SUPPLEMENTARY DATA

Online Supplementary Material

Insulin-like growth factor binding protein 2 (IGFBP-2) and the risk of developing type 2 diabetes

Clemens Wittenbecher, PhD, Meriem Ouni, PhD, Olga Kuxhaus, MSc, Markus Jähnert, MSc, Pascal Gottmann, MSc, Andrea Teichmann, Karina Meidtner, PhD, Jennifer Kriebel, PhD, Tobias Pischon, MD, MPH, Heiner Boeing, PhD, Matthias B. Schulze, DrPH, Annette Schürmann, PhD

Supplementary Note 1

Analysis of cis regulatory elements of *IGFBP2* gene

For the identification of putative regulatory elements located within or in close proximity to the *IGFBP-2* gene, hepatic H3K27ac, H3K4me3, H3K9ac histone marks known to be associated with active promoters were downloaded from the ENCODE-Project. BAM-files were converted to bed files using BEDTools and then filtered for chromosomes. Bed-files were loaded into R (version 3.4.3) by use of Gviz (version 1.22.3), rtracklayer (version 1.38.3) and chipseq (version 1.28.0) packages. Chip-Seq scores were calculated within a size of 100 base pairs and plotted with the plot Tracks function. The resulting tracks of the single tissues were overlapped with help of Gimp (version 2.8).

SUPPLEMENTARY DATA

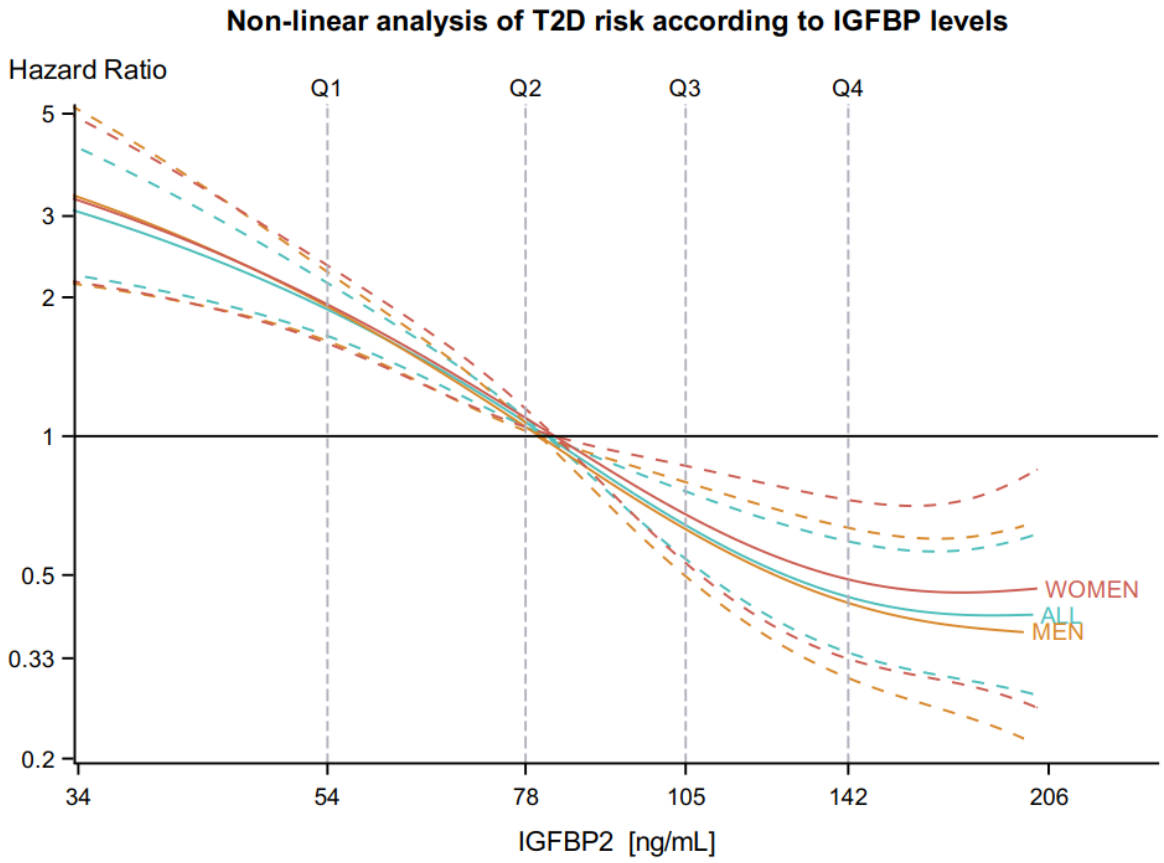
Supplementary Table 1. Baseline characteristics in cases and controls of the nested case-control sample with methylation data.

All (n=580)	**Controls** n=290	**Cases** n=290
IGFBP-2 [ng/ml]	100 (67, 142)	60 (44, 87)
Gender (% female)	48	48
Age at baseline [y]	56 (49, 60)	56 (49, 61)
BMI [kg/m²]	26 (24, 28)	30 (27, 33)
Waist [cm]	89 (81, 95)	100 (92, 107)
Smoking Status [%]		
never smoker	47	33
ex-smoker, < 20 u./d.	23	26
ex-smoker, ≥ 20 u./d.	11	19
smoker, < 20 u./d.	13	11
smoker, ≥ 20 u./d.	7	12
Education [%]		
vocational training or lower	37	47
technical college	24	22
university	39	31
Alcohol [g/day]	9.7 (3.3, 22.3)	8.2 (2.5 19.6)
FLI [%]	37 (13, 62)	79 (54, 90)
IGF-1 [ng/ml]	157 (126, 197)	153 (125, 194)
IGFBP-3 [µg/ml]	3 (2.7, 3.5)	3.1 (2.7, 3.5)
Triglyceride [mg/dl]	117 (84, 168)	168 (123, 241)
Fetuin [µg/ml]	257 (223, 302)	279 (236, 317)
GPT [U/L]	22 (16, 30)	29.5 (21, 42)
gamma-GT [U/L]	20 (13, 36)	34 (21, 53)
Adiponectin [µg/ml]	7.6 (5.4, 9.8)	5.3 (4, 7.5)
Glucose [mg/dl]	103 (95, 113)	118 (105, 140)
HbA1c [%]	5.4 (5.1, 5.7)	6.1 (5.7, 6.7)

Comparison of the baseline characteristics between randomly selected incident type 2 diabetes cases and 1:1 matched controls; matching criteria were age (±6 months), sex, and fasting time (<3h, 3h to <6h, and ≥6 h before blood draw), time of day of blood sampling (±2 h), and season at blood sampling.

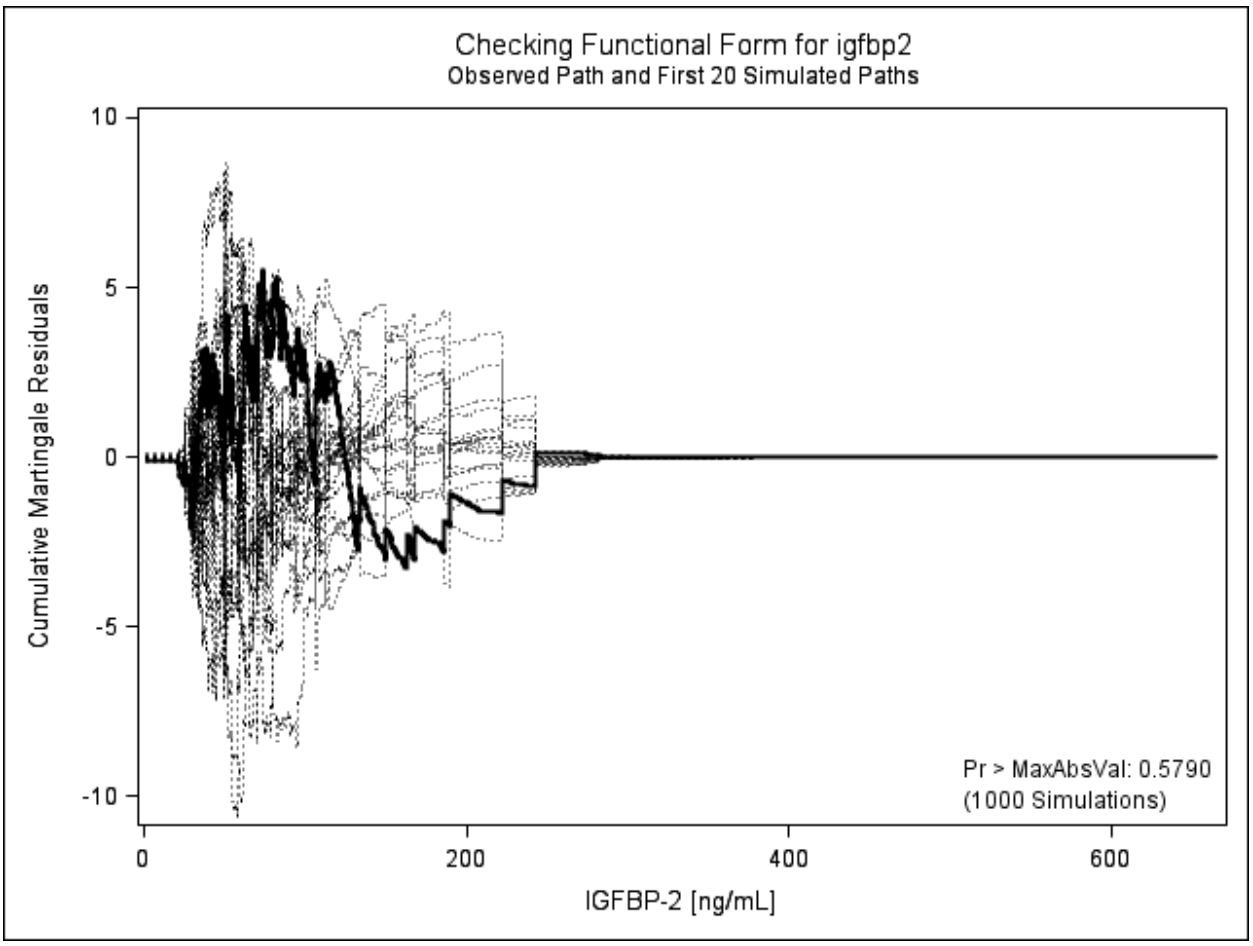
SUPPLEMENTARY DATA

Supplementary Figure 1. Restricted cubic spline analysis of the non-linear relation between circulating IGFBP2 concentrations and type 2 diabetes (Q1-Q4: Quintiles of the IGFBP2 distribution)



SUPPLEMENTARY DATA

Supplemental Figure 2. Testing potential interactions with follow-up time



Supremum Test for Functional Form

Variable	*Replications*	*Seed*	*Pr > MaxAbsVal*
igfbp2	1000	48680156	0.579

SUPPLEMENTARY DATA

Supplemental Figure 3. Checking proportional hazards assumption