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Original research

Haploinsufficiency of the *NF1* gene is associated with protection against diabetes

Roope A Kallionpää,[1] Sirkku Peltonen,[2,3] Jussi Leppävirta,[4,5] Minna Pöyhönen,[4,5] Kari Auranen,[6] Hannu Järveläinen,[1,7] Juha Peltonen [1]

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/jmedgenet-2020-107062).

[1]Institute of Biomedicine, University of Turku, Turku, Finland
[2]Department of Dermatology and Venereology, University of Turku, Turku, Finland
[3]Department of Dermatology, Turku University Hospital, Turku, Finland
[4]Department of Clinical Genetics, HUSLAB, Helsinki University Hospital (HUS) Diagnostic Center, Helsinki, Finland
[5]Department of Medical and Clinical Genetics, University of Helsinki, Helsinki, Finland
[6]Department of Mathematics and Statistics and Department of Clinical Medicine, University of Turku, Turku, Finland
[7]Department of Internal Medicine, Satakunta Central Hospital, Pori, Finland

Correspondence to
Professor Juha Peltonen, Institute of Biomedicine, University of Turku, Turku FI 20520, Finland; juhpel@utu.fi

Received 1 April 2020
Revised 8 May 2020
Accepted 11 May 2020
Published Online First 22 June 2020

To cite: Kallionpää RA, Peltonen S, Leppävirta J, *et al*. *J Med Genet* 2021;**58**:378–384.

ABSTRACT

Background The hereditary predisposition to diabetes is only partially explained by genes identified so far. Neurofibromatosis type 1 (NF1) is a rare monogenic dominant syndrome caused by aberrations of the *NF1* gene. Here, we used a cohort of 1410 patients with NF1 to study the association of the *NF1* gene with type 1 (T1D) and type 2 diabetes (T2D).

Methods A total of 1410 patients were confirmed to fulfil the National Institutes of Health diagnostic criteria for NF1 by individually reviewing their medical records. The patients with NF1 were compared with 14 017 controls matched for age, sex and area of residence as well as 1881 non-NF1 siblings of the patients with NF1. Register-based information on purchases of antidiabetic medication and hospital encounters related to diabetes were retrieved. The Cox proportional hazards model was used to calculate the relative risk for diabetes in NF1.

Results Patients with NF1 showed a lower rate of T2D when compared with a 10-fold control cohort (HR 0.27, 95% CI 0.17 to 0.43) or with their siblings without NF1 (HR 0.28, 95% CI 0.16 to 0.47). The estimates remained practically unchanged after adjusting the analyses for history of obesity and dyslipidaemias. The rate of T1D in NF1 was decreased although statistically non-significantly (HR 0.58, 95% CI 0.27 to 1.25).

Conclusion Haploinsufficiency of the *NF1* gene may protect against T2D and probably T1D. Since *NF1* negatively regulates the Ras signalling pathway, the results suggest that the Ras pathway may be involved in the pathogenesis of diabetes.

INTRODUCTION

Diabetes is characterised by impaired insulin production or action, leading to chronically increased blood glucose concentrations. Type 2 diabetes (T2D) is the most common form of diabetes.[1] It typically manifests in adulthood and is strongly linked to lifestyle factors such as obesity and lack of exercise.[1 2] The development of T2D involves decreased insulin sensitivity and commonly leads to impaired insulin production.[1 3] Type 1 diabetes (T1D) is an autoimmune disorder characterised by destruction of the insulin-producing β-cells in the pancreas.[4] T1D is usually diagnosed already in childhood. While T2D can be treated with a variety of medications and lifestyle changes, patients with T1D are fully dependent on insulin treatment. Both types of diabetes are associated with increased mortality.[1 4]

Familial aggregation and twin studies clearly indicate a genetic component in diabetes risk.[2 4–6] However, the genetic risk for especially T2D is highly complex with relatively limited contributions from individual genes. Genome-wide association studies (GWAS) and sequencing have identified numerous variants associated with T2D.[7–11] These are mostly common variants with small or modest effects or low frequency variants with larger effects.[9 12] Variants protective against T2D have been described in, for example, *CCND2*,[8 9] *SLC30A8*,[11 13] *TCF2*,[14] *MC4R*[15] and *PPARG*,[16] some of which can also harbour risk-increasing variants.[8 15] Regarding T1D, up to 50% of its heredity can be explained by human leucocyte antigen (HLA) genes, especially with HLA-DR3-DQ2 and HLA-DR4-DQ8 conferring increased risk as well as certain HLA haplotypes providing protection against T1D.[5 17 18] Other major T1D-associated genes include *INS*, *PTPN22* and *IL2RA* while most of the known non-HLA risk genes only confer minor increases in T1D susceptibility.[18] The genes contributing to the initial development of autoimmunity may differ from those involved in the progression from islet autoantibodies to clinical T1D, which further complicates the genetics of T1D.[19] Since family history of T1D associates with higher risk for T2D and vice versa, these diseases are thought to have also shared risk genes but only a few (eg, *MTNR1B* and *HNF1A*) have been identified.[20–22] The identification of the genetic underpinnings of diabetes elucidates the disease pathogenesis, and knowledge of the protective variants may also suggest novel therapeutic approaches.[12]

Neurofibromatosis type 1 (NF1; MIM 162200) is a dominantly inherited monogenic multiorgan syndrome caused by aberrations of the *NF1* gene in chromosome 17q11.2. NF1 has a prevalence of 1/3000–1/2000 (0.03%–0.05%).[23] The diagnosis is based on the National Institutes of Health (NIH) diagnostic criteria,[24] which include café-au-lait macules of the skin, cutaneous and plexiform neurofibromas, skinfold freckling, Lisch nodules of the eye, optic glioma, distinct osseous lesions and NF1 in a family member. The *NF1* gene encodes a tumour suppressor protein neurofibromin that downregulates Ras activity.[25] Thus, NF1 is a Rasopathy. The disease-causing variants are spread throughout the gene, and only few mutational hotspots have been identified.[26] NF1 is associated with increased mortality and a variety of comorbidities including predisposition to many

types of cancers and cardiovascular complications.[27–32] Lower fasting blood glucose concentrations have been reported in NF1 compared with control persons.[33–35] A reduced rate of gestational diabetes was observed in one cohort of patients with NF1[36] but not in another.[37] An analysis of insurance claims reported OR of 0.4 for diabetes among patients with NF1 in the USA.[38] In addition, two previous studies based on death certificates have noted a lower than expected number of diabetes-related deaths among patients with NF1.[39 40] However, these studies have not excluded premature mortality associated with NF1 as the cause for the lower rate of diabetes among patients with NF1. Interestingly, a recent Danish cohort study reported a significantly reduced rate of T1D-related hospitalisations and non-significantly reduced rate of T2D-related hospitalisations among patients with NF1.[32] Here, we aimed at further dissecting the risk for diabetes in NF1.

METHODS

Research permissions

Research permissions were obtained from the Finnish Institute for Health and Welfare, The Social Insurance Institution of Finland, Finnish Population Register Centre and all participating hospitals. The study was register-based and exempt from obtaining informed consent from participants.

Patients

The collection of the NF1 cohort has previously been described.[29] In brief, the cohort was collected by searching for neurofibromatosis-related hospital visits in the 5 University Hospitals and 15 Central Hospitals of mainland Finland in 1987–2011. The medical records of the patients identified in this initial search were individually reviewed to confirm NF1 by the NIH diagnostic criteria.[24] Thus, the cohort is fully ascertained and based on the complete population of mainland Finland. The procedure yielded a total of 1410 patients with confirmed NF1 and availability of valid minimum necessary information. All Finnish residents have a unique personal identity code that can be used as a key for retrieving information from national registers.

The patients with NF1 were compared with two cohorts that were formed using data from the Finnish Population Register Centre: (1) For each patient with NF1, 10 controls matched for date of birth, sex and place of residence were retrieved. First-degree relatives of patients with NF1 were excluded. Due to the small size of some municipalities, a total of 14 017 controls were obtained. (2) Siblings of patients with NF1, defined by at least one shared parent, were retrieved and persons with known or suspected NF1 diagnosis were excluded. A total of 1881 non-NF1 siblings of patients with NF1 were available.

Data sources

Dates of death and emigration were retrieved from the Finnish Population Register Centre, yielding complete follow-up.

The Finnish Institute for Health and Welfare maintains the Care Register for Health Care. The register was founded in 1969 as Hospital Discharge Register covering inpatient care. Since 1998, specialised public outpatient care has also been included. The hospital visits and stays have been recorded using International Classification of Diseases, 9th revision (ICD-9) diagnosis codes in 1987–1995 and ICD-10 diagnosis codes since 1996. Each hospital visit and stay can be associated with up to six diagnoses, all of which were taken into account in the present study. These diagnoses do not necessarily reflect the primary reason for the hospital encounter but may also be used to record pre-existing chronic diseases. Hospital visits and stays associated with diabetes in overall were identified using ICD-10 codes E10–E14. In order to identify T1D, ICD-10 code E10 was used. For T2D, ICD-10 code E11 was used in 1996–2014 and ICD-9 code 250xA, where x can be any digit, was used pre-1996. The ICD-10 code E66 was used to retrieve diagnoses of obesity, E78 for disorders of lipoprotein metabolism and other lipidaemias, and I10 for primary hypertension.

The Social Insurance Institution of Finland is a government agency that reimburses part of medication costs for most prescription drugs bought by Finnish residents. The reimbursements are paid directly to pharmacies when the drug is dispensed, and the patient only pays the remaining part of the price. Since the reimbursement does not require application by the patient, the system encompasses the vast majority of all prescription drug purchases in Finland. Reimbursed medication purchases have been recorded since 1995 using Anatomical Therapeutic Chemical (ATC) coding. Insulins and analogues are included in class A10A and blood glucose lowering drugs other than insulins in class A10B. Patients may also receive a higher special reimbursement of drugs prescribed for certain conditions, including insulin-dependent diabetes, where an application by the physician is required.

Statistical methods

The primary study period was from 1 January 1998 to 31 December 2014 since all data types were available starting from 1998. The follow-up of patients with NF1 started at the age of their first neurofibromatosis-related hospital visit or at their age at the beginning of the study period, whichever was later. The follow-up of controls started at the latter of the age at the first neurofibromatosis-related hospital visit of the respective patient with NF1 or the age at the beginning of the study period. The follow-up of the siblings without NF1 started at birth, age at the first neurofibromatosis-related hospital visit of their NF1 sibling(s) or the age at the beginning of the study period, whichever occurred last. For all study subjects, the follow-up ended at the age of the first occurrence of a diagnosis of interest, death, emigration or end of study period, whichever came first.

When all types of diabetes or T2D only were analysed, the first occurrence of diabetes was defined by the first hospital visit or stay with a relevant diagnosis code or the first purchase of drugs classified as ATC A10A or A10B. In the T2D analysis adjusted by the features of metabolic syndrome, the history of having had a diagnosis of obesity or dyslipidaemias at any time during the follow-up was included as a covariate. In the T1D analysis, only the hospital visits and stays were considered when defining the follow-up, but since T1D always progresses to insulin-dependency, having purchased insulin or its analogue (ATC A10A) at any time was also required to exclude any coding errors. Patients with T1D were excluded from the analyses of T2D. As a sensitivity analysis, the rate of T2D was also assessed in the years 1987–2014 but medication purchases were not included due to the availability of this information only since 1995. Another sensitivity analysis restricted the age range of follow-up to 0–50 years.

Rates were estimated using the Poisson distribution. The estimation of HRs was based on the Cox proportional hazard models with delayed entry. In these analyses, the proportional hazards assumption was fulfilled as assessed using scaled Schoenfeld residuals. To allow heterogeneity between subgroups formed by each patient with NF1 and the respective controls, a subgroup-specific frailty term was included in the models. Similarly, in the

Table 1 Characteristics of the study cohorts in the primary study period 1998–2014 and in the sensitivity analysis study period 1987–2014

	Follow-up 1998–2014			Follow-up 1987–2014		
	Patients with NF1	Controls	Siblings without NF1	Patients with NF1	Controls	Siblings without NF1
n	1349	13870	1871	1410	14017	1881
Sex						
Females, n (%)	694 (51.4)	7189 (51.8)	893 (47.7)	732 (51.9)	7256 (51.8)	895 (47.6)
Males, n (%)	655 (48.6)	6681 (48.2)	978 (52.3)	678 (48.1)	6761 (48.2)	986 (52.4)
Year of birth, mean (SD)	1975.2 (21.6)	1974.4 (22.0)	1976.5 (18.5)	1974.0 (22.4)	1974.0 (22.4)	1976.4 (18.5)
Start of follow-up (cohort entry)						
Age, mean (SD)	25.7 (20.7)	26.3 (21.0)	24.4 (17.4)	23.6 (20.7)	23.6 (20.7)	21.5 (16.7)
Year, mean (SD)	2001.1 (4.2)	2001.0 (4.1)	2001.1 (4.1)	1997.6 (7.3)	1997.6 (7.3)	1997.9 (7.2)
End of follow-up						
Age, mean (SD)	38.3 (20.8)	39.7 (21.5)	37.8 (18.4)	38.6 (21.0)	40.0 (21.6)	37.7 (18.4)
Year, mean (SD)	2013.1 (2.8)	2013.7 (1.8)	2013.7 (1.5)	2012.2 (4.9)	2013.5 (2.7)	2013.6 (2.1)
Follow-up time, mean (SD)	12.7 (4.9)	13.4 (4.5)	13.4 (4.4)	15.0 (7.5)	16.3 (7.4)	16.2 (7.2)

NF1, neurofibromatosis type 1.

analyses comparing patients with NF1 and their siblings without NF1, each family was included as a frailty term. Results are reported as point estimates and 95% CIs. Statistical significance is defined at the two-sided 5% level. The R software (V.3.6.1) and package survival (V.3.1–8) were used in the analyses.

RESULTS

We explored the incidence of diabetes in NF1 using a cohort of 1410 Finnish patients with NF1 (table 1). Patients with NF1 were compared with 14017 control subjects individually matched for age, sex and place of residence. Another comparison cohort consisted of 1881 non-NF1 siblings of the patients with NF1. We used register-based information on hospital visits and stays and purchases of antidiabetic medication in 1998–2014 to identify those with diabetes. The overall rate of diabetes among patients with NF1 was 1.7 cases per 1000 person-years (95% CI 1.1 to 2.4) while the rate was 5.1 (95% CI 4.8 to 5.4) in the control cohort and 4.3 (95% CI 3.5 to 5.2) among the non-NF1 siblings. Consequently, the relative rate of diabetes was markedly lower among patients with NF1 when compared with controls (HR 0.34, 95% CI 0.23 to 0.49) or the siblings without NF1 (HR 0.35, 95% CI 0.23 to 0.55).

Type 1 diabetes

We next analysed T1D and T2D separately. Seven persons with T1D were observed in the NF1 cohort during the follow-up 1998–2014, while there were 129 persons with T1D in the control cohort and 19 siblings with T1D (tables 2 and 3). The HR for T1D in NF1 was 0.58 (95% CI 0.27 to 1.25) when compared with controls and 0.55 (95% CI 0.23 to 1.33) when compared with the siblings without NF1 but the associations were not statistically significant (table 2). One patient with NF1 and eight control persons were observed to have both T1D and coeliac disease, yet no cases of autoimmune thyroiditis or Addison's disease were seen among the patients with T1D. No complication of T1D was particularly common among patients with NF1 (online supplementary table S1).

Type 2 diabetes

During the follow-up 1998–2014, T2D was significantly less common among patients with NF1 than among controls (HR 0.27, 95% CI 0.17 to 0.43) or siblings without NF1 (HR 0.28, 95% CI 0.16 to 0.47) and the HRs for T2D in NF1 were even lower than for T1D (table 2). Although statistically significant in both sexes, the reduction in the rate was more pronounced among males (HR 0.14, 95% CI 0.06 to 0.35 in comparison NF1 versus controls) than among females (HR 0.40, 95% CI 0.23 to 0.68). Approximately 90%–98% of the persons had purchased antidiabetic medication while only 33%–40% had a T2D-related hospital encounter, indicating that the treatment was primarily carried out in primary care setting (table 4). Moreover, 5/7 patients with NF1 and T2D-related hospital encounters had at least one hospital encounter due to T2D without complications (online supplementary table S2).

To assess whether the lower rate of T2D among patients with NF1 was affected by premature mortality, the analysis was restricted to ages<50 years. The decreased rate of T2D in NF1 persisted at ages<50 years with HR 0.35 (95% CI 0.16 to 0.79) in comparison with controls and HR 0.30 (95% CI 0.13 to 0.71) in comparison with the siblings without NF1. In this analysis, T2D was observed in six patients with NF1, 184 control subjects and 36 siblings without NF1. We had to restrict the time range of the primary analysis to 1998–2014 because outpatient hospital visits were only available since 1998 and medication purchases since 1995. However, the relative risk estimates remained stable when the study period was extended to 1987–2014 and only hospital visits and stays were considered (NF1 versus controls: 7 and 338 persons with T2D, respectively; HR 0.26, 95% CI 0.12 to 0.54; NF1 versus siblings: 7 and 28 persons with T2D, respectively; HR 0.20, 95% CI 0.07 to 0.53).

We next explored whether the reduced rate of T2D in NF1 was related to metabolic syndrome and overweight. The HR for a diagnosis of obesity in NF1 was 0.94 (95% CI 0.59 to 1.48) compared with the matched controls and 1.44 (95% CI 0.76 to 2.72) compared with the siblings without NF1. When the rate of any of the features of metabolic syndrome other than hyperglycaemia were considered, that is, obesity, primary hypertension and disorders of lipoprotein metabolism and other lipidaemias, their HR in NF1 was 1.37 (95% CI 1.14 to 1.66) compared with controls and 1.66 (95% CI 1.25 to 2.21) compared with the siblings without NF1. After exclusion of hypertension, which is known to be more prevalent among patients with NF1, the HRs were 1.04 (95% CI 0.76 to 1.43) and 1.20 (95% CI 0.77 to 1.87), respectively. Thus, the other components of metabolic syndrome do not seem to be less frequent among patients with NF1 despite the decreased rate of T2D. Consequently, after adjusting the

Table 2 Observed follow-up times, diabetes cases during the follow-up and the resulting HRs in NF1 as compared with controls without NF1 or siblings without NF1 in 1998–2014

			Patients with NF1 vs controls			Patients with NF1 vs siblings without NF1		
			All	Females	Males	All	Females	Males
Type 1 diabetes		HR (95% CI)	0.58 (0.27 to 1.25)	0.95 (0.38 to 2.36)	0.29 (0.07 to 1.2)	0.55 (0.23 to 1.33)	1.11 (0.35 to 3.52)	0.26 (0.06 to 1.16)
	NF1	Number of diabetes cases	7	5	2	7	5	2
		Follow-up (person-years)	17 005.9	8814.0	8191.9	17 005.9	8814.0	8191.9
		Rate (n/1000 person-years) (95% CI)	0.41 (0.17 to 0.85)	0.57 (0.18 to 1.32)	0.24 (0.03 to 0.88)	0.41 (0.17 to 0.85)	0.57 (0.18 to 1.32)	0.24 (0.03 to 0.88)
	Reference	Number of diabetes cases	129	57	72	19	7	12
		Follow-up (person-years)	184 594.4	97 064.6	87 529.8	24 928.7	12 138.2	12 790.5
		Rate (n/1000 person-years) (95% CI)	0.7 (0.58 to 0.83)	0.59 (0.44 to 0.76)	0.82 (0.64 to 1.04)	0.76 (0.46 to 1.19)	0.58 (0.23 to 1.19)	0.94 (0.48 to 1.64)
Type 2 diabetes		HR (95% CI)	0.27 (0.17 to 0.43)	0.40 (0.23 to 0.68)	0.14 (0.06 to 0.35)	0.28 (0.16 to 0.47)	0.37 (0.19 to 0.71)	0.15 (0.06 to 0.41)
	NF1	Number of diabetes cases	19	14	5	19	14	5
		Follow-up (person-years)	16 877.2	8714.6	8162.6	16 877.2	8714.6	8162.6
		Rate (n/1000 person-years) (95% CI)	1.13 (0.68 to 1.76)	1.61 (0.88 to 2.70)	0.61 (0.20 to 1.43)	1.13 (0.68 to 1.76)	1.61 (0.88 to 2.70)	0.61 (0.20 to 1.43)
	Reference	Number of diabetes cases	779	402	377	83	42	41
		Follow-up (person-years)	179 213.0	94 286.5	84 926.6	24 337.8	11 890.2	12 447.6
		Rate (n/1000 person-years) (95% CI)	4.35 (4.05 to 4.66)	4.26 (3.86 to 4.70)	4.44 (4.00 to 4.91)	3.41 (2.72 to 4.23)	3.53 (2.55 to 4.77)	3.29 (2.36 to 4.47)

NF1, neurofibromatosis type 1.

T2D analyses for both history of obesity and dyslipidaemias, the relative rate for T2D among patients with NF1 remained low with HRs of 0.28 (95% CI 0.18 to 0.44) in comparison to the control cohort and 0.27 (95% CI 0.16 to 0.46) in comparison to the siblings without NF1.

DISCUSSION

Here, we have shown that NF1 is associated with a reduced rate of T2D and a statistically non-significant reduction in the rate of T1D. Since NF1 is caused by pathogenic variants of the *NF1* gene with full penetrance, the results suggest that germline aberrations of the *NF1* gene and the resulting deficiency of neurofibromin protein confer protection against diabetes. NF1 syndrome is rare with the prevalence <0.05%,[23] and any individual pathogenic variant of the *NF1* gene is even rarer. Rare variants are generally hard to detect in GWAS,[12] and the case of *NF1* is even more difficult as the variants are spread throughout the gene. Variants of *NF1* are also associated with reduced fitness, further decreasing their prevalence in older age groups.[23] It is thus not surprising that the association between the *NF1* gene and T1D or T2D has not been recognised in the previous studies looking for genes associated with diabetes. Our approach of studying a curated clinical cohort of patients who share aberrations of the *NF1* gene overcomes some of these limitations. The clinical features of NF1 allow combining a variety of different variants with similar functional consequences. Assuming a prevalence of 1/2000–3000 for NF1, observing as many persons with *NF1* haploinsufficiency and diabetes as in our cohort would require an unselected population of 2.8–4.2 million persons, which is much more than in the largest diabetes-GWAS published so far.[9]

Only few genes affecting the risk of both T1D and T2D have been identified despite their overlapping heritability patterns.[20 21] Although the ages of onset and risk factors of T1D and T2D are largely different, the two types of diabetes share pathological processes. Both types may involve inflammatory loss of pancreatic β-cell mass, which is due to autoimmunity in T1D and toxic metabolites in T2D.[3 4 20 21] Moreover, T1D may present with insulin resistance.[20 21] The association between NF1 and T1D reported here was not statistically significant, yet a reduced rate of T1D-related hospitalisations among persons with NF1 was also observed in a recent cohort study.[32] Together, these results suggest that *NF1* aberrations may be a mechanism affecting both types of diabetes. Unfortunately, our register-based data do not allow dissecting whether the observed low HRs for T1D and T2D in NF1 are related to, for example, inflammation or metabolic activity. Previous data show NF1-associated alterations in the immune system,[41] and gene expression data from Schwann cells suggest association of *NF1* gene dosage with HLA class II expression (NCBI GEO database, accession GSE32029), which may speak in favour of an inflammation-mediated mechanism. NF1 is known to affect especially the cells of the neural crest during development. Interestingly, neural crest cells have been observed to regulate the size of the β-cell population in a murine model during embryonic development.[42]

Previous publications have suggested lower fasting blood glucose levels, increased insulin sensitivity and higher resting energy expenditure in NF1.[34 35 43] Hypothetically, the voluminous benign neurofibroma tumour mass could be one mechanism increasing the energy consumption of patients with NF1. The increased energy expenditure in NF1 is in concordance with results from other Rasopathies, as high resting energy expenditure has also been reported in patients with Costello syndrome,[44] and a murine model of Noonan syndrome with multiple

Table 3 Descriptive characteristics of patients with T1D during follow-up 1998–2014

	Patients with NF1	Controls	Siblings without NF1
n	7	129	19
Sex			
Female, n (%)	5 (71.4)	57 (44.2)	7 (36.8)
Male, n (%)	2 (28.6)	72 (55.8)	12 (63.2)
Age at first T1D-related encounter during follow-up, mean (SD)	31.05 (19.15)	30.17 (20.42)	33.16 (20.99)
Number of T1D-related encounters/patient, median (range)	25 (2 to 72)	21 (1 to 1195)	22 (1 to 62)
Number of insulin purchases (ATC A10A)/patient, median (range)	50 (2 to 115)	66 (1 to 152)	62 (15 to 118)
Special drug reimbursement for insulin, n (%)	7 (100)	129 (100)	19 (100)

ATC, Anatomical Therapeutic Chemical classification; NF1, neurofibromatosis type 1; T1D, type 1 diabetes.

lentigines showed increased energy expenditure.[45] Costello and Noonan syndromes and Noonan syndrome with multiple lentigines have been reported to associate with lower weight and a decreased rate of obesity.[44–46] These findings suggest that the present observation of reduced risk of T2D in NF1 may be related to Ras signalling pathway activity. Interestingly, NF1 of the child has been found to increase birth weight,[37] while low birth weight is known to be associated with increased risk for T2D.[47] Therefore, the increased birth weight of children with NF1 may be an underlying factor of the lower risk for T2D in NF1, or both these observations may reflect the same biological process. Our present analysis of obesity and other components of metabolic syndrome do not suggest lower incidence of these risk factors among patients with NF1, nor did we previously find altered rates of gestational diabetes in NF1.[37]

Table 4 Descriptive characteristics of patients with T2D during follow-up 1998–2014

	Patients with NF1	Controls	Siblings without NF1
n	19	779	83
Sex			
Female, n (%)	14 (73.7)	402 (51.6)	42 (50.6)
Male, n (%)	5 (26.3)	377 (48.4)	41 (49.4)
Age at first T2D-related encounter or drug purchase during follow-up, mean (SD)	56.49 (17.45)	58.64 (14.18)	49.48 (12.63)
T2D-related encounters			
Patients, n (%)	7 (36.8)	312 (40.1)	27 (32.5)
Number/patient among those with at least one encounter, median (range)	2 (1 to 8)	2 (1 to 190)	2 (1 to 504)
Purchases of insulins and analogues (ATC A10A)			
Patients, n (%)	6 (31.6)	201 (25.8)	17 (20.5)
Number/patient among those with at least one purchase, median (range)	15 (2 to 22)	17 (1 to 99)	10 (1 to 85)
Purchases of other antidiabetic medication (ATC A10B)			
Patients, n (%)	17 (89.5)	723 (92.8)	76 (91.6)
Number/patient among those with at least one purchase, median (range)	10 (1 to 81)	18 (1 to 128)	15 (1 to 104)
Special drug reimbursement for insulin, n (%)	10 (52.6)	554 (71.1)	56 (67.5)

ATC, Anatomical Therapeutic Chemical classification; NF1, neurofibromatosis type 1; T2D, type 2 diabetes.

In light of the previous studies,[48] the association between the *NF1* gene and T2D highlights the role of the Ras pathway in the pathogenesis of T2D. The increased phosphorylation of Erk1/2 has previously been linked to protection against T2D by gain-of-function variants of *MC4R*,[15] and the protection conferred by *AKNRD55* is linked to *MAP3K1* (MEK kinase).[7] The lean phenotype observed in a mouse model of the Noonan syndrome with multiple lentigines was observed to revert on MEK inhibition.[45] Moreover, a study using mouse embryonic fibroblasts reported altered mitochondrial function attributed to ERK1/2 activity induced by *NF1* loss.[49] While the pharmacological inhibition of the tumour suppressor protein neurofibromin is not a feasible therapeutic approach, downstream components of the Ras pathway may provide targets for interfering with the pathogenesis of T2D.

The present findings are corroborated by previous studies, which suggested a lower rate of diabetes in NF1.[32 38–40] Our total population-based approach reduces biases inherent in hospital-based patient ascertainment and the individual curation of NF1 diagnoses ensures reliability. Since we assessed the rates of diabetes using patient-specific person times conditionally on survival, the premature mortality associated with NF1 cannot explain the findings. This conclusion is further supported by the robustness of the decreased rate of T2D in NF1 also among persons aged <50 years. Moreover, we were able to analyse T1D and T2D separately to show that especially the rate of T2D is reduced among patients with NF1. A limitation of the present study is the lack of biospecimens that would allow elucidating the reasons of the decreased rate of diabetes, and T2D in particular, seen in NF1. The use of register-based data also leaves some uncertainty in the diabetes diagnoses as a large proportion of T2D is known to be undiagnosed in the population. However, patients with NF1 are not expected to have a higher rate of missing diagnoses than persons without NF1, since NF1 and its comorbidities are likely to increase the number of healthcare contacts. Furthermore, the comprehensive data on medication purchases should allow identification of all patients undergoing treatment.

All the patients with NF1 included in the present study fulfilled the diagnostic criteria of NF1 and thus carried a pathogenic variant of the *NF1* gene. Comparing patients with NF1 with both the matched controls and siblings allows ruling out confounding related to, for example, calendar time, age, parental education or hereditary factors other than *NF1*. Thus, we conclude that the lower rate of at least T2D and probably T1D in NF1 is indeed associated with the *NF1* gene.

Acknowledgements Turku University Hospital is a member of ERN GENTURIS.

Contributors Data acquisition: RAK, SP, JL, MP, JP. Data analysis: RAK, JL, KA. Drafting the manuscript: RAK. All authors have made contributions to the conception

and design of the study, taken part in data interpretation, revised the manuscript and approved the submitted version.

Funding The study was funded with grants from the Finnish Cancer Foundation, Turku University Hospital, University of Turku, Turku University Foundation, Orion Research Foundation and Ida Montin Foundation.

Competing interests JP, consultant to AstraZeneca.

Patient consent for publication Not required.

Ethics approval The study was approved by the Ethics Committee of the Hospital District of Southwest Finland and adhered to the principles of the Declaration of Helsinki.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data may be obtained from a third party and are not publicly available. Data are available for researchers with appropriate research permissions from Finnish Institute for Health and Welfare, The Social Insurance Institution of Finland and Finnish Population Register Centre.

ORCID iD

Juha Peltonen http://orcid.org/0000-0002-5732-4167

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