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Diabetes, metformin and cancer risk in myotonic dystrophy type I

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Myotonic dystrophy type I (DM1) is an autosomal dominant multisystem disorder characterized by myotonia and muscle weakness. Type 2 diabetes (T2D) and cancer have been shown to be part of the DM1 phenotype. Metformin, a well-established agent for the management of T2D, is thought to have cancer-preventive effects in the general population. In our study, we aimed to assess the association between T2D, metformin use and the risk of cancer in DM1 patients. We identified a cohort of 913 DM1 patients and an age-, sex- and clinic-matched cohort of 12,318 DM1-free controls from the UK Clinical Practice Research Datalink, a large primary care records database. We used Cox regression models to assess cancer risk in T2D patients who were metformin users or nonusers compared to patients without T2D. Separate analyses were conducted for DM1 patients and controls. T2D was more prevalent in DM1 than in controls (8% *vs.* 3%, $p < 0.0001$). DM1 patients with T2D, compared to those without T2D, were more likely to develop cancer (hazard ratio [HR] = 3.60, 95% confidence interval [CI] = 1.18–10.97; $p = 0.02$), but not if they were treated with metformin (HR = 0.43, 95% CI = 0.06–3.35; $p = 0.42$). Among controls, we observed no significant associations between T2D and cancer risk in either users or nonusers of Metformin (HR = 1.28, 95% CI = 0.91–1.79; $p = 0.16$ and HR = 1.13, 95% CI = 0.72–1.79; $p = 0.59$, respectively). These results show an association between T2D and cancer risk in DM1 patients and may provide new insights into the possible benefits of Metformin use in DM1.

Introduction

Myotonic dystrophy type I (*dystrophia myotonica* I; DM1; Steinert's disease) is a dominantly inherited neuromuscular disorder that affects multiple organ systems, primarily skeletal muscles.[1] DM1 is caused by an unstable CTG-repeat expansion in the 3′ untranslated region of the dystrophia myotonica protein kinase (*DMPK*) gene on chromosome 19.[2,3] Although myotonia and progressive muscle weakness are the primary symptoms of DM1, prevalent extramuscular manifestations include early-onset cataracts, cognitive impairment, cardiac conduction defects, thyroid dysfunction and insulin resistance.[1,4] More recently, DM1 patients have been shown to have excess risks of certain cancers including those originating in the endometrium, brain, ovary, thyroid, skin and possibly colon, testes and eye.[5–12] Accumulating evidence has shown that DM1 pathogenesis is largely RNA-mediated; the transcription of the expanded repeats produces mutant RNA that alters the splicing of various target transcripts leading to the complex clinical manifestations of DM1.[1,13] For instance, spliceopathy of the insulin receptor gene (*INSR*) in DM1 induces insulin resistance, leading to glucose intolerance, hyperinsulinemia and increased risk of type 2 diabetes (T2D).[14,15] The prevalence of T2D in DM1 is not well-known, with published estimates ranging

Key words: myotonic dystrophy, diabetes, cancer, metformin, CPRD

Abbreviations: BMI: body mass index; CI: confidence interval; CPRD: Clinical Practice Research Datalink; *DMPK*: *dystrophia myotonica* protein kinase; HES: hospital episodes statistics; HR: hazard ratio; ICD-10: International classification of diseases, 10th edition; IMD: index of multiple deprivation; *INSR*: insulin receptor gene; IR: insulin receptor; IRB: Institutional Review Board; ISAC: Independent Scientific Advisory Committee; NHS: National Health Services; NIH: National Institutes of Health; NMSC: nonmelanoma skin cancers; ONS: Office of National Statistics; PPV: positive predictive value; RNA: ribonucleic acid; SD: standard deviation; T1D: type 1 diabetes; T2D: type 2 diabetes; UK: United Kingdom

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What's new?

Recent evidence suggests that myotonic dystrophy type I (DM1), an inherited nucleotide repeat disorder, is a cancer predisposition syndrome. However, the underlying pathological mechanisms and cancer-predisposing risk factors remain unknown. Here, type 2 diabetes (T2D) and metformin were investigated for potential associations with cancer risk in DM1 patients. T2D was found to be more prevalent in DM1 patients than controls. DM1 patients with T2D were at increased risk of developing cancer. By contrast, cancer risk was not elevated in DM1-T2D patients taking metformin. Further investigation of metformin use and cancer in DM1 patients could yield important insights into DM1-related cancer prevention.

from 5% to 17%, in studies with varying age, sex and disease subtype distributions.[16–18]

In the general population, diabetes mellitus (primarily T2D) is associated with increased risk of several cancers including those arising in the breast, colorectum, endometrium, liver and pancreas; some of which are also common in DM1.[19] On the other hand, the use of metformin, a well-established first-line therapy for T2D management, has been inconsistently suggested to reduce the risk of cancer in various populations.[20,21] In DM1, metformin was shown to be effective in treating insulin resistance and, more recently, suggested to improve mobility in DM1 patients without T2D.[22,23] A recent *in vitro* study of DM1 showed that metformin produced a corrective effect on DM1-related alternative splicing defects.[24] In light of recent evidence of excess cancer risks in DM1 and T2D, and as the indications for metformin in DM1 continue to grow, it is important to understand the interplay between diabetes, metformin use and cancer risk in DM1 patients. In our study, we aimed to estimate the prevalence of diabetes and to assess the association between T2D, metformin use and the risk of cancer in a large cohort of DM1 patients compared to a matched cohort of DM1-free controls.

Methods

Data sources and study population

Data sources and the study population were described previously.[6,11] Briefly, we utilized the United Kingdom (UK) Clinical Practice Research Datalink (CPRD) October 2016 data release (data collected from June 1987 to October 2016, however, clinics were permitted to submit prior patient records [before the start of CPRD], all of which were available for our study). CPRD is a large electronic primary care records database with linkage (for ~57% of the CPRD clinics) to additional patient-level data sources, including hospitalization records from the Hospital Episodes Statistics (HES; data coverage from April 1997 to February 2016), and mortality data from the Office of National Statistics (ONS; data coverage from January 1988 to March 2016).[25] We identified all DM1 patients (n = 1,061) using Read Codes (F392011: Steinert's disease, and F392000: Dystrophia myotonica [Steinert's disease]), a clinical classification system used by the UK National Health Services (NHS). For each DM1 patient, we randomly selected up to 20 DM1-free controls (never had a record of DM1; n = 15,130) matched on year of birth ($\pm$2 years), sex, clinic and clinic registration year ($\pm$1 year), who were alive and actively registered at the index date (later of first DM1 record or clinic registration dates) of their respective DM1 patient. Patients with prevalent cancers at baseline, less than 6 months of follow-up and those who were taking metformin but did not have a record of diabetes were excluded from the present study. We also excluded type 1 diabetes (T1D) patients because of their small number (<1% in each cohort). The final analysis included 913 DM1 patients and 12,318 DM1-free controls (Fig. 1).

Our study was approved by the CPRD Independent Scientific Advisory Committee (ISAC; Protocol # 16_005RA2). The use of the CPRD database was exempt from the full Institutional Review Board (IRB) review by the National Institutes of Health (NIH) Office of Human Subject Research and the University of Maryland's IRB because of the irrevocably anonymized nature of the data.

Outcome and study variables

Incident cancer cases were identified from CPRD, HES or ONS as described previously (HES and ONS data were available for linkage-eligible patients only [n = 7,867; 59%]). The date of an incident cancer diagnosis was defined as the earliest cancer record, excluding nonmelanoma skin cancer (NMSC), from CPRD (Read codes available upon request) or HES (ICD-10 codes C00 to C96, excluding NMSC codes C44 and C4A). Individuals lacking cancer records within CPRD or HES, but for whom the primary cause of death, per ONS, was cancer, were considered as incident cancer cases with the date of death used as the cancer diagnosis date (<2% of cancers).

Diabetes status and metformin use were determined from primary care clinical and prescription records, using the date of first CPRD record as a proxy for the date of diagnosis or first use, respectively. Similarly, information on baseline covariates ($\pm$2 years from start of follow-up) including body mass index (BMI), smoking status and alcohol consumption were ascertained from CPRD. We used multiple imputations for missing information on smoking status, alcohol consumption and BMI (imputed height and weight, separately). The frequencies of missing values ranged from 27% to 54% in both cohorts. We used the SAS IVEware software to generate five imputed datasets, from which results were combined according to the method of Rubin using the MIANALYZE procedure (SAS 9.4).[26]

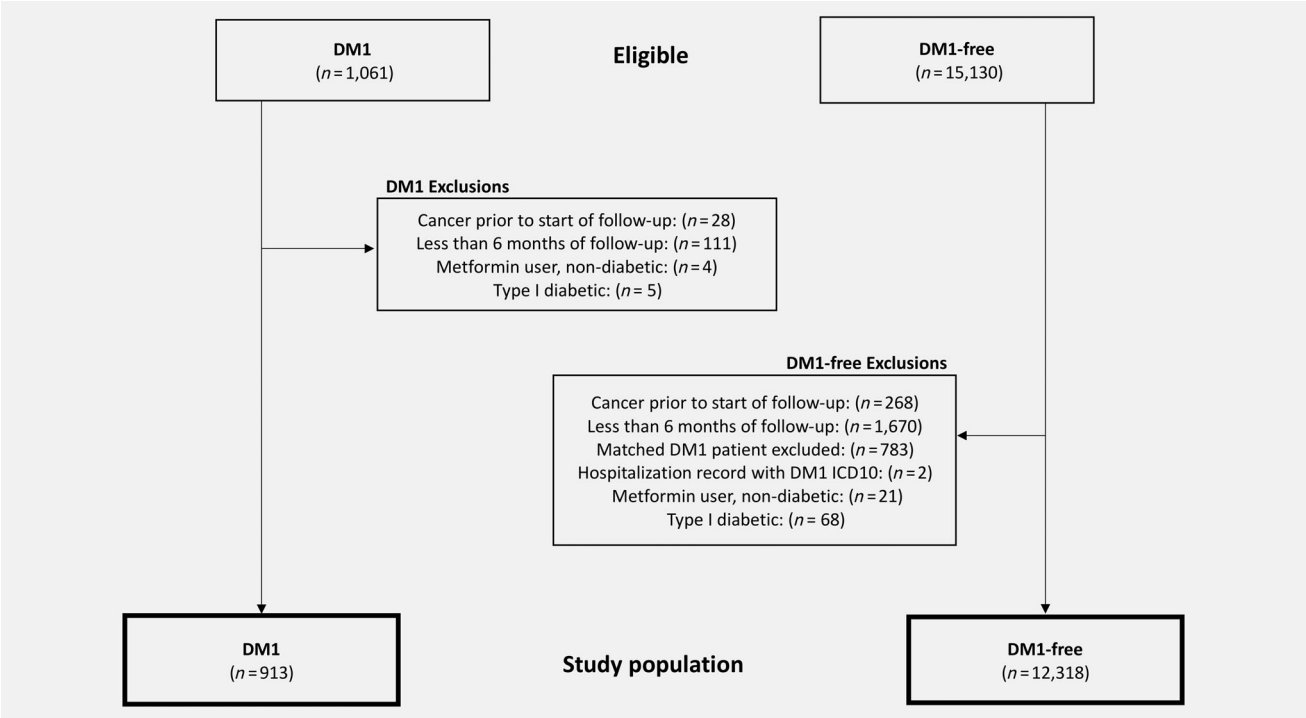

Figure 1. Flow diagram of the study population.

Statistical analysis

Cox regression models were used to calculate hazard ratios (HRs) and 95% confidence intervals (Cis) for the associations of diabetes in metformin users or nonusers with cancer incidence in the DM1 and DM1-free cohorts, separately. Age was the time scale used for all analyses. Follow-up started at the latest of 18 years of age (since diabetes and cancer are both adult-phenotypes in DM1), age at first DM1 record (as a proxy for age at DM1-diagnosis) or DM1-free selection, clinic registration or study start date (January 1, 1988; 6 months after the start of CPRD, to maximize reporting

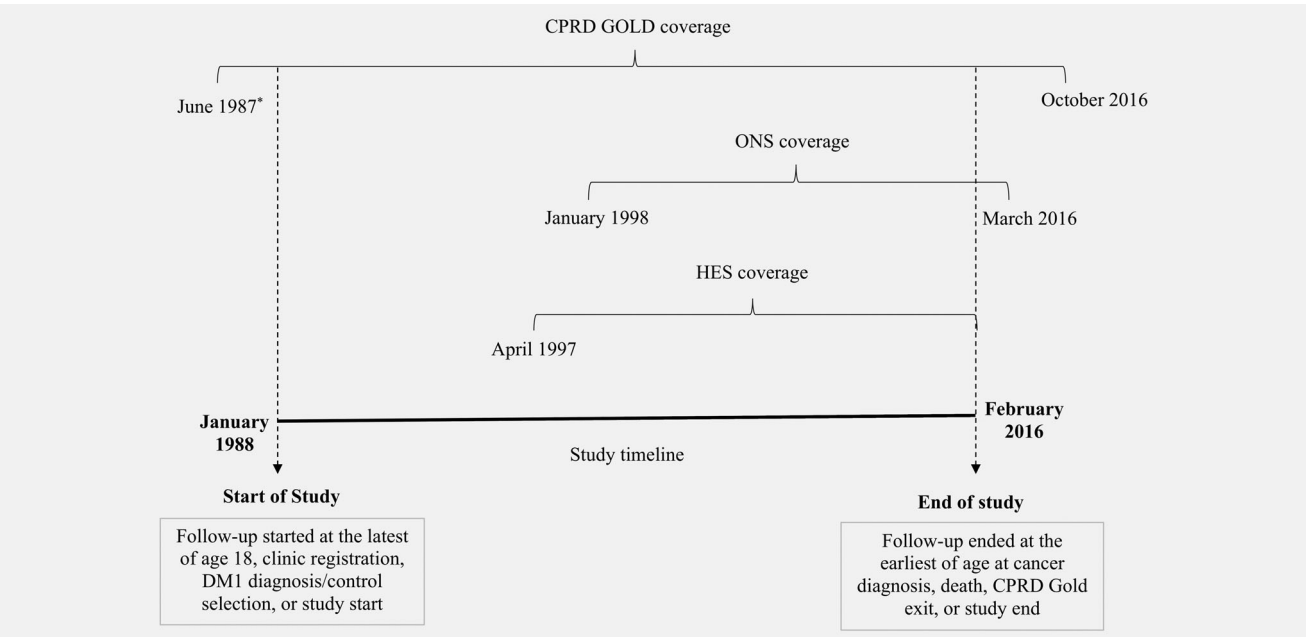

Figure 2. Study timeline and database coverage periods. *CPRD began collecting data from participating clinics in June of 1987, however, clinics were permitted to submit prior patient records (before 1987), which were available for our study.

accuracy and completeness). A three-level, time-dependent categorical exposure variable was used to classify patients with diabetes as metformin users or nonusers, and patients with no record of diabetes as comparators (reference group). For example, a patient with no record of T2D at the start of follow-up, a T2D record 1 year after follow up, and metformin use at Year 2 would contribute person-time of follow-up in the "no diabetes" group from start of follow-up to Year 1, the "T2D metformin nonuser" group from Year 1 to 2, and the "T2D metformin user" group after Year 2. Patients who developed diabetes or used metformin prior to start of follow-up were considered exposed at the start of follow-up.

Table 1. Characteristics of final analysis set of 913 DM1 patients and 12,318 DM1-free controls by type 2 diabetes status

	DM1 Cohort (n = 913)		DM1-free Cohort (n = 12,318)	
Characteristic	**Type II diabetes (n = 75)**	**No diabetes (n = 838)**	**Type II diabetes (n = 412)**	**No diabetes (n = 11,906)**
Age at DM1 diagnosis (years)				
Median (range)	43.6 (0–64)	31.0 (0–91)	–	–
Age at start of follow-up (years)				
Median (range)	50.4 (23–71)	36.5 (18–96)	52.9 (23–87)	34.6 (18–97)
Gender, *n* (%)				
Male	37 (49.3%)	411 (49.1%)	251 (60.9%)	5,800 (48.7%)
Female	38 (50.7%)	427 (50.9%)	161 (39.1%)	6,106 (51.3%)
Metformin use				
Yes, *n* (%)	56 (74.7%)	–	276 (67.0%)	–
Time from diabetes to first prescription in years, median (range)	1.5 (0–23.5)	–	1.5 (0–16.5)	–
Cancer				
Yes, *n* (%)	7 (9.3%)	34 (4.1%)	70 (17.0%)	631 (5.3%)
Age at diagnosis, median (range)	57.4 (44–62)	56.4 (34–96)	65.2 (48–87)	60.7 (24–97)
BMI (kg/m^2)				
‹25	9 (12.0%)	279 (33.3%)	44 (10.7%)	3,179 (26.7%)
25–29.9	16 (21.3%)	99 (11.8%)	79 (19.2%)	1,721 (14.5%)
30+	28 (37.3%)	66 (7.9%)	161 (39.1%)	922 (7.7%)
Missing	22 (29.3%)	394 (47.0%)	128 (31.1%)	6,084 (51.1%)
Mean (SD)	31.0 (6.5)	24.4 (6.0)	31.5 (7.6%)	25.4 (5.2)
Smoking, *n* (%)				
Never	22 (29.3%)	218 (26.0%)	78 (18.9%)	2,459 (20.7%)
Former	25 (33.3%)	158 (18.9%)	116 (28.2%)	1,997 (16.8%)
Current	8 (10.7%)	149 (17.8%)	88 (21.4%)	2,517 (21.1%)
Missing	20 (26.7%)	313 (37.4%)	130 (31.6%)	4,933 (41.4%)
Alcohol Consumption, *n* (%)				
Never	13 (17.3%)	84 (10.0%)	46 (11.2%)	982 (8.3%)
Ever	30 (40.0%)	312 (37.2%)	180 (43.7%)	4,468 (37.5%)
Missing	32 (42.7%)	442 (52.7%)	186 (45.1%)	6,456 (54.2%)
Death during follow-up				
Yes, *n* (%)	39 (52.0%)	228 (27.2%)	177 (43.0%)	1,118 (9.4%)
Age at death, *median (range)*	59.9 (38–73)	56.1 (23–97)	68.1 (41–93)	63.0 (20–104)
Practice-level index of multiple deprivations quintiles				
1 (most affluent)	7 (9.3%)	121 (14.4%)	44 (10.7%)	1,646 (13.8%)
2	17 (22.7%)	145 (17.3%)	52 (12.6%)	2,051 (17.2%)
3	14 (18.7%)	151 (18.0%)	88 (21.4%)	2,198 (18.5%)
4	10 (13.3%)	180 (21.5%)	76 (18.4%)	2,545 (21.4%)
5 (most deprived)	27 (36.0%)	241 (28.8%)	152 (36.9%)	3,466 (29.1%)
Healthcare visits during follow-up, mean (SD)	15.3 (11.9)	9.6 (9.3)	12.2 (13.7)	3.9 (6.1)
Follow-up years, total (median)	681.9 (7.6)	8,024.2 (8.0)	4,375.2 (9.3)	113,051.4 (8.1)

Follow-up ended at the earliest of either age at cancer diagnosis, death, exit from CPRD (transfer-out of the CPRD clinic or the clinic's last data collection date), or end of study (February 29, 2016; the end of HES which was the database among those used in our study for which coverage ended earliest). Linkage-eligible patients with hospitalization records in HES after CPRD exit remained in the cohort. Figure 2 describes the study timeline relative to data coverage periods for CPRD, HES and ONS.

To adjust for cancer ascertainment from multiple databases for linkage-eligible patients, all models were adjusted for linkage-eligibility as a time-dependent covariate (based on the start date of HES data collection [April 1997]). To address the possibility of detection bias, we adjusted all models for the number of healthcare encounters from CPRD or HES as a continuous, time-varying covariate. We included a one-year time lag from cancer diagnosis or end of follow-up to avoid adjusting for visits due to early symptoms of cancer itself, and counted healthcare encounters in 12-month intervals between start and end of follow-up. Final models were adjusted for sex, BMI, linkage status and healthcare encounters. No confounding (>10% change in the hazard ratios of the associations between diabetes or metformin and cancer risk) by age at DM1 diagnosis (continuous), year of patient registration with the CPRD clinic (categories: <1988 [included patients who registered with their clinic prior to the start of CPRD, but continued to be actively registered and met the study inclusion criteria]; 1988–2000; >2000), region (England *vs*. others), practice-level index of multiple deprivation (IMD) quintiles (as a proxy for socioeconomic status; available for all CPRD practices), smoking (current, former, never) or alcohol consumption (current, former, never) was observed. In a sensitivity analysis, we required patients to have a minimum of two metformin prescription records to be considered "users."

All tests were two-sided, with statistical significance defined as p-value < 0.05. Analyses were conducted using SAS version 9.4 (Cary, NC).

Data availability

Our study is based on data from the CPRD GOLD database October 2016 release (ISAC; Protocol # 16_005RA2; details in the Acknowledgments). The data used in our study are administered by the United Kingdom Clinical Practice Research Datalink; access to CPRD data is subject to protocol approval by the Independent Scientific Advisory Committee (https://www.cprd.com/research-applications). All relevant data and methods are reported in the article.

Results

The characteristics of DM1 patients and DM1-free controls involved in our study were described earlier[6,11,27] Briefly, 503 (55%) of 918 DM1 patients (prior to excluding those with T1D) were first diagnosed between ages 11–40 years, 313 (34%) after age 40 and 102 (11%) by age 10, with an overall average age at DM1 diagnosis of 32.8 years. Seventy-five (8%) DM1 patients had T2D (median age at T2D diagnosis = 48 years), a statistically significant higher prevalence than in the controls (3%, p Chi-Square < 0.001; median age at diagnosis = 54 years). On the other hand, the prevalence of T1D was similar in DM1 patients and DM1-free controls (0.5% *vs*. 0.6%, respectively, p Chi-Square = 0.99; median age at T1D diagnosis = 28 and 22 years, respectively). Seventy-five percent of T2D DM1 patients and 67% of T2D DM1-free controls were metformin users. DM1 patients who had T2D but did not use metformin (N = 19) were either prescribed other oral antidiabetic drugs or insulin (37%), were on diet-only control (21%), or had no treatment but multiple diagnostic records (42%). DM1 patients with T2D were more likely to have late-onset DM1 (46 of 75 [61%], median age at DM1 diagnosis = 44 years) compared to DM1 patient without T2D (267 of 881 [30%], median age at DM1 diagnosis = 31 years). No sex differences were observed among DM1 T2D patients and those without T2D (51% female *vs*. 49% male in both groups); however, among DM1-free controls, patients with T2D were more likely to be male compared to patients without T2D (61% *vs*. 49%, respectively). In both cohorts, patients with T2D were more likely to be

Table 2. Unadjusted and adjusted analyses of cancer risk comparing patients with type 2 diabetes who are metformin users or nonusers with those without diabetes, within the DM1 and DM1-free cohorts

	DM1 cohort (n = 913)		DM1-free cohort (n = 12,318)	
Analysis	**HR (95% CI)**	***p*-value**	**HR (95% CI)**	***p*-value**
Unadjusted				
No Diabetes	Reference	Reference		
T2D (metformin nonusers)	3.74 (1.28–10.91)	0.02	1.36 (0.87–2.12)	0.18
T2D (metformin users)	0.58 (0.08–4.25)	0.59	1.54 (1.11–2.12)	0.01
Adjusted[1]				
No Diabetes	Reference	Reference		
T2D (metformin nonusers)	3.60 (1.18–10.97)	0.02	1.13 (0.72–1.79)	0.59
T2D (metformin users)	0.43 (0.06–3.35)	0.42	1.28 (0.91–1.79)	0.16

[1]Adjusted for sex, baseline BMI (continuous), linkage status (time-dependent starting April 1997) and number of healthcare encounters (in CPRD and/or HES prior to outcome/end of follow-up date, with a 1-year time lag).

Table 3. Sensitivity analysis of cancer risk comparing patients with type 2 diabetes who are metformin users or nonusers with those without diabetes, within the DM1 and DM1-free cohorts, requiring a minimum of two metformin prescriptions to be considered a user

	DM1 cohort (n = 913)		DM1-free cohort (n = 12,318)	
Analysis[1]	HR (95% CI)	p-value	HR (95% CI)	p-value
No diabetes	Reference	Reference		
T2D (metformin nonusers)	3.49 (1.15–10.6)	0.03	1.16 (0.74–1.81)	0.51
T2D (metformin users)	0.44 (0.06–3.40)	0.43	1.26 (0.90–1.78)	0.18

[1]Adjusted for sex, baseline BMI (continuous), linkage status (time-dependent starting April 1997) and number of healthcare encounters (in CPRD and/or HES prior to outcome/end of follow-up date, with a 1-year time lag).

obese (mean BMI in DM1 and DM1-free T2D patients = 32 and 31 *vs.* 24 and 25 in DM1 and DM1-free patients without T2D, respectively) and had a higher number of healthcare encounters compared to patients without T2D (average number of visits per year in DM1 = 15 *vs.* 10; DM1-free controls = 12 *vs.* 4). Table 1 summarizes the characteristics of the DM1 and DM1-free cohorts by T2D status.

Cancer profiles in the DM1 and DM1-free cohorts have been previously reported.[11] In multivariable analyses, we found that DM1 patients with T2D who do not use metformin had a higher risk of developing cancer compared to patients without T2D (HR = 3.60, 95% confidence interval [CI] = 1.18–10.97; p = 0.02). On the other hand, cancer risk among DM1 patients with T2D who used metformin was similar to that observed in patients without T2D, or possibly lower (HR = 0.43, 95% CI = 0.06–3.35; p = 0.42; Table 2). Among controls, we observed no significant associations between T2D with or without metformin use and cancer risk (HR = 1.28, 95% CI = 0.91–1.79, p = 0.16 and HR = 1.13, 95% CI = 0.72–1.79, p = 0.59, respectively). Table 3 shows the sensitivity analysis in which a minimum of two metformin prescriptions was required to define "users"; the results were consistent with the main findings.

Discussion

In a large cohort of 913 DM1 patients and 12,318 DM1-free controls, we found: (*i*) the prevalence of T2D in DM1 (8%) was approximately three times higher than in matched DM1-free controls (3%); (*ii*) in DM1, T2D was associated with a statistically significant three-fold increase in cancer risk compared to those without T2D, an excess not observed among metformin users; and (*iii*) no significant associations between diabetes, metformin use and cancer risk among DM1-free controls.

Although T2D has long been associated with DM1, its relative prevalence compared to the general population has been little studied over the years. The most recent (2012) report of 97 Norwegian DM1 subjects documented a 5% prevalence of T2D in DM1 *vs.* 3% in the general population, compared to 8 and 3% in our study, respectively.[16] More recently, a large study of 1,409 French DM1 patients reported frequencies of diabetes in adult males and females (9.4 and 8%, respectively) similar to those observed in our study (8% in both sexes).[18]

The relationship between T2D and the risk of cancer in DM1 patients is unknown. A small cross-sectional study of 255 Italian DM1 patients has previously reported no association between T2D and cancer.[28] It is possible that the association was masked by the use of metformin in those patients. In our study, the excess cancer risk among T2D DM1 patients remained significant even after adjusting for risk factors common to both cancer and T2D (e.g., age, sex, BMI, alcohol and smoking). If validated, this novel observation may help elucidate the pathological mechanisms underlying carcinogenesis in DM1. It is possible that the aberrant expression of the insulin receptor (IR) gene could play a role in cancer development among DM1 patients.[29] Normally, splicing of the IR gene results in two isoforms to which insulin can bind: IR-A and IR-B; while all cells express both isoforms, insulin responsive tissue such as adipose, liver and muscle tissue predominately express IR-B.[30,31] In DM1 patients, the IR-A isoform is overly expressed in muscle tissue, distorting the ratio between A and B isoforms and contributing to insulin resistance and hyperinsulinemia.[32] Because many cancer cell types also overexpress IR-A, this isoform is thought to provide a selective growth advantage to malignant cells when exposed to insulin, thereby increasing the risk of cancer and its progression.[33]

Our data suggest that the use of metformin in DM1 patients may be associated with cancer risk that is similar to that observed in patients without T2D, or possibly lower. The biologic mechanisms underlying metformin's chemopreventive effects are not completely understood. Proposed hypotheses include modulation of insulin and insulin-like growth factor levels in the blood that may affect cellular proliferation.[34] Metformin has been shown to be effective in treating insulin resistance in DM1 by improving glucose utilization in peripheral tissue and increasing insulin sensitivity.[23] Additionally, metformin has been shown, *in vitro*, to correct splicing defects in the IR gene and other genes known to be affected in DM1.[24] Thus, it is biologically plausible that DM1 patients may be sensitive to the chemopreventive effects of metformin.

Although we did not observe a statistically significant increase in cancer risk among T2D DM1-free controls, our observed HR of 1.13 is similar in magnitude to previously reported statistically significant risk estimates linking T2D and cancer within the general population.[19] It is possible that our study was underpowered to detect a statistically significant effect of this magnitude.

Regarding the association between metformin and cancer risk, evidence from observational studies has been inconsistent despite substantial preclinical data in support of metformin's chemopreventive effects.[35–41] Our results in the DM1-free

population are consistent with a null association between metformin and cancer.[42–44] However, it is important to note that our DM1-free cohort was matched to DM1 patients and is, therefore, a much younger population than those in previous studies (median age at start of follow-up in the DM1-free cohort = 35.3 years) and are less likely to have T2D (prevalence of T2D in the DM1-free cohort = 3% *vs*. 5% in the general UK population).[45] Thus, our results may not be directly comparable to those in the existing literature.

Our study is the first to investigate the associations between T2D, metformin use and cancer risk in DM1 patients. We utilized one of the largest electronic medical records databases in the world, which enabled us to identify a large number of DM1 patients and matched DM1-free controls, with detailed information on clinical, therapeutic and lifestyle variables. This allowed for time-dependent analyses and permitted adjustment for potential confounding variables. The use of CPRD does not rely on self-report, thereby avoiding recall bias and CPRD patients have been shown to be representative of the UK population with respect to age, sex and ethnicity,[25] improving the generalizability of our results.

We also acknowledge several study limitations. Although we included a large cohort of DM1 patients, the frequency of cancers was relatively low (4%), not surprising given that our prior analysis of Swedish Population Registry data suggested that absolute cancer risks after DM diagnosis were 1.6, 5 and 9% at ages 40, 50 and 60 years, respectively.[46] This is an unavoidable constraint when studying rare events in an uncommon disease and may have affected our ability to detect a statistically significant difference in the effect of metformin on cancer risk. It also prevented us from conducting analyses by metformin dosage, DM subtype, or cancer site. Additionally, it is possible that patients with T2D were more likely to be diagnosed with cancer due to their underlying chronic condition and potentially increased surveillance. To address this possibility, we adjusted all models for the number of healthcare encounters. In addition, analyses were conducted in the DM1 and DM1-free cohorts separately, minimizing this possibility. Also, the lack of association between diabetes and cancer risk in the DM1-free controls argues against detection bias. Although misclassification of our study variables is possible, studies of CPRD have shown high validity in the reporting of chronic conditions including neoplasms (of 26 studies, the median proportion of cases confirmed = 95.25%) and diabetes (positive predictive value [PPV] = 98.6%), hence the potential for misclassification of these variables was reduced.[47–49] Furthermore, to address the high frequency of missing values for smoking, alcohol consumption and BMI, we used multiple imputation, a known robust statistical method to handle large missing data.[50] Additionally, BMI was the only imputed variable retained in the final model, and it did not significantly change the observed relationship between diabetes, metformin and cancer risk in either cohort. While it is plausible that death may be a competing risk, using cause-specific hazard models, we found that our results remained unchanged (DM1: $HR_{diabetes}$ = 3.6, 95% CI = 1.18–10.97, *p*-value = 0.02 and $HR_{metformin}$ = 0.43, 95% CI = 0.06–3.35, *p*-value = 0.42; DM1-free $HR_{diabetes}$ = 1.13, 95% CI = 0.72–1.79, *p*-value = 0.59 and $HR_{metformin}$ = 1.28, 95% CI = 0.91–1.79, *p*-value = 0.16). This was also true using the Fine and Gray method (DM1: $HR_{diabetes}$ = 3.3, 95% CI = 1.12–9.68, *p*-value = 0.03 and $HR_{metformin}$ = 0.31, 95% CI = 0.04–2.32, *p*-value = 0.26; DM1-free $HR_{diabetes}$ = 1.03, 95% CI = 0.64–1.65, *p*-value = 0.91 and $HR_{metformin}$ = 1.07, 95% CI = 0.75–1.51, *p*-value = 0.71). Finally, to better capture metformin use, we conducted a sensitivity analysis requiring patients to have a minimum of two metformin prescription records to be classified as "users," and the results were consistent with our main findings.

Our findings need further investigation. Recently, a small randomized controlled study showed that metformin improved mobility in DM1 patients.[22] If validated, our results add to the potential benefits of expanding metformin use in DM1, outside of the management of T2D, to include cancer prevention. These results may also provide insight into cancer etiology in DM1. Continued efforts to understand carcinogenic mechanisms in DM1 and to identify specific patients at risk of developing cancer are warranted.

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