A Systematic Review of Variations in Circadian Rhythm Genes and Type 2 Diabetes

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Abstract

Background: Type 2 diabetes (T2D) is a chronic disease that has severe individual and societal consequences, which is forecast to worsen in future. A new field of investigation is variations in circadian rhythm (CR) genes, in conjunction with diet and sleep variables, associations with, and effects on, T2D development.

Objective: This systematic review aimed to analyse all current literature regarding CR gene variations and T2D, and explore their interplay with diet and sleep variables on T2D outcomes. This review was registered with PROSPERO (CRD42021259682).

Methodology: Embase and Pubmed were searched on 6/8/2021 / 11/8/2021 for studies of all designs, including participants from both sexes, all ethnicities, ages, and geographic locations. Participants with risk alleles / genotypes were compared with the wildtype regarding T2D outcomes. Studies risk of bias were scored according to the ROBINS I/E criteria.

Results: 31 studies were found (association n=29 / intervention n=2) including >600,000 participants from various ethnicities, sexes, and ages. Variations in the melatonin receptor 1b (MTNR1B), brain and muscle arnt-like 1 (BMAL1) and period circadian regulator (PER) genes were consistently associated with T2D outcomes.

Conclusions: Individuals with variations in MTNR1B, BMAL1 and PER may be at higher risk of T2D. Further research is needed regarding other CR genes. More longitudinal studies and randomised trials are required before clinical recommendations can be made.

Keywords: Type 2 Diabetes; Circadian Rhythm; Genetics; Diet; Sleep.

Introduction

Type 2 diabetes (T2D) is a chronic disease that is forecasted to be associated with 1.59 million deaths per year by 2025 (Lin *et al.*, 2020). The estimated cost of diabetes to the global economy was $1.31 trillion in 2015 and is predicted to rise to $2.5 trillion by 2030, equivalent to 2.2% of global gross domestic product (Zhang and Gregg, 2017; Bommer *et al.*, 2018). Approximately 90% of diabetes cases are type 2 and currently 422 million people aged between 20 and 79 years have diabetes, forecast to rise to 629 million by 2045, which is about 6.3% of global population (Khan *et al.*, 2020; *Diabetes*, no date).

The main symptom of T2D is hyperglycaemia caused by ineffective insulin secretion and/or action, characterised by eventual pancreatic β-cell failure (Olokoba, Obateru and Olokoba, 2012). T2D is most frequently onset in adults aged 45 years and over, but its prevalence is increasing in younger populations (Lascar *et al.*, 2018). The aetiology of T2D includes obesity, lack of physical activity (PA), age, family history, genetics, high consumption of sugar sweetened beverages and red and processed meats, and low consumption of fruits and vegetables (Ali, 2013; Forouhi and Wareham, 2014).

Recently, circadian rhythm (CR) genes have been implicated in the development of T2D (Javeed and Matveyenko, 2018). CR genes are mostly expressed in the suprachiasmatic nucleus (SCN) of the hypothalamus and in peripheral tissues including pancreatic β-cells. They influence physiological processes, including the sleep-wake cycle, metabolism and the immune system, by variable expression over general diurnal / nocturnal phases (Rijo-Ferreira and Takahashi, 2019). CR processes can interact with hormones including insulin and

74 melatonin, and processes including gluconeogenesis, which may increase T2D risk (Rijo-
75 Ferreira and Takahashi, 2019; Dashti *et al.*, 2020). Single nucleotide polymorphisms (SNPs)
76 in CR genes may therefore further modify this risk. Transcription in CR genes oscillates via
77 an autoregulatory feedback loop, triggered by external cues including light exposure, PA, and
78 diet (Cagampang and Bruce, 2012). CR gene expression is mediated by the retinoic acid-
79 related orphan receptor alpha (RORa) gene, which triggers a central positive feedback loop
80 consisting of a circadian locomotor output cycles kaput (CLOCK) and brain and muscle arnt-
81 like 1 (BMAL1) heterodimer. The central positive feedback loop results in expression of
82 tissue specific genes including melatonin receptor 1B (MTNR1B), as well as triggering a
83 negative feedback loop consisting of period circadian regulator (PER) 1/2, cryptochrome
84 circadian regulator (CRY) 1/2, and nuclear receptor subfamily 1 group D member (NR1D)
85 1/2. NR1D1/2 mediate transcription of REV-ERBα/β proteins which repress transcription of
86 BMAL1, ultimately halting CR gene expression (Jakubowicz *et al.*, 2017; Rijo-Ferreira and
87 Takahashi, 2019). A greater understanding of the circadian cycle's relationship with T2D
88 may lead to clinical strategies which limit T2D prevalence.
89
90 Previous research has revealed that CR disruptions, sometimes due to modifiable lifestyle
91 zeitgebers diet and sleep, can further modify T2D risk (Dashti *et al.*, 2015; Javeed and
92 Matveyenko, 2018; Poggiogalle, Jamshed and Peterson, 2018; Sinturel, Petrenko and Dibner,
93 2020). Poor dietary regulation, including breakfast-skipping, consumption of a traditional
94 'Western' diet and night-time feeding can dysregulate secretion of CR controlled hormones,
95 including glucagon-like peptide 1 (GLP-1), which is key for glucose-dependent insulin release,
96 therefore increasing T2D risk (Froy, 2010; Jakubowicz *et al.*, 2017; Rijo-Ferreira and
97 Takahashi, 2019). Sleeping patterns can also moderate CR gene expression via changes in
98 light-dark cycle stimuli (Jakubowicz *et al.*, 2017). Sleep disruptors such as excessive light

exposure, shift work and enforced clinical laboratory settings can limit secretion of CR gene mediated anti-diabetic hormones including GLP-1, and also limit insulin sensitivity and β-cell function, leading to metabolic dysregulation and increased T2D risk (Jakubowicz *et al.*, 2017; Javeed and Matveyenko, 2018). A previous meta-analysis has been conducted of associations and interactions between CR gene variations, diet, sleep and T2D using studies from the cohorts for heart and aging research in genomic epidemiology (CHARGE) consortium (Dashti *et al.*, 2015). However, to our knowledge no systematic reviews encompassing all existing literature have taken place. Therefore, the primary aim of this systematic review was to determine whether variations in CR genes had an association with, or effect on T2D outcomes. And secondly, to determine whether diet and sleep moderate CR gene variations associations / effects on T2D outcomes.

Methodology

This systematic review was reported according to the preferred reporting items for systematic reviews and meta-analysis (PRISMA) guidelines (Page *et al.*, 2021).

Search Strategy

Search terms for Pubmed were formulated by HS, GV and LL, and were modified for Embase by CG. CG, LL and GV conducted searches of Embase (6/8/2021), and Pubmed (11/8/2021). English-language, human studies from any date prior to the search were included.

Criteria for Study Inclusion (PI/ECOS)

Population: Studies regarding CR genes and T2D and/or T2D related metabolic traits were searched for in populations including both sexes, all ethnicities, ages, and geographic locations. Participants were recruited from various methods, including from existing cohorts, from the general population, or from hospitals and medical registers. Participants remained included if they were suffering from comorbidities including but not limited to cardiovascular disease (CVD), obesity and metabolic syndrome (MS), or if they were taking T2D medication. Studies were required to state the number of participants included. Pregnant females and participants suffering from other forms of diabetes were excluded.

Intervention / Exposure: The intervention group were participants with T2D risk alleles and/or genotypes in CR genes. Accepted genotyping methods included DNA isolated from samples including blood and saliva, and genotyped via methods including TaqMan and Biobank Axiom arrays (*Axiom™ Biobank Plus Genotyping Array*, no date; *Real-Time PCR Assays - UK*, no date). Studies were required to report all CR genes and SNPs that were analysed. Secondary interventions included study-specific dietary and sleep variables (controlled and uncontrolled). Dietary variables included diet patterns (e.g., Mediterranean diet) and individual nutrients (e.g., fat consumption). Dietary patterns were measured via any method, including lab observations and self-reports such as 24-hour food frequency questionnaires and food diaries. Sleep variables were measured by any method, including controlled sleeping hours under clinical conditions and actigraphy, as well as self-reported sleeping habits recorded via questionnaire.

Comparison: The primary comparison group were wildtype participants in the aforementioned CR genes. Secondary comparisons included diet and sleep variables (controlled and uncontrolled).

Outcomes: Primary outcomes were incidence of T2D and T2D-related metabolic traits, measured by methods including medical records, self-report, and metabolic tests (OGTT, fasting glucose and HbA1c data). Studies could report T2D outcomes from a diverse number of measures, including relative risk (RR), odds ratio (OR), or comparison of metabolic outcomes. All outcomes related to T2D were collected. Studies were required to include statistical analysis at a significance value $p<0.05$ prior to multiple comparisons. Studies were not required to have carried out corrections for multiple testing. Studies which included no T2D outcome data were excluded.

Study Design: Both intervention and association studies were included. A non-exhaustive list of study designs considered were randomised controlled trials (RCTs), case-controls, cross-sectional studies, and genome-wide association studies (GWAS). Only peer-reviewed, published studies were accepted. Review articles, pre-proofs and conference documents were excluded.

Study Selection

All identified studies were manually screened by HS and LL, and duplicates were removed. Remaining studies were exported to Rayyan (Ouzzani *et al.*, 2016), and were screened according to title and abstract by HS, LL and GV. After, remaining studies were full text screened by HS, LL and GV. Full texts were accessed via St Mary's University library

services. Remaining studies underwent reference screening by HS, LL and GV for an additional literature search. All conflicts were discussed and resolved by HS, LL and GV.

Data Extraction

Data was extracted using a checklist formulated by HS, LL and GV, by two researchers per study. Data included author's details, study characteristics (location, setting etc.), methodology data, participant characteristics, interventions, comparisons, results, strengths and limitations, conclusions, and areas for future research. All conflicts were discussed and resolved by HS, LL and GV.

Quality Assessment

Studies were scored according to criteria from the risk of bias in non-randomised studies – interventions / exposures (ROBINS-I/E) tools (Sterne *et al.*, 2016; *Risk of bias tools - ROBINS-E tool*, no date), by HS, LL and GV. Criteria included control of confounders, participant recruitment, classification of exposures / interventions, deviations from original protocol, missing data, measurement of outcomes, and selection of reported results. Studies were assigned a low / moderate / serious / critical risk of bias (ROB) for each category, and overall, according to the ROBINS-I/E guidelines.

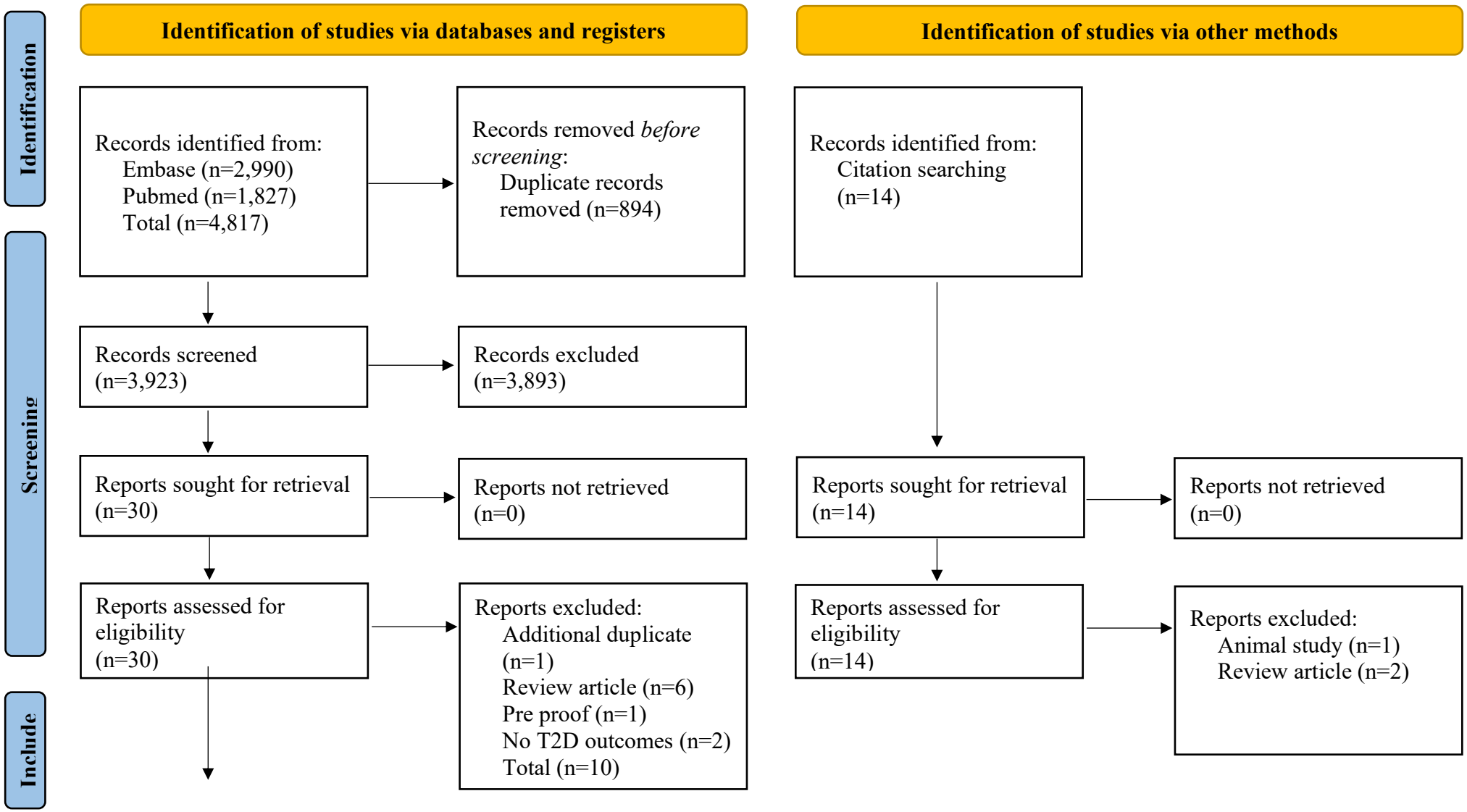
For ease of comparison, remaining studies were grouped by 1) genes and SNPs, 2) study design (e.g., intervention / association), and 3) study quality (ROB).

Registration

This systematic review was submitted to PROSPERO ('PROSPERO International prospective register of systematic reviews', 2021) for registration on 27/7/2021, and its protocol was accepted on 13/8/2021 (registration: CRD42021259682). Modifications were made to the registration regarding use of Rayyan software on 23/9/2021.

Results

Figure 1. Search Results Flow Diagram (Page *et al.*, 2021).



Search Results

Figure 1: On 6/8/2021 / 11/8/2021, the aforementioned search terms applied to Embase and Pubmed returned 2,990 and 1,827 results respectively (n=4,817). Following title screening, 894 duplicates were excluded (n=3,923). Following export to Rayyan, title and abstract screening excluded a further 3,983 studies (n=30). After full-text screening, a further 10 studies were excluded (n=20). Following citation searching of the remaining studies, 14 further studies were included (n=34). Following full text screening of studies identified via citation searching, 3 were excluded (n=31).

Table 1. Study Characteristics Including Genes, Participant Variables, Study Design, Genotyping Method, and T2D Outcome Measure.

	Total
Genes	MTNR1B (n*=19) CLOCK (n=8) BMAL (n=4) PER (n=6) CRY (n=5) REV-ERBα/β (n=1)
Participants	
Number	604,825 (Median = 1,675)
Sex**	Male (51.5%) Female (48.5%)
Ethnicity	Bosnia and Herzegovina (n=1) Caucasian UK (n=1) Chinese (n=1) European (n=5) European Caucasian (n=2) German (n=2) Greek (n=1) Han Chinese (n=5) Indian (n=3) Japanese (n=2) Mediterranean (n=2) Norwegian (n=1) Pakistani (n=1) Sri Lankan (n=1) Taiwanese (n=1) Turkish (n=3) UK (n=1) USA (n=3)
Recruitment Method	Biobank (n=2) Birth cohort (n=1) Health register (n=1) Medical facilities (n=12) Previous cohorts (n=13) Stratified cluster sample (n=1)
Study Design	Case-control (n=13) Cross-sectional (n=13) GWAS (n=2) Multiple designs (n=2) RCT (n=1)
Genotyping	
Tissue**	Blood (n=18) No Data (n=13)
Method	BeadChip (n=1) BiLEVE array (n=2) Illumina array (n=1) KASPar (n=1) Light-SNiP Genotyping assay (n=1) Mass Spectrometry (n=1) MassARRAY (n=1) Next Generation Sequencing (n=1) SNPstream (n=2) TaqMan assay (n=15) Tetra-ARMS (n=1)
T2D Outcome Measure	Metabolic data*** (n=21) T2D Incidence (n=10)

* Number of studies

** Results calculated from studies with published participant characteristics.

*** Oral glucose tolerance test (OGTT) results, fasting glucose (FBG), insulin resistance (IR), β-cell function, HbA1c data.

Study Characteristics

Table 1: 31 remaining studies (MTNR1B: n=19 / CLOCK: n=8 / BMAL: n=4 / PER n=6 / CRY n=5 / REV-ERBα/β n=1) included 604,825 participants (median n=1,675). Of studies that provided requisite participant data, the mean participant age was 46.1 years / 51.5% male. Participants were from 18 different ethnicities. Study designs were case-controls (n=13), cross-sectional studies (n=13), GWAS (n=2), a RCT (n=1), and studies of mixed design (n=2) (association n=29, intervention n=2). T2D outcome measures included

metabolic data (n=21) (OGTT results / fasting glucose / insulin resistance / β-cell function / HbA1c data) and T2D incidence (n=10). Sleep and dietary variables were included in n=5 and n=4 studies respectively.

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280 ***Table 2.*** *Quality Assessment of 31 Studies Using the ROBINS-I/E Guidelines (Sterne et al., 2016; Risk of bias tools - ROBINS-E tool, no date).*
281

Gene / Author	***SNP***	***Risk of Bias Score***	***Reason for Score***
MTNR1B			
(Arikoglu *et al.*, 2021)	rs1387153 / rs10830963	Serious	Lack of Hardy-Weinberg equilibrium (HWE).
(Barragan *et al.*, 2018)	rs10830963	Low	
(Chambers *et al.*, 2009)	rs2166706	Critical	Lack of participant controls. Missing data regarding diabetic participants. Lack of control regarding lifestyle variables. Lack of HWE.
(Dashti *et al.*, 2020)	rs10830963	Low	
(Gao *et al.*, 2016)	rs10830963	Low	
(Kan *et al.*, 2010)	rs10830963	Critical	Missing recruitment data. Missing genotyping data. Limited participant data.
(Lane *et al.*, 2016)	rs10830963	Low	
(Langenberg *et al.*, 2009)	rs10830963	Low	
(Ling *et al.*, 2011)	rs3781637	Low	
(Liu *et al.*, 2010)	rs10830963	Low	
(Lopez Minguez *et al.*, 2016)	rs1387153	Low	
(Olsson *et al.*, 2011)	rs10830963	Low	
(Patel *et al.*, 2018)	rs4753426, rs10830962, rs10830963	Moderate	Lack of participant controls.
(Reinehr *et al.*, 2011)	rs18030963	Critical	Missing results data. Results collected via questionnaire. Lack of

			follow-up during lifestyle intervention.
(Rönn *et al.*, 2009)	rs10830963	Moderate	Missing data regarding selection of participants.
(Semiz *et al.*, 2014)	rs10830963	Low	
(Staiger *et al.*, 2008)	rs10830962, rs4753426, rs12804291, rs10830963, rs3781638	Moderate	Limited participant data.
(Takeuchi *et al.*, 2009)	rs1387153, rs10830963	Moderate	Limited participant data. Limited inclusion / exclusion criteria.
(Tan *et al.*, 2020)	rs10830963	Low	
CLOCK			
(Garaulet *et al.*, 2009)	rs1464490, rs3749474, rs4864584, rs4580704, rs18012602	Moderate	No adjustment for multiple comparisons for significant results.
(Scott, Carter and Grant, 2008)	rs4864548, rs3736544, rs1801260	Low	
BMAL			
(Yamaguchi *et al.*, 2015)	BMAL1 rs11022775, rs2290035 / BMAL2 rs7958822	Low	
PER			
(Karthikeyan *et al.*, 2014)	PER3 exon 18 tandem repeat sequences	Serious	Lack of potential confounder controls.
CRY			
(Dashti *et al.*, 2014)	rs2287161	Low	
REV-ERBα/β			
(Tokat *et al.*, 2020)	rs38253751, rs72836608, rs2314339, rs2102928, rs24003765, rs924403442	Low	
Multiple SNPs			
(Chang, Chiu and Chuang, 2013)	20 SNPs from CLOCK, BMAL1, PER 1/2, CRY1/2	Serious	Lack of potential confounder controls.
(Englund *et al.*, 2009)	CLOCK rs35878285, rs2412646, rs11240, rs2412648, rs3805151 /	Moderate	Lack of some confounder controls.

	PER2 rs934945, rs2304672 / CRY2 rs2863712		
(Kelly *et al.*, 2012)	CLOCK rs11133373 / BMAL1 rs7950226, rs11022775 / PER1, rs885747, rs2289591 / PER2 rs7602358 / PER3 rs1012477 / CRY1 rs12315175 / CRY2 rs2292912	Critical	Lack of inclusion / exclusion criteria.T2D diagnosis not described. Potential T1D participant inclusion.
(Li, Wang and Chen, 2020)	CLOCK rs1801260 / BMAL1 rs7950226	Low	
(Machicao *et al.*, 2016)	121 SNPs from CLOCK, CRY1, CRY2, PER1, PER2 and PER3	Low	
(Tsekmekidou *et al.*, 2021)	CLOCK rs11943456 / PER1 rs2278637 / PER2 rs6744132 / PER3 rs2859389	Low	

Quality Assessment

Table 2: A quality assessment undertaken by HS, GV and LL, shows 18 studies had a low; 6 had a moderate; 3 had a serious; and 4 had a critical ROB. Common reasons for bias were lack of HWE, lack of control of confounders, missing data regarding participant characteristics and recruitment data, and possible T1D participant inclusion.

292

293 **Findings**

294

295 ***Table 3.*** Case-Control Studies Including Genes and SNPs / Authors / Design / Participant Numbers / Inclusion of Diet and Sleep Variables /
296 Outcomes Measured.
297

Gene / Author	***Design***	***SNP***	***Participants*** Cases / Controls	***Diet / Sleep Variables***	***Outcomes***
MTNR1B					
Arikoglu et al (2021)	Case-control	rs1387153, rs10830963	454 / 370 Turkish	N/A	Significant association between rs1387153 and T2D (OR 2.07 1.03-4.15, p=0.041).
Gao et al (2016)	Case-control	rs10830963	736 / 768 Han Chinese	N/A	No significant association between rs10830963 and T2D. However, determined a significant SNP-SNP interaction with GCKR rs780094 and reduced association with T2D (OR 0.392 0.157-0.982, p=0.046).
Kan et al (2010)	Case-control	rs10830963, rs1387153	1,912 / 2,014 Han Chinese	N/A	Significant association between rs10830963 and T2D (OR 1.12 1.02-1.23, p=0.024).

					No significant association with rs1387153.
Ling et al (2011)	Case-control	rs3781637, rs10830963, rs1562444	1,118 / 1,161 Han Chinese	N/A	Significant association between rs3781637 and T2D (OR 2.281 1.28-6.17, p=0.01).
Olsson et al (2011)	Case-control	rs10830963	1,322 / 1,447 Norwegian	Sleep variables including quality and duration	Significant association between rs10830963 and T2D (OR 1.21 1.03-1.41, p=0.0190). No significant association between rs10830963, sleep variables, and T2D.
Patel et al (2018)	Case-Control	rs4753426, rs10830962, rs10830963	478 / 502 Indian	N/A	Significant association between rs10830963 and FBG (p=0.02). No significant associations with rs4753426 and rs10830962.
Ronn et al (2009)	Case-control	rs10830963	1,165 / 1,105 Han Chinese	N/A	Significant association between rs10830963, T2D (OR 1.16 1.03-1.31 p=0.015) and FBG (0.068mmol/l per risk allele).
Semiz et al (2014)	Case-control	rs10830963	162 / 106 Bosnia and Herzegovina	N/A	Significant association between rs10830963, FBG (OR 0.29mmol/l

					per risk allele) and HbA1c (p=0.04). No significant association with T2D incidence.
CLOCK					
Kelly et al (2012)	Case-control	rs11133373	892 / 471 UK 840 / 1,309 Pakistani	N/A	No significant associations.
Li et al (2020)	Case-control	rs1801260	103 / 231 Chinese	N/A	Significant association between rs1801260 and IR (p=<0.05).
Tsekmekidou et al (2021)	Case-control	rs11943456	716 / 569 Greek	N/A	Significant association between rs11943456 and reduced T2D (OR 0.78, p=0.01).
BMAL					
Kelly et al (2012)	Case-control	BMAL1 rs7950226 rs11022775	892 / 471 UK 840 / 1,309 Pakistani	N/A	Significant association between rs1102275 and reduced T2D (OR 0.78, p=0.01).
Li et al (2020)	Case-control	BMAL1 rs7950226	103 / 231 Chinese	N/A	Significant association between rs7950226 and IR (p=0.015).
PER					

Karthikeyan et al (2014)	Case-control	PER3 exon 18 tandem repeat sequences	302 / 330 Indian	N/A	Significant association between five repeat allele and T2D (OR 1.95 1.21-3.15, p=0.006)
Kelly et al (2012)	Case-control	PER1 rs885747 rs2289591 / PER2 rs7602358 / PER3 rs1012477	892 / 471 UK 840 / 1,309 Pakistani	N/A	Significant association between rs7602358 and reduced T2D (p=<0.05).
Tsekmekidou et al (2021)	Case-control	PER1 rs2278637 / PER2 rs6744132 / PER3 rs2859389	716 / 569 Greek	N/A	Significant associations between rs6744132 and T2D (OR 1.18, p=0.04).
CRY					
Kelly et al (2012)	Case-control	CRY1 rs12315175 / CRY2 rs2292912	892 / 471 UK 840 / 1,309 Pakistani	N/A	No significant associations.
REV-ERBα/β					
Tokat et al (2020)	Case-control	rs38253751, rs72836608, rs2314339, rs2102928, rs24003765, rs924403442	42 / 66 Turkish	N/A	Significant association between rs38253751 and increased HbA1c in controls (p=0.002). Significant association between rs2102928 and increased FBG (p<0.05).

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301 ***Table 4.*** Cross-Sectional Studies Including Genes and SNPs / Authors / Design / Participant Numbers / Inclusion of Diet and Sleep Variables /
302 Outcomes Measured.
303

Gene / Author	*Design*	*SNP*	*Participants*	*Diet / Sleep Variables*	*Outcomes*
MTNR1B					
Barragan et al (2018)	Cross-sectional	rs10830963	2,823 Mediterranean	N/A	Significant association between rs10830963 and increased FBG (p=0.009). However, results were no longer significant in an older cohort (>41 years old).
Dashti et al (2020)	Cross-sectional	rs10830963	189,488 European	Sleep variables including shift work and morning and evening preference	Significant association between rs10830963 and T2D (OR 1.1 1.05-1.15, p<0.05 per allele), HbA1c (0.26mmol/l per allele), and 'definitely morning' chronotype (OR 1.17 1.07-1.28, p<0.05).
Langberg et al (2009)	Cross-sectional	rs10830963	1,276 European	N/A	Significant association between rs10830963 and FBG (0.17 0.085-0.25mmol/l), reduced insulin response (-0.19 -0.28--0.1pmol/l), and increased glucose

					sensitivity (-0.11 -0.2--0.027pmol/m).
Liu et al (2010)	Cross-sectional	rs10830963	3,210 Han Chinese	Sleep variables including quality and duration	Significant association between rs10830963 and increased FBG (0.11 0.03-0.18mmol/l, p=0.005), HbA1c (0.07 0.02-0.12%, p=0.004) and reduced β-cell function (-5.01 -8.24--1.77%, p=0.003) in Shanghai participants, but not in Beijing Hans. No significant association with any sleep variables.
Staiger et al (2008)	Cross-sectional	rs10830962, rs4753426, rs12804291, rs10830963, rs3781638	1,598 German	N/A	Significant associations between rs10830962, rs4753426, and rs10830963 with increased FBG (p=0.0001) and reduced insulin release (p=<0.0007). Significant associations between rs3781638 and reduced FBG (p=0.0001), higher insulin release (p=0.0002), insulin sensitivity (p=0.0021)

					and hepatic insulin clearance (p=0.0199).
Takeuchi et al (2019)	Cross-sectional	rs1387153, rs10830963	4,813 Japanese 2,319 Sri-Lankan	N/A	Significant association between rs1387153, rs10830963 and FBG (p<0.05). Significant association between rs10830963 and reduced β-cell function (p=<0.05).
Tan et al (2020)	Cross-sectional	rs10830963	337,083 Caucasian British	Chronotype data	Significant association between rs10830963 and T2D (OR 1.1 1.07-1.14, p=<0.05).
CLOCK					
Chang et al (2013)	Cross-sectional	rs3736544 rs12504300	1,510 Chinese	N/A	No significant associations.
Garaulet et al (2009)	Cross-sectional	rs1464490, rs3749474, rs4864584, rs4580704, rs18012602	1,100 European	Monounsaturated fatty acid (MUFA) consumption	Significant associations found between all SNPs and T2D (p=<0.05). Protective effects of wildtype genes were only present when monounsaturated fatty acid (MUFA) intake was >13.2% total calories (p=>0.05).
Machicao et al (2016)	Cross-sectional	121 SNPs from various CR genes including CLOCK	1,715 German	N/A	No significant associations.

Scott et al (2008)	Cross-sectional	rs4864548, rs3736544, rs1801260	537 European	N/A	CAT haplotype was associated with metabolic syndrome (MS) (p=0.020).
BMAL					
Chang et al (2013)	Cross-sectional	BMAL1 rs6486120 rs7396943 rs11022769 rs2278749 rs2290035	1,510 Chinese	N/A	Significant association between rs2290035, nucleoid occlusion factor (NOC) SNP rs9684900 and FBG (p=0.001).
Yamaguchi et al (2015)	Cross-sectional	BMAL1 rs11022775, rs2290035 / BMAL2 rs7958822	2,467 Japanese	Overall dietary pattern	No significant associations found. However, among obese participants, rs7958822 was significantly associated with increased T2D (OR 2.2 1.1-4.6 males / 2.7 1.1-6.7 females p=<0.05).
PER					
Chang et al (2013)	Cross-sectional	PER1 rs2304911 / PER2 rs2304676 rs238853208	1,510 Chinese	N/A	No significant associations.
Machicao et al (2016)	Cross-sectional	121 SNPs from various CR genes including PER1/2/3	1,715 German	N/A	No significant associations.
CRY					
Chang et al (2013)	Cross-sectional	CRY1 rs11829762 rs11113181 / CRY2	1,510 Chinese	N/A	No significant associations.

		rs4756034 rs7945565 rs17787136			
Dashti et al (2014)	Cross-sectional	CRY1 rs2287161	1,548 USA	Carbohydrate consumption	Significant association between rs2287161, high carbohydrate consumption, and increased FBG (p=0.007) and IR (p=0.002).
Machicao et al (2016)	Cross-sectional	121 SNPs from various CR genes including CRY1/2	1,715 German	N/A	CRY2 SNP rs11605924 significantly associated with increased FBG (p=0.0005).

Table 5. Other Study Designs Including Genes and SNPs / Authors / Design / Participant Numbers / Inclusion of Diet and Sleep Variables / Outcomes Measured.

Gene / Author	***Design***	***SNP***	***Participants***	***Diet / Sleep Variables***	***Outcomes***
MTNR1B					
Chambers et al (2009)	GWAS	rs2166706, rs3847554, rs1387153	11,936 Indian / European Caucasian	N/A	Significant association between rs2166706, rs3847554, rs1387153 and increased FBG (0.05mmol/l per allele). Significant association between rs2166706 and T2D incidence (OR 1.21 1.06-1.38

					p=<0.05) A1C and HOMA-B (p<0.05).
Lane et al (2016)	Mixed	rs10830963	10,525 Mixed ethnicity	Sleep variables including melatonin levels and sleep timing	Significant association between rs10830963, T2D (OR 1.08 1.01-1.16, p=0.01), and FBG (1.52 1.3-1.74mmol/l, p=<0.05). Significant association between rs10830963, early sleep timing and T2D (p=0.024).
Lopez-Minguez et al (2016)	Randomised Controlled Crossover Trial	rs1387153	40 USA	Meal timings	Significant association between rs1387153, late meal timing and T2D (p=<0.05).
Reinehr et al (2011)	Mixed	rs10830963	1,118 German	Effect of lifestyle intervention including 'Obeldicks' dietary education programme	Significant association between rs10830963 and basal glucose levels (1.101 0.316-1.886mg/dL per risk allele, p=0.006). A 1-year lifestyle intervention improved metabolic phenotype with no association to rs10830963 (p=0.355).
CLOCK					
Englund et al (2009)	GWAS	rs35878285, rs2412646, rs11240, rs2412648, rs3805151	7,979 Turkish	N/A	No significant associations.

PER					
Englund et al (2009)	GWAS	PER2 rs934945, 10870, rs2304672	7,979 Turkish	N/A	PER2 SNP rs10870 significantly associated with increased FBG (p=0.049).
CRY					
Englund et al (2009)	GWAS	CRY2 rs2863712	7,979 Turkish	N/A	No significant associations.

Associations / Effects of MTNR1B Gene Variations on T2D Outcomes: All 19 MTNR1B studies determined significant associations / effects of genetic variations on T2D outcomes (Tables 1, 2, and 3). SNPs that were associated with increased T2D outcomes were: rs1387153, 10830963, rs3781637, rs1083096, rs10830962, rs2166706, rs3847554. SNPs associated with reduced T2D outcomes were: rs780094 and rs3781638. However, one study only found significant results in participants aged <41 (Barragan *et al.*, 2018) (Table 4.). There were differences due to ethnicity, including a significantly greater association amongst Indian and Shanghai populations in comparison with Caucasian European and Beijing populations (Chambers *et al.*, 2009; Liu *et al.*, 2010). Also, rs10830963 association with reduced T2D was dependent on the rs780094 SNP in the glucokinase regulatory protein (GKCR) (Gao *et al.*, 2016) (Table 3.). *Diet:* A significant association between rs1387153, late meal timing and T2D was found (Lopez Minguez *et al.*, 2016) (Table 5.). *Sleep:* A significant association was found between rs10830963 and sleep variables including shift work, a 'definitely morning' chronotype, early rising, early sleep timing and T2D (Lane *et al.*, 2016; Dashti *et*

322 *al.*, 2020). Other studies found no association between MTNR1B genetic variations, sleep variables and T2D (Liu *et al.*, 2010; Olsson *et al.*,
323 2011).

Associations / Effects of CLOCK Gene Variations on T2D Outcomes: Four of eight studies determined variations in CLOCK are associated with T2D outcomes (Tables 3, 4, and 5). SNPs that were associated with increased T2D outcomes were rs1801260, rs1464490, rs3749474, rs4864584, rs4580704, rs18012602 and rs3736544. However, rs11943456 was associated with reduced T2D outcomes. *Diet:* In one cross-sectional study, a protective association of wild type CLOCK genes was only established when participants consumed >13.2% of calories from MUFAs (Garaulet *et al.*, 2009) (Table 4.).

Associations / Effects of BMAL Gene Variations on T2D Outcomes: Four BMAL studies found significant associations with T2D outcomes (Tables 3 and 4). SNPs that were associated with increased T2D outcomes were: rs7950226, rs2290035 and rs7958822. Conversely, rs1102275 was associated with reduced T2D outcomes. However, rs7958822 was only significantly associated with T2D in obese participants (Yamaguchi *et al.*, 2015) (Table 4.); and rs2290035 was only associated with FBG in the presence of the polymorphism rs9684900 in the NOC gene (Chang, Chiu and Chuang, 2013) (Table 4.). *Diet:* No significant associations were found between overall dietary habits, BMAL variations and T2D outcomes (Yamaguchi *et al.*, 2015) (Table 4.).

Associations / Effects of PER Gene Variations on T2D Outcomes: Four of six PER studies found significant associations with T2D outcomes (Tables 3, 4 and 5). SNPs that were associated with T2D were: a PER3 exon 18 tandem repeat sequence, rs6744132, and rs10870. However, rs7602358 was associated with reduced T2D.

Associations / Effects of CRY Gene Variations on T2D Outcomes: Two of five CRY studies found significant associations with T2D outcomes (Tables 3, 4, and 5). SNPs that were

associated with increased T2D were rs2287161 and rs11605924. *Diet:* One study determined that high carbohydrate consumption (as determined by greater than median consumption in two cohorts: >/<41.65% Mediterranean / >/<49.14% USA) and the CRY1 SNP rs2287161 was significantly associated with higher levels of IR and FBG compared to the low carbohydrate consumption group (Dashti *et al.*, 2014) (Table 4.).

Associations / Effects of REV-ERBα/β Gene Variations on T2D Outcomes: The only REV-ERBα/β study determined rs38253751 was significantly associated with increased HbA1c amongst the controls, and there was a significant association between rs2102928 and increased FBG (Tokat *et al.*, 2020) (Table 3.).

Discussion

The current systematic review aimed to determine whether variations in CR genes had an association with, or effect on T2D outcomes. The evidence suggests variations in MTNR1B, BMAL and PER are associated with T2D, whilst more evidence is needed for other CR genes, including CLOCK, CRY and REV-ERBα/β. The systematic review found no consistent associations or effects of CR gene variations in combination with diet and sleep on T2D outcomes.

Variations in MTNR1B were consistently associated with increased T2D (Tables 3, 4, and 5). Gain-of-function MTNR1B variations can lead to increased melatonin release, a hormone which regulates the sleep-wake cycle, but which has been associated with increased IR, decreased glucose-stimulated insulin response, and increased T2D risk (Dashti *et al.*, 2020). The SNPs rs10830963, rs10830962 and rs1387153 were consistently associated with T2D

outcomes, whereas rs4753426, rs3781637, rs3781638, rs2166706 and rs3847554 had mixed results or a limited number of studies. More replication is needed for consistent results regarding MTNR1B variations interaction with diet and sleep factors, including shift work, late chronotypes, and early rising. Future MTNR1B studies should investigate location and ethnicity differences, including between Caucasian and Indian, and Shanghai and Beijing populations. Differences between these populations may be due to genetics, lifestyle variables that were uncontrolled during baseline comparisons, or seasonality and light exposure, particularly in the Caucasian population from Northern Europe. The SNPs rs780094 and rs3781638 require further investigation, as they were associated with reduced T2D, which was contrary to the majority of the evidence.

Variations in CLOCK genes had inconsistent links to T2D outcomes (Tables 3, 4, and 5). However, rs1801260 was associated with T2D outcomes in multiple studies (Scott, Carter and Grant, 2008; Garaulet *et al.*, 2009; Li, Wang and Chen, 2020). Other SNPs associated with T2D outcomes that require more replications are rs1464490, rs3749474, rs4864584, rs4580704, rs18012602 and rs3736544. Variations in CLOCK downregulate sirtuin 1 and limit adipocytokine expression which may lead to T2D. However, the SNP rs11943456 requires further investigation, as it was associated with reduced T2D. An area for future research is if sleep and CLOCK variations can modify T2D outcomes, and the effects of MUFA, as one study found there may be a protective effect of high levels of consumption (Garaulet *et al.*, 2009) (Table 4.).

Variations in BMAL were consistently associated with T2D outcomes (Tables 3 and 4). However, replication studies are needed regarding rs7950226, rs2290035 and rs7958822 - all SNPs with an association - as none were repeated across multiple studies. Also, rs1102275

399 requires further study, as it was the only SNP associated with reduced T2D (Kelly *et al.*,
400 2012) (Table 3.). BMAL variations have been associated with lower β-cell function and
401 decreased pancreatic islet development (Kelly *et al.*, 2012; Chang, Chiu and Chuang, 2013).
402 Also, similar to the CLOCK gene, BMAL also regulates sirtuin 1 expression, which has been
403 associated with decreased β-cell function and IR (Li, Wang and Chen, 2020), which may
404 increase T2D outcomes. More studies of variations in BMAL and T2D should be an area of
405 future research, as currently only four have taken place. Further studies involving diet and
406 sleep variables are also required, as currently only one study with a dietary variable has taken
407 place, with no significant findings (Yamaguchi *et al.*, 2015) (Table 4.). Current BMAL
408 studies were also of low quality, due to lack of control of confounders, lack of a clear
409 inclusion and exclusion criteria, poor T2D diagnosis methodology, and possible inclusion of
410 T1D participants (Table 2.). Therefore, current evidence regarding BMAL may have low
411 reliability, and improved quality studies should be a focus of future research.
412
413 The majority of PER studies found associations with T2D outcomes (Tables 3, 4, and 5).
414 However, the variations associated with T2D outcomes – a PER3 exon 18 tandem repeat
415 sequence, rs644123 and rs10870 have not been replicated in multiple studies. Also,
416 rs7602358 requires further investigation, as it was associated with reduced T2D (Kelly *et al.*,
417 2012) (Table 3.). Gain-of-function PER variants may increase risk of T2D via repressing the
418 CLOCK/BMAL heterodimer, which has been associated with reduced pancreatic β-cell
419 function, islet development and insulin release (Englund *et al.*, 2009). Another area for future
420 study is if PER variations interaction with diet and sleep variables modify T2D outcomes, as
421 currently, none have taken place.
422

The majority of CRY studies found no association with T2D outcomes (Tables 3, 4, and 5). However, when rs2287161 was paired with high carbohydrate consumption, it was associated with increased FBG and IR (Dashti *et al.*, 2014) (Table 4.). Also, rs11605924 was associated with increased FBG (Machicao *et al.*, 2016) (Table 4.). The CRY gene can limit gluconeogenesis via downregulating cyclic adenosine monophosphate (cAMP) / cAMP response element binding (CREB) protein signalling, and repressing glucocorticoid receptor and nuclear forkhead box protein O1 (FOXO1) (Rijo-Ferreira and Takahashi, 2019). Reduced expression of CRY due to genetic variations therefore upregulate gluconeogenesis, increasing T2D risk (Rijo-Ferreira and Takahashi, 2019). Future CRY studies should further investigate its associations with high carbohydrate consumption, as this could lead to actionable changes to reduce T2D risk. Also, future studies could investigate CRY variations interactions with sleep variables to modify T2D outcomes, as currently none have taken place.

The singular REV-ERBα/β study found significant associations between rs38253751, rs2102928 and T2D outcomes (Table 3.). Gain-of-function REV-ERBα/β variations increase phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase expression, therefore upregulating hepatic gluconeogenesis (Jakubowicz *et al.*, 2017; Tokat *et al.*, 2020). REV-ERBα/β variations may also increase hepatosteatosis, which may increase T2D risk (Jakubowicz *et al.*, 2017; Tokat *et al.*, 2020). However, only one study regarding REV-ERBα/β variations and T2D has taken place; therefore, more are needed to increase the quality of evidence.

Literature Evaluation

A limitation of current research is a lack of longitudinal studies. All but two included data measurements from only one time point (Reinehr *et al.*, 2011; Lopez Minguez *et al.*, 2016). T2D is a chronic disease that develops over long periods, therefore, longitudinal designs that include variables such as diet and sleep are essential to produce robust results (Forouhi and Wareham, 2014). In addition, a lack of intervention trials (n=2) (Reinehr *et al.*, 2011; Lopez Minguez *et al.*, 2016) mean causal links between CR genes, lifestyle factors and T2D are yet to be established. The majority of included literature was case-control or cross-sectional studies (n=26). A strength of case-control and cross-sectional studies is many variables, including diet, sleep, and participant characteristics can be studied simultaneously. Also, they can generate hypothesis for future intervention studies. However, case-control and cross-sectional studies are liable to suffer recall bias and cannot establish cause and effect. Another limitation was that data regarding diet and sleep were frequently recorded via questionnaire. Questionnaire data is susceptible to bias and may limit reliability of results (Resnicow *et al.*, 2000). Intervention studies with more strict controls over diet and sleep are therefore required.

Another limitation of current research was thirteen studies only found differences in metabolic traits, rather than T2D incidence. Therefore, the associations / effects of CR gene variations on T2D risk cannot be established. For example, one study determined rs10830963 was associated with higher FBG and HbA1c, but not increased T2D risk (Semiz *et al.*, 2014). This suggests the overall effect size of variations in CR genes and T2D outcomes may be negligible, reducing clinical and practical relevance. Another limitation was a number of studies included participants taking T2D medication. This may have confounded studies that measured T2D status via metabolic phenotypes. However, excluding all participants taking T2D medication would have been too limiting for the current systematic review. A strength

of current literature was similar male (51.5%) female (49.5%) inclusion, as results can be generalised to both sexes.

An interesting finding was the BMAL and PER SNPs (rs1102275 / rs7602358) associated with reduced T2D were in the same study, which had a critical ROB due to a lack of inclusion / exclusion criteria, lack of description of T2D diagnosis methods, and possible T1D participant inclusion (Kelly *et al.*, 2012) (Tables 1 and 3.). Therefore, these SNPs require further investigation to confirm their interaction with T2D.

Another area for future research is increased replication in east and south-east Asian, and Caucasian European populations, as current literature indicates populations in these locations may have significantly different outcomes to variations in CR genes (Chambers *et al.*, 2009; Liu *et al.*, 2010).

Conclusion

T2D is a chronic disease that places a huge burden on individual's lives and health services globally, which is forecast to worsen in coming years. Recently, the effects and associations between genetic variations in CR genes and T2D have been investigated. This novel systematic review aimed to assess the current literature to determine CR genes associations and/or effects on T2D, as well as the modifying effects of diet and sleep. The results of this systematic review suggest consistent associations of variations in MTNR1B, BMAL and PER with T2D outcomes. For confirmatory results, and before practical and clinical recommendations can be made, further longitudinal and intervention studies and further diet and sleep studies need to be performed.

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Availability of Data and Materials

Supplementary data is published on the Figshare repository platform.

Consent for Publication

All authors have expressed consent for publication.

Ethical Approval

All reported studies including human participants, human data, or human tissue include a statement on ethical approval and consent. This systematic review was registered with PROSPERO on 13/8/2021 (registration: CRD42021259682).

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