess mortality caused by NF1 throughout the lifetime (Study I) is concordant with prior evidence (Wilding et al., 2012). The poor survival associated with NF1 had major methodological implications for the present study. The competing risk for death was taken into account when estimating the risks for breast cancer and contralateral breast cancer in Studies II and III. The results of Study III highlight the consequences of taking the competing risk for death into account, as the plain estimate for the risk of contralateral breast cancer was 27% within 20 years,

yet the estimated risk was 16% allowing for the competing risk for death. The 27% risk applies to those who live 20 years after their first breast cancer, yet since the risk for death is marked among individuals with NF1, a woman with NF1 and breast cancer faces a 16% risk for contralateral breast cancer plus a significant risk for death within 20 years. In the case of diabetes, in Study IV, the excess mortality associated with NF1 could introduce bias due to the scarcity of older individuals who are at the greatest risk for type 2 diabetes, but the age-specific follow-up overcame this bias, which was further confirmed in a sensitivity analysis restricted to individuals younger than 50 years of age.

6.1 Implications for the care of individuals with NF1

The results regarding the prevalence of NF1 (Study I) and breast cancer (Studies II and III) have clear clinical implications for the care of individuals with NF1. In contrast, the decreased risk for diabetes observed among individuals with NF1 in Study IV cannot be used to guide clinical practice. As type 2 diabetes is a relatively common disorder, it is clear that the risk remains clinically significant also in NF1. Individuals with NF1 should undergo similar testing for blood glucose concentrations as the general population, and they also need to be educated about the risks related to type 2 diabetes irrespective of their NF1.

Study V focused on exploring the association of NF1 and cfDNA concentration. The results are insufficient to advise the clinical care of individuals with NF1. Even though the results suggest that NF1 does not markedly interfere with plasma cfDNA levels, the sample size was too small, and the cohort included, for example, no individuals with MPNST. Importantly, another recent trial did not observe a significant tumor fraction in cfDNA among individuals with plexiform neurofibromas (Szymanski et al., 2021). However, further studies are called for to assess how plexiform neurofibromas affect the sensitivity of cfDNA-based assays for non-invasive prenatal testing or for cancer screening or monitoring. An ideal study would compare serial samples of plasma cfDNA between individuals with and without plexiform neurofibromas and encompass a sample size and follow-up time sufficient for observing cancers and pregnancies in the course of the study.

6.1.1 Prevalence and access to specialized health care

The estimated prevalence of NF1 (1/2,052) was markedly higher than the observed prevalence of NF1 (1/4,088) in Finland. The difference between the estimated and observed prevalence was particularly high in the older age groups. The prevalence estimate of 1/2,052 is also high compared to most previous estimates of overall

prevalence of NF1 (Huson et al., 1989; Clementi et al., 1990; Poyhonen et al., 2000; Lammert et al., 2005; McKeever et al., 2008; Evans et al., 2010).

Individuals who have not visited specialized health care were missed in the present study, since the individuals included in the Finnish NF1 cohort were searched from central and university hospitals. However, it is currently recommended that all individuals with NF1 should undergo surveillance in specialized health care (Peltonen et al., 2014; Stewart et al., 2018). The gap between the estimated and observed prevalence of NF1 indicates that a marked proportion of individuals with NF1 are not undergoing the surveillance that they would need. The lack of proper surveillance may postpone the detection of NF1-related health concerns, such as cancers and cardiovascular disease, which complicates their management. Indeed, the survival of individuals with NF1 was poor even among those who had been diagnosed. Morbidity and mortality caused by NF1 could be reduced by encompassing a higher proportion of individuals with NF1 in surveillance by a multidisciplinary team of specialists. The education of individuals with NF1 and primary care physicians is needed to achieve this goal.

Since the results indicate that the youngest age groups have been diagnosed more comprehensively than their parents, the situation is already likely improving, and the observed prevalence of NF1 will likely be higher a decade later. However, most studies on the prevalence of NF1, including the present Study I, are based on hospital-based ascertainment and may therefore be inclined to include individuals with the most severe NF1-related disease manifestations. As the diagnostic sensitivity is increasing in the youngest age groups, also the milder NF1 may be diagnosed more often. When treating these individuals, it is important to remember that the risk estimates based on older NF1 cohorts may overestimate the risks for comorbidities due to the selection bias.

6.1.2 Breast cancer risk associated with NF1

The results related to the breast cancer risk of individuals with NF1 have clinical implications. A marked breast cancer risk was observed among women with NF1 already after 30 years of age, as the risk for being diagnosed with breast cancer at age 30–39 was 4.7% (95% CI 1.5–7.9%). The result is in concordance with other studies showing a significantly increased risk for breast cancer among women with NF1 after 30 years of age (Walker et al., 2006; Sharif et al., 2007; Madanikia et al., 2012; Wang et al., 2012; Seminog and Goldacre, 2015). Notably, the 18% absolute risk for breast cancer by 85 years of age is higher than the lifetime risk of 7.5–16% for MPNST in NF1 (McGaughran et al., 1999; Evans et al., 2002; Ingham et al., 2011; Uusitalo et al., 2016). Moreover, the risk for contralateral breast cancer was markedly high among individuals with NF1 after the first breast cancer. Given the

high breast cancer incidence in NF1, it remains to be elucidated whether having a history of breast cancer indicates an increased risk for a second breast cancer, or whether the rate of contralateral breast cancer only reflects the high baseline incidence of breast cancer in NF1.

In accordance with other studies (Uusitalo et al., 2016; Yap et al., 2018; Suarez-Kelly et al., 2019), also the survival after NF1-associated breast cancer was poor. The comparison cohort of Study III consisted of women undergoing enhanced screening due to familial risk for breast cancer in Manchester and it does not represent breast cancer in the general population. However, the combined 5-year survival rate of 65% in the four unscreened NF1 cohorts was also poor compared to previous reports of a 5-year survival of 80–87% after breast cancer in the general population (Allemani et al., 2018). The tumors often displayed poor prognostic factors, and it is thus evident that breast cancers in individuals with NF1 need to be detected at an earlier stage to improve the prognosis. Unfortunately, the relapse-free survival after NF1-related breast cancer could not be examined in Studies II and III due to the lack of sufficiently detailed follow-up data.

It has been suggested that the breast cancer screening of individuals with breast cancer-predisposing gene variants should be started at the age when the risk exceeds that of the general population being routinely screened (Tung et al., 2016). In Finland, women aged 50–69 years undergo mammography screening (Sarkeala et al., 2008a), and the risk for being diagnosed with breast cancer at ages 50–59 years is 2.6% in the general population (Study II). Consequently, the results suggest that women with NF1 should be screened with mammography starting at the age of 30. However, the efficacy of mammography screening of young women with NF1 is currently poorly known (Stewart et al., 2018; Maani et al., 2019). Young women have radiologically dense breasts which reduces the diagnostic sensitivity of mammography (Burton et al., 2017). Cutaneous neurofibromas may further interfere with image analysis (Zhou et al., 2012; Da Silva et al., 2015; Howell et al., 2017). The cumulative dose of ionizing radiation caused by repeated mammography has also been a concern in NF1 (Sharif et al., 2006; Evans, 2012). Biennial screening would reduce the radiation dose compared to annual examinations, yet longer screening interval may lead to more interval cancers and a higher tumor stage at diagnosis especially if the cancers display aggressive phenotype.

A simulation study estimated that the ionizing radiation associated with annual screening mammography at the ages 30–80 causes 36–46 breast cancer deaths per 100,000 screened women in the general population, while the corresponding mortality rates are 27–35 and 10–12 for women screened at the ages of 35–80 and 50–80 years, respectively (Hendrick, 2010). Thus, the start of annual screening mammography at the age of 30 instead of 50 years would cause 26–34 breast cancer-related deaths per 100,000 women in the general population. Similarly, the start of

screening at 35 years of age could be related to 17–23 additional deaths. Another simulation estimated rates of 16 and 7 breast cancer deaths per 100,000 women in the general population, if annual mammography screening is performed at the ages of 40–74 or 50–74 years, respectively (Miglioretti et al., 2016). Importantly, the modeling estimated that the rates of breast cancer deaths avoided owing to the screening are 968 and 819 per 100,000 women in the age ranges of 40–74 and 50–74 years, respectively. Therefore, these simulations clearly show the benefit of mammography screening and support its initiation already at 40 years of age. Extrapolating these figures for women with NF1 is difficult, yet the large gap between the numbers of radiation-induced and screen-prevented deaths suggests that the screening is likely beneficial also in NF1. Cutaneous neurofibromas may increase the need for follow-up imaging (Howell et al., 2017; Maani et al., 2019), which further increases the radiation dose and thereby the risk for radiation-induced carcinogenesis (Miglioretti et al., 2016). Moreover, data from animal models suggest that NF1 may increase radiation-induced tumorigenesis (Chao et al., 2005; Nakamura et al., 2011; Choi et al., 2012).

Nevertheless, mammography screening for women with NF1 at ages of greater than 30 years is already recommended in, for example, the USA (Daly et al., 2018). In Finland, annual breast MRI is recommended for women with NF1 at ages 30–50 and mammography starting at age 35 (Suomen Rintasyöpäryhmä Ry, 2021). The accumulating experience will elucidate the effectiveness of the different screening approaches. The excellent breast cancer-specific survival observed in the Padua cohort with enhanced screening since the age of 45 years (Study III) suggests that earlier start of screening may indeed be beneficial. However, to verify the benefit of screening, a reduction in breast cancer mortality should be observed. Women with NF1 can also be encouraged to perform regular breast self-examination, yet the efficacy of this practice remains to be elucidated (Kösters and Gøtzsche, 2003; Nelson et al., 2009). Nevertheless, women with NF1 should be educated about their breast cancer risk. Both the patients and their physicians need to bear in mind that a new breast lump always requires proper examination. This is particularly important since cutaneous and subcutaneous neurofibromas are frequent in women with NF1 and may confuse the evaluation.

The true risk for breast cancer likely varies between women with NF1, since the NF1 phenotype overall is highly variable. The risk conferred by different pathogenic variants of breast cancer risk genes may differ even among protein truncating variants, and the effects of missense variants are even more challenging to predict (Easton et al., 2015). Identification of the germline *NF1* variants causing the greatest increase in breast cancer risk would allow targeting of screening efforts and would thus reduce the harms associated with radiation and the psychological burden of cancer screening. However, currently available evidence does not allow stratification

of women with NF1 by their breast cancer risk. The overall risk for breast cancer among women with NF1 can be termed moderate, and the risk is smaller than, for example, the risk for breast cancer among carriers of pathogenic variants of *PALB2*, *CHEK2*, or *BRCA1/2* (Easton et al., 2015). Common variants of other genes, as measured by a polygenic risk score, significantly modify the breast cancer risk of women with a pathogenic variant of *PALB2* (Mars et al., 2020). Genes other than *NF1* may also modify the breast cancer risk in the case of NF1. While the roles of polygenic risk scores and specific modifier-genes in NF1-associated breast cancer risk have not been identified, family history needs to be taken into account when considering, for example, a contralateral risk-reducing mastectomy.

6.2 The role of the *NF1* gene in breast cancer evolution

In Study II, somatic *NF1* alterations in sporadic primary breast cancers were associated with a similar pattern of poor survival, decreased hormone receptor positivity, and an increased frequency of *ERBB2* amplifications as breast cancers occurring in women with NF1. The concordance of these observations suggests that they are due to the loss of *NF1* in breast cancer cells. While it is plausible that the *NF1*-haploinsufficient microenvironment, present in individuals with NF1 due to the pathogenic germline *NF1* variant, can also affect cancer progression, the microenvironment does not seem to be a necessary contributor to the poor prognosis and prognostic factors observed in Study II. The association between NF1 and HER2 positivity was also observed in the five combined NF1 cohorts in Study III, yet the rate of estrogen receptor positivity was only non-significantly decreased. However, the association of NF1 with hormone receptor negativity and HER2 positivity of breast cancer has been reported also in other studies (Wang et al., 2016; Yap et al., 2018; Landry et al., 2021), and taken together, the phenomenon seems to be reproducible and real. Similar to *NF1*, also the pathogenic germline variants of *BRCA1* are associated with hormone receptor-negative breast cancer (Foulkes et al., 2004; Lakhani et al., 2005), and pathogenic germline variants of *TP53* are linked to *ERBB2* amplifications (Wilson et al., 2010; Masciari et al., 2012).

6.2.1 The *NF1* gene and resistance to hormonal therapy

In addition to the present study, also others have reported that somatic alterations of the *NF1* gene are frequent in breast cancers of the general population (Wallace et al., 2012; Yap et al., 2018). Moreover, the observation of breast cancers with somatically acquired *NF1* deficiency being often hormone receptor-negative and HER2-positive has been reproduced in multiple studies (Dischinger et al., 2018; Griffith et al., 2018;

Razavi et al., 2018; Yap et al., 2018). While the TCGA dataset represents primary breast cancers, somatic *NF1* mutations have often been observed especially in advanced breast cancers (Razavi et al., 2018; Angus et al., 2019; Bertucci et al., 2019; Sokol et al., 2019; Pareja et al., 2020; Pearson et al., 2020; Huang et al., 2021). The higher frequency of *NF1* mutations in a metastatic than in a primary setting could be related to, for example, an initial treatment resistance or an aggressive phenotype (Sokol et al., 2019). However, several studies have found a higher frequency of *NF1* alterations at metastatic sites than in matched primary tumors (Keene et al., 2018; Razavi et al., 2018; Fumagalli et al., 2020; Richard et al., 2020; Akcakanat et al., 2021). This suggests that somatic *NF1* loss is associated with the acquisition of treatment resistance in sporadic breast cancer (Yates et al., 2017; Razavi et al., 2018; Fumagalli et al., 2020). While *NF1* alterations are apparently associated with hormone receptor negativity in primary breast cancer, especially hormone receptor-positive cancers seem to acquire *NF1* mutations during cancer progression (Yates et al., 2017; Angus et al., 2019; Pearson et al., 2020). It has also been suggested that *NF1* mutations would be more frequent in advanced lobular breast cancers than in ductal carcinomas, which may be related to the high frequency of hormone receptor positivity in lobular tumors (Sokol et al., 2019; Pareja et al., 2020). These observations suggest that the treatment of hormone receptor-positive breast cancers with anti-estrogen therapy provides *NF1*-deficient cells an evolutionary benefit. Thus, an initial *NF1* deficiency often yields the primary breast cancer as hormone receptor-negative, yet an initially hormone receptor-positive breast cancer with normal *NF1* may acquire *NF1* alterations during disease progression.

Somatic *NF1* deficiency has indeed been reported to be associated with gene expression patterns related to endocrine therapy resistance (Dischinger et al., 2018; Pearson et al., 2020). Mutations of the *ESR1* gene encoding the estrogen receptor are a mechanism of resistance to endocrine therapy (Razavi et al., 2018; Pearson et al., 2020), and *NF1* deficiency has been reported to be mutually exclusive with *ESR1* mutations (Razavi et al., 2018; Sokol et al., 2019; Angus et al., 2019; Fumagalli et al., 2020; Pareja et al., 2020; Pearson et al., 2020). This suggests that *NF1*-mutant cancer cells gain no further benefit of *ESR1* mutations, and vice versa, indicating a role for *NF1* loss in the acquisition of endocrine therapy resistance in breast cancer (Griffith et al., 2018). Supporting this hypothesis, a study reported that the prevalence of somatic *NF1* mutations in breast cancer after endocrine therapy such as treatment with tamoxifen or aromatase inhibitor is twice as high as prior to hormonal therapy (Razavi et al., 2018). Somatic *NF1* mutations may therefore be a mechanism of tamoxifen resistance in breast cancer (Griffith et al., 2018; Razavi et al., 2018; Sokol et al., 2019; Pearson et al., 2020; Zheng et al., 2020).

An *in vitro* study found that *NF1* silencing yields resistance to tamoxifen and to estrogen deprivation (Pearson et al., 2020). *NF1* knockdown reduces estrogen receptor expression, while it increases MAPK pathway activation and upregulates cyclin D1 expression driving resistance to endocrine therapy such as tamoxifen or estrogen deprivation (Pearson et al., 2020). Another mechanistic study found that 4-hydroxytamoxifen stimulates the growth of estradiol-deprived breast cancer cells both *in vitro* and when xenografted into nude mice, if the *NF1* gene is silenced (Zheng et al., 2020). The effect is only seen in estrogen receptor-positive breast cancer cells. Normally, 4-hydroxytamoxifen acts as an antagonist of cell growth. The *NF1* knockdown also modulates the effects of estradiol and 4-hydroxytamoxifen on gene expression. Neurofibromin protein contains estrogen receptor co-repressor motifs that normally downregulate the response to estrogen (Zheng et al., 2020). Consequently, *NF1* deficiency reduces the downregulation of estrogen receptor activity. The results suggest that tamoxifen treatment of *NF1*-deficient breast cancer can even be detrimental (Zheng et al., 2020).

In concordance with the idea of endocrine therapy resistance induced by somatic *NF1* mutations, somatic *NF1* mutations have been found to be associated with lower survival compared with *NF1*-wild-type tumors in sporadic, hormone receptor-positive breast cancer (Griffith et al., 2018; Bertucci et al., 2019; Pearson et al., 2020), independent of tumor grade and disease stage (Griffith et al., 2018). Moreover, *NF1* deletions are also associated with a high tumor grade, stage, and size in addition to worse survival (Dischinger et al., 2018). Low *NF1* expression detected in biopsies taken prior to the initiation of tamoxifen therapy is associated with an increased risk for distant metastasis independent of other prognostic markers (Mendes-Pereira et al., 2012). In addition to the signaling contributing to endocrine therapy resistance, *NF1* loss has been suggested to be associated with epithelial-to-mesenchymal transition in cell models (Zheng et al., 2020). *NF1* silencing increases the expression of transcription factors inducing epithelial-to-mesenchymal transition in MCF7 and T47D breast cancer cell lines (Arima et al., 2010).

Taken together, initially hormone receptor-positive breast cancer can acquire resistance to anti-estrogen therapy *via* somatic *NF1* mutations during the disease progression, which suggests that *NF1*-deficient breast cancer cells can prosper without or at low levels of hormone stimulation. This phenomenon likely explains the high rate of hormone receptor negativity in breast cancers of individuals with NF1, which was observed in Study II. Since individuals with NF1 are born with a germline pathogenic variant of the *NF1* gene, their breast cancers are *NF1*-deficient already at the beginning of tumorigenesis. This enables a relative independence of hormone receptors leading to low estrogen and progesterone receptor expression already in the primary tumor. The findings highlight that the order of mutations leading to malignant transformation guide the tumor phenotype, as an initial *NF1*

deficiency leads to hormone-independent tumors, while initially *NF1*-wild-type tumor expressing the estrogen receptor may gain an evolutionary benefit upon *NF1* loss, especially when endocrine therapy leads to the selection of hormone-independent cells. As observed in Studies II and III focusing on breast cancer in NF1, individuals with NF1 can also have hormone receptor-positive breast cancers, which suggests that estrogen receptor expression may still be beneficial for the cancer cells in some genetic contexts, or *NF1* variants may differ in terms of their effects on hormone receptor signaling. The poor survival observed in association with both somatically *NF1*-deficient sporadic breast cancer, and breast cancer of women with NF1 may be partly explained by the effects of *NF1* deficiency on estrogen signaling, as suggested by the agonistic action of tamoxifen in *NF1*-deficient cells reported by Zheng and colleagues (2020). Understanding the causal role of the *NF1* gene in breast cancer evolution should receive a high priority in future research, since it can affect the treatment of *NF1*-deficient breast cancers both in association with the NF1 syndrome and in sporadic settings.

6.2.2 MAPK pathway activation and resistance to hormonal therapy

The effects of *NF1* deficiency on hormone signaling are partly mediated by the MAPK pathway (Razavi et al., 2018; Pearson et al., 2020; Zheng et al., 2020). Similar to the downregulation of estrogen receptor expression by *NF1* deficiency (Pearson et al., 2020), such an effect can also be caused by other mechanisms of MAPK pathway activation, such as constitutively active Ras or MEK (Creighton et al., 2006). Tamoxifen, fulvestrant, and estradiol deprivation fail to reduce the ERK and Akt phosphorylation induced by *NF1* knockdown in breast cancer cell lines (Mendes-Pereira et al., 2012; Pearson et al., 2020). The Ras–Raf–MEK–ERK activation resulting from *NF1* loss confers resistance to anti-estrogen therapy, and Raf, MEK, or ERK inhibition can restore the sensitivity to hormonal therapy (Mendes-Pereira et al., 2012; Razavi et al., 2018; Pearson et al., 2020; Zheng et al., 2020). MEK inhibition for recovering sensitivity to endocrine therapy can be achieved *via*, for example, trametinib or selumetinib (Pearson et al., 2020; Zheng et al., 2020).

Mutations of also MAPK pathway genes other than *NF1*, such as *ERBB3*, *KRAS*, *HRAS*, *BRAF*, and *MAP2K1*, have been found to be mutually exclusive with *ESR1* mutations and to enrich after endocrine therapy of hormone receptor-positive breast cancer (Razavi et al., 2018). Alterations of the MAPK pathway are associated with poor progression-free survival compared with wild-type cancers when treated with an aromatase inhibitor or selective estrogen receptor degrader (Razavi et al., 2018). Moreover, epidermal growth factor receptor overexpression confers resistance to

fulvestrant (Razavi et al., 2018). When a MCF7 breast cancer cell line was modified to display MAPK hyperactivation, the expression of several genes involved in estrogen receptor signaling and estradiol response was similarly altered irrespective of the genetic event leading to MAPK hyperactivation (Creighton et al., 2006).

6.2.3 The interplay between *NF1* and *ERBB2*

In Study II, a positive association between *NF1* alterations and *ERBB2* amplifications was observed in breast cancers of women with NF1 and in sporadic primary breast cancers included in the TCGA dataset. A later study found that somatic *NF1* deficiency is also associated with higher HER2 expression among tumors with *ERBB2* amplification as compared to *ERBB2*-amplified tumors with normal *NF1* (Wang et al., 2018b). The association between *NF1* deficiency and HER2 expression remained unclear in tumors without *ERBB2* amplification, since the *NF1* deficiency was often caused by deletions spanning also the *ERBB2* locus (Wang et al., 2018b). Another study reported that somatic *NF1* frameshift or nonsense mutations are frequent in HER2-enriched breast cancer without *ERBB2* amplification (Griffith et al., 2018). These observations further highlight that *NF1*-deficient breast cancer often upregulates HER2 expression either through the amplification of the *ERBB2* gene, as in Study II, or increased transcription.

Neurofibromin is a negative regulator of the MAPK pathway, and the pathway can be activated by HER2 (Figure 1). It can thus be hypothesized that early *NF1* loss, such as a germline pathogenic *NF1* variant or somatically acquired *NF1* deficiency that is already present in the primary tumor, molds the cancer cells dependent on the MAPK pathway. Such MAPK-dependent cells would gain a growth benefit by HER2 amplification or overexpression. On the other hand, an initially *ERBB2*-amplified breast cancer may acquire resistance to HER2 inhibition *via* loss of *NF1* (Smith et al., 2021). This implies that the somatic loss of *NF1* can make cell growth independent of HER2, which is discordant with the idea of an evolutionary benefit upon HER2 upregulation in cells with an initially high MAPK pathway activation. However, the apparent discrepancy may be related to the neurofibromin dose: cells with the loss of one *NF1* allele may gain a growth advantage upon increased HER2 activity, yet biallelic loss of *NF1* can render HER2 activation obsolete.

6.2.4 Alterations of the *NF1* gene in breast carcinogenesis

While it seems clear that *NF1* deficiency has profound effects on the cell signaling in breast cancer and breast cancer evolution, reflected in the hormone receptor and HER2 status, the high risk for breast cancer of women with NF1 observed in Studies II and III calls for further explanations. One obvious contributor is the germline loss

of one allele of the *NF1* tumor suppressor gene that takes the breast cells one step towards tumorigenesis. In the tumorigenesis of cutaneous and plexiform neurofibromas, a somatic *NF1* second-hit mutation in the healthy allele or loss-of-heterozygosity is a crucial step (Maertens et al., 2006a). Studies examining the *NF1* status of NF1-associated breast cancer are scarce, yet *NF1* second-hit mutations or loss-of-heterozygosity have been observed in some breast cancers of individuals with NF1 (Güran and Safali, 2005; Yap et al., 2018). A research group observed somatic *NF1* loss-of-heterozygosity in 4/9 NF1-related breast cancers studied (Wang et al., 2018b). This is well in accordance with the 2–4-fold overall risk for breast cancer associated with the NF1 syndrome in Study II and in previous reports (Table 5). Since there is no reason to believe that individuals with NF1 would be protected from the pathogenic mechanisms occurring in sporadic breast cancers, 25–50% of their breast tumors would occur irrespective of the NF1 syndrome. The NF1 syndrome can therefore be considered the underlying reason of approximately half of the tumors, which is consistent with the 44% rate of *NF1* loss-of-heterozygosity observed by Wang and co-workers (2018b).

While the somatic inactivation of the healthy *NF1* allele is considered a necessary step in the pathogenesis of neurofibromas (Jouhilahti et al., 2011), a mouse study reporting recurrent spontaneous *Nf1* deletions in mammary tumors found a homozygous deletion only in half of the cases suggesting that already the loss of one *Nf1* allele drives tumorigenesis (Wallace et al., 2012). This conclusion is supported by a study on *NF1* mutations in sporadic breast cancer, where the *NF1* loss was heterozygous in 20% of *NF1*-mutant cancers (Sokol et al., 2019). Experiments based on breast cancer cells have demonstrated that partial *NF1* silencing affects the cell phenotype highlighting the contribution of heterozygous *NF1* loss (Pearson et al., 2020). Obviously, the pathogenicity of heterozygous *NF1* loss is also supported by the various consequences of germline loss of one *NF1* allele in individuals with the NF1 syndrome. Frayling and co-workers (2019) suggested that the dimerization of neurofibromin could explain why already the loss of one *NF1* allele leads to haploinsufficiency and has major consequences. A faulty protein product can dimerize with unaffected neurofibromin and yield inactive dimers, thereby theoretically reducing the protein activity down to 25% instead of the 50% expected after loss of one allele (Frayling et al., 2019; Sherekar et al., 2020).

6.3 *NF1* deficiency and energy metabolism

Study IV revealed that individuals with NF1 are at a decreased risk for diabetes, in general, and for type 2 diabetes in particular. These findings are corroborated by a previous Danish study that reported a significantly decreased risk for hospitalizations related to type 1 diabetes and a non-significantly decreased risk for hospitalizations

related to type 2 diabetes (Kenborg et al., 2020). The results are also concordant with the previously reported low proportions of diabetes-related deaths in NF1 (Rasmussen et al., 2001; Masocco et al., 2011) and the decreased rate of diabetes-related insurance claims among individuals with NF1 (Madubata et al., 2015). The decreased risk for type 2 diabetes likely reflects the same process as the previous reports on lower fasting blood glucose levels, increased insulin sensitivity, and a higher resting energy expenditure in NF1 compared to controls (de Souza et al., 2015, 2019; Martins et al., 2016, 2018). These phenomena could be partly due to NF1-related tumors since even the benign tumor mass likely consumes energy. However, similar phenotypes have been reported in other RASopathies, which suggests a mechanism unrelated to neurofibromas. Individuals with Costello syndrome, who have an activating variant of the *HRAS* gene, show increased resting energy expenditure compared to age- and sex-matched controls (Leoni et al., 2016). Moreover, individuals with Costello syndrome, Noonan syndrome, or Noonan syndrome with multiple lentigines show a decreased mean weight and a reduced rate of obesity (Binder et al., 2012; Tajan et al., 2014; Leoni et al., 2016). Mouse models of NF1 and Noonan syndrome with multiple lentigines have demonstrated higher insulin sensitivity and better glucose tolerance than seen in control mice (Tajan et al., 2014; Tritz et al., 2021).

Together, these findings suggest that the low risk for type 2 diabetes observed in NF1 likely represents a more general association of the Ras pathway with energy metabolism, and that the lower risk for diabetes observed in NF1 is related to the Ras-GAP function of neurofibromin. This is fully concordant with the known pathways of downstream signaling activated by receptor tyrosine kinases (Figure 1). However, the role of Ras activation has not received as much attention as the direct signaling route through PI3K in the regulation of insulin sensitivity (Saltiel, 2021). *NF1* haploinsufficiency in NF1; activating variants of *HRAS* in Costello syndrome; and activating variants of, for example, *SOS1*, *KRAS*, or *NRAS* in the Noonan syndrome have a role upstream of Raf and PI3K, and thus their effects on insulin sensitivity can be ultimately mediated through PI3K. However, the lean phenotype observed in the murine model of Noonan syndrome with multiple lentigines was partially reversed upon MEK inhibition (Tajan et al., 2014). In mouse embryonic fibroblasts, loss of neurofibromin led to altered mitochondrial function *via* ERK phosphorylation (Masgras et al., 2017). The role of ERK phosphorylation was also highlighted in a study that identified an association between the gain-of-function variants of the *MC4R* gene and a reduced risk for type 2 diabetes (Lotta et al., 2019). In another study, MAPK pathway proteins mediated the decreased risk for type 2 diabetes conferred by *AKNRD55* variants (Morris et al., 2012).

The presence of similar changes in energy metabolism in multiple conditions associated with Ras pathway hyperactivation, the reversal of these characteristics

upon MEK inhibition in a murine model, and other reports on the association of the MAPK pathway with protection against type 2 diabetes suggest that also the Raf–MEK–ERK pathway mediates the effect of, for example, *NF1* deficiency on insulin sensitivity and not only the PI3K–Akt–mTOR pathway. Interestingly, a study on HER2-positive breast cancer found that the somatic loss of *NF1* can drive a shift of pathway dependence from PI3K–Akt–mTOR to the MAPK pathway (Smith et al., 2021). The hypothesis regarding the important role of the MAPK pathway in mediating the effects of insulin is also supported by data from the clinical trials of the MEK inhibitor, selumetinib, in the treatment of tumors in individuals with NF1. Weight gain has been reported as an adverse effect of selumetinib (Klesse et al., 2020; Fangusaro et al., 2019, 2021), and grade 1–2 hyperglycemia was reported in 20% of children treated for low-grade glioma (Fangusaro et al., 2019). Among the clinically approved MEK inhibitors, selumetinib provides a particularly interesting case, since it is used for the treatment of NF1-associated benign tumors and is therefore free of the bias caused by cancer cachexia, which complicates the evaluation of weight gain in the treatment of malignant tumors.

Taken together, the findings of Study IV and previous reports in NF1 and other RASopathies, the murine experiments, and the adverse events observed in selumetinib trials highlight the role of the Ras pathway and also the MAPK route in the regulation of insulin sensitivity. While this may be due to crosstalk with PI3K or directly mediated by the MAPK cascade, the risks for weight gain and hyperglycemia need to be considered in future clinical trials of Raf, MEK, and ERK inhibitors.

Type 2 diabetes and breast cancer have shared environmental and genetic risk factors (Boyle et al., 2012; Barnard et al., 2015; Rojas and Stuckey, 2016; Zhao et al., 2016; Zheng et al., 2018). Interestingly, NF1 decreased the risk for diabetes and increased the risk for breast cancer. These opposite effects further support the hypothesis that NF1 affects specific pathogenic processes instead of environmental factors affecting both diabetes and breast cancer. Similar opposite effects on the risk for breast cancer and diabetes have previously been reported for the *FTO* and *PRC1* genes (Zhao et al., 2016).

6.4 NF1-related alterations in circulating free plasma DNA

The analysis of plasma cfDNA in general holds a great promise for cancer screening, targeting of therapy, and monitoring of treatment response (Cristiano et al., 2019; Ignatiadis et al., 2021). In addition to oncology, non-invasive prenatal testing has become an important application of cfDNA analysis. The benefits of cfDNA-based methods in the context of NF1 could be multiple if the method was proven feasible

and reliable despite the NF1 syndrome. Individuals with NF1 are at a highly increased risk for cancer (Uusitalo et al., 2016), and new methods for the earlier detection of developing tumors are urgently needed to improve the prognosis. This is the case in common cancers frequently seen in NF1, such as breast cancer, yet especially the transformation of a benign plexiform neurofibroma into an MPNST is a long-standing problem. Traditional biopsies of plexiform neurofibromas are problematic, since the localization of the tumors in the close vicinity of nerves increases the risk for adverse events. Moreover, traditional biopsy likely misses the intratumor heterogeneity, i.e., most of the tumor may still be benign, while some cells have undergone malignant transformations. Assays based on cfDNA could mitigate these problems. Interestingly, a recent trial found differing tumor fractions in cfDNA in individuals with MPNST and individuals with benign plexiform neurofibroma only (Szymanski et al., 2021). Moreover, the authors could differentiate individuals with MPNST from those with plexiform neurofibroma using copy-number alterations detected in cfDNA.

As shown by the breast cancer characteristics in Studies II and III, NF1-associated cancers may follow a specific evolutionary path due to the germline *NF1* variant (Section 6.2), which highlights the need for studying cancer somatic mutations in both clinical and research settings. The analysis of cfDNA would allow a comprehensive characterization of the full tumor mutational spectrum in the patient (Heitzer et al., 2015; Ignatiadis et al., 2021).

While Study V only focused on cfDNA concentration, an ideal study would characterize specific genetic alterations, such as somatic mutations and copy number alterations. This would allow answering questions regarding malignant transformation and tumor burden. However, the sensitivity of the molecular analysis of cfDNA depends on the baseline concentration of cfDNA that represents the background for the detection of tumor-specific signals (Ignatiadis et al., 2021). Therefore, knowing the baseline level of cfDNA is a prerequisite of detailed cfDNA-based assays. Since cfDNA concentration may not increase only because of tumors, but it may be associated with, for example, inflammation (Schwarzenbach et al., 2011; Heitzer et al., 2015), the concentration assay alone is not specific to tumors. The length distribution of tumor-derived cfDNA has been suggested to differ from cfDNA from healthy individuals, yet the direction of association may be tumor type-specific (Wu et al., 2002; Wang et al., 2003; Umetani et al., 2006a, 2006b; Thierry et al., 2010; Mouliere et al., 2018; Ossandon et al., 2018; Cristiano et al., 2019). Analysis of the fragment length and its distribution could improve the specificity of cfDNA analysis even if sequencing was not possible.

The small sample size in the present study did not allow conclusive evidence regarding the contribution of NF1 overall or plexiform neurofibromas on cfDNA concentration. Another caveat was relying only on clinical information about the

presence of plexiform neurofibromas. The association of cfDNA and plexiform neurofibromas should be explored in a larger sample where all participants would have been imaged with whole-body MRI at some point of their life, which would allow reliable stratification by the presence of plexiform neurofibromas.

6.5 Limitations of the study

Publications I–V of the present thesis were based on individuals who have visited secondary or tertiary health care because of their NF1. The hospital-based ascertainment may bias the material towards those with severe disease manifestations. Individuals with very mild NF1-related manifestations may not have been diagnosed with the disease, or they have only been treated in primary care. Moreover, individuals can also be missed because of an unusual disease phenotype and not fulfilling the diagnostic criteria for NF1, which may be the case in, for example, the spinal subtype of NF1 (Poyhonen et al., 1997; Ruggieri et al., 2015; Legius et al., 2021). Studying this cohort may therefore result in overestimation of the NF1-related risks. In the study focusing on the prevalence of NF1 (Study I), it was estimated that approximately half of all the individuals with NF1 in Finland are included in the cohort. If the risk for various comorbidities was at the general population level in the missing half of individuals with NF1, for instance, the point estimate of the SIR for breast cancer would be 1.91, which still indicates an almost two-fold risk. It is, however, unlikely that only the individuals with mild NF1 phenotype would be missing from the cohort or that those with mild phenotype would carry no excess risk for NF1-related complications. The results regarding diabetes (Study IV) indicate that the Finnish NF1 cohort is not extensively biased, because if the individuals included in the cohort were highly morbid, it would be difficult to detect a decreased risk for any condition. It should also be noted that the results represent a description of the NF1 population seen in specialized health care and can therefore be used, as such, for purposes like genetic counselling. Despite the hospital-based ascertainment, these population-based results are likely more representative of NF1 as a whole than estimates obtained using material from single very specialized NF1 clinics.

Studies I–IV were register-based, while the data for Study V were generated with laboratory measurements and extracted from medical records. Register-based data can always be subject to bias related to recording of the information. The prevalence of NF1 was studied using the comprehensive Finnish Population Register Centre data on births, deaths, and emigration, yet the NF1 cohort may show bias towards more severe phenotypes, as described above. The breast cancer risk in NF1 was examined using data from the Finnish Cancer Registry. The Finnish Cancer Registry collects information on cancer diagnoses from clinicians, pathology laboratories, and

death certificates. As a result, the register is highly comprehensive in most cancer types, including breast cancer (Leinonen et al., 2017). In contrast, the Care Register for Health Care, that was utilized for studying diabetes, is not curated, as it directly records the clinical diagnosis codes collected from hospitals. Consequently, the material may contain misregistered diagnosis codes, such as inaccurate codes and typographical errors. If a patient has a severe and acute disorder, former and non-acute diseases may not be recorded. The diagnosis codes may also represent a mere suspicion of a disease. During the time period examined in this study, the Care Register for Health Care did not cover primary outpatient care where most patients with diabetes are treated. To overcome these uncertainties, diagnosis of type 1 diabetes was confirmed with a purchase of insulin, and individuals with any type of diabetes or type 2 diabetes were also searched based on purchases of anti-diabetic medication. The Drug Reimbursement Register is relatively reliable for identifying individuals with relevant prescriptions, and it is reasonable to assume that a prescription indicates the diagnosis of a related disease. On the other hand, the Drug Reimbursement Register does not reveal whether the patients have actually used the prescribed medication.

The early mortality associated with NF1, demonstrated in Study I, complicates the comparisons between individuals with NF1 and the general population. Since individuals with NF1 die on average at a younger age than the general population, detecting diseases that become more common at an older age is difficult. For example, prior observations of a decreased risk for diabetes in NF1 were made in cross-sectional settings and could therefore have been caused by the lower number of live individuals with NF1 in the age groups where type 2 diabetes becomes common. However, the problem can be overcome by computing SIR as in the case of breast cancer (Study II) and by performing age-dependent analysis using patient-specific person-time as in the studies on prevalence and diabetes (Studies I and IV).

In Study III, five cohorts were combined to estimate the prognosis of breast cancer and the risk for contralateral breast cancer in NF1. International collaboration was necessary to obtain a sufficient number of patients for a reliable estimation of contralateral breast cancer risk. However, the five cohorts were highly heterogeneous and caution is therefore needed when interpreting the findings. Two of the cohorts, the Finnish and Manchester cohorts, are population-based and encompass individuals with NF1 who have been treated in secondary or tertiary health care. The other three cohorts – Paris, Hamburg, and Padua – are derived from specialized NF1 clinics and may not be equally representative of the whole population with NF1. Patients seen in these clinics may be more aware of the risks associated with NF1 than other individuals with NF1, they may receive targeted surveillance, have more frequent health care contacts, and an overall better socioeconomic status. Thus, although no deaths due to breast cancer were observed

in the Padua cohort with enhanced breast cancer screening, the result is not necessarily merely representative of the effects of the enhanced screening protocol but may also be due to biased selection of patients or overall better awareness of the breast cancer risk.

The limitations of Study V on cfDNA are related to both the sample size and methods of cfDNA measurement. Because of the small numbers of individuals with NF1 and controls enrolled in the study, the full spectrum of NF1 manifestations potentially affecting cfDNA concentration could not be examined. Moreover, the small cohort had insufficient statistical power for detecting associations of cfDNA level and specific NF1 features, such as plexiform neurofibromas or optic pathway gliomas. It also remains to be explored whether the interaction of NF1 and age is a coincidence observed in this small-scale study, or due to, for example, age-related alterations in the immune system or neurofibroma tumor burden. Laboratory measurements are always subject to uncertainty. Each sample was measured in duplicate, yet no technical replicates of cfDNA extraction were included. The cfDNA measurements were solely fluorescence based. The assay method is specific to double-stranded DNA, yet the integrity or purity of cfDNA were not assessed. While fluorescence-based methods for the quantification of double-stranded DNA allow the detection of non-amplifiable DNA, future studies should confirm the findings using quantitative real-time polymerase chain reaction. The cfDNA concentrations were normalized relative to the total plasma protein concentration in order to control for plasma dilution during sample processing. While this normalization can correct for variation in sample processing, it could also introduce bias in cases where plasma protein concentration and cfDNA correlate. However, the analysis results were highly concordant using both the normalized and plain cfDNA.

6.6 Future perspectives

The characteristic disease manifestations related to NF1 such as neurofibromas, café-au-lait macules, and skeletal defects are well known. However, the incidence of many common diseases among individuals with NF1 is only beginning to emerge. The effect of NF1 on the risk for breast cancer is not surprising given the known function of neurofibromin as a tumor suppressor protein, yet NF1 also alters the risks for dementia (Uusitalo et al., 2015; Kallionpää et al., 2021a), behavioral and cognitive disorders (Vogel et al., 2017; Johansson et al., 2021), and diabetes (Study IV). More epidemiological studies using well-defined cohorts of individuals with NF1 are needed to better describe the risks associated with NF1. For example, hypertension is often mentioned in association with NF1 (Gutmann et al., 2017), yet estimates of relative risk are scarce and the contributions of different disease

mechanisms have not been fully elucidated. Given the interplay between socioeconomic well-being and health, the effects of NF1 on all aspects of life need to be taken into account in future epidemiological research (Doser et al., 2019; Johansson et al., 2021, 2022).

The high risk for breast cancer observed among women with NF1 calls for further evidence regarding optimal management. While breast cancer screening is already widely recommended for young women with NF1 (Daly et al., 2018; Suomen Rintasyöpäryhmä Ry, 2021), more research is needed to establish the efficacy and best practices of screening, such as the optimal imaging modality, screening interval, sensitivity, and the risk for false positives. Since the current screening practices for women with NF1 have been only recently introduced, it will take time for the evidence regarding their efficacy to accumulate. In the meantime, analysis of the Finnish Mass Screening Registry and similar resources related to the population-level breast cancer screening programs could elucidate the risks for false positives and interval cancers in women with NF1 older than 50 years. Further studies on the role of family history in predicting the breast cancer risk associated with NF1 and on the associations of *NF1* genotype and the risk for breast cancer might also contribute to the better targeting of screening efforts. While Study III described the absolute risk for a second contralateral breast cancer after an initial breast cancer diagnosis, it is essential to know how this risk relates to the baseline risk for breast cancer in NF1, that is, whether the history of breast cancer can be used to predict the risk for a new breast cancer. Overall, the aggregation of cancers to the same individuals with NF1 also needs to be studied in further detail, as the history of cancer could help to identify those at the greatest risk for new cancers. Three males with breast cancer were included in the international NF1 cohorts of Study III, yet the risk for male breast cancer in NF1 remains to be formally evaluated in future studies.

The results demonstrated that breast cancer in NF1 has specific characteristics that likely stem from the changes in cell signaling caused by the germline *NF1* deficiency. The role of the *NF1* gene in breast cancer evolution calls for therapies specifically tailored for *NF1*-deficient breast cancers. The MEK inhibitor, selumetinib, has been approved for the treatment of plexiform neurofibromas (Gross et al., 2020), and cell models have demonstrated its efficacy in overcoming *NF1*-associated endocrine treatment resistance in breast cancer (Pearson et al., 2020; Zheng et al., 2020). The use of selumetinib or other MEK inhibitors for the treatment of *NF1*-deficient breast cancer is a feasible therapeutic option to be explored in preclinical models and in clinical trials. Since the survival after breast cancer was worse among women with NF1 compared to controls even after matching for estrogen receptor status in Study II, resistance to endocrine therapy may not solely explain the poor prognosis of breast cancer in NF1. Further studies are therefore

required to establish any effects of NF1 on, for example, the efficacy of traditional chemotherapeutic agents.

The age-specific estimates of NF1 prevalence reported in Study I can be used to compute an age-adjusted expected prevalence in a given population, yet the estimates are not fully reliable when applied to other geographical areas, and future studies should establish the prevalence of NF1 in different societies and frequently update the prevalence estimates. The survival of individuals with NF1 and therefore also the prevalence of NF1 is dependent on the quality of care and availability of efficient therapies as evidenced by the improvements observed in the prognosis of NF1-associated MPNST (Ingham et al., 2011; Kolberg et al., 2013). The treatment of NF1 is constantly evolving, and the availability of new drugs (Gross et al., 2020) and better surveillance practices (Daly et al., 2018) may improve the survival of individuals with NF1. Therefore, even if the birth incidence of NF1 was constant worldwide, the overall prevalence of NF1 depends on the time period and geographical area of interest. A major source of variation in the overall prevalence is the age structure of the population. If the mean age of a population is low, the overall prevalence of NF1 is likely to be higher than in an area with an older population. On the other hand, it is likely that access to modern medical care reduces the excess mortality associated with NF1, which may increase the overall prevalence of NF1 in developed countries compared to developing countries, and in the modern times compared to prior decades. Nevertheless, the results of Study I are likely representative of the prevalence of NF1 in developed countries where the availability of medical treatment is similar as in Finland. The age-specific prevalence estimates provide a tool for future studies assessing whether individuals with NF1 are overrepresented in cohorts defined by other features, such as intracranial aneurysms (Kurtelius et al., 2017) or JMML (Bader and Miller, 1978).

The evidence from NF1 and other RASopathies clearly highlights the roles of Ras and the MAPK pathway in the pathogenesis of diabetes. However, mechanistic studies are required to dissect the direct signaling through the MAPK pathway and the indirect crosstalk with the PI3K pathway. The non-significant observation of a decreased risk for type 1 diabetes observed in Study IV and the previously reported reduced rate of hospitalizations related to type 1 diabetes in NF1 (Kenborg et al., 2020) still require further verification and mechanistic explanation. Since type 1 diabetes is an autoimmune disease, its pathogenesis is expected to be largely different than in type 2 diabetes. It is thus possible that the decreased risks for the two types of diabetes observed in NF1 are a mechanistically distinct coincidence or that NF1 may affect a pathogenic process involved in both type 1 and type 2 diabetes. The results also suggested that sex may contribute to the interplay between NF1 and the risk for diabetes, as only a few men with NF1 had type 1 or type 2 diabetes.

However, the size of the Finnish NF1 cohort was insufficient to formally demonstrate such an interaction, and the topic requires further studies.

As one potential tool for cancer screening, the use of cfDNA-based assays should be explored for the detection of NF1-related malignancies in clinical studies with a sufficiently long follow-up. Given the experience from the current imaging-based screening tools, such as mammography (Singh et al., 2016; Maani et al., 2019), the specificity of cfDNA-based assays represents a major challenge, as false-positive findings may prompt extensive further studies and anxiety in the patients.

7 Conclusions

Most parts of the present study were based on the Finnish NF1 cohort of more than 1,400 individuals with NF1. This epidemiological study of relatively common diseases, breast cancer and diabetes, in a nationwide and population-based cohort has implications relevant to the clinical management of NF1, and the results highlight the need for efficient surveillance of NF1-related comorbidities. On the other hand, the monogenic etiology of NF1 allows drawing biochemical conclusions based on the epidemiologic findings. Taken together, the results imply that:

1. NF1 is more common than previously thought. Many individuals with NF1 are still lacking the health care services that they would need for the optimal management of the disorder. Cohort studies on NF1 may not capture the full phenotypic spectrum of NF1.
2. women with NF1 are at an increased risk for breast cancer and a contralateral second breast cancer, and the prognosis of NF1-related breast cancer is poor. Raising awareness of the breast cancer risk in NF1 and providing enhanced surveillance to the affected individuals may help to improve the prognosis.
3. variants of the *NF1* gene affect the evolutionary course of breast cancer.
4. the germline haploinsufficiency of the *NF1* gene protects against diabetes, apparently *via* the hyperactivation of the Ras pathway.
5. the NF1 syndrome, as such, may not significantly affect the baseline concentration of cfDNA, yet further studies are required to establish the analytical and clinical validity of cfDNA-based assays in the context of NF1.

Acknowledgements

The research described in this thesis has been carried out in the Institute of Biomedicine within the former Department of Cell Biology and Anatomy and in the Department of Dermatology and Venereology in the University of Turku between the years 2014 and 2022. I wish to thank the head of the former Department of Cell Biology and Anatomy, Professor Juha Peltonen; the head of the Department of Dermatology and Venereology, Professor Veli-Matti Kähäri; and the former and present heads of the Institute of Biomedicine, Professor Pirkko Härkönen and Professor Sari Mäkelä, respectively, for the opportunity to work in these departments.

My supervisors, Professor Juha Peltonen and Professor Sirkku Peltonen, hired me to work as a summer employee over ten years ago. Since then, I have been fortunate to enjoy their vast knowledge on NF1, and I am thankful for all their guidance and encouragement. I am grateful to them for allowing me to take part in a variety of different projects. Not only have they taught me how to do research, but they have also allowed me to get a glimpse of the many other aspects of the academic world. Sirkku and Juha have always been available to help and discuss any questions. Over the many hours spent in Juha's office, Juha has shared his views on NF1 and cell biology, and ensured that I understand the broader context of our research. Sirkku's account of her clinical experience has substantially improved my understanding of NF1 and the aspects that need to be considered in epidemiological research.

The official reviewers of this thesis, Professor Jukka-Pekka Mecklin and Professor Arto Mannermaa, made a great effort reading and commenting on this thesis amid all their other commitments. I am grateful for their valuable feedback that significantly improved this thesis. The members of my follow-up committee, Professor Markku Koulu and Docent Tiina Laitala, have provided great comments, asked interesting questions, and encouraged me throughout the process. They always found the time needed for the annual follow-up meetings that were inspiring and helpful.

I wish to thank all my co-authors: Elina Uusitalo, Jussi Leppävirta, Paula Pennanen, Kaisa Ahramo, Marianna Aaltonen, Samu Kurki, Matti Rantanen, Janne

Pitkäniemi, Pauliina Kronqvist, Pirkko Härkönen, Riikka Huovinen, Olli Carpén, Kari Auranen, Hannu Järveläinen, Minna Pöyhönen, Sirkku Peltonen, Juha Peltonen, Gareth Evans, Maurizio Clementi, Eva Trevisson, Victor-Felix Mautner, Sacha Howell, Lauren Lewis, Ouidad Zehou, Antonella Brunello, Elaine Harkness, and Pierre Wolkenstein. Obviously, this research would not have been possible without their contribution.

I wish to thank Professor Minna Pöyhönen, who has always been willing to help and answer my questions. Her guidance, networks, and great company have been a major part in my experiences of attending conferences abroad. The clinical insights and positive attitude of Professor Hannu Järveläinen have been of a great help during the process. Professor Kari Auranen has patiently helped me in performing time-to-event analyses while also sharing his knowledge on a variety of other topics. I thank Professor Janne Pitkäniemi for introducing me to cancer registration and the related statistics.

I am thankful for having had the opportunity to work with all the past and present members of the NF1 group. Being a member of such a great team has made going to work fun every day.

Elina Uusitalo introduced me to using registry data and the Finnish NF1 cohort. We shared the office for several years, and in the daily conversations, I learned a lot about NF1, breast cancer, and doing research. It has been great to always be able to ask Elina for help. Without all that guidance, this thesis would be very different.

Paula Pennanen is an expert in cell culture, and while I have learned about laboratory skills and research from Paula, she has also always brightened up the day. Whenever I have been frustrated with some problem, a conversation with Paula has helped.

I have learned a lot from Jussi Leppävirta both in and outside the office. Jussi has a great skill to dissect complex questions into smaller, answerable pieces, and to evaluate them systematically. When learning to use and analyze the different types of registry data, it was great to explore them together with him. Jussi has a great understanding of research, life, and the world, and I have truly enjoyed all the conversations with him.

Solving problems and making plans together with Eija Martikkala has always been enjoyable. The conversations, sometimes quite long, could meander between research and almost anything else, and, while productive in terms of science, talking with Eija has also provided much needed breaks from research.

I am thankful to Heli Ylä-Outinen for sharing her great clinical insights, understanding of diseases, and long experience in NF1-related research. We have had all kinds of conversations with Niina Loponen, and I especially wish to thank her for guiding me with all the issues related to teaching and anatomy. Maria Alanne,

Vivian Reinhold, Eeva-Mari Jouhilahti, and Eetu Heervä have always kindly shared their expertise and answered my various questions.

I was supposed to look after Kim Le, Sini Viitamäki, and Kaisa Ahramo when they joined the group as summer interns, but I ended up learning a lot from them all, as they set up our protocols for culturing breast cancer cells and explored image analysis methods. Working with Juha Määttänen has been a great pleasure, as he has an in-depth understanding of what is important and a great ability to manage tangled issues. The cheerfulness of Mari Hellén made every day of working together fun.

Miso Immonen initially introduced me to servicing pipettes and culturing cells. It has been wonderful to have someone who usually knows about all the practicalities and can solve a variety of problems, irrespective of whether it is the lack of a suitable pipette, hanging something on the wall, or the need for a special tool. And not only has he always been there to help and replied to my messages also on the evenings and weekends, but he has a great skill for anticipating any needs and solving problems beforehand. Most importantly, Miso always keeps up a good spirit and delivers the news of the day.

In addition to the present thesis, I have had an opportunity to work on many other projects. I am grateful to Professor Edvard Johansson and Professor Petri Böckerman for the many fruitful discussions regarding the diverse mechanisms of the socioeconomic consequences of NF1. I wish to thank Docent Erik Wallén, Professor Jari Yli-Kauhaluoma, and Docent Moshe Finel for their supervision and encouragement during my first steps in science at the University of Helsinki.

I wish to acknowledge all my co-workers in the former Department of Cell Biology and Anatomy and in the C3 and C4 floors of Medisiina, who are too numerous to be listed. I am grateful for all the guidance and help, and I have enjoyed the conversations with them. The efforts of the Laboratory Manager, Docent Anna-Marja Säämänen, through the renovations and relocations, have been instrumental for maintaining good working conditions, and I admire her patience while answering the different needs of all the stakeholders. She has always been available for valuable advice and help. Liudmila Shumskaya, Pia Tuominen, Merja Lakkisto, Taina Malinen, and Annika Kankare have provided valuable practical help and advice on numerous occasions.

I wish to acknowledge Robert M. Badeau from Aura Professional English Consulting for reviewing the language of this thesis and delivering his excellent revisions within a tight schedule. I thank artist Kristian Krokfors for allowing me to use his work in the cover of this thesis.

My work with this thesis has been made possible by the funding granted by the Cancer Foundation Finland, the Finnish Cultural Foundation, the Finnish Dermatological Society, the Ida Montin Foundation, the Instrumentarium Science Foundation, the Jalmari and Rauha Ahokas Foundation, the Orion Research

Foundation, the Turku University Foundation, and the University of Turku. I am thankful for this support. Attending international conferences has been a major part of my growth as a researcher and has been a great inspiration. The Cancer Foundation Finland, the Emil Aaltonen Foundation, the Lifespan Research Program, the Turku Doctoral Programme of Molecular Medicine, and the Turku University Foundation have supported my conference travel expenses during the formation of this thesis.

I wish to thank all my friends. Erkka and Mikko have ensured that I do not forget pharmacy and chemistry and have also asked many critical questions regarding research. Olli has always had time for great discussions about the world. Emma, Henrik, Valentina, Arttu, Kristiina, and Mikko have been great company over numerous evenings. The friendship of Karri and Juuso has shaped me in many ways.

I am fortunate to have a close family. I thank Marjo, Kari, Reetta, and Eero for all their support and interest. I am grateful to Santtu, Elina, Lenni, and Sauli. Santtu has always been an inspiring example for me. Having seen him go through all those different phases of school and growing up has paved the way for me to follow. Santtu has shared his experience and tips whenever I have started something new, which is a great demonstration of the way he has been taking care of me. My parents, Liisa and Tuomo, have always trusted me and supported me in whatever I have been doing, be it fishing, assembling computers, or science. They never pushed me during the school years but helped and encouraged at every stage. The way they have been broadly interested in the world and followed the news has set a good example to follow and probably inspired my curiosity in research. I am thankful for having always had such a great and supportive home.

Lastly, I wish to express my deep gratitude to Roosa. She has supported me in both life and research over all these years: from ensuring that I remember to eat, to giving great advice, asking important scientific questions, and commenting on this thesis. It is wonderful to have such a kind, skilled, and wise partner with whom I can share anything. Thank you for being there!

Turku, April 2022
Roope Kallionpää

References

Ahlawat S, Blakeley JO, Langmead S, Belzberg AJ, Fayad LM. 2020. Current status and recommendations for imaging in neurofibromatosis type 1, neurofibromatosis type 2, and schwannomatosis. Skeletal Radiol. 49: 199–219.

Airewele GE, Sigurdson AJ, Wiley KJ, Frieden BE, Caldarera LW, Riccardi VM, Lewis RA, Chintagumpala MM, Ater JL, Plon SE, Bondy ML. 2001. Neoplasms in neurofibromatosis 1 are related to gender but not to family history of cancer. Genet. Epidemiol. 20: 75–86.

Akcakanat A, Zheng X, Cruz Pico CX, Kim T-B, Chen K, Korkut A, Sahin A, Holla V, Tarco E, Singh G, Damodaran S, Mills GB, Gonzalez-Angulo AM, Meric-Bernstam F. 2021. Genomic, Transcriptomic, and Proteomic Profiling of Metastatic Breast Cancer. Clin. Cancer Res. 27: 3243–3252.

Allemani C, Matsuda T, Di Carlo V, Harewood R, Matz M, Nikšić M, Bonaventure A, Valkov M, Johnson CJ, Estève J, Ogunbiyi OJ, Azevedo E Silva G, Chen W-Q, Eser S, Engholm G, Stiller CA, Monnereau A, Woods RR, Visser O, Lim GH, Aitken J, Weir HK, Coleman MP, CONCORD Working Group. 2018. Global surveillance of trends in cancer survival 2000-14 (CONCORD-3): analysis of individual records for 37 513 025 patients diagnosed with one of 18 cancers from 322 population-based registries in 71 countries. Lancet 391: 1023–1075.

Allison KH, Hammond MEH, Dowsett M, McKernin SE, Carey LA, Fitzgibbons PL, Hayes DF, Lakhani SR, Chavez-MacGregor M, Perlmutter J, Perou CM, Regan MM, Rimm DL, Symmans WF, Torlakovic EE, Varella L, Viale G, Weisberg TF, McShane LM, Wolff AC. 2020. Estrogen and Progesterone Receptor Testing in Breast Cancer: ASCO/CAP Guideline Update. J. Clin. Oncol. 38: 1346–1366.

Amant F, Verheecke M, Wlodarska I, Dehaspe L, Brady P, Brison N, Van Den Bogaert K, Dierickx D, Vandecaveye V, Tousseyn T, Moerman P, Vanderstichele A, Vergote I, Neven P, Berteloot P, Putseys K, Danneels L, Vandenberghe P, Legius E, Vermeesch JR. 2015. Presymptomatic Identification of Cancers in Pregnant Women During Noninvasive Prenatal Testing. JAMA Oncol. 1: 814–9.

André F, Ciruelos E, Rubovszky G, Campone M, Loibl S, Rugo HS, Iwata H, Conte P, Mayer IA, Kaufman B, Yamashita T, Lu Y-S, Inoue K, Takahashi M, Pápai Z, Longin A-S, Mills D, Wilke C, Hirawat S, Juric D, SOLAR-1 Study Group. 2019. Alpelisib for PIK3CA-Mutated, Hormone Receptor-Positive Advanced Breast Cancer. N. Engl. J. Med. 380: 1929–1940.

Angus L, Smid M, Wilting SM, van Riet J, Van Hoeck A, Nguyen L, Nik-Zainal S, Steenbruggen TG, Tjan-Heijnen VCG, Labots M, van Riel JMGH, Bloemendal HJ, Steeghs N, Lolkema MP, Voest EE, van de Werken HJG, Jager A, Cuppen E, Sleijfer S, Martens JWM. 2019. The genomic landscape of metastatic breast cancer highlights changes in mutation and signature frequencies. Nat. Genet. 51: 1450–1458.

Aoki Y, Niihori T, Inoue S, Matsubara Y. 2016. Recent advances in RASopathies. J. Hum. Genet. 61: 33–9.

Arima Y, Hayashi H, Kamata K, Goto TM, Sasaki M, Kuramochi A, Saya H. 2010. Decreased expression of neurofibromin contributes to epithelial-mesenchymal transition in neurofibromatosis type 1. Exp. Dermatol. 19: e136-41.

Asthagiri AR, Parry DM, Butman JA, Kim HJ, Tsilou ET, Zhuang Z, Lonser RR. 2009. Neurofibromatosis type 2. Lancet 373: 1974–86.

Azzopardi JG, Chepick OF, Hartmann WH, Jafarey NA, Llombart-Bosch A, Ozzello L, Rilke F, Sasano N, Sobin LH, Sommers SC, Stalsberg H, Sugar J, Williams AO. 1982. The World Health Organization Histological Typing of Breast Tumors—Second Edition. Am. J. Clin. Pathol. 78: 806–816.

Bader JL, Miller RW. 1978. Neurofibromatosis and childhood leukemia. J. Pediatr. 92: 925–9.

Barbaric D, Stevens M, Dalla-Pozza L. 2003. Neurofibromatosis type 1 and multiple primary malignancies. Med. Pediatr. Oncol. 41: 568–9.

Barnard ME, Boeke CE, Tamimi RM. 2015. Established breast cancer risk factors and risk of intrinsic tumor subtypes. Biochim. Biophys. Acta 1856: 73–85.

Bates JE, Peterson CR, Dhakal S, Giampoli EJ, Constine LS. 2014. Malignant peripheral nerve sheath tumors (MPNST): a SEER analysis of incidence across the age spectrum and therapeutic interventions in the pediatric population. Pediatr. Blood Cancer 61: 1955–60.

Ben-Shachar S, Dubov T, Toledano-Alhadef H, Mashiah J, Sprecher E, Constantini S, Leshno M, Messiaen LM. 2017. Predicting neurofibromatosis type 1 risk among children with isolated café-au-lait macules. J. Am. Acad. Dermatol. 76: 1077-1083.e3.

Berg WA, Zhang Z, Lehrer D, Jong RA, Pisano ED, Barr RG, Böhm-Vélez M, Mahoney MC, Evans WP, Larsen LH, Morton MJ, Mendelson EB, Farria DM, Cormack JB, Marques HS, Adams A, Yeh NM, Gabrielli G, ACRIN 6666 Investigators. 2012. Detection of breast cancer with addition of annual screening ultrasound or a single screening MRI to mammography in women with elevated breast cancer risk. JAMA 307: 1394–404.

Bergqvist C, Servy A, Valeyrie-Allanore L, Ferkal S, Combemale P, Wolkenstein P, NF France Network. 2020. Neurofibromatosis 1 French national guidelines based on an extensive literature review since 1966. Orphanet J. Rare Dis. 15: 37.

Bertucci F, Ng CKY, Patsouris A, Droin N, Piscuoglio S, Carbuccia N, Soria JC, Dien AT, Adnani Y, Kamal M, Garnier S, Meurice G, Jimenez M, Dogan S, Verret B, Chaffanet M, Bachelot T, Campone M, Lefeuvre C, Bonnefoi H, Dalenc F, Jacquet A, De Filippo MR, Babbar N, Birnbaum D, Filleron T, Le Tourneau C, André F. 2019. Genomic characterization of metastatic breast cancers. Nature 569: 560–564.

Bhatia S, Chen Y, Wong FL, Hageman L, Smith K, Korf B, Cannon A, Leidy DJ, Paz A, Andress JE, Friedman GK, Metrock K, Neglia JP, Arnold M, Turcotte LM, de Blank P, Leisenring W, Armstrong GT, Robison LL, Clapp DW, Shannon K, Nakamura JL, Fisher MJ. 2019. Subsequent Neoplasms After a Primary Tumor in Individuals With Neurofibromatosis Type 1. J. Clin. Oncol. 37: 3050–3058.

Binder G, Grathwol S, von Loeper K, Blumenstock G, Kaulitz R, Freiberg C, Webel M, Lissewski C, Zenker M, Paul T. 2012. Health and Quality of Life in Adults with Noonan Syndrome. J. Pediatr. 161: 501-505.e1.

de Blank P, Li N, Fisher MJ, Ullrich NJ, Bhatia S, Yasui Y, Sklar CA, Leisenring W, Howell R, Oeffinger K, Hardy K, Okcu MF, Gibson TM, Robison LL, Armstrong GT, Krull KR. 2020. Late morbidity and mortality in adult survivors of childhood glioma with neurofibromatosis type 1: report from the Childhood Cancer Survivor Study. Genet. Med. 22: 1794–1802.

Boddy JL, Gal S, Malone PR, Harris AL, Wainscoat JS. 2005. Prospective study of quantitation of plasma DNA levels in the diagnosis of malignant versus benign prostate disease. Clin. Cancer Res. 11: 1394–1399.

Bonner ER, Bornhorst M, Packer RJ, Nazarian J. 2018. Liquid biopsy for pediatric central nervous system tumors. NPJ Precis. Oncol. 2: 29.

Boyle P, Boniol M, Koechlin A, Robertson C, Valentini F, Coppens K, Fairley L-L, Boniol M, Zheng T, Zhang Y, Pasterk M, Smans M, Curado MP, Mullie P, Gandini S, Bota M, Bolli GB, Rosenstock J, Autier P. 2012. Diabetes and breast cancer risk: a meta-analysis. Br. J. Cancer 107: 1608–17.

Brems H, Chmara M, Sahbatou M, Denayer E, Taniguchi K, Kato R, Somers R, Messiaen L, De Schepper S, Fryns J-P, Cools J, Marynen P, Thomas G, Yoshimura A, Legius E. 2007. Germline loss-of-function mutations in SPRED1 cause a neurofibromatosis 1-like phenotype. Nat. Genet. 39: 1120–6.

Bucksch K, Zachariae S, Aretz S, Büttner R, Holinski-Feder E, Holzapfel S, Hüneburg R, Kloor M, von Knebel Doeberitz M, Morak M, Möslein G, Nattermann J, Perne C, Rahner N, Schmiegel W, Schulmann K, Steinke-Lange V, Strassburg CP, Vangala DB, Weitz J, Loeffler M, Engel C, German Consortium for Familial Intestinal Cancer. 2020. Cancer risks in Lynch syndrome, Lynch-like syndrome, and familial colorectal cancer type X: a prospective cohort study. BMC Cancer 20: 460.

Burstein HJ, Curigliano G, Thürlimann B, Weber WP, Poortmans P, Regan MM, Senn HJ, Winer EP, Gnant M, Panelists of the St Gallen Consensus Conference. 2021. Customizing local and systemic therapies for women with early breast cancer: the St. Gallen International Consensus Guidelines for treatment of early breast cancer 2021. Ann. Oncol. 32: 1216–1235.

Burton A, Maskarinec G, Perez-Gomez B, Vachon C, Miao H, Lajous M, López-Ridaura R, Rice M, Pereira A, Garmendia ML, Tamimi RM, Bertrand K, Kwong A, Ursin G, Lee E, Qureshi SA, Ma H, Vinnicombe S, Moss S, Allen S, Ndumia R, Vinayak S, Teo S-H, Mariapun S, Fadzli F, Peplonska B, Bukowska A, Nagata C, Stone J, Hopper J, Giles G, Ozmen V, Aribal ME, Schüz J, Van Gils CH, Wanders JOP, Sirous R, Sirous M, Hipwell J, Kim J, Lee JW, Dickens C, Hartman M, Chia K-S, Scott C, Chiarelli AM, Linton L, Pollan M, Flugelman AA, Salem D, Kamal R, Boyd N, Dos-Santos-Silva I, McCormack V. 2017. Mammographic density and ageing: A collaborative pooled analysis of cross-sectional data from 22 countries worldwide. PLoS Med. 14: e1002335.

Cancer Genome Atlas Network. 2012. Comprehensive molecular portraits of human breast tumours. Nature 490: 61–70.

Carli M, Ferrari A, Mattke A, Zanetti I, Casanova M, Bisogno G, Cecchetto G, Alaggio R, De Sio L, Koscielniak E, Koscielniak E, Sotti G, Treuner J. 2005. Pediatric malignant peripheral nerve sheath tumor: the Italian and German soft tissue sarcoma cooperative group. J. Clin. Oncol. 23: 8422–30.

Carnes RM, Kesterson RA, Korf BR, Mobley JA, Wallis D. 2019. Affinity Purification of NF1 Protein-Protein Interactors Identifies Keratins and Neurofibromin Itself as Binding Partners. Genes (Basel). 10.

Cecen E, Ince D, Uysal KM, Ozer E, Cetingoz R, Ozguven AA, Cakmakci H, Sarialioglu F, Olgun N. 2011. Soft tissue sarcomas and central nervous system tumors in children with neurofibromatosis type 1. Childs. Nerv. Syst. 27: 1885–93.

Chao RC, Pyzel U, Fridlyand J, Kuo Y-M, Teel L, Haaga J, Borowsky A, Horvai A, Kogan SC, Bonifas J, Huey B, Jacks TE, Albertson DG, Shannon KM. 2005. Therapy-induced malignant neoplasms in Nf1 mutant mice. Cancer Cell 8: 337–48.

Cheng J, Holland-Letz T, Wallwiener M, Surowy H, Cuk K, Schott S, Trumpp A, Pantel K, Sohn C, Schneeweiss A, Burwinkel B. 2018. Circulating free DNA integrity and concentration as independent prognostic markers in metastatic breast cancer. Breast Cancer Res. Treat. 169: 69–82.

Cheuk DKL, Chiang AKS, Ha SY, Chan GCF. 2013. Malignancies in Chinese patients with neurofibromatosis type 1. Hong Kong Med. J. 19: 42–9.

Choi G, Huang B, Pinarbasi E, Braunstein SE, Horvai AE, Kogan S, Bhatia S, Faddegon B, Nakamura JL. 2012. Genetically mediated Nf1 loss in mice promotes diverse radiation-induced tumors modeling second malignant neoplasms. Cancer Res. 72: 6425–34.

Chompret A, Brugières L, Ronsin M, Gardes M, Dessarps-Freichey F, Abel A, Hua D, Ligot L, Dondon MG, Bressac-de Paillerets B, Frébourg T, Lemerle J, Bonaïti-Pellié C, Feunteun J. 2000. P53 germline mutations in childhood cancers and cancer risk for carrier individuals. Br. J. Cancer 82: 1932–7.

Chun FK-H, Müller I, Lange I, Friedrich MG, Erbersdobler A, Karakiewicz PI, Graefen M, Pantel K, Huland H, Schwarzenbach H. 2006. Circulating tumour-associated plasma DNA represents an independent and informative predictor of prostate cancer. BJU Int. 98: 544–8.

Clementi M, Barbujani G, Turolla L, Tenconi R. 1990. Neurofibromatosis-1: a maximum likelihood estimation of mutation rate. Hum. Genet. 84: 116–8.

Cnossen MH, van der Est MN, Breuning MH, van Asperen CJ, Breslau-Siderius EJ, van der Ploeg AT, de Goede-Bolder A, van den Ouweland AM, Halley DJ, Niermeijer MF. 1997. Deletions spanning the neurofibromatosis type 1 gene: implications for genotype-phenotype correlations in neurofibromatosis type 1? Hum. Mutat. 9: 458–64.

Coyte A, Morrison DS, McLoone P. 2014. Second primary cancer risk - the impact of applying different definitions of multiple primaries: results from a retrospective population-based cancer registry study. BMC Cancer 14: 272.

Creighton CJ, Hilger AM, Murthy S, Rae JM, Chinnaiyan AM, El-Ashry D. 2006. Activation of mitogen-activated protein kinase in estrogen receptor alpha-positive breast cancer cells in vitro induces an in vivo molecular phenotype of estrogen receptor alpha-negative human breast tumors. Cancer Res. 66: 3903–11.

Cristiano S, Leal A, Phallen J, Fiksel J, Adleff V, Bruhm DC, Jensen SØ, Medina JE, Hruban C, White JR, Palsgrove DN, Niknafs N, Anagnostou V, Forde P, Naidoo J, Marrone K, Brahmer J, Woodward BD, Husain H, van Rooijen KL, Ørntoft M-BW, Madsen AH, van de Velde CJH, Verheij M, Cats A, Punt CJA, Vink GR, van Grieken NCT, Koopman M, Fijneman RJA, Johansen JS, Nielsen HJ, Meijer GA, Andersen CL, Scharpf RB, Velculescu VE. 2019. Genome-wide cell-free DNA fragmentation in patients with cancer. Nature 570: 385–389.

Cristofanilli M, Gonzalez-Angulo A, Sneige N, Kau S-W, Broglio K, Theriault RL, Valero V, Buzdar AU, Kuerer H, Buchholz TA, Buccholz TA, Hortobagyi GN. 2005. Invasive lobular carcinoma classic type: response to primary chemotherapy and survival outcomes. J. Clin. Oncol. 23: 41–8.

Crook A, Kwa R, Ephraums S, Wilding M, Thiyagarajan L, Fleming J, Moore K, Berman Y. 2022. The psychological impact and experience of breast cancer screening in young women with an increased risk of breast cancer due to neurofibromatosis type 1. Fam. Cancer 21: 241–253.

Crowell V, Houghton R, Tomar A, Fernandes T, Squitieri F. 2021. Modeling Manifest Huntington's Disease Prevalence Using Diagnosed Incidence and Survival Time. Neuroepidemiology 55: 361–368.

Crowley E, Di Nicolantonio F, Loupakis F, Bardelli A. 2013. Liquid biopsy: Monitoring cancer-genetics in the blood. Nat. Rev. Clin. Oncol. 10: 472–484.

Daly MB, Pilarski R, Berry MP, Buys SS, Friedman S, Garber JE, Hutton ML, Kauff ND, Khan S, Klein C, Kohlmann W, Kurian AW, Laronga C, Litton JK, Madlensky L, Mak JS, Merajver SD, Offit K, Pal T, Pederson HJ, Reiser G, Mahoney Shannon K, Thaker P, Visvanathan K, Weitzel JN, Wick MJ, Wisinski KB, Darlow S, Dwyer M. 2018. NCCN guidelines version 2.2019: Genetic/Familial High-Risk Assessment: Breast and Ovarian. National Comprehensive Cancer Network.

Dare AJ, Gupta AA, Thipphavong S, Miettinen M, Gladdy RA. 2020. Abdominal neoplastic manifestations of neurofibromatosis type 1. Neuro-oncology Adv. 2: i124–i133.

Dawson SJ, Tsui DWY, Murtaza M, Biggs H, Rueda OM, Chin SF, Dunning MJ, Gale D, Forshew T, Mahler-Araujo B, Rajan S, Humphray S, Becq J, Halsall D, Wallis M, Bentley D, Caldas C, Rosenfeld N. 2013. Analysis of circulating tumor DNA to monitor metastatic breast cancer. N. Engl. J. Med. 368: 1199–1209.

DeBella K, Szudek J, Friedman JM. 2000. Use of the national institutes of health criteria for diagnosis of neurofibromatosis 1 in children. Pediatrics 105: 608–14.

Deeb SS, Fajas L, Nemoto M, Pihlajamäki J, Mykkänen L, Kuusisto J, Laakso M, Fujimoto W, Auwerx J. 1998. A Pro12Ala substitution in PPARgamma2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. Nat. Genet. 20: 284–7.

Diaz-Valencia PA, Bougnères P, Valleron A-J. 2015. Global epidemiology of type 1 diabetes in young adults and adults: a systematic review. BMC Public Health 15: 255.

DiMeglio LA, Evans-Molina C, Oram RA. 2018. Type 1 diabetes. Lancet 391: 2449–2462.

Dischinger PS, Tovar EA, Essenburg CJ, Madaj ZB, Gardner EE, Callaghan ME, Turner AN, Challa AK, Kempston T, Eagleson B, Kesterson RA, Bronson RT, Bowman MJ, Graveel CR, Steensma MR. 2018. NF1 deficiency correlates with estrogen receptor signaling and diminished survival in breast cancer. NPJ breast cancer 4: 29.

Doorn PF, Molenaar WM, Buter J, Hoekstra HJ. 1995. Malignant peripheral nerve sheath tumors in patients with and without neurofibromatosis. Eur. J. Surg. Oncol. 21: 78–82.

Doser K, Kenborg L, Andersen EW, Bidstrup PE, Kroyer A, Hove H, Østergaard J, Sørensen SA, Johansen C, Mulvihill J, Winther JF, Dalton SO. 2019. Educational delay and attainment in persons with neurofibromatosis 1 in Denmark. Eur. J. Hum. Genet. 27: 857–868.

Dunzendorfer-Matt T, Mercado EL, Maly K, McCormick F, Scheffzek K. 2016. The neurofibromin recruitment factor Spred1 binds to the GAP related domain without affecting Ras inactivation. Proc. Natl. Acad. Sci. U. S. A. 113: 7497–502.

Duong TA, Sbidian E, Valeyrie-Allanore L, Vialette C, Ferkal S, Hadj-Rabia S, Glorion C, Lyonnet S, Zerah M, Kemlin I, Rodriguez D, Bastuji-Garin S, Wolkenstein P. 2011. Mortality associated with neurofibromatosis 1: a cohort study of 1895 patients in 1980-2006 in France. Orphanet J. Rare Dis. 6: 18.

Dyba T, Randi G, Bray F, Martos C, Giusti F, Nicholson N, Gavin A, Flego M, Neamtiu L, Dimitrova N, Negrão Carvalho R, Ferlay J, Bettio M. 2021. The European cancer burden in 2020: Incidence and mortality estimates for 40 countries and 25 major cancers. Eur. J. Cancer 157: 308–347.

Easton DF, Ponder MA, Huson SM, Ponder BA. 1993. An analysis of variation in expression of neurofibromatosis (NF) type 1 (NF1): evidence for modifying genes. Am. J. Hum. Genet. 53: 305–13.

Easton DF, Pharoah PDP, Antoniou AC, Tischkowitz M, Tavtigian S V, Nathanson KL, Devilee P, Meindl A, Couch FJ, Southey M, Goldgar DE, Evans DGR, Chenevix-Trench G, Rahman N, Robson M, Domchek SM, Foulkes WD. 2015. Gene-panel sequencing and the prediction of breast-cancer risk. N. Engl. J. Med. 372: 2243–57.

Elefteriou F, Kolanczyk M, Schindeler A, Viskochil DH, Hock JM, Schorry EK, Crawford AH, Friedman JM, Little D, Peltonen J, Carey JC, Feldman D, Yu X, Armstrong L, Birch P, Kendler DL, Mundlos S, Yang F-C, Agiostratidou G, Hunter-Schaedle K, Stevenson DA. 2009. Skeletal abnormalities in neurofibromatosis type 1: approaches to therapeutic options. Am. J. Med. Genet. A 149A: 2327–38.

Emanuel PD. 2008. Juvenile myelomonocytic leukemia and chronic myelomonocytic leukemia. Leukemia 22: 1335–42.

Eulo V, Lesmana H, Doyle LA, Nichols KE, Hirbe AC. 2020. Secondary Sarcomas: Biology, Presentation, and Clinical Care. Am. Soc. Clin. Oncol. Educ. book. Am. Soc. Clin. Oncol. Annu. Meet. 40: 1–12.

Evans DGR, Baser ME, McGaughran J, Sharif S, Howard E, Moran A. 2002. Malignant peripheral nerve sheath tumours in neurofibromatosis 1. J. Med. Genet. 39: 311–4.

Evans DGR, Birch JM, Ramsden RT, Sharif S, Baser ME. 2006. Malignant transformation and new primary tumours after therapeutic radiation for benign disease: substantial risks in certain tumour prone syndromes. J. Med. Genet. 43: 289–94.

Evans DG, Howard E, Giblin C, Clancy T, Spencer H, Huson SM, Lalloo F. 2010. Birth incidence and prevalence of tumor-prone syndromes: estimates from a UK family genetic register service. Am. J. Med. Genet. A 152A: 327–32.

Evans DGR, O'Hara C, Wilding A, Ingham SL, Howard E, Dawson J, Moran A, Scott-Kitching V, Holt F, Huson SM. 2011. Mortality in neurofibromatosis 1: in North West England: an assessment of actuarial survival in a region of the UK since 1989. Eur. J. Hum. Genet. 19: 1187–1191.

Evans DGR, Huson SM, Birch JM. 2012. Malignant peripheral nerve sheath tumours in inherited disease. Clin. Sarcoma Res. 2: 17.

Evans DG. 2012. Are we ready for targeted early breast cancer detection strategies in women with NF1 aged 30-49 years? Am. J. Med. Genet. A 158A: 3054–5.

Evans DG, Bowers N, Burkitt-Wright E, Miles E, Garg S, Scott-Kitching V, Penman-Splitt M, Dobbie A, Howard E, Ealing J, Vassalo G, Wallace AJ, Newman W, Northern UK NF1 Research Network, Huson SM. 2016. Comprehensive RNA Analysis of the NF1 Gene in Classically Affected NF1 Affected Individuals Meeting NIH Criteria has High Sensitivity and Mutation Negative Testing is Reassuring in Isolated Cases With Pigmentary Features Only. EBioMedicine 7: 212–20.

Evans DGR, Salvador H, Chang VY, Erez A, Voss SD, Schneider KW, Scott HS, Plon SE, Tabori U. 2017. Cancer and Central Nervous System Tumor Surveillance in Pediatric Neurofibromatosis 1. Clin. Cancer Res. 23: e46–e53.

Fackenthal JD, Olopade OI. 2007. Breast cancer risk associated with BRCA1 and BRCA2 in diverse populations. Nat. Rev. Cancer 7: 937–48.

Fangusaro J, Onar-Thomas A, Young Poussaint T, Wu S, Ligon AH, Lindeman N, Banerjee A, Packer RJ, Kilburn LB, Goldman S, Pollack IF, Qaddoumi I, Jakacki RI, Fisher PG, Dhall G, Baxter P, Kreissman SG, Stewart CF, Jones DTW, Pfister SM, Vezina G, Stern JS, Panigrahy A, Patay Z, Tamrazi B, Jones JY, Haque SS, Enterline DS, Cha S, Fisher MJ, Doyle LA, Smith M, Dunkel IJ, Fouladi M. 2019. Selumetinib in paediatric patients with BRAF-aberrant or neurofibromatosis type 1-associated recurrent, refractory, or progressive low-grade glioma: a multicentre, phase 2 trial. Lancet. Oncol. 20: 1011–1022.

Fangusaro J, Onar-Thomas A, Poussaint TY, Wu S, Ligon AH, Lindeman N, Campagne O, Banerjee A, Gururangan S, Kilburn LB, Goldman S, Qaddoumi I, Baxter P, Vezina G, Bregman C, Patay Z, Jones JY, Stewart CF, Fisher MJ, Doyle LA, Smith M, Dunkel IJ, Fouladi M. 2021. A phase II trial of selumetinib in children with recurrent optic pathway and hypothalamic low-grade glioma without NF1: a Pediatric Brain Tumor Consortium study. Neuro. Oncol. 23: 1777–1788.

Fazii P, Passamonti M, Lappa G, Di Mascio R, Ballone E. 1998. Prevalence of neurofibromatosis type 1 among the young adult population of central Italy. Arch. Dermatol. 134: 1303–4.

Fernando MR, Jiang C, Krzyzanowski GD, Ryan WL. 2017. New evidence that a large proportion of human blood plasma cell-free DNA is localized in exosomes. PLoS One 12: e0183915.

Ferner RE, Huson SM, Thomas N, Moss C, Willshaw H, Evans DG, Upadhyaya M, Towers R, Gleeson M, Steiger C, Kirby A. 2007. Guidelines for the diagnosis and management of individuals with neurofibromatosis 1. J. Med. Genet. 44: 81–8.

Ferner RE, Golding JF, Smith M, Calonje E, Jan W, Sanjayanathan V, O'Doherty M. 2008. [18F]2-fluoro-2-deoxy-D-glucose positron emission tomography (FDG PET) as a diagnostic tool for neurofibromatosis 1 (NF1) associated malignant peripheral nerve sheath tumours (MPNSTs): a long-term clinical study. Ann. Oncol. 19: 390–4.

Flannick J, Thorleifsson G, Beer NL, Jacobs SBR, Grarup N, Burtt NP, Mahajan A, Fuchsberger C, Atzmon G, Benediktsson R, Blangero J, Bowden DW, Brandslund I, Brosnan J, Burslem F, Chambers J, Cho YS, Christensen C, Douglas DA, Duggirala R, Dymek Z, Farjoun Y, Fennell T, Fontanillas P, Forsén T, Gabriel S, Glaser B, Gudbjartsson DF, Hanis C, Hansen T, Hreidarsson AB, Hveem K, Ingelsson E, Isomaa B, Johansson S, Jørgensen T, Jørgensen ME, Kathiresan S, Kong A, Kooner J, Kravic J, Laakso M, Lee J-Y, Lind L, Lindgren CM, Linneberg A, Masson G, Meitinger T, Mohlke KL, Molven A, Morris AP, Potluri S, Rauramaa R, Ribel-Madsen R, Richard A-M, Rolph T, Salomaa V, Segrè A V, Skärstrand H, Steinthorsdottir V, Stringham HM, Sulem P, Tai ES, Teo YY, Teslovich T, Thorsteinsdottir U, Trimmer JK, Tuomi T, Tuomilehto J, Vaziri-Sani F, Voight BF, Wilson JG, Boehnke M, McCarthy MI, Njølstad PR, Pedersen O, Groop L, Cox DR, Stefansson K, Altshuler D, Stefansson K, Altshuler D. 2014. Loss-of-function mutations in SLC30A8 protect against type 2 diabetes. Nat. Genet. 46: 357–363.

Flannick J, Mercader JM, Fuchsberger C, Udler MS, Mahajan A, Wessel J, Teslovich TM, Caulkins L, Koesterer R, Barajas-Olmos F, Blackwell TW, Boerwinkle E, Brody JA, Centeno-Cruz F, Chen L, Chen S, Contreras-Cubas C, Córdova E, Correa A, Cortes M, DeFronzo RA, Dolan L, Drews KL, Elliott A, Floyd JS, Gabriel S, Garay-Sevilla ME, García-Ortiz H, Gross M, Han S, Heard-Costa NL, Jackson AU, Jørgensen ME, Kang HM, Kelsey M, Kim B-J, Koistinen HA, Kuusisto J, Leader JB, Linneberg A, Liu C-T, Liu J, Lyssenko V, Manning AK, Marcketta A, Malacara-

Hernandez JM, Martínez-Hernández A, Matsuo K, Mayer-Davis E, Mendoza-Caamal E, Mohlke KL, Morrison AC, Ndungu A, Ng MCY, O'Dushlaine C, Payne AJ, Pihoker C, Broad Genomics Platform WS, Post WS, Preuss M, Psaty BM, Vasan RS, Rayner NW, Reiner AP, Revilla-Monsalve C, Robertson NR, Santoro N, Schurmann C, So WY, Soberón X, Stringham HM, Strom TM, Tam CHT, Thameem F, Tomlinson B, Torres JM, Tracy RP, van Dam RM, Vujkovic M, Wang S, Welch RP, Witte DR, Wong T-Y, Atzmon G, Barzilai N, Blangero J, Bonnycastle LL, Bowden DW, Chambers JC, Chan E, Cheng C-Y, Cho YS, Collins FS, de Vries PS, Duggirala R, Glaser B, Gonzalez C, Gonzalez ME, et al. 2019. Exome sequencing of 20,791 cases of type 2 diabetes and 24,440 controls. Nature 570: 71–76.

Fletcher B, Gulanick M, Lamendola C. 2002. Risk factors for type 2 diabetes mellitus. J. Cardiovasc. Nurs. 16: 17–23.

Forde C, King AT, Rutherford SA, Hammerbeck-Ward C, Lloyd SK, Freeman SR, Pathmanaban ON, Stapleton E, Thomas OM, Laitt RD, Stivaros S, Kilday J-P, Vassallo G, McBain C, Kerrigan S, Smith MJ, McCabe MG, Harkness EF, Evans DG. 2021. Disease course of neurofibromatosis type 2: a 30-year follow-up study of 353 patients seen at a single institution. Neuro. Oncol. 23: 1113–1124.

Foulkes WD, Metcalfe K, Sun P, Hanna WM, Lynch HT, Ghadirian P, Tung N, Olopade OI, Weber BL, McLennan J, Olivotto IA, Bégin LR, Narod SA. 2004. Estrogen receptor status in BRCA1- and BRCA2-related breast cancer: the influence of age, grade, and histological type. Clin. Cancer Res. 10: 2029–34.

Frayling IM, Mautner V-F, van Minkelen R, Kallionpaa RA, Aktaş S, Baralle D, Ben-Shachar S, Callaway A, Cox H, Eccles DM, Ferkal S, LaDuca H, Lázaro C, Rogers MT, Stuenkel AJ, Summerour P, Varan A, Yap YS, Zehou O, Peltonen J, Evans DG, Wolkenstein P, Upadhyaya M. 2019. Breast cancer risk in neurofibromatosis type 1 is a function of the type of NF1 gene mutation: a new genotype-phenotype correlation. J. Med. Genet. 56: 209–219.

Friedrich RE, Nuding MA. 2016. Optic Pathway Glioma and Cerebral Focal Abnormal Signal Intensity in Patients with Neurofibromatosis Type 1: Characteristics, Treatment Choices and Follow-up in 134 Affected Individuals and a Brief Review of the Literature. Anticancer Res. 36: 4095–121.

Fumagalli C, Ranghiero A, Gandini S, Corso F, Taormina S, De Camilli E, Rappa A, Vacirca D, Viale G, Guerini-Rocco E, Barberis M. 2020. Inter-tumor genomic heterogeneity of breast cancers: comprehensive genomic profile of primary early breast cancers and relapses. Breast Cancer Res. 22: 107.

Garty BZ, Laor A, Danon YL. 1994. Neurofibromatosis type 1 in Israel: survey of young adults. J. Med. Genet. 31: 853–7.

Grabuschnig S, Bronkhorst AJ, Holdenrieder S, Rosales Rodriguez I, Schliep KP, Schwendenwein D, Ungerer V, Sensen CW. 2020. Putative Origins of Cell-Free DNA in Humans: A Review of Active and Passive Nucleic Acid Release Mechanisms. Int. J. Mol. Sci. 21: 8062.

Granström S, Langenbruch A, Augustin M, Mautner V-F. 2012. Psychological burden in adult neurofibromatosis type 1 patients: impact of disease visibility on body image. Dermatology 224: 160–7.

Griffith OL, Spies NC, Anurag M, Griffith M, Luo J, Tu D, Yeo B, Kunisaki J, Miller CA, Krysiak K, Hundal J, Ainscough BJ, Skidmore ZL, Campbell K, Kumar R, Fronick C, Cook L, Snider JE, Davies S, Kavuri SM, Chang EC, Magrini V, Larson DE, Fulton RS, Liu S, Leung S, Voduc D, Bose R, Dowsett M, Wilson RK, Nielsen TO, Mardis ER, Ellis MJ. 2018. The prognostic effects of somatic mutations in ER-positive breast cancer. Nat. Commun. 9: 3476.

Gross AM, Wolters PL, Dombi E, Baldwin A, Whitcomb P, Fisher MJ, Weiss B, Kim A, Bornhorst M, Shah AC, Martin S, Roderick MC, Pichard DC, Carbonell A, Paul SM, Therrien J, Kapustina O, Heisey K, Clapp DW, Zhang C, Peer CJ, Figg WD, Smith M, Glod J, Blakeley JO, Steinberg SM, Venzon DJ, Doyle LA, Widemann BC. 2020. Selumetinib in Children with Inoperable Plexiform Neurofibromas. N. Engl. J. Med. 382: 1430–1442.

Gudmundsson J, Sulem P, Steinthorsdottir V, Bergthorsson JT, Thorleifsson G, Manolescu A, Rafnar T, Gudbjartsson D, Agnarsson BA, Baker A, Sigurdsson A, Benediktsdottir KR, Jakobsdottir M, Blondal T, Stacey SN, Helgason A, Gunnarsdottir S, Olafsdottir A, Kristinsson KT, Birgisdottir B, Ghosh S, Thorlacius S, Magnusdottir D, Stefansdottir G, Kristjansson K, Bagger Y, Wilensky RL, Reilly MP, Morris AD, Kimber CH, Adeyemo A, Chen Y, Zhou J, So W-Y, Tong PCY, Ng MCY, Hansen T, Andersen G, Borch-Johnsen K, Jorgensen T, Tres A, Fuertes F, Ruiz-Echarri M, Asin L, Saez B, van Boven E, Klaver S, Swinkels DW, Aben KK, Graif T, Cashy J, Suarez BK, van Vierssen Trip O, Frigge ML, Ober C, Hofker MH, Wijmenga C, Christiansen C, Rader DJ, Palmer CNA, Rotimi C, Chan JCN, Pedersen O, Sigurdsson G, Benediktsson R, Jonsson E, Einarsson G V, Mayordomo JI, Catalona WJ, Kiemeney LA, Barkardottir RB, Gulcher JR, Thorsteinsdottir U, Kong A, Stefansson K. 2007. Two variants on chromosome 17 confer prostate cancer risk, and the one in TCF2 protects against type 2 diabetes. Nat. Genet. 39: 977–83.

Guillamo J-S, Creange A, Kalifa C, Grill J, Rodriguez D, Doz F, Barbarot S, Zerah M, Sanson M, Bastuji-Garin S, Wolkenstein P. 2003. Prognostic factors of CNS tumours in Neurofibromatosis 1 (NF1): A retrospective study of 104 patients. Brain 126: 152–160.

Güran S, Safali M. 2005. A case of neurofibromatosis and breast cancer: loss of heterozygosity of NF1 in breast cancer. Cancer Genet. Cytogenet. 156: 86–8.

Gutmann DH, Ferner RE, Listernick RH, Korf BR, Wolters PL, Johnson KJ. 2017. Neurofibromatosis type 1. Nat. Rev. Dis. Prim. 3: 17004.

Haber DA, Velculescu VE. 2014. Blood-based analyses of cancer: Circulating tumor cells and circulating tumor DNA. Cancer Discov. 4: 650–661.

Hanahan D, Weinberg RA. 2000. The hallmarks of cancer. Cell 100: 57–70.

Hanahan D, Weinberg RA. 2011. Hallmarks of cancer: the next generation. Cell 144: 646–74.

Harder A. 2022. Do non-pathogenic variants of DNA mismatch repair genes modify neurofibroma load in neurofibromatosis type 1? Childs. Nerv. Syst. 38: 705–713.

Heervä E, Alanne MH, Peltonen S, Kuorilehto T, Hentunen T, Väänänen K, Peltonen J. 2010. Osteoclasts in neurofibromatosis type 1 display enhanced resorption capacity, aberrant morphology, and resistance to serum deprivation. Bone 47: 583–90.

Heervä E, Koffert A, Jokinen E, Kuorilehto T, Peltonen S, Aro HT, Peltonen J. 2012. A controlled register-based study of 460 neurofibromatosis 1 patients: increased fracture risk in children and adults over 41 years of age. J. Bone Miner. Res. 27: 2333–7.

Heervä E, Leinonen P, Kuorilehto T, Peltonen S, Pöyhönen M, Väänänen K, Peltonen J. 2013. Neurofibromatosis 1-related osteopenia often progresses to osteoporosis in 12 years. Calcif. Tissue Int. 92: 23–7.

Heitzer E, Ulz P, Geigl JB. 2015. Circulating tumor DNA as a liquid biopsy for cancer. Clin. Chem. 61: 112–123.

Hendrick RE. 2010. Radiation doses and cancer risks from breast imaging studies. Radiology 257: 246–53.

Hodis E, Watson IR, Kryukov G V, Arold ST, Imielinski M, Theurillat J-P, Nickerson E, Auclair D, Li L, Place C, Dicara D, Ramos AH, Lawrence MS, Cibulskis K, Sivachenko A, Voet D, Saksena G, Stransky N, Onofrio RC, Winckler W, Ardlie K, Wagle N, Wargo J, Chong K, Morton DL, Stemke-Hale K, Chen G, Noble M, Meyerson M, Ladbury JE, Davies MA, Gershenwald JE, Wagner SN, Hoon DSB, Schadendorf D, Lander ES, Gabriel SB, Getz G, Garraway LA, Chin L. 2012. A landscape of driver mutations in melanoma. Cell 150: 251–63.

Hofvind S, Vacek PM, Skelly J, Weaver DL, Geller BM. 2008. Comparing screening mammography for early breast cancer detection in Vermont and Norway. J. Natl. Cancer Inst. 100: 1082–91.

Howell SJ, Hockenhull K, Salih Z, Evans DG. 2017. Increased risk of breast cancer in neurofibromatosis type 1: current insights. Breast cancer (Dove Med. Press) 9: 531–536.

Huang RSP, Haberberger J, McGregor K, Mata DA, Decker B, Hiemenz MC, Lechpammer M, Danziger N, Schiavone K, Creeden J, Graf RP, Strowd R, Lesser GJ, Razis ED, Bartsch R, Giannoudis A, Bhogal T, Lin NU, Pusztai L, Ross JS, Palmieri C, Ramkissoon SH. 2021.

Clinicopathologic and Genomic Landscape of Breast Carcinoma Brain Metastases. Oncologist 26: 835–844.

Huson SM, Compston DA, Clark P, Harper PS. 1989. A genetic study of von Recklinghausen neurofibromatosis in south east Wales. I. Prevalence, fitness, mutation rate, and effect of parental transmission on severity. J. Med. Genet. 26: 704–11.

Ignatiadis M, Sledge GW, Jeffrey SS. 2021. Liquid biopsy enters the clinic - implementation issues and future challenges. Nat. Rev. Clin. Oncol. 18: 297–312.

Ingham S, Huson SM, Moran A, Wylie J, Leahy M, Evans DGR. 2011. Malignant peripheral nerve sheath tumours in NF1: improved survival in women and in recent years. Eur. J. Cancer 47: 2723–8.

Inshaw JRJ, Cutler AJ, Crouch DJM, Wicker LS, Todd JA. 2020. Genetic Variants Predisposing Most Strongly to Type 1 Diabetes Diagnosed Under Age 7 Years Lie Near Candidate Genes That Function in the Immune System and in Pancreatic β-Cells. Diabetes Care 43: 169–177.

Jacks T, Shih TS, Schmitt EM, Bronson RT, Bernards A, Weinberg RA. 1994. Tumour predisposition in mice heterozygous for a targeted mutation in Nf1. Nat. Genet. 7: 353–61.

Jahr S, Hentze H, Englisch S, Hardt D, Fackelmayer FO, Hesch RD, Knippers R. 2001. DNA fragments in the blood plasma of cancer patients: Quantitations and evidence for their origin from apoptotic and necrotic cells. Cancer Res. 61: 1659–1665.

Jégu J, Colonna M, Daubisse-Marliac L, Trétarre B, Ganry O, Guizard A-V, Bara S, Troussard X, Bouvier V, Woronoff A-S, Velten M. 2014. The effect of patient characteristics on second primary cancer risk in France. BMC Cancer 14: 94.

Jerram S, Leslie RD. 2017. The Genetic Architecture of Type 1 Diabetes. Genes (Basel). 8: 209.

Johansson E, Kallionpää RA, Böckerman P, Peltonen J, Peltonen S. 2021. A rare disease and education: Neurofibromatosis type 1 decreases educational attainment. Clin. Genet. 99: 529–539.

Johansson E, Kallionpää RA, Böckerman P, Peltonen S, Peltonen J. 2022. The rare disease neurofibromatosis 1 as a source of hereditary economic inequality: Evidence from Finland. Genet. Med. 24: 870–879.

Jones J, Cain S, Pesic-Smith J, Choong PFM, Morokoff AP, Drummond KJ, Dabscheck G. 2021. Circulating tumor DNA for malignant peripheral nerve sheath tumors in neurofibromatosis type 1. J. Neurooncol. 154: 265–274.

Jouhilahti EM, Peltonen S, Heape AM, Peltonen J. 2011. The pathoetiology of neurofibromatosis 1. Am. J. Pathol. 178: 1932–1939.

Jung M, Klotzek S, Lewandowski M, Fleischhacker M, Jung K. 2003. Changes in concentration of DNA in serum and plasma during storage of blood samples. Clin. Chem. 49: 1028–9.

Kallionpää RA, Valtanen M, Auranen K, Uusitalo E, Rinne JO, Peltonen S, Peltonen J. 2021a. Increased risk for dementia in neurofibromatosis type 1. Genet. Med. 23: 2219–2222.

Kallionpää RA, Ahramo K, Martikkala E, Fazeli E, Haapaniemi P, Rokka A, Leivo I, Harvima IT, Peltonen J, Peltonen S. 2021b. Mast Cells in Human Cutaneous Neurofibromas: Density, Subtypes, and Association with Clinical Features in Neurofibromatosis 1. Dermatology: 1–11.

Kamat AA, Baldwin M, Urbauer D, Dang D, Han LY, Godwin A, Karlan BY, Simpson JL, Gershenson DM, Coleman RL, Bischoff FZ, Sood AK. 2010. Plasma cell-free DNA in ovarian cancer: an independent prognostic biomarker. Cancer 116: 1918–25.

Kammesheidt A, Tonozzi TR, Lim SW, Braunstein GD. 2018. Mutation detection using plasma circulating tumor DNA (ctDNA) in a cohort of asymptomatic adults at increased risk for cancer. Int. J. Mol. Epidemiol. Genet. 9: 1–12.

Keene KS, King T, Hwang ES, Peng B, McGuire KP, Tapia C, Zhang H, Bae S, Nakhlis F, Klauber-Demore N, Meszoely I, Sabel MS, Willey SC, Eterovic AK, Hudis C, Wolff AC, De Los Santos J, Thompson A, Mills GB, Meric-Bernstam F. 2018. Molecular determinants of post-mastectomy breast cancer recurrence. NPJ breast cancer 4: 34.

Kenborg L, Duun-Henriksen AK, Dalton SO, Bidstrup PE, Doser K, Rugbjerg K, Pedersen C, Krøyer A, Johansen C, Andersen KK, Østergaard JR, Hove H, Sørensen SA, Riccardi VM, Mulvihill JJ,

Winther JF. 2020. Multisystem burden of neurofibromatosis 1 in Denmark: registry- and population-based rates of hospitalizations over the life span. Genet. Med. 22: 1069–1078.

Kim ET, Namgung H, Shin HD, Lee S Il, Kwon JE, Chang MC, Park DG. 2012. Oncologic manifestations of neurofibromatosis type 1 in Korea. J. Korean Surg. Soc. 82: 205–10.

Kim K, Shin DG, Park MK, Baik SH, Kim TH, Kim S, Lee S. 2014. Circulating cell-free DNA as a promising biomarker in patients with gastric cancer: diagnostic validity and significant reduction of cfDNA after surgical resection. Ann. Surg. Treat. Res. 86: 136–42.

King M-C, Marks JH, Mandell JB, New York Breast Cancer Study Group. 2003. Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. Science 302: 643–6.

Klesse LJ, Jordan JT, Radtke HB, Rosser T, Schorry E, Ullrich N, Viskochil D, Knight P, Plotkin SR, Yohay K. 2020. The Use of MEK Inhibitors in Neurofibromatosis Type 1-Associated Tumors and Management of Toxicities. Oncologist 25: e1109–e1116.

Klinger B, Sieber A, Fritsche-Guenther R, Witzel F, Berry L, Schumacher D, Yan Y, Durek P, Merchant M, Schäfer R, Sers C, Blüthgen N. 2013. Network quantification of EGFR signaling unveils potential for targeted combination therapy. Mol. Syst. Biol. 9: 673.

Koczkowska M, Chen Y, Callens T, Gomes A, Sharp A, Johnson S, Hsiao M-C, Chen Z, Balasubramanian M, Barnett CP, Becker TA, Ben-Shachar S, Bertola DR, Blakeley JO, Burkitt-Wright EMM, Callaway A, Crenshaw M, Cunha KS, Cunningham M, D'Agostino MD, Dahan K, De Luca A, Destrée A, Dhamija R, Eoli M, Evans DGR, Galvin-Parton P, George-Abraham JK, Gripp KW, Guevara-Campos J, Hanchard NA, Hernández-Chico C, Immken L, Janssens S, Jones KJ, Keena BA, Kochhar A, Liebelt J, Martir-Negron A, Mahoney MJ, Maystadt I, McDougall C, McEntagart M, Mendelsohn N, Miller DT, Mortier G, Morton J, Pappas J, Plotkin SR, Pond D, Rosenbaum K, Rubin K, Russell L, Rutledge LS, Saletti V, Schonberg R, Schreiber A, Seidel M, Siqveland E, Stockton DW, Trevisson E, Ullrich NJ, Upadhyaya M, van Minkelen R, Verhelst H, Wallace MR, Yap Y-S, Zackai E, Zonana J, Zurcher V, Claes K, Martin Y, Korf BR, Legius E, Messiaen LM. 2018. Genotype-Phenotype Correlation in NF1: Evidence for a More Severe Phenotype Associated with Missense Mutations Affecting NF1 Codons 844-848. Am. J. Hum. Genet. 102: 69–87.

Koczkowska M, Callens T, Chen Y, Gomes A, Hicks AD, Sharp A, Johns E, Uhas KA, Armstrong L, Bosanko KA, Babovic-Vuksanovic D, Baker L, Basel DG, Bengala M, Bennett JT, Chambers C, Clarkson LK, Clementi M, Cortés FM, Cunningham M, D'Agostino MD, Delatycki MB, Digilio MC, Dosa L, Esposito S, Fox S, Freckmann ML, Fauth C, Giugliano T, Giustini S, Goetsch A, Goldberg Y, Greenwood RS, Griffis C, Gripp KW, Gupta P, Haan E, Hachen RK, Haygarth TL, Hernández-Chico C, Hodge K, Hopkin RJ, Hudgins L, Janssens S, Keller K, Kelly-Mancuso G, Kochhar A, Korf BR, Lewis AM, Liebelt J, Lichty A, Listernick RH, Lyons MJ, Maystadt I, Martinez Ojeda M, McDougall C, McGregor LK, Melis D, Mendelsohn N, Nowaczyk MJM, Ortenberg J, Panzer K, Pappas JG, Pierpont ME, Piluso G, Pinna V, Pivnick EK, Pond DA, Powell CM, Rogers C, Ruhrman Shahar N, Rutledge SL, Saletti V, Sandaradura SA, Santoro C, Schatz UA, Schreiber A, Scott DA, Sellars EA, Sheffer R, Siqveland E, Slopis JM, Smith R, Spalice A, Stockton DW, Streff H, Theos A, Tomlinson GE, Tran G, Trapane PL, Trevisson E, Ullrich NJ, Van den Ende J, Schrier Vergano SA, Wallace SE, Wangler MF, Weaver DD, Yohay KH, et al. 2020. Clinical spectrum of individuals with pathogenic NF1 missense variants affecting p.Met1149, p.Arg1276, and p.Lys1423: genotype–phenotype study in neurofibromatosis type 1. Hum. Mutat. 41: 299–315.

Kodra Y, Giustini S, Divona L, Porciello R, Calvieri S, Wolkenstein P, Taruscio D. 2009. Health-related quality of life in patients with neurofibromatosis type 1: A survey of 129 Italian patients. Dermatology 218: 215–220.

Kolberg M, Høland M, Agesen TH, Brekke HR, Liestøl K, Hall KS, Mertens F, Picci P, Smeland S, Lothe RA. 2013. Survival meta-analyses for >1800 malignant peripheral nerve sheath tumor patients with and without neurofibromatosis type 1. Neuro. Oncol. 15: 135–47.

Koskenvuo L, Malila N, Pitkäniemi J, Miettinen J, Heikkinen S, Sallinen V. 2019. Sex differences in faecal occult blood test screening for colorectal cancer. Br. J. Surg. 106: 436–447.

Kösters JP, Gøtzsche PC. 2003. Regular self-examination or clinical examination for early detection of breast cancer. Cochrane database Syst. Rev.: CD003373.

Krauthammer M, Kong Y, Bacchiocchi A, Evans P, Pornputtapong N, Wu C, McCusker JP, Ma S, Cheng E, Straub R, Serin M, Bosenberg M, Ariyan S, Narayan D, Sznol M, Kluger HM, Mane S, Schlessinger J, Lifton RP, Halaban R. 2015. Exome sequencing identifies recurrent mutations in NF1 and RASopathy genes in sun-exposed melanomas. Nat. Genet. 47: 996–1002.

Krischer JP, Liu X, Lernmark Å, Hagopian WA, Rewers MJ, She J-X, Toppari J, Ziegler A-G, Akolkar B, TEDDY Study Group. 2017. The Influence of Type 1 Diabetes Genetic Susceptibility Regions, Age, Sex, and Family History on the Progression From Multiple Autoantibodies to Type 1 Diabetes: A TEDDY Study Report. Diabetes 66: 3122–3129.

Kuorilehto T, Pöyhönen M, Bloigu R, Heikkinen J, Väänänen K, Peltonen J. 2005. Decreased bone mineral density and content in neurofibromatosis type 1: lowest local values are located in the load-carrying parts of the body. Osteoporos. Int. 16: 928–36.

Kurtelius A, Kallionpää RA, Huttunen J, Huttunen TJ, Helin K, Koivisto T, Frösen J, von und zu Fraunberg M, Peltonen S, Peltonen J, Jääskeläinen JE, Lindgren AE. 2017. Neurofibromatosis type 1 is not associated with subarachnoid haemorrhage. PLoS One 12: e0178711.

Lakhani SR, Reis-Filho JS, Fulford L, Penault-Llorca F, van der Vijver M, Parry S, Bishop T, Benitez J, Rivas C, Bignon Y-J, Chang-Claude J, Hamann U, Cornelisse CJ, Devilee P, Beckmann MW, Nestle-Krämling C, Daly PA, Haites N, Varley J, Lalloo F, Evans G, Maugard C, Meijers-Heijboer H, Klijn JGM, Olah E, Gusterson BA, Pilotti S, Radice P, Scherneck S, Sobol H, Jacquemier J, Wagner T, Peto J, Stratton MR, McGuffog L, Easton DF, Breast Cancer Linkage Consortium. 2005. Prediction of BRCA1 status in patients with breast cancer using estrogen receptor and basal phenotype. Clin. Cancer Res. 11: 5175–80.

Lakhani S, Ellis I, Schnitt S, Tan P, van de Vijver M. 2012. WHO Classification of Tumours of the Breast, Fourth Edition. Lyon: International Agency for Research on Cancer.

Lam NYL, Rainer TH, Chiu RWK, Lo YMD. 2004. EDTA Is a Better Anticoagulant than Heparin or Citrate for Delayed Blood Processing for Plasma DNA Analysis. Clin. Chem. 50: 256–257.

Lammert M, Friedman JM, Kluwe L, Mautner VF. 2005. Prevalence of neurofibromatosis 1 in German children at elementary school enrollment. Arch. Dermatol. 141: 71–4.

Lamminaho M, Kujala J, Peltonen H, Tengström M, Kosma V-M, Mannermaa A. 2021. High Cell-Free DNA Integrity Is Associated with Poor Breast Cancer Survival. Cancers (Basel). 13: 4679.

Landry JP, Schertz KL, Chiang Y-J, Bhalla AD, Yi M, Keung EZ, Scally CP, Feig BW, Hunt KK, Roland CL, Guadagnolo A, Bishop AJ, Lazar AJ, Slopis JM, McCutcheon IE, Torres KE. 2021. Comparison of Cancer Prevalence in Patients With Neurofibromatosis Type 1 at an Academic Cancer Center vs in the General Population From 1985 to 2020. JAMA Netw. open 4: e210945.

Lauby-Secretan B, Vilahur N, Bianchini F, Guha N, Straif K, International Agency for Research on Cancer Handbook Working Group. 2018. The IARC Perspective on Colorectal Cancer Screening. N. Engl. J. Med. 378: 1734–1740.

Legius E, Messiaen L, Wolkenstein P, Pancza P, Avery RA, Berman Y, Blakeley J, Babovic-Vuksanovic D, Cunha KS, Ferner R, Fisher MJ, Friedman JM, Gutmann DH, Kehrer-Sawatzki H, Korf BR, Mautner V-F, Peltonen S, Rauen KA, Riccardi V, Schorry E, Stemmer-Rachamimov A, Stevenson DA, Tadini G, Ullrich NJ, Viskochil D, Wimmer K, Yohay K, International Consensus Group on Neurofibromatosis Diagnostic Criteria (I-NF-DC), Huson SM, Evans DG, Plotkin SR. 2021. Revised diagnostic criteria for neurofibromatosis type 1 and Legius syndrome: an international consensus recommendation. Genet. Med. 23: 1506–1513.

Leinonen MK, Miettinen J, Heikkinen S, Pitkäniemi J, Malila N. 2017. Quality measures of the population-based Finnish Cancer Registry indicate sound data quality for solid malignant tumours. Eur. J. Cancer 77: 31–39.

Leon SA, Shapiro B, Sklaroff DM, Yaros MJ. 1977. Free DNA in the Serum of Cancer Patients and the Effect of Therapy. Cancer Res. 37: 646–650.

Leoni C, Onesimo R, Giorgio V, Diamanti A, Giorgio D, Martini L, Rossodivita A, Tartaglia M, Zampino G. 2016. Understanding Growth Failure in Costello Syndrome: Increased Resting Energy Expenditure. J. Pediatr. 170: 322–4.

Leppävirta J, Kallionpää RA, Uusitalo E, Vahlberg T, Pöyhönen M, Peltonen J, Peltonen S. 2019. Neurofibromatosis type 1 of the child increases birth weight. Am. J. Med. Genet. Part A 179: ajmg.a.61161.

Listernick R, Charrow J, Greenwald M, Mets M. 1994. Natural history of optic pathway tumors in children with neurofibromatosis type 1: a longitudinal study. J. Pediatr. 125: 63–6.

Listernick R, Louis DN, Packer RJ, Gutmann DH. 1997. Optic pathway gliomas in children with neurofibromatosis 1: consensus statement from the NF1 Optic Pathway Glioma Task Force. Ann. Neurol. 41: 143–9.

Listernick R, Ferner RE, Liu GT, Gutmann DH. 2007. Optic pathway gliomas in neurofibromatosis-1: Controversies and recommendations. Ann. Neurol. 61: 189–198.

Lönnberg S, Anttila A, Luostarinen T, Nieminen P. 2012. Age-specific effectiveness of the Finnish cervical cancer screening programme. Cancer Epidemiol. Biomarkers Prev. 21: 1354–61.

Lotta LA, Mokrosiński J, Mendes de Oliveira E, Li C, Sharp SJ, Luan J, Brouwers B, Ayinampudi V, Bowker N, Kerrison N, Kaimakis V, Hoult D, Stewart ID, Wheeler E, Day FR, Perry JRB, Langenberg C, Wareham NJ, Farooqi IS. 2019. Human Gain-of-Function MC4R Variants Show Signaling Bias and Protect against Obesity. Cell 177: 597-607.e9.

Lousdal ML, Kristiansen IS, Møller B, Støvring H. 2016. Effect of organised mammography screening on stage-specific incidence in Norway: population study. Br. J. Cancer 114: 590–6.

Lyssenko V, Jonsson A, Almgren P, Pulizzi N, Isomaa B, Tuomi T, Berglund G, Altshuler D, Nilsson P, Groop L. 2008. Clinical Risk Factors, DNA Variants, and the Development of Type 2 Diabetes. N. Engl. J. Med. 359: 2220–2232.

Maani N, Westergard S, Yang J, Scaranelo AM, Telesca S, Thain E, Schachter NF, McCuaig JM, Kim RH. 2019. NF1 Patients Receiving Breast Cancer Screening: Insights from The Ontario High Risk Breast Screening Program. Cancers (Basel). 11: 707.

Madanikia SA, Bergner A, Ye X, Blakeley JO. 2012. Increased risk of breast cancer in women with NF1. Am. J. Med. Genet. A 158A: 3056–60.

Madubata CC, Olsen MA, Stwalley DL, Gutmann DH, Johnson KJ. 2015. Neurofibromatosis type 1 and chronic neurological conditions in the United States: an administrative claims analysis. Genet. Med. 17: 36–42.

Maertens O, Brems H, Vandesompele J, De Raedt T, Heyns I, Rosenbaum T, De Schepper S, De Paepe A, Mortier G, Janssens S, Speleman F, Legius E, Messiaen L. 2006a. Comprehensive NF1 screening on cultured Schwann cells from neurofibromas. Hum. Mutat. 27: 1030–1040.

Maertens O, Prenen H, Debiec-Rychter M, Wozniak A, Sciot R, Pauwels P, De Wever I, Vermeesch JR, de Raedt T, De Paepe A, Speleman F, van Oosterom A, Messiaen L, Legius E. 2006b. Molecular pathogenesis of multiple gastrointestinal stromal tumors in NF1 patients. Hum. Mol. Genet. 15: 1015–23.

Maertens O, Johnson B, Hollstein P, Frederick DT, Cooper ZA, Messiaen L, Bronson RT, McMahon M, Granter S, Flaherty K, Wargo JA, Marais R, Cichowski K. 2013. Elucidating distinct roles for NF1 in melanomagenesis. Cancer Discov. 3: 338–49.

Mahajan A, Taliun D, Thurner M, Robertson NR, Torres JM, Rayner NW, Payne AJ, Steinthorsdottir V, Scott RA, Grarup N, Cook JP, Schmidt EM, Wuttke M, Sarnowski C, Mägi R, Nano J, Gieger C, Trompet S, Lecoeur C, Preuss MH, Prins BP, Guo X, Bielak LF, Below JE, Bowden DW, Chambers JC, Kim YJ, Ng MCY, Petty LE, Sim X, Zhang W, Bennett AJ, Bork-Jensen J, Brummett CM, Canouil M, Ec Kardt K-U, Fischer K, Kardia SLR, Kronenberg F, Läll K, Liu C-T, Locke AE, Luan J, Ntalla I, Nylander V, Schönherr S, Schurmann C, Yengo L, Bottinger EP, Brandslund I, Christensen C, Dedoussis G, Florez JC, Ford I, Franco OH, Frayling TM, Giedraitis

V, Hackinger S, Hattersley AT, Herder C, Ikram MA, Ingelsson M, Jørgensen ME, Jørgensen T, Kriebel J, Kuusisto J, Ligthart S, Lindgren CM, Linneberg A, Lyssenko V, Mamakou V, Meitinger T, Mohlke KL, Morris AD, Nadkarni G, Pankow JS, Peters A, Sattar N, Stančáková A, Strauch K, Taylor KD, Thorand B, Thorleifsson G, Thorsteinsdottir U, Tuomilehto J, Witte DR, Dupuis J, Peyser PA, Zeggini E, Loos RJF, Froguel P, Ingelsson E, Lind L, Groop L, Laakso M, Collins FS, Jukema JW, Palmer CNA, et al. 2018. Fine-mapping type 2 diabetes loci to single-variant resolution using high-density imputation and islet-specific epigenome maps. Nat. Genet. 50: 1505–1513.

Mahdi J, Shah AC, Sato A, Morris SM, McKinstry RC, Listernick R, Packer RJ, Fisher MJ, Gutmann DH. 2017. A multi-institutional study of brainstem gliomas in children with neurofibromatosis type 1. Neurology 88: 1584–1589.

Mai PL, Best AF, Peters JA, DeCastro RM, Khincha PP, Loud JT, Bremer RC, Rosenberg PS, Savage SA. 2016. Risks of first and subsequent cancers among TP53 mutation carriers in the National Cancer Institute Li-Fraumeni syndrome cohort. Cancer 122: 3673–3681.

Mann RM, Cho N, Moy L. 2019. Breast MRI: State of the Art. Radiology 292: 520–536.

Marchuk DA, Saulino AM, Tavakkol R, Swaroop M, Wallace MR, Andersen LB, Mitchell AL, Gutmann DH, Boguski M, Collins FS. 1991. cDNA cloning of the type 1 neurofibromatosis gene: complete sequence of the NF1 gene product. Genomics 11: 931–40.

Maris JM, Wiersma SR, Mahgoub N, Thompson P, Geyer RJ, Hurwitz CG, Lange BJ, Shannon KM. 1997. Monosomy 7 myelodysplastic syndrome and other second malignant neoplasms in children with neurofibromatosis type 1. Cancer 79: 1438–46.

Mars N, Widén E, Kerminen S, Meretoja T, Pirinen M, Della Briotta Parolo P, Palta P, FinnGen, Palotie A, Kaprio J, Joensuu H, Daly M, Ripatti S. 2020. The role of polygenic risk and susceptibility genes in breast cancer over the course of life. Nat. Commun. 11: 6383.

Martin GA, Viskochil D, Bollag G, McCabe PC, Crosier WJ, Haubruck H, Conroy L, Clark R, O'Connell P, Cawthon RM. 1990. The GAP-related domain of the neurofibromatosis type 1 gene product interacts with ras p21. Cell 63: 843–9.

Martin E, Coert JH, Flucke UE, Slooff W-BM, van de Sande MAJ, van Noesel MM, Grünhagen DJ, Wijnen MHWA, Verhoef C. 2020. Neurofibromatosis-associated malignant peripheral nerve sheath tumors in children have a worse prognosis: A nationwide cohort study. Pediatr. Blood Cancer 67: e28138.

Martins AS, Jansen AK, Rodrigues LOC, Matos CM, Souza MLR, de Souza JF, Diniz M de FHS, Barreto SM, Diniz LM, de Rezende NA, Riccardi VM. 2016. Lower fasting blood glucose in neurofibromatosis type 1. Endocr. Connect. 5: 28–33.

Martins AS, Jansen AK, Rodrigues LOC, Matos CM, Souza MLR, Miranda DM, Rezende NA de. 2018. Increased insulin sensitivity in individuals with neurofibromatosis type 1. Arch. Endocrinol. Metab. 62: 41–46.

Masciari S, Dillon DA, Rath M, Robson M, Weitzel JN, Balmana J, Gruber SB, Ford JM, Euhus D, Lebensohn A, Telli M, Pochebit SM, Lypas G, Garber JE. 2012. Breast cancer phenotype in women with TP53 germline mutations: a Li-Fraumeni syndrome consortium effort. Breast Cancer Res. Treat. 133: 1125–30.

Masgras I, Ciscato F, Brunati AM, Tibaldi E, Indraccolo S, Curtarello M, Chiara F, Cannino G, Papaleo E, Lambrughi M, Guzzo G, Gambalunga A, Pizzi M, Guzzardo V, Rugge M, Vuljan SE, Calabrese F, Bernardi P, Rasola A. 2017. Absence of Neurofibromin Induces an Oncogenic Metabolic Switch via Mitochondrial ERK-Mediated Phosphorylation of the Chaperone TRAP1. Cell Rep. 18: 659–672.

Masocco M, Kodra Y, Vichi M, Conti S, Kanieff M, Pace M, Frova L, Taruscio D. 2011. Mortality associated with neurofibromatosis type 1: a study based on Italian death certificates (1995-2006). Orphanet J. Rare Dis. 6: 11.

Mautner V-F, Asuagbor FA, Dombi E, Fünsterer C, Kluwe L, Wenzel R, Widemann BC, Friedman JM. 2008. Assessment of benign tumor burden by whole-body MRI in patients with neurofibromatosis 1. Neuro. Oncol. 10: 593–598.

McCaughan JA, Holloway SM, Davidson R, Lam WWK. 2007. Further evidence of the increased risk for malignant peripheral nerve sheath tumour from a Scottish cohort of patients with neurofibromatosis type 1. J. Med. Genet. 44: 463–6.

McGaughran JM, Harris DI, Donnai D, Teare D, MacLeod R, Westerbeek R, Kingston H, Super M, Harris R, Evans DG. 1999. A clinical study of type 1 neurofibromatosis in north west England. J. Med. Genet. 36: 197–203.

McKeever K, Shepherd CW, Crawford H, Morrison PJ. 2008. An epidemiological, clinical and genetic survey of neurofibromatosis type 1 in children under sixteen years of age. Ulster Med. J. 77: 160–3.

von Mehren M, Joensuu H. 2018. Gastrointestinal Stromal Tumors. J. Clin. Oncol. 36: 136–143.

Mendes-Pereira AM, Sims D, Dexter T, Fenwick K, Assiotis I, Kozarewa I, Mitsopoulos C, Hakas J, Zvelebil M, Lord CJ, Ashworth A. 2012. Genome-wide functional screen identifies a compendium of genes affecting sensitivity to tamoxifen. Proc. Natl. Acad. Sci. U. S. A. 109: 2730–5.

Messiaen LM, Callens T, Mortier G, Beysen D, Vandenbroucke I, Van Roy N, Speleman F, Paepe AD. 2000. Exhaustive mutation analysis of the NF1 gene allows identification of 95% of mutations and reveals a high frequency of unusual splicing defects. Hum. Mutat. 15: 541–55.

Messiaen L, Yao S, Brems H, Callens T, Sathienkijkanchai A, Denayer E, Spencer E, Arn P, Babovic-Vuksanovic D, Bay C, Bobele G, Cohen BH, Escobar L, Eunpu D, Grebe T, Greenstein R, Hachen R, Irons M, Kronn D, Lemire E, Leppig K, Lim C, McDonald M, Narayanan V, Pearn A, Pedersen R, Powell B, Shapiro LR, Skidmore D, Tegay D, Thiese H, Zackai EH, Vijzelaar R, Taniguchi K, Ayada T, Okamoto F, Yoshimura A, Parret A, Korf B, Legius E. 2009. Clinical and mutational spectrum of neurofibromatosis type 1-like syndrome. JAMA 302: 2111–8.

Miao R, Wang H, Jacobson A, Lietz AP, Choy E, Raskin KA, Schwab JH, Deshpande V, Nielsen GP, DeLaney TF, Cote GM, Hornicek FJ, Chen Y-LE. 2019. Radiation-induced and neurofibromatosis-associated malignant peripheral nerve sheath tumors (MPNST) have worse outcomes than sporadic MPNST. Radiother. Oncol. 137: 61–70.

Miettinen M, Fetsch JF, Sobin LH, Lasota J. 2006. Gastrointestinal stromal tumors in patients with neurofibromatosis 1: a clinicopathologic and molecular genetic study of 45 cases. Am. J. Surg. Pathol. 30: 90–6.

Miglioretti DL, Lange J, van den Broek JJ, Lee CI, van Ravesteyn NT, Ritley D, Kerlikowske K, Fenton JJ, Melnikow J, de Koning HJ, Hubbard RA. 2016. Radiation-Induced Breast Cancer Incidence and Mortality From Digital Mammography Screening: A Modeling Study. Ann. Intern. Med. 164: 205–14.

Miller DT, Freedenberg D, Schorry E, Ullrich NJ, Viskochil D, Korf BR, Council on Genetics, American College of Medical Genetics and Genomics. 2019. Health Supervision for Children With Neurofibromatosis Type 1. Pediatrics 143: e20190660.

Molina-Montes E, Requena M, Sánchez-Cantalejo E, Fernández MF, Arroyo-Morales M, Espín J, Arrebola JP, Sánchez M-J. 2015. Risk of second cancers cancer after a first primary breast cancer: a systematic review and meta-analysis. Gynecol. Oncol. 136: 158–71.

Møller P, Seppälä T, Bernstein I, Holinski-Feder E, Sala P, Evans DG, Lindblom A, Macrae F, Blanco I, Sijmons R, Jeffries J, Vasen H, Burn J, Nakken S, Hovig E, Rødland EA, Tharmaratnam K, de Vos Tot Nederveen Cappel WH, Hill J, Wijnen J, Green K, Lalloo F, Sunde L, Mints M, Bertario L, Pineda M, Navarro M, Morak M, Renkonen-Sinisalo L, Frayling IM, Plazzer J-P, Pylvanainen K, Sampson JR, Capella G, Mecklin J-P, Möslein G, Mallorca Group (http://mallorca-group.eu). 2017. Cancer incidence and survival in Lynch syndrome patients receiving colonoscopic and gynaecological surveillance: first report from the prospective Lynch syndrome database. Gut 66: 464–472.

Morris AP, Voight BF, Teslovich TM, Ferreira T, Segrè A V, Steinthorsdottir V, Strawbridge RJ, Khan H, Grallert H, Mahajan A, Prokopenko I, Kang HM, Dina C, Esko T, Fraser RM, Kanoni S, Kumar A, Lagou V, Langenberg C, Luan J, Lindgren CM, Müller-Nurasyid M, Pechlivanis S, Rayner NW, Scott LJ, Wiltshire S, Yengo L, Kinnunen L, Rossin EJ, Raychaudhuri S, Johnson AD, Dimas AS, Loos RJF, Vedantam S, Chen H, Florez JC, Fox C, Liu C-T, Rybin D, Couper DJ, Kao WHL, Li M, Cornelis MC, Kraft P, Sun Q, van Dam RM, Stringham HM, Chines PS, Fischer K, Fontanillas P, Holmen OL, Hunt SE, Jackson AU, Kong A, Lawrence R, Meyer J, Perry JRB, Platou CGP, Potter S, Rehnberg E, Robertson N, Sivapalaratnam S, Stančáková A, Stirrups K, Thorleifsson G, Tikkanen E, Wood AR, Almgren P, Atalay M, Benediktsson R, Bonnycastle LL, Burtt N, Carey J, Charpentier G, Crenshaw AT, Doney ASF, Dorkhan M, Edkins S, Emilsson V, Eury E, Forsen T, Gertow K, Gigante B, Grant GB, Groves CJ, Guiducci C, Herder C, Hreidarsson AB, Hui J, James A, Jonsson A, Rathmann W, Klopp N, Kravic J, Krjutškov K, Langford C, Leander K, Lindholm E, et al. 2012. Large-scale association analysis provides insights into the genetic architecture and pathophysiology of type 2 diabetes. Nat. Genet. 44: 981–990.

Morris SM, Gupta A, Kim S, Foraker RE, Gutmann DH, Payne PRO. 2021. Predictive Modeling for Clinical Features Associated with Neurofibromatosis Type 1. Neurol. Clin. Pract.: 10.1212/CPJ.0000000000001089.

Mouliere F, Chandrananda D, Piskorz AM, Moore EK, Morris J, Ahlborn LB, Mair R, Goranova T, Marass F, Heider K, Wan JCM, Supernat A, Hudecova I, Gounaris I, Ros S, Jimenez-Linan M, Garcia-Corbacho J, Patel K, Østrup O, Murphy S, Eldridge MD, Gale D, Stewart GD, Burge J, Cooper WN, Van Der Heijden MS, Massie CE, Watts C, Corrie P, Pacey S, Brindle KM, Baird RD, Mau-Sørensen M, Parkinson CA, Smith CG, Brenton JD, Rosenfeld N. 2018. Enhanced detection of circulating tumor DNA by fragment size analysis. Sci. Transl. Med. 10: eaat4921.

Mussi C, Schildhaus H-U, Gronchi A, Wardelmann E, Hohenberger P. 2008. Therapeutic consequences from molecular biology for gastrointestinal stromal tumor patients affected by neurofibromatosis type 1. Clin. Cancer Res. 14: 4550–5.

Nakamura JL, Phong C, Pinarbasi E, Kogan SC, Vandenberg S, Horvai AE, Faddegon BA, Fiedler D, Shokat K, Houseman BT, Chao R, Pieper RO, Shannon K. 2011. Dose-dependent effects of focal fractionated irradiation on secondary malignant neoplasms in Nf1 mutant mice. Cancer Res. 71: 106–15.

Namløs HM, Zaikova O, Bjerkehagen B, Vodák D, Hovig E, Myklebost O, Boye K, Meza-Zepeda LA. 2017. Use of liquid biopsies to monitor disease progression in a sarcoma patient: a case report. BMC Cancer 17: 29.

National Institutes of Health Consensus Development Conference. 1988. Neurofibromatosis. Conference statement. Arch. Neurol. 45: 575–8.

Neglia JP, Friedman DL, Yasui Y, Mertens AC, Hammond S, Stovall M, Donaldson SS, Meadows AT, Robison LL. 2001. Second malignant neoplasms in five-year survivors of childhood cancer: childhood cancer survivor study. J. Natl. Cancer Inst. 93: 618–29.

Nejentsev S, Walker N, Riches D, Egholm M, Todd JA. 2009. Rare variants of IFIH1, a gene implicated in antiviral responses, protect against type 1 diabetes. Science 324: 387–9.

Nelson HD, Tyne K, Naik A, Bougatsos C, Chan BK, Humphrey L, U.S. Preventive Services Task Force. 2009. Screening for breast cancer: an update for the U.S. Preventive Services Task Force. Ann. Intern. Med. 151: 727–37, W237-42.

Niell BL, Freer PE, Weinfurtner RJ, Arleo EK, Drukteinis JS. 2017. Screening for Breast Cancer. Radiol. Clin. North Am. 55: 1145–1162.

Nissan MH, Pratilas CA, Jones AM, Ramirez R, Won H, Liu C, Tiwari S, Kong L, Hanrahan AJ, Yao Z, Merghoub T, Ribas A, Chapman PB, Yaeger R, Taylor BS, Schultz N, Berger MF, Rosen N, Solit DB. 2014. Loss of NF1 in cutaneous melanoma is associated with RAS activation and MEK dependence. Cancer Res. 74: 2340–50.

Noble JA, Valdes AM. 2011. Genetics of the HLA Region in the Prediction of Type 1 Diabetes. Curr. Diab. Rep. 11: 533–542.

Nyström L, Andersson I, Bjurstam N, Frisell J, Nordenskjöld B, Rutqvist LE. 2002. Long-term effects of mammography screening: updated overview of the Swedish randomised trials. Lancet 359: 909–19.

Oda K, Matsuoka Y, Funahashi A, Kitano H. 2005. A comprehensive pathway map of epidermal growth factor receptor signaling. Mol. Syst. Biol. 1: 2005.0010.

Orraca M, Morejón G, Cabrera N, Menéndez R, Orraca O. 2014. Neurofibromatosis 1 prevalence in children aged 9-11 years, Pinar del Río Province, Cuba. MEDICC Rev. 16: 22–6.

Ortonne N, Wolkenstein P, Blakeley JO, Korf B, Plotkin SR, Riccardi VM, Miller DC, Huson S, Peltonen J, Rosenberg A, Carroll SL, Verma SK, Mautner V, Upadhyaya M, Stemmer-Rachamimov A. 2018. Cutaneous neurofibromas: Current clinical and pathologic issues. Neurology 91: S5–S13.

Ossandon MR, Agrawal L, Bernhard EJ, Conley BA, Dey SM, Divi RL, Guan P, Lively TG, McKee TC, Sorg BS, Tricoli J V. 2018. Circulating Tumor DNA Assays in Clinical Cancer Research. J. Natl. Cancer Inst. 110: 929–934.

Ottenhoff MJ, Rietman AB, Mous SE, Plasschaert E, Gawehns D, Brems H, Oostenbrink R, van Minkelen R, Nellist M, Schorry E, Legius E, Moll HA, Elgersma Y. 2020. Examination of the genetic factors underlying the cognitive variability associated with neurofibromatosis type 1. Genet. Med. 22: 889–897.

Pal A, Barber TM, Van de Bunt M, Rudge SA, Zhang Q, Lachlan KL, Cooper NS, Linden H, Levy JC, Wakelam MJO, Walker L, Karpe F, Gloyn AL. 2012. PTEN mutations as a cause of constitutive insulin sensitivity and obesity. N. Engl. J. Med. 367: 1002–11.

Pareja F, Ferrando L, Lee SSK, Beca F, Selenica P, Brown DN, Farmanbar A, Da Cruz Paula A, Vahdatinia M, Zhang H, Zoppoli G, Wen HY, Brogi E, Robson ME, Razavi P, Chandarlapaty S, Weigelt B, Reis-Filho JS. 2020. The genomic landscape of metastatic histologic special types of invasive breast cancer. NPJ breast cancer 6: 53.

Passmore SJ, Chessells JM, Kempski H, Hann IM, Brownbill PA, Stiller CA. 2003. Paediatric myelodysplastic syndromes and juvenile myelomonocytic leukaemia in the UK: a population-based study of incidence and survival. Br. J. Haematol. 121: 758–67.

Patterson CC, Harjutsalo V, Rosenbauer J, Neu A, Cinek O, Skrivarhaug T, Rami-Merhar B, Soltesz G, Svensson J, Parslow RC, Castell C, Schoenle EJ, Bingley PJ, Dahlquist G, Jarosz-Chobot PK, Marčiulionytė D, Roche EF, Rothe U, Bratina N, Ionescu-Tirgoviste C, Weets I, Kocova M, Cherubini V, Rojnic Putarek N, deBeaufort CE, Samardzic M, Green A. 2019. Trends and cyclical variation in the incidence of childhood type 1 diabetes in 26 European centres in the 25 year period 1989-2013: a multicentre prospective registration study. Diabetologia 62: 408–417.

Pearson A, Proszek P, Pascual J, Fribbens C, Shamsher MK, Kingston B, O'Leary B, Herrera-Abreu MT, Cutts RJ, Garcia-Murillas I, Bye H, Walker BA, Gonzalez De Castro D, Yuan L, Jamal S, Hubank M, Lopez-Knowles E, Schuster EF, Dowsett M, Osin P, Nerurkar A, Parton M, Okines AFC, Johnston SRD, Ring A, Turner NC. 2020. Inactivating NF1 Mutations Are Enriched in Advanced Breast Cancer and Contribute to Endocrine Therapy Resistance. Clin. Cancer Res. 26: 608–622.

Peltonen J, Jaakkola S, Lebwohl M, Renvall S, Risteli L, Virtanen I, Uitto J. 1988. Cellular differentiation and expression of matrix genes in type 1 neurofibromatosis. Lab. Invest. 59: 760–771.

Peltonen S, Pöyhönen M, Koillinen H, Valanne L, Peltonen J. 2014. Miten tunnistan neurofibromatoosin? Duodecim 130: 619–25.

Peltonen S, Kallionpää RA, Peltonen J. 2017. Neurofibromatosis type 1 (NF1) gene: Beyond café au lait spots and dermal neurofibromas. Exp. Dermatol. 26: 645–648.

Peltonen S, Kallionpää RA, Rantanen M, Uusitalo E, Lähteenmäki PM, Pöyhönen M, Pitkäniemi J, Peltonen J. 2019. Pediatric malignancies in neurofibromatosis type 1: A population-based cohort study. Int. J. cancer 145: 2926–2932.

Pennanen P, Kallionpää RA, Peltonen S, Nissinen L, Kähäri V-M, Heervä E, Peltonen J. 2021. Signaling pathways in human osteoclasts differentiation: ERK1/2 as a key player. Mol. Biol. Rep. 48: 1243–1254.

Perou CM, Sørlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, Fluge O, Pergamenschikov A, Williams C, Zhu SX, Lønning PE, Børresen-Dale AL, Brown PO, Botstein D. 2000. Molecular portraits of human breast tumours. Nature 406: 747–52.

Pitkäniemi J, Malila N, Tanskanen T, Degerlund H, Heikkinen S, Seppä K. 2021. Syöpä 2019. Helsinki: Suomen Syöpäyhdistys.

Pociot F, Lernmark Å. 2016. Genetic risk factors for type 1 diabetes. Lancet 387: 2331–2339.

Pociot F. 2017. Type 1 diabetes genome-wide association studies: not to be lost in translation. Clin. Transl. Immunol. 6: e162.

Poyhonen M, Leisti EL, Kytölä S, Leisti J. 1997. Hereditary spinal neurofibromatosis: a rare form of NF1? J. Med. Genet. 34: 184–7.

Poyhonen M, Kytölä S, Leisti J. 2000. Epidemiology of neurofibromatosis type 1 (NF1) in northern Finland. J. Med. Genet. 37: 632–6.

De Raedt T, Brems H, Wolkenstein P, Vidaud D, Pilotti S, Perrone F, Mautner V, Frahm S, Sciot R, Legius E. 2003. Elevated risk for MPNST in NF1 microdeletion patients. Am. J. Hum. Genet. 72: 1288–92.

Raikhlin A, Curpen B, Warner E, Betel C, Wright B, Jong R. 2015. Breast MRI as an adjunct to mammography for breast cancer screening in high-risk patients: retrospective review. AJR Am. J. Roentgenol. 204: 889–97.

Rasmussen SA, Yang Q, Friedman JM. 2001. Mortality in neurofibromatosis 1: an analysis using U.S. death certificates. Am. J. Hum. Genet. 68: 1110–8.

Ratner N, Miller SJ. 2015. A RASopathy gene commonly mutated in cancer: the neurofibromatosis type 1 tumour suppressor. Nat. Rev. Cancer 15: 290–301.

Rauen KA. 2013. The RASopathies. Annu. Rev. Genomics Hum. Genet. 14: 355–69.

Razavi P, Chang MT, Xu G, Bandlamudi C, Ross DS, Vasan N, Cai Y, Bielski CM, Donoghue MTA, Jonsson P, Penson A, Shen R, Pareja F, Kundra R, Middha S, Cheng ML, Zehir A, Kandoth C, Patel R, Huberman K, Smyth LM, Jhaveri K, Modi S, Traina TA, Dang C, Zhang W, Weigelt B, Li BT, Ladanyi M, Hyman DM, Schultz N, Robson ME, Hudis C, Brogi E, Viale A, Norton L, Dickler MN, Berger MF, Iacobuzio-Donahue CA, Chandarlapaty S, Scaltriti M, Reis-Filho JS, Solit DB, Taylor BS, Baselga J. 2018. The Genomic Landscape of Endocrine-Resistant Advanced Breast Cancers. Cancer Cell 34: 427-438.e6.

Redondo MJ, Hagopian WA, Oram R, Steck AK, Vehik K, Weedon M, Balasubramanyam A, Dabelea D. 2020. The clinical consequences of heterogeneity within and between different diabetes types. Diabetologia 63: 2040–2048.

Riccardi VM. 1993. A Controlled Multiphase Trial of Ketotifen to Minimize Neurofibroma-Associated Pain and Itching. Arch. Dermatol. 129: 577–581.

Richard F, Majjaj S, Venet D, Rothé F, Pingitore J, Boeckx B, Marchio C, Clatot F, Bertucci F, Mariani O, Galant C, Eynden G van den, Salgado R, Biganzoli E, Lambrechts D, Vincent-Salomon A, Pruneri G, Larsimont D, Sotiriou C, Desmedt C. 2020. Characterization of Stromal Tumor-infiltrating Lymphocytes and Genomic Alterations in Metastatic Lobular Breast Cancer. Clin. Cancer Res. 26: 6254–6265.

Risberg B, Tsui DWY, Biggs H, Ruiz-Valdepenas Martin de Almagro A, Dawson S-J, Hodgkin C, Jones L, Parkinson C, Piskorz A, Marass F, Chandrananda D, Moore E, Morris J, Plagnol V, Rosenfeld N, Caldas C, Brenton JD, Gale D. 2018. Effects of Collection and Processing Procedures on Plasma Circulating Cell-Free DNA from Cancer Patients. J. Mol. Diagn. 20: 883–892.

Rojas K, Stuckey A. 2016. Breast Cancer Epidemiology and Risk Factors. Clin. Obstet. Gynecol. 59: 651–672.

Rojnueangnit K, Xie J, Gomes A, Sharp A, Callens T, Chen Y, Liu Y, Cochran M, Abbott M-A, Atkin J, Babovic-Vuksanovic D, Barnett CP, Crenshaw M, Bartholomew DW, Basel L, Bellus G, Ben-

Shachar S, Bialer MG, Bick D, Blumberg B, Cortes F, David KL, Destree A, Duat-Rodriguez A, Earl D, Escobar L, Eswara M, Ezquieta B, Frayling IM, Frydman M, Gardner K, Gripp KW, Hernández-Chico C, Heyrman K, Ibrahim J, Janssens S, Keena BA, Llano-Rivas I, Leppig K, McDonald M, Misra VK, Mulbury J, Narayanan V, Orenstein N, Galvin-Parton P, Pedro H, Pivnick EK, Powell CM, Randolph L, Raskin S, Rosell J, Rubin K, Seashore M, Schaaf CP, Scheuerle A, Schultz M, Schorry E, Schnur R, Siqveland E, Tkachuk A, Tonsgard J, Upadhyaya M, Verma IC, Wallace S, Williams C, Zackai E, Zonana J, Lazaro C, Claes K, Korf B, Martin Y, Legius E, Messiaen L. 2015. High Incidence of Noonan Syndrome Features Including Short Stature and Pulmonic Stenosis in Patients carrying NF1 Missense Mutations Affecting p.Arg1809: Genotype-Phenotype Correlation. Hum. Mutat. 36: 1052–63.

Roman M, Skaane P, Hofvind S. 2014. The cumulative risk of false-positive screening results across screening centres in the Norwegian Breast Cancer Screening Program. Eur. J. Radiol. 83: 1639–44.

Rosenfeld A, Listernick R, Charrow J, Goldman S. 2010. Neurofibromatosis type 1 and high-grade tumors of the central nervous system. Childs. Nerv. Syst. 26: 663–7.

Rouleau GA, Merel P, Lutchman M, Sanson M, Zucman J, Marineau C, Hoang-Xuan K, Demczuk S, Desmaze C, Plougastel B. 1993. Alteration in a new gene encoding a putative membrane-organizing protein causes neuro-fibromatosis type 2. Nature 363: 515–21.

Ruggieri M, Polizzi A, Spalice A, Salpietro V, Caltabiano R, D'Orazi V, Pavone P, Pirrone C, Magro G, Platania N, Cavallaro S, Muglia M, Nicita F. 2015. The natural history of spinal neurofibromatosis: a critical review of clinical and genetic features. Clin. Genet. 87: 401–10.

Saltiel AR. 2021. Insulin signaling in health and disease. J. Clin. Invest. 131.

Salz T, DeFrank JT, Brewer NT. 2011. False positive mammograms in Europe: do they affect reattendance? Breast Cancer Res. Treat. 127: 229–31.

Sarkeala T, Heinävaara S, Anttila A. 2008a. Breast cancer mortality with varying invitational policies in organised mammography. Br. J. Cancer 98: 641–5.

Sarkeala T, Heinävaara S, Anttila A. 2008b. Organised mammography screening reduces breast cancer mortality: a cohort study from Finland. Int. J. cancer 122: 614–9.

Sarkeala T, Färkkilä M, Anttila A, Hyöty M, Kairaluoma M, Rautio T, Voutilainen M, Helander S, Jäntti M, Lehtinen M, Patrikka L, Malila N, Heinävaara S. 2021. Piloting gender-oriented colorectal cancer screening with a faecal immunochemical test: population-based registry study from Finland. BMJ Open 11: e046667.

Scheffzek K, Shivalingaiah G. 2019. Ras-Specific GTPase-Activating Proteins-Structures, Mechanisms, and Interactions. Cold Spring Harb. Perspect. Med. 9.

De Schepper S, Maertens O, Callens T, Naeyaert J-M, Lambert J, Messiaen L. 2008. Somatic mutation analysis in NF1 café au lait spots reveals two NF1 hits in the melanocytes. J. Invest. Dermatol. 128: 1050–3.

Schneider M, Obringer AC, Zackai E, Meadows AT. 1986. Childhood neurofibromatosis: risk factors for malignant disease. Cancer Genet. Cytogenet. 21: 347–54.

Schwarzenbach H, Stoehlmacher J, Pantel K, Goekkurt E. 2008. Detection and monitoring of cell-free DNA in blood of patients with colorectal cancer. Ann. N. Y. Acad. Sci. 1137: 190–196.

Schwarzenbach H, Hoon DSB, Pantel K. 2011. Cell-free nucleic acids as biomarkers in cancer patients. Nat. Rev. Cancer 11: 426–437.

Seminog OO, Goldacre MJ. 2013. Risk of benign tumours of nervous system and of malignant neoplasms, in people with neurofibromatosis: population-based record-linkage study. Br. J. Cancer 108: 193–198.

Seminog OO, Goldacre MJ. 2015. Age-specific risk of breast cancer in women with neurofibromatosis type 1. Br. J. Cancer 112: 1546–8.

Seppälä TT, Latchford A, Negoi I, Sampaio Soares A, Jimenez-Rodriguez R, Sánchez-Guillén L, Evans DG, Ryan N, Crosbie EJ, Dominguez-Valentin M, Burn J, Kloor M, Knebel Doeberitz M von, Duijnhoven FJB van, Quirke P, Sampson JR, Møller P, Möslein G, European Hereditary Tumour

Group (EHTG) and European Society of Coloproctology (ESCP). 2021. European guidelines from the EHTG and ESCP for Lynch syndrome: an updated third edition of the Mallorca guidelines based on gene and gender. Br. J. Surg. 108: 484–498.

Shah JS, Soon PS, Marsh DJ. 2016. Comparison of Methodologies to Detect Low Levels of Hemolysis in Serum for Accurate Assessment of Serum microRNAs. PLoS One 11: e0153200.

Shain AH, Garrido M, Botton T, Talevich E, Yeh I, Sanborn JZ, Chung J, Wang NJ, Kakavand H, Mann GJ, Thompson JF, Wiesner T, Roy R, Olshen AB, Gagnon A, Gray JW, Huh N, Hur JS, Busam KJ, Scolyer RA, Cho RJ, Murali R, Bastian BC. 2015. Exome sequencing of desmoplastic melanoma identifies recurrent NFKBIE promoter mutations and diverse activating mutations in the MAPK pathway. Nat. Genet. 47: 1194–9.

Shalem O, Sanjana NE, Hartenian E, Shi X, Scott DA, Mikkelson T, Heckl D, Ebert BL, Root DE, Doench JG, Zhang F. 2014. Genome-scale CRISPR-Cas9 knockout screening in human cells. Science 343: 84–87.

Sharif S, Ferner R, Birch JM, Gillespie JE, Gattamaneni HR, Baser ME, Evans DGR. 2006. Second primary tumors in neurofibromatosis 1 patients treated for optic glioma: substantial risks after radiotherapy. J. Clin. Oncol. 24: 2570–5.

Sharif S, Moran A, Huson SM, Iddenden R, Shenton A, Howard E, Evans DGR. 2007. Women with neurofibromatosis 1 are at a moderately increased risk of developing breast cancer and should be considered for early screening. J. Med. Genet. 44: 481–4.

Shearer P, Parham D, Kovnar E, Kun L, Rao B, Lobe T, Pratt C. 1994. Neurofibromatosis type I and malignancy: review of 32 pediatric cases treated at a single institution. Med. Pediatr. Oncol. 22: 78–83.

Sherekar M, Han S-W, Ghirlando R, Messing S, Drew M, Rabara D, Waybright T, Juneja P, O'Neill H, Stanley CB, Bhowmik D, Ramanathan A, Subramaniam S, Nissley D V, Gillette W, McCormick F, Esposito D. 2020. Biochemical and structural analyses reveal that the tumor suppressor neurofibromin (NF1) forms a high-affinity dimer. J. Biol. Chem. 295: 1105–1119.

Siena S, Sartore-Bianchi A, Garcia-Carbonero R, Karthaus M, Smith D, Tabernero J, Van Cutsem E, Guan X, Boedigheimer M, Ang A, Twomey B, Bach BA, Jung AS, Bardelli A. 2018. Dynamic molecular analysis and clinical correlates of tumor evolution within a phase II trial of panitumumab-based therapy in metastatic colorectal cancer. Ann. Oncol. 29: 119–126.

Da Silva AV, Rodrigues FR, Pureza M, Lopes VGS, Cunha KS. 2015. Breast cancer and neurofibromatosis type 1: a diagnostic challenge in patients with a high number of neurofibromas. BMC Cancer 15: 183.

Simanshu DK, Nissley D V, McCormick F. 2017. RAS Proteins and Their Regulators in Human Disease. Cell 170: 17–33.

Singh D, Malila N, Pokhrel A, Anttila A. 2015. Association of symptoms and breast cancer in population-based mammography screening in Finland. Int. J. cancer 136: E630-7.

Singh D, Pitkäniemi J, Malila N, Anttila A. 2016. Cumulative risk of false positive test in relation to breast symptoms in mammography screening: a historical prospective cohort study. Breast Cancer Res. Treat. 159: 305–13.

Singhal S, Birch JM, Kerr B, Lashford L, Evans GR. 2002. Neurofibromatosis type 1 and sporadic optic gliomas. Arch Dis Child 87: 65–70.

Sivendran S, Agarwal N, Gartrell B, Ying J, Boucher KM, Choueiri TK, Sonpavde G, Oh WK, Galsky MD. 2014. Metabolic complications with the use of mTOR inhibitors for cancer therapy. Cancer Treat. Rev. 40: 190–6.

Smith AE, Ferraro E, Safonov A, Morales CB, Lahuerta EJA, Li Q, Kulick A, Ross D, Solit DB, de Stanchina E, Reis-Filho J, Rosen N, Arribas J, Razavi P, Chandarlapaty S. 2021. HER2 + breast cancers evade anti-HER2 therapy via a switch in driver pathway. Nat. Commun. 12: 6667.

Sokol ES, Feng YX, Jin DX, Basudan A, Lee A V, Atkinson JM, Chen J, Stephens PJ, Frampton GM, Gupta PB, Ross JS, Chung JH, Oesterreich S, Ali SM, Hartmaier RJ. 2019. Loss of function of

NF1 is a mechanism of acquired resistance to endocrine therapy in lobular breast cancer. Ann. Oncol. 30: 115–123.

Sørensen SA, Mulvihill JJ, Nielsen A. 1986. Long-term follow-up of von Recklinghausen neurofibromatosis. Survival and malignant neoplasms. N. Engl. J. Med. 314: 1010–5.

Sørlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, Hastie T, Eisen MB, van de Rijn M, Jeffrey SS, Thorsen T, Quist H, Matese JC, Brown PO, Botstein D, Lønning PE, Børresen-Dale AL. 2001. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc. Natl. Acad. Sci. U. S. A. 98: 10869–74.

de Souza MLR, Jansen AK, Martins AS, Rodrigues LOC, de Rezende NA. 2015. Nutrient intake in neurofibromatosis type 1: A cross-sectional study. Nutrition 31: 858–62.

de Souza MLR, Jansen AK, Rodrigues LOC, Vilela DL de S, Kakehasi AM, Martins AS, de Souza JF, de Rezende NA. 2019. Increased resting metabolism in neurofibromatosis type 1. Clin. Nutr. ESPEN 32: 44–49.

Spitale A, Mazzola P, Soldini D, Mazzucchelli L, Bordoni A. 2009. Breast cancer classification according to immunohistochemical markers: clinicopathologic features and short-term survival analysis in a population-based study from the South of Switzerland. Ann. Oncol. 20: 628–35.

Steinthorsdottir V, Thorleifsson G, Sulem P, Helgason H, Grarup N, Sigurdsson A, Helgadottir HT, Johannsdottir H, Magnusson OT, Gudjonsson SA, Justesen JM, Harder MN, Jørgensen ME, Christensen C, Brandslund I, Sandbæk A, Lauritzen T, Vestergaard H, Linneberg A, Jørgensen T, Hansen T, Daneshpour MS, Fallah M-S, Hreidarsson AB, Sigurdsson G, Azizi F, Benediktsson R, Masson G, Helgason A, Kong A, Gudbjartsson DF, Pedersen O, Thorsteinsdottir U, Stefansson K. 2014. Identification of low-frequency and rare sequence variants associated with elevated or reduced risk of type 2 diabetes. Nat. Genet. 46: 294–298.

Stewart DR, Korf BR, Nathanson KL, Stevenson DA, Yohay K. 2018. Care of adults with neurofibromatosis type 1: A clinical practice resource of the American College of Medical Genetics and Genomics (ACMG). Genet. Med. 20: 671–682.

Stiller CA, Chessells JM, Fitchett M. 1994. Neurofibromatosis and childhood leukaemia/lymphoma: a population-based UKCCSG study. Br. J. Cancer 70: 969–72.

Stucky C-CH, Johnson KN, Gray RJ, Pockaj BA, Ocal IT, Rose PS, Wasif N. 2012. Malignant peripheral nerve sheath tumors (MPNST): the Mayo Clinic experience. Ann. Surg. Oncol. 19: 878–85.

Suarez-Kelly LP, Yu L, Kline D, Schneider EB, Agnese DM, Carson WE. 2019. Increased breast cancer risk in women with neurofibromatosis type 1: a meta-analysis and systematic review of the literature. Hered. Cancer Clin. Pract. 17: 12.

Sunami E, Vu AT, Nguyen SL, Giuliano AE, Hoon DSB. 2008. Quantification of LINE1 in circulating DNA as a molecular biomarker of breast cancer. Ann. N. Y. Acad. Sci. 1137: 171–174.

Sung H, Hyland PL, Pemov A, Sabourin JA, Baldwin AM, Bass S, Teshome K, Luo W, Frederick National Laboratory for Cancer Research, Widemann BC, Stewart DR, Wilson AF. 2020. Genome-wide association study of café-au-lait macule number in neurofibromatosis type 1. Mol. Genet. genomic Med. 8: e1400.

Suomen Rintasyöpäryhmä Ry. 2021. Rintasyövän Valtakunnallinen Diagnostiikka- ja Hoitosuositus.

Swedish Organised Service Screening Evaluation Group. 2006. Reduction in breast cancer mortality from organized service screening with mammography: 1. Further confirmation with extended data. Cancer Epidemiol. Biomarkers Prev. 15: 45–51.

Szpechcinski A, Struniawski R, Zaleska J, Chabowski M, Orlowski T, Roszkowski K, Chorostowska-Wynimko J. 2008. Evaluation of fluorescence-based methods for total vs. amplifiable DNA quantification in plasma of lung cancer patients. J. Physiol. Pharmacol. 59: 675–681.

Szudek J, Birch P, Riccardi VM, Evans DG, Friedman JM. 2000. Associations of clinical features in neurofibromatosis 1 (NF1). Genet. Epidemiol. 19: 429–39.

Szudek J, Joe H, Friedman JM. 2002. Analysis of intrafamilial phenotypic variation in neurofibromatosis 1 (NF1). Genet. Epidemiol. 23: 150–64.

Szymanski JJ, Sundby RT, Jones PA, Srihari D, Earland N, Harris PK, Feng W, Qaium F, Lei H, Roberts D, Landeau M, Bell J, Huang Y, Hoffman L, Spencer M, Spraker MB, Ding L, Widemann BC, Shern JF, Hirbe AC, Chaudhuri AA. 2021. Cell-free DNA ultra-low-pass whole genome sequencing to distinguish malignant peripheral nerve sheath tumor (MPNST) from its benign precursor lesion: A cross-sectional study. PLoS Med. 18: e1003734.

Tajan M, Batut A, Cadoudal T, Deleruyelle S, Le Gonidec S, Saint Laurent C, Vomscheid M, Wanecq E, Tréguer K, De Rocca Serra-Nédélec A, Vinel C, Marques M-A, Pozzo J, Kunduzova O, Salles J-P, Tauber M, Raynal P, Cavé H, Edouard T, Valet P, Yart A. 2014. LEOPARD syndrome-associated SHP2 mutation confers leanness and protection from diet-induced obesity. Proc. Natl. Acad. Sci. U. S. A. 111: E4494-503.

Tajan M, Paccoud R, Branka S, Edouard T, Yart A. 2018. The RASopathy Family: Consequences of Germline Activation of the RAS/MAPK Pathway. Endocr. Rev. 39: 676–700.

Teppo L, Pukkala E, Saxén E. 1985. Multiple cancer--an epidemiologic exercise in Finland. J. Natl. Cancer Inst. 75: 207–17.

Terry AR, Barker FG, Leffert L, Bateman BT, Souter I, Plotkin SR. 2013. Neurofibromatosis type 1 and pregnancy complications: a population-based study. Am. J. Obstet. Gynecol. 209: 46.e1–8.

Thierry AR, Mouliere F, Gongora C, Ollier J, Robert B, Ychou M, Del Rio M, Molina F. 2010. Origin and quantification of circulating DNA in mice with human colorectal cancer xenografts. Nucleic Acids Res. 38: 6159–6175.

Thierry AR, Mouliere F, El Messaoudi S, Mollevi C, Lopez-Crapez E, Rolet F, Gillet B, Gongora C, Dechelotte P, Robert B, Del Rio M, Lamy P-J, Bibeau F, Nouaille M, Loriot V, Jarrousse A-S, Molina F, Mathonnet M, Pezet D, Ychou M. 2014. Clinical validation of the detection of KRAS and BRAF mutations from circulating tumor DNA. Nat. Med. 20: 430–5.

Tissot C, Toffart A-C, Villar S, Souquet P-J, Merle P, Moro-Sibilot D, Pérol M, Zavadil J, Brambilla C, Olivier M, Couraud S. 2015. Circulating free DNA concentration is an independent prognostic biomarker in lung cancer. Eur. Respir. J. 46: 1773–80.

Tofighi D, MacKinnon DP. 2011. RMediation: an R package for mediation analysis confidence intervals. Behav. Res. Methods 43: 692–700.

Tritz R, Benson T, Harris V, Hudson FZ, Mintz J, Zhang H, Kennard S, Chen W, Stepp DW, Csanyi G, Belin de Chantemèle EJ, Weintraub NL, Stansfield BK. 2021. Nf1 heterozygous mice recapitulate the anthropometric and metabolic features of human neurofibromatosis type 1. Transl. Res. 228: 52–63.

Tung N, Domchek SM, Stadler Z, Nathanson KL, Couch F, Garber JE, Offit K, Robson ME. 2016. Counselling framework for moderate-penetrance cancer-susceptibility mutations. Nat. Rev. Clin. Oncol. 13: 581–8.

Tuomi T. 2005. Type 1 and type 2 diabetes: what do they have in common? Diabetes 54 Suppl 2: S40-5.

Umetani N, Giuliano AE, Hiramatsu SH, Amersi F, Nakagawa T, Martino S, Hoon DSB. 2006a. Prediction of breast tumor progression by integrity of free circulating DNA in serum. J. Clin. Oncol. 24: 4270–6.

Umetani N, Kim J, Hiramatsu S, Reber HA, Hines OJ, Bilchik AJ, Hoon DSB. 2006b. Increased integrity of free circulating DNA in sera of patients with colorectal or periampullary cancer: Direct quantitative PCR for ALU repeats. Clin. Chem. 52: 1062–1069.

Upadhyaya M, Huson SM, Davies M, Thomas N, Chuzhanova N, Giovannini S, Evans DG, Howard E, Kerr B, Griffiths S, Consoli C, Side L, Adams D, Pierpont M, Hachen R, Barnicoat A, Li H, Wallace P, Van Biervliet JP, Stevenson D, Viskochil D, Baralle D, Haan E, Riccardi V, Turnpenny P, Lazaro C, Messiaen L. 2007. An absence of cutaneous neurofibromas associated with a 3-bp inframe deletion in exon 17 of the NF1 gene (c.2970-2972 delAAT): evidence of a clinically significant NF1 genotype-phenotype correlation. Am. J. Hum. Genet. 80: 140–51.

Uusitalo E, Hammais A, Palonen E, Brandt A, Mäkelä V-V, Kallionpää R, Jouhilahti E-M, Pöyhönen M, Soini J, Peltonen J, Peltonen S. 2014. Neurofibromatosis type 1 gene mutation analysis using sequence capture and high-throughput sequencing. Acta Derm. Venereol. 94: 663–6.

Uusitalo E, Leppävirta J, Koffert A, Suominen S, Vahtera J, Vahlberg T, Pöyhönen M, Peltonen J, Peltonen S. 2015. Incidence and mortality of neurofibromatosis: a total population study in Finland. J. Invest. Dermatol. 135: 904–906.

Uusitalo E, Rantanen M, Kallionpää RA, Pöyhönen M, Leppävirta J, Ylä-Outinen H, Riccardi VM, Pukkala E, Pitkäniemi J, Peltonen S, Peltonen J. 2016. Distinctive Cancer Associations in Patients With Neurofibromatosis Type 1. J. Clin. Oncol. 34: 1978–86.

de Vathaire F, Hawkins M, Campbell S, Oberlin O, Raquin MA, Schlienger JY, Shamsaldin A, Diallo I, Bell J, Grimaud E, Hardiman C, Lagrange JL, Daly-Schveitzer N, Panis X, Zucker JM, Sancho-Garnier H, Eschwège F, Chavaudra J, Lemerle J. 1999. Second malignant neoplasms after a first cancer in childhood: temporal pattern of risk according to type of treatment. Br. J. Cancer 79: 1884–93.

Vogel AC, Gutmann DH, Morris SM. 2017. Neurodevelopmental disorders in children with neurofibromatosis type 1. Dev. Med. Child Neurol. 59: 1112–1116.

Walker L, Thompson D, Easton D, Ponder B, Ponder M, Frayling I, Baralle D. 2006. A prospective study of neurofibromatosis type 1 cancer incidence in the UK. Br. J. Cancer 95: 233–8.

Wallace MR, Marchuk DA, Andersen LB, Letcher R, Odeh HM, Saulino AM, Fountain JW, Brereton A, Nicholson J, Mitchell AL, Brownstein BH, Collins FS. 1990. Type 1 neurofibromatosis gene: Identification of a large transcript disrupted in three NF1 patients. Science 249: 181–186.

Wallace MD, Pfefferle AD, Shen L, McNairn AJ, Cerami EG, Fallon BL, Rinaldi VD, Southard TL, Perou CM, Schimenti JC. 2012. Comparative oncogenomics implicates the neurofibromin 1 gene (NF1) as a breast cancer driver. Genetics 192: 385–96.

Wang BG, Huang H-Y, Chen Y-C, Bristow RE, Kassauei K, Cheng C-C, Roden R, Sokoll LJ, Chan DW, Shih I-M. 2003. Increased plasma DNA integrity in cancer patients. Cancer Res. 63: 3966–8.

Wang X, Levin AM, Smolinski SE, Vigneau FD, Levin NK, Tainsky MA. 2012. Breast cancer and other neoplasms in women with neurofibromatosis type 1: a retrospective review of cases in the Detroit metropolitan area. Am. J. Med. Genet. A 158A: 3061–4.

Wang E, Batey A, Struble C, Musci T, Song K, Oliphant A. 2013. Gestational age and maternal weight effects on fetal cell-free DNA in maternal plasma. Prenat. Diagn. 33: 662–666.

Wang X, Tousignant RN, Levin AM, Niell B, Blakeley JO, Acosta MT. 2016. Indicator exploration for cancers in women with neurofibromatosis type 1–a multi-centre retrospective study. J Gen Syndr Gene Ther 7: 292.

Wang X, Teer JK, Tousignant RN, Levin AM, Boulware D, Chitale DA, Shaw BM, Chen Z, Zhang Y, Blakeley JO, Acosta MT, Messiaen LM, Korf BR, Tainsky MA. 2018a. Breast cancer risk and germline genomic profiling of women with neurofibromatosis type 1 who developed breast cancer. Genes. Chromosomes Cancer 57: 19–27.

Wang X, Kallionpää RA, Gonzales PR, Chitale DA, Tousignant RN, Crowley JP, Chen Z, Yoder SJ, Blakeley JO, Acosta MT, Korf BR, Messiaen LM, Tainsky MA. 2018b. Germline and Somatic NF1 Alterations Are Linked to Increased HER2 Expression in Breast Cancer. Cancer Prev. Res. (Phila). 11: 655–664.

Wang J, Xu B. 2019. Targeted therapeutic options and future perspectives for HER2-positive breast cancer. Signal Transduct. Target. Ther. 4: 34.

Watson KL, Al Sannaa GA, Kivlin CM, Ingram DR, Landers SM, Roland CL, Cormier JN, Hunt KK, Feig BW, Ashleigh Guadagnolo B, Bishop AJ, Wang W-L, Slopis JM, McCutcheon IE, Lazar AJ, Torres KE. 2017. Patterns of recurrence and survival in sporadic, neurofibromatosis Type 1-associated, and radiation-associated malignant peripheral nerve sheath tumors. J. Neurosurg. 126: 319–329.

Well L, Jaeger A, Kehrer-Sawatzki H, Farschtschi S, Avanesov M, Sauer M, de Sousa MT, Bannas P, Derlin T, Adam G, Mautner VF, Salamon JM. 2020. The effect of pregnancy on growth-dynamics of neurofibromas in Neurofibromatosis type 1. PLoS One 15: e0232031.

Whittaker SR, Theurillat J-P, Van Allen E, Wagle N, Hsiao J, Cowley GS, Schadendorf D, Root DE, Garraway LA. 2013. A genome-scale RNA interference screen implicates NF1 loss in resistance to RAF inhibition. Cancer Discov. 3: 350–62.

Wilding A, Ingham SL, Lalloo F, Clancy T, Huson SM, Moran A, Evans DG. 2012. Life expectancy in hereditary cancer predisposing diseases: an observational study. J. Med. Genet. 49: 264–9.

Williams LA, Moertel CL, Richardson M, Marcotte EL. 2020. Incidence of second malignancies in individuals diagnosed with malignant peripheral nerve sheath tumors. J. Neurooncol. 147: 701–709.

Wilson CH, Griffith CDM, Shrimankar J, Douglas F. 2004. Gynaecomastia, neurofibromatosis and breast cancer. Breast 13: 77–9.

Wilson JRF, Bateman AC, Hanson H, An Q, Evans G, Rahman N, Jones JL, Eccles DM. 2010. A novel HER2-positive breast cancer phenotype arising from germline TP53 mutations. J. Med. Genet. 47: 771–4.

Wu TL, Zhang D, Chia JH, Tsao KC, Sun CF, Wu JT. 2002. Cell-free DNA: Measurement in various carcinomas and establishment of normal reference range. Clin. Chim. Acta 321: 77–87.

Xu G, O'Connell P, Viskochil D, Cawthon R, Robertson M, Culver M, Dunn D, Stevens J, Gesteland R, White R, Weiss R. 1990. The neurofibromatosis type 1 gene encodes a protein related to GAP. Cell 62: 599–608.

Xue A, Wu Y, Zhu Z, Zhang F, Kemper KE, Zheng Z, Yengo L, Lloyd-Jones LR, Sidorenko J, Wu Y, eQTLGen Consortium AF, McRae AF, Visscher PM, Zeng J, Yang J. 2018. Genome-wide association analyses identify 143 risk variants and putative regulatory mechanisms for type 2 diabetes. Nat. Commun. 9: 2941.

Yap Y-S, Munusamy P, Lim C, Chan CHT, Prawira A, Loke S-Y, Lim S-H, Ong K-W, Yong W-S, Ng SBH, Tan IBH, Callen DF, Lim JCT, Thike A-A, Tan P-H, Lee ASG. 2018. Breast cancer in women with neurofibromatosis type 1 (NF1): a comprehensive case series with molecular insights into its aggressive phenotype. Breast Cancer Res. Treat. 171: 719–735.

Yates LR, Knappskog S, Wedge D, Farmery JHR, Gonzalez S, Martincorena I, Alexandrov LB, Van Loo P, Haugland HK, Lilleng PK, Gundem G, Gerstung M, Pappaemmanuil E, Gazinska P, Bhosle SG, Jones D, Raine K, Mudie L, Latimer C, Sawyer E, Desmedt C, Sotiriou C, Stratton MR, Sieuwerts AM, Lynch AG, Martens JW, Richardson AL, Tutt A, Lønning PE, Campbell PJ. 2017. Genomic Evolution of Breast Cancer Metastasis and Relapse. Cancer Cell 32: 169-184.e7.

Ylä-Outinen H, Loponen N, Kallionpää RA, Peltonen S, Peltonen J. 2019. Intestinal tumors in neurofibromatosis 1 with special reference to fatal gastrointestinal stromal tumors (GIST). Mol. Genet. Genomic Med. 7: e927.

Yu D, Tong Y, Guo X, Feng L, Jiang Z, Ying S, Jia J, Fang Y, Yu M, Xia H, Shi L, Lou J. 2019. Diagnostic Value of Concentration of Circulating Cell-Free DNA in Breast Cancer: A Meta-Analysis. Front. Oncol. 9: 95.

Zhang M, Bhat T, Gutmann DH, Johnson KJ. 2019. Melanoma in individuals with neurofibromatosis type 1: a retrospective study. Dermatol. Online J. 25.

Zhao Z, Wen W, Michailidou K, Bolla MK, Wang Q, Zhang B, Long J, Shu X-O, Schmidt MK, Milne RL, García-Closas M, Chang-Claude J, Lindstrom S, Bojesen SE, Ahsan H, Aittomäki K, Andrulis IL, Anton-Culver H, Arndt V, Beckmann MW, Beeghly-Fadiel A, Benitez J, Blomqvist C, Bogdanova N V, Børresen-Dale A-L, Brand J, Brauch H, Brenner H, Burwinkel B, Cai Q, Casey G, Chenevix-Trench G, Couch FJ, Cox A, Cross SS, Czene K, Dörk T, Dumont M, Fasching PA, Figueroa J, Flesch-Janys D, Fletcher O, Flyger H, Fostira F, Gammon M, Giles GG, Guénel P, Haiman CA, Hamann U, Harrington P, Hartman M, Hooning MJ, Hopper JL, Jakubowska A, Jasmine F, John EM, Johnson N, Kabisch M, Khan S, Kibriya M, Knight JA, Kosma V-M, Kriege M, Kristensen V, Le Marchand L, Lee E, Li J, Lindblom A, Lophatananon A, Luben R, Lubinski

J, Malone KE, Mannermaa A, Manoukian S, Margolin S, Marme F, McLean C, Meijers-Heijboer H, Meindl A, Miao H, Muir K, Neuhausen SL, Nevanlinna H, Neven P, Olson JE, Perkins B, Peterlongo P, Phillips K-A, Pylkäs K, Rudolph A, Santella R, Sawyer EJ, Schmutzler RK, Schoemaker M, Shah M, Shrubsole M, Southey MC, Swerdlow AJ, et al. 2016. Association of genetic susceptibility variants for type 2 diabetes with breast cancer risk in women of European ancestry. Cancer Causes Control 27: 679–93.

Zheng Y, Ley SH, Hu FB. 2018. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. Nat. Rev. Endocrinol. 14: 88–98.

Zheng Y, Pan D. 2019. The Hippo Signaling Pathway in Development and Disease. Dev. Cell 50: 264–282.

Zheng Z-Y, Anurag M, Lei JT, Cao J, Singh P, Peng J, Kennedy H, Nguyen N-C, Chen Y, Lavere P, Li J, Du X-H, Cakar B, Song W, Kim B-J, Shi J, Seker S, Chan DW, Zhao G-Q, Chen X, Banks KC, Lanman RB, Shafaee MN, Zhang XH-F, Vasaikar S, Zhang B, Hilsenbeck SG, Li W, Foulds CE, Ellis MJ, Chang EC. 2020. Neurofibromin Is an Estrogen Receptor-α Transcriptional Co-repressor in Breast Cancer. Cancer Cell 37: 387-402.e7.

Zhou Y, Pan B, Mao F, Zhu Q, Huo Z, Wang H, Cai F, Liang Z, Wang C, Zhang Y, Sun Q. 2012. A hidden breast lump covered by nipple appendices in a patient with von recklinghausen disease: a case report and review of the literature. Clin. Breast Cancer 12: 71–5.

Zöller M, Rembeck B, Akesson HO, Angervall L. 1995. Life expectancy, mortality and prognostic factors in neurofibromatosis type 1. A twelve-year follow-up of an epidemiological study in Göteborg, Sweden. Acta Derm. Venereol. 75: 136–40.

Zöller ME, Rembeck B, Odén A, Samuelsson M, Angervall L. 1997. Malignant and benign tumors in patients with neurofibromatosis type 1 in a defined Swedish population. Cancer 79: 2125–31.

TURUN
YLIOPISTO
UNIVERSITY
OF TURKU

Painosalama, Turku, Finland 2022

ISBN 978-951-29-8854-9 (PRINT)
ISBN 978-951-29-8853-2 (PDF)
ISSN 0355-9483 (Print)
ISSN 2343-3213 (Online)