Detailed Physiologic Characterization Reveals Diverse Mechanisms for Novel Genetic Loci Regulating Glucose and Insulin Metabolism in Humans: Supplementary Notes and Tables

Supplementary Note

Evaluation of OGTT-Derived Insulin Sensitivity Indices

In order to confirm the physiological validity of the various insulin sensitivity indices, we examined the correlations among them and in reference to the gold standard (euglycemic clamp-derived M/I) in non-diabetic participants in the ULSAM and RISC cohorts. The correlations of OGTT-derived measures of insulin sensitivity with M/I were highly significant (all $P<0.0001$) and ranged from 0.67 to 0.76 among 922 individuals in ULSAM who did not have diabetes (**Supplementary Table 4**). As expected, all four OGTT-derived insulin sensitivity indexes were also highly correlated with each other. These indices were less well correlated (r ranging from -0.54 to -0.59) with the commonly used fasting measure of insulin resistance by homeostasis model assessment (HOMA-IR) (14), with the exception of the Matsuda index (r=-0.88). This likely reflects the more dynamic assessment of insulin sensitivity in extrahepatic tissues provided by the OGTT. As expected, these correlations were inverse since HOMA-IR is a measure of insulin *resistance*, whereas the other indices are measures of insulin *sensitivity*. Of note, the correlation between HOMA-IR and euglycemic-clamp derived insulin sensitivity (r=-0.53) was clearly weaker than that of all the other OGTT-derived measures of insulin sensitivity. The strength of the correlation between OGTT-derived measures of insulin sensitivity and M/I, though still statistically significant, was lower in the 1,319 participants of the RISC cohort (r=0.49, 0.44, 0.49 and 0.36 for the Stumvoll, Matsuda, Belfiore and Gutt indices, respectively).

For the derived insulin sensitivity indices, we performed two additional validation analyses to accommodate cohorts in which the 2-hour OGTT had been performed with multi-point sampling, but critical sampling points had not been obtained for a given index. First, we examined the effects of using alternate intermediate time points (30 or 60 minutes instead of 90 minutes) in the calculation of the Stumvoll index. In ULSAM, where all time points had been collected, the correlation between the original Stumvoll index (with the intermediate time point set at 90 minutes) and the modified Stumvoll indices (with the intermediate time points set at 30 or 60 minutes) were 0.96 and 0.98 respectively, providing reassurance that the original and modified indices were largely interchangeable. Second, we examined the effect of calculating the Matsuda index with only three measurements of glucose and insulin, instead of five. In ULSAM, the correlation between these two ways of calculating the Matsuda index was 0.99, again reassuring us that indices calculated from 3- and 5-point OGTTs were largely comparable. Also, the correlations of these two alternative indices (modified Stumvoll and Matsuda) with euglycemic-derived insulin sensitivity were almost identical with the original versions of the indices (0.7554 vs. 0.7627 or 0.7490 [Stumvoll with 30 or 60 minutes measurement] and 0.7057 vs. 0.7048 [Matsuda], for original vs. modified, respectively).

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Author Contributions

Writing group: E.I., C.Lan., M-F.H., I.P., V.L., J.D., J.B.M., M.I.M., L.G., R.M.W., J.C.F.

Project design, management and coordination: (Botnia) L.G.; (DIAGEN) S.R.B., P.S.; (Ely) N.J.W.; (ENGAGE) M.I.M.; (Framingham Heart Study) J.B.M.; (FUSION) M.B., L.J.S., R.N.B., F.S.C., K.L.M., J.T., R.M.W.; (Hertfordshire) C.C.C.; (METSIM) J.K., M.L.; (NHANES III) J.B.M.; (Partners/Roche) J.B.M.; (PIVUS) E.I.; (RISC) E.F.; (Sorbs) M.S.; (Stanford IST) T.Q.; (ULSAM) E.I.

Sample collection and phenotyping: (Botnia) B.I., T.T., L.G.; (DIAGEN) A.B., J.G., P.S.; (Ely) N.J.W.; (Framingham Heart Study) J.B.M., C.S.F.; (FUSION) R.N.B., T.A.B., J.T., T.T.V.; (Hertfordshire) C.C.C., E.M.D., K.A.J., A.A.S.; (METSIM) J.K., M.L.; (NHANES III) J.B.M.; (Partners/Roche) D.M.N., G.H.W., J.B.M.; (PIVUS) B.Z., L.L.; (RISC) J.R.P., M.W.; (Sorbs) P.K., A.T.; (Stanford IST) F.A.A., T.Q.; (ULSAM) E.I., B.Z., C.B.

Genotyping: (Botnia) V.L.; (DIAGEN) P.C.; A.J.S.; (Ely) C.Lan., S.B., F.P., I.B., N.J.W.; (Framingham Heart Study) J.C.F.; (FUSION) L.L.B., M.R.E.; (Hertfordshire) C.Lan., S.B., F.P., I.B., N.J.W.; (METSIM) M.A.M., N.N.; (NHANES III) J.C.F.; (Partners/Roche) J.C.F.; (PIVUS) E.I., A-C.S., L.L.; (RISC) L.P., M.W.; (Sorbs) Y.B., P.K.; (Stanford IST) T.L.A., J.W.K., K.H.; (ULSAM) E.I., B.Z., C.B., A-C.S.

Data analysis: (Botnia) V.L., C.Lad.; (DIAGEN) A.U.J., H.M.S.; (Ely) C.Lan., S.S.; (Framingham Heart Study) M-F.H., J.D.; (FUSION) A.U.J., H.M.S.; (Hertfordshire) C.Lan., S.S.; (METSIM) A.U.J., H.M.S.; (NHANES III) P.S.; (Partners/Roche) P.S.; (PIVUS) E.I.;

(RISC) C.L., S.S.; (Sorbs) I.P., R.M.; (Stanford IST) T.L.A., J.W.K., F.A.A., K.H.; (ULSAM) E.I.

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V.L. is a consultant for Tethys Bioscience. K.H. is an employee at Merck & Co. I.B. and spouse own stock in Incyte and GlaxoSmithKline. J.B.M. currently has research grants from GlaxoSmithKline and sanofi-aventis, and serves on consultancy boards for Eli Lilly and Interleukin Genetics. R.M.W. has received consulting honoraria from Merck & Co., receives research funding from Merck & Co., and receives research material support from Takeda Pharmaceuticals North America. J.C.F. has received consulting honoraria from Merck, Pfizer, bioStrategies, XOMA and Publicis Healthcare Communications Group, a global advertising agency engaged by Amylin Pharmaceuticals.

RISC investigators and recruiting centers

Amsterdam, The Netherlands: RJ Heine, J Dekker, S de Rooij, G Nijpels, W Boorsma
Athens, Greece: A Mitrakou, S Tournis, K Kyriakopoulou, P Thomakos
Belgrade, Serbia: N Lalic, K Lalic, A Jotic, L Lukic, M Civcic
Dublin, Ireland: J Nolan, TP Yeow, M Murphy, C DeLong, G Neary, MP Colgan, M Hatunic
Frankfurt, Germany: T Konrad, H Böhles, S Fuellert, F Baer, H Zuchhold
Geneva, Switzerland: A Golay, E Harsch Bobbioni,V. Barthassat, V. Makoundou, TNO Lehmann, T Merminod
Glasgow, Scotland: JR Petrie (now Dundee), C Perry, F Neary, C MacDougall, K Shields, L Malcolm
Kuopio, Finland: M Laakso, U Salmenniemi, A Aura, R Raisanen, U Ruotsalainen, T Sistonen, M Laitinen, H Saloranta
London, England: SW Coppack, N McIntosh, J Ross, L Pettersson, P Khadobaksh
Lyon, France: M Laville, F. Bonnet (now Rennes), A Brac de la Perriere, C Louche-Pelissier, C Maitrepierre, J Peyrat, S Beltran, A Serusclat
Madrid, Spain: R. Gabriel, EM Sánchez, R. Carraro, A Friera, B. Novella
Malmö, Sweden (1): P Nilsson, M Persson, G Östling, (2): O Melander, P Burri
Milan, Italy: PM Piatti, LD Monti, E Setola, E Galluccio, F Minicucci, A Colleluori
Newcastle-upon-Tyne, England: M Walker, IM Ibrahim, M Jayapaul, D Carman, C Ryan, K Short, Y McGrady, D Richardson, L Pascoe
Odense, Denmark: H Beck-Nielsen, P Staehr, K Hojlund, V Vestergaard, C Olsen, L Hansen
Perugia, Italy: GB Bolli, F Porcellati, C Fanelli, P Lucidi, F Calcinaro, A Saturni
Pisa, Italy: E Ferrannini, A Natali, E Muscelli, S Pinnola, M Kozakova, A Casolaro, BD Astiarraga
Rome, Italy: G Mingrone, C Guidone, A Favuzzi. P Di Rocco
Vienna, Austria: C Anderwald, M Bischof, M Promintzer, M Krebs, M Mandl, A Hofer, A Luger, W Waldhäusl, M Roden

Project Management Board: B Balkau (Villejuif, France), SW Coppack (London, England), JM Dekker (Amsterdam, The Netherlands), E Ferrannini (Pisa, Italy), A Mari (Padova, Italy), A Natali (Pisa, Italy), M Walker (Newcastle, England)

RISC core laboratories and reading centres
Lipids Dublin, Ireland: P Gaffney, J Nolan, G Boran
Hormones Odense, Denmark: C Olsen, L Hansen, H Beck-Nielsen

Albumin:creatinine Amsterdam, The Netherlands: A Kok, J Dekker
Genetics Newcastle-upon-Tyne, England: S Patel, M Walker
Stable isotope laboratory Pisa, Italy: A Gastaldelli, D Ciociaro
Adiponectin, CRP , MBL Odense, Denmark: Allan Flyvbjerg
Ultrasound reading centre Pisa, Italy: M Kozakova
ECG reading, Villejuif, France: MT Guillanneuf
Actigraph, Villejuif, France: B Balkau, L Mhamdi
Data Management Villejuif, France, Padova, and Pisa, Italy**:** B Balkau, A Mari, L Mhamdi, L Landucci, S Hills, L Mota
Mathematical modelling and website management Padova, Italy: A Mari, G Pacini, C Cavaggion
Coordinating office: Pisa, Italy: SA Hills, L Landucci. L Mota

Further information on the RISC Study and participating centres can be found on www.egir.org.

Supplementary Table 1: Cohort Descriptions and Baseline Characteristics

and

Supplementary Table 5: Associations of 19 SNPs previously associated with fasting glucose, fasting insulin and/or 2-h glucose on detailed physiologic measures of insulin processing, secretion and sensitivity – complete results including numbers in each analysis

Are available as online Excel files.

Supplementary Table 2: Genetic Markers Included in the Present Study

Lead SNP	Nearest gene	Effect allele*	Non-effect allele	Freq†	Proxy used	r^2 with lead SNP‡	Studies where the proxy was used
Glucose/HOMA-B selected SNPs							
rs560887	*G6PC2*	C	T	0.70	rs573225	0.96	NHANES, Partners/Roche
rs10830963	*MTNR1B*	G	C	0.29			
rs4607517	*GCK*	A	G	0.19	rs917793	1	Ely, Hertfordshire, NHANES, Partners/Roche
rs2191349	*DGKB*	T	G	0.52			
rs780094	*GCKR*	C	T	0.61	rs1260326	0.93	BotniaPPP, RISC
rs11708067	*ADCY5*	A	G	0.79	rs2877716	0.82	RISC
rs7944584	*MADD*	A	T	0.74			
rs10885122	*ADRA2A*	G	T	0.88			
rs11605924	*CRY2*	A	C	0.49			
rs174550	*FADS1*	T	C	0.64			
rs340874	*PROX1*	C	T	0.52			
rs11920090	*SLC2A2*	T	A	0.84			
rs7034200	*GLIS3*	A	C	0.50			
rs13266634	*SLC30A8*	C	T	0.64	rs11558471	0.96	BotniaPPP, RISC
rs7903146	*TCF7L2*	T	C	0.31			
rs11071657	*FAM148B*	A	G	0.63	rs11635220	1	RISC
Insulin/HOMA-IR selected SNPs							
rs35767	*IGF1*	G	A	0.81	rs855228	0.92	DIAGEN, FUSION, METSIM, NHANES, Partners/Roche
2 hour glucose selected SNPs							
rs10423928	*GIPR*	A	T	0.18	rs11672660	0.95	Ely, Hertfordshire
rs17271305	*VPS13C*	G	A	0.42			

* Effect allele was defined as the 2-h glucose-raising alleles for *GIPR* rs10423928 and *VPS13C* rs17271305; the fasting insulin-raising allele for *IGF1* rs35767; and the fasting glucose-raising allele for all other SNPs.

† Effect allele frequencies from the initial MAGIC discovery studies (1, 2)

‡ r^2 values based on HapMap CEU, release 22

Supplementary Table 3: Definitions of Insulin Sensitivity Index Equations Used in the Present Study

Index	Equation	Reference
Belfiore	2/[({[0.5*fasting PG (mmol/L)] + OGTT 1-h PG + (0.5*OGTT 2-h PG)} / 11.36) * ({[0.5*fasting PI (pmol/L)] + OGTT 1-h PI + (0.5*OGTT 2-h PI)} / 638) + 1]	(3)
Gutt	{75000 + [fasting PG (mg/L) - OGTT 2-h PG] * 0.19 * body weight (kg)/120} / [(fasting PG + OGTT 2-h PG) / 2] / $\log_{10}${[fasting PI (mU/L) + OGTT 2-h PI] / 2}	(4)
Matsuda	10 000√{fasting PG (mg/dL) * fasting PI (μU/mL) * [mean OGTT PG * mean OGTT PI]}	(5)
Stumvoll	0.226 - [0.0032 * BMI (kg/m^2)] - [0.0000645 * OGTT 2-h PI (pmol/L)] - [0.00375 * OGTT 1.5-h PG (mmol/L)]	(6)

Abbreviations: PG, plasma glucose; OGTT, oral glucose tolerance test; PI, plasma insulin;

Supplementary Table 4: Correlations of Insulin Sensitivity Indices from Oral Glucose Tolerance Tests with Euglycemic Clamp-Derived Insulin Sensitivity

	M/I	BMI	Fasting glucose	Fasting insulin	HOMA-IR	Belfiore	Gutt	Matsuda	Stumvoll
Uppsala Longitudinal Study of Adult Men (ULSAM; n=922)[*]									
M/I	1.0000								
BMI	-0.5609	1.0000							
Fasting glucose	-0.3144	0.2974	1.0000						
Fasting insulin	-0.4954	0.4139	0.2231	1.0000					
HOMA-IR	-0.5259	0.4461	0.3924	0.9794	1.0000				
Belfiore	0.6970	-0.4269	-0.3999	-0.5508	-0.5944	1.0000			
Gutt	0.6702	-0.3976	-0.5240	-0.4680	-0.5401	0.7373	1.0000		
Matsuda	0.7057	-0.4991	-0.4369	-0.8391	-0.8756	0.8827	0.7387	1.0000	
Stumvoll	0.7554	-0.6638	-0.4395	-0.5023	-0.5548	0.7921	0.8971	0.7857	1.0000
RISC (n=1,319)[*]									
M/I	1.0000								
BMI	-0.3689	1.0000							
Fasting glucose	-0.1080	0.2771	1.0000						
Fasting insulin	-0.3920	0.5404	0.2904	1.0000					
HOMA-IR	-0.3840	0.5438	0.4580	0.9777	1.0000				
Belfiore	0.4908	-0.4345	-0.3369	-0.6195	-0.6314	1.0000			
Gutt	0.3637	-0.3267	-0.4275	-0.4738	-0.5094	0.7142	1.0000		
Matsuda	0.4393	-0.4827	-0.4179	-0.7071	-0.7086	0.7850	0.7443	1.0000	
Stumvoll	0.4888	-0.7553	-0.3078	-0.6399	-0.6473	0.7333	0.6582	0.6441	1.0000

All values are Spearman correlation coefficients. All correlations were significant (P<0.0001).
[*] The number of individuals in the correlation matrix is slightly lower than in the main analyses, since the correlation analyses were only performed in individuals with data on all variables.

Supplementary Table 6: Associations of 19 SNPs on fasting split proinsulin levels in 3 studies (Ely, Hertfordshire, ULSAM)

Lead SNP*	Nearest gene	Effect allele†	Non-effect allele	Effect allele freq	N	Beta	SE	P-value
Glucose/HOMA-B selected SNPs								
rs560887	*G6PC2*	C	T	0.69	3444	0.0004	0.0152	0.98
rs10830963	*MTNR1B*	G	C	0.27	3934	-0.0066	0.0149	0.66
rs4607517	*GCK*	A	G	0.17	3573	-0.0016	0.0186	0.93
rs2191349	*DGKB*	T	G	0.53	3437	0.0075	0.0141	0.59
rs780094	*GCKR*	C	T	0.63	3511	0.0092	0.0147	0.53
rs11708067	*ADCY5*	A	G	0.76	3519	0.0384	0.0161	0.017
rs7944584	*MADD*	A	T	0.74	3531	0.1291	0.0156	1.24e-16
rs10885122	*ADRA2A*	G	T	0.88	3513	-0.0177	0.0213	0.41
rs11605924	*CRY2*	A	C	0.48	3557	0.0186	0.0137	0.18
rs174550	*FADS1*	T	C	0.66	3524	0.0033	0.0148	0.82
rs340874	*PROX1*	C	T	0.55	3524	0.0145	0.0139	0.30
rs11920090	*SLC2A2*	T	A	0.87	3537	-0.0395	0.0209	0.059
rs7034200	*GLIS3*	A	C	0.48	3543	-0.0062	0.0139	0.66
rs13266634	*SLC30A8*	C	T		n.a.			
rs7903146	*TCF7L2*	T	C	0.26	908	0.0332	0.0317	0.29
rs11071657	*FAM148B*	A	G	0.64	3490	0.0087	0.0147	0.56
Insulin/HOMA-IR selected SNPs								
rs35767	*IGF1*	G	A	0.85	3575	0.0344	0.0196	0.078
2 hour glucose selected SNPs								
rs10423928	*GIPR*	A	T	0.21	3464	0.0342	0.0172	0.047
rs17271305	*VPS13C*	G	A	0.40	879	-0.0273	0.0294	0.35

* Proxy rs917793 used for rs4607517, and proxy rs11672660 used for rs10423928 in Ely and Hertfordshire studies.

† Effect allele was defined as the 2-h glucose-raising alleles for GIPR rs10423928 and VPS13C rs17271305; the fasting insulin-raising allele for IGF1 rs35767; and the fasting glucose-raising allele for all other SNPs.

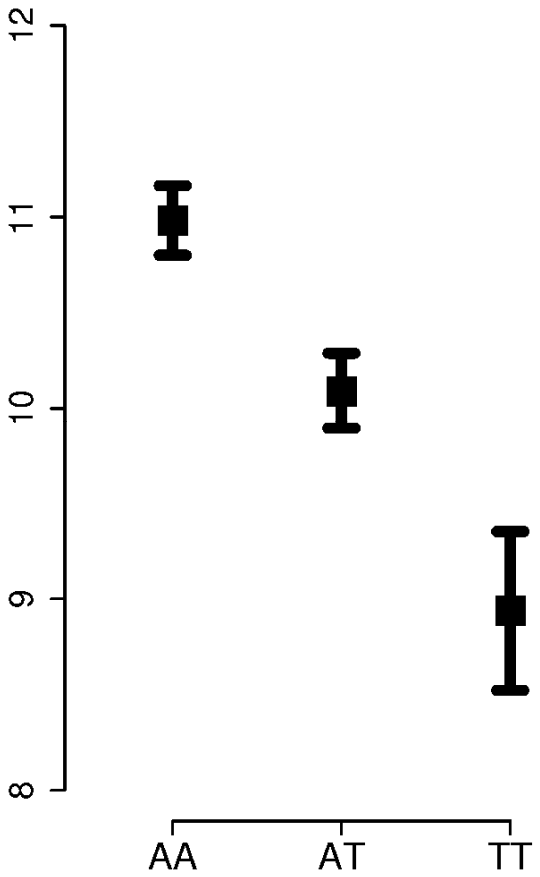
Supplementary Table 7: Associations of 19 SNPs on fasting C-peptide levels in 7 studies (BotniaPPP, DIAGEN, Ely, FUSION, NHANES, RISC, and SORBS)

Lead SNP*	Nearest gene	Effect allele†	Non-effect allele	Effect allele freq	N	Beta	SE	P-value
Glucose/HOMA-B selected SNPs								
rs560887	*G6PC2*	C	T	0.70	6775	0.0019	0.0073	0.80
rs10830963	*MTNR1B*	G	C	0.29	7044	-0.0029	0.0071	0.69
rs4607517	*GCK*	A	G	0.13	7158	-0.0093	0.0101	0.36
rs2191349	*DGKB*	T	G	0.53	6958	-0.006	0.0063	0.34
rs780094	*GCKR*	C	T	0.61	7138	0.0168	0.0064	0.0086
rs11708067	*ADCY5*	A	G	0.80	7013	-0.0025	0.0082	0.76
rs7944584	*MADD*	A	T	0.75	6977	0.0098	0.0075	0.19
rs10885122	*ADRA2A*	G	T	0.88	6978	-0.0014	0.0099	0.89
rs11605924	*CRY2*	A	C	0.49	7071	0.0002	0.0063	0.98
rs174550	*FADS1*	T	C	0.66	6973	-0.0033	0.0068	0.63
rs340874	*PROX1*	C	T	0.50	7093	0.0074	0.0063	0.24
rs11920090	*SLC2A2*	T	A	0.80	6984	-0.0133	0.0093	0.15
rs7034200	*GLIS3*	A	C	0.54	4979	-0.0058	0.0069	0.39
rs13266634	*SLC30A8*	C	T	0.69	3232	-0.0049	0.0082	0.55
rs7903146	*TCF7L2*	T	C	0.25	4934	-0.0256	0.0083	0.0020
rs11071657	*FAM148B*	A	G	0.61	5531	-0.0112	0.0077	0.15
Insulin/HOMA-IR selected SNPs								
rs35767	*IGF1*	G	A	0.83	6972	-0.001	0.0085	0.91
2 hour glucose selected SNPs								
rs10423928	*GIPR*	A	T	0.23	6877	-0.0015	0.0092	0.87
rs17271305	*VPS13C*	G	A	0.42	3399	0.0072	0.008	0.37

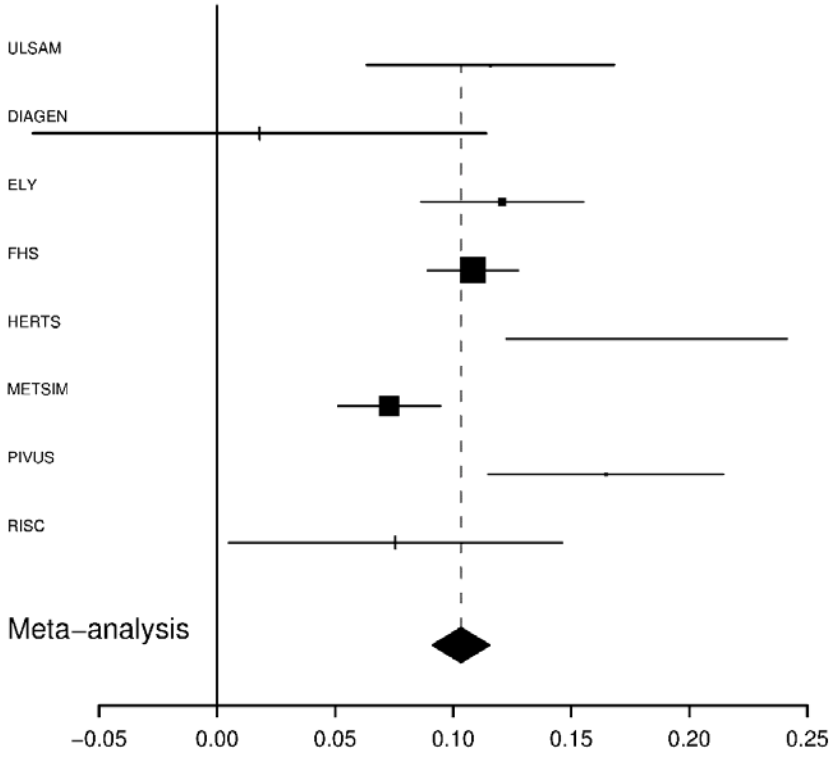
* Proxy rs855228 used for rs35767 in DIAGEN, FUSION and NHANES; proxy rs573225 used for rs560887 in NHANES; proxy rs917793 used for rs4607517 in NHANES and Ely; proxy rs1260326 used for rs780094 in RISC and BotniaPPP; proxy rs11558471 used for rs13266634 in RISC and BotniaPPP; proxy rs2877716 used for rs11708067 in RISC; proxy rs11672660 used for rs10423928 in Ely.

† Effect allele was defined as the 2-h glucose-raising alleles for GIPR rs10423928 and VPS13C rs17271305; the fasting insulin-raising allele for IGF1 rs35767; and the fasting glucose-raising allele for all other SNPs.

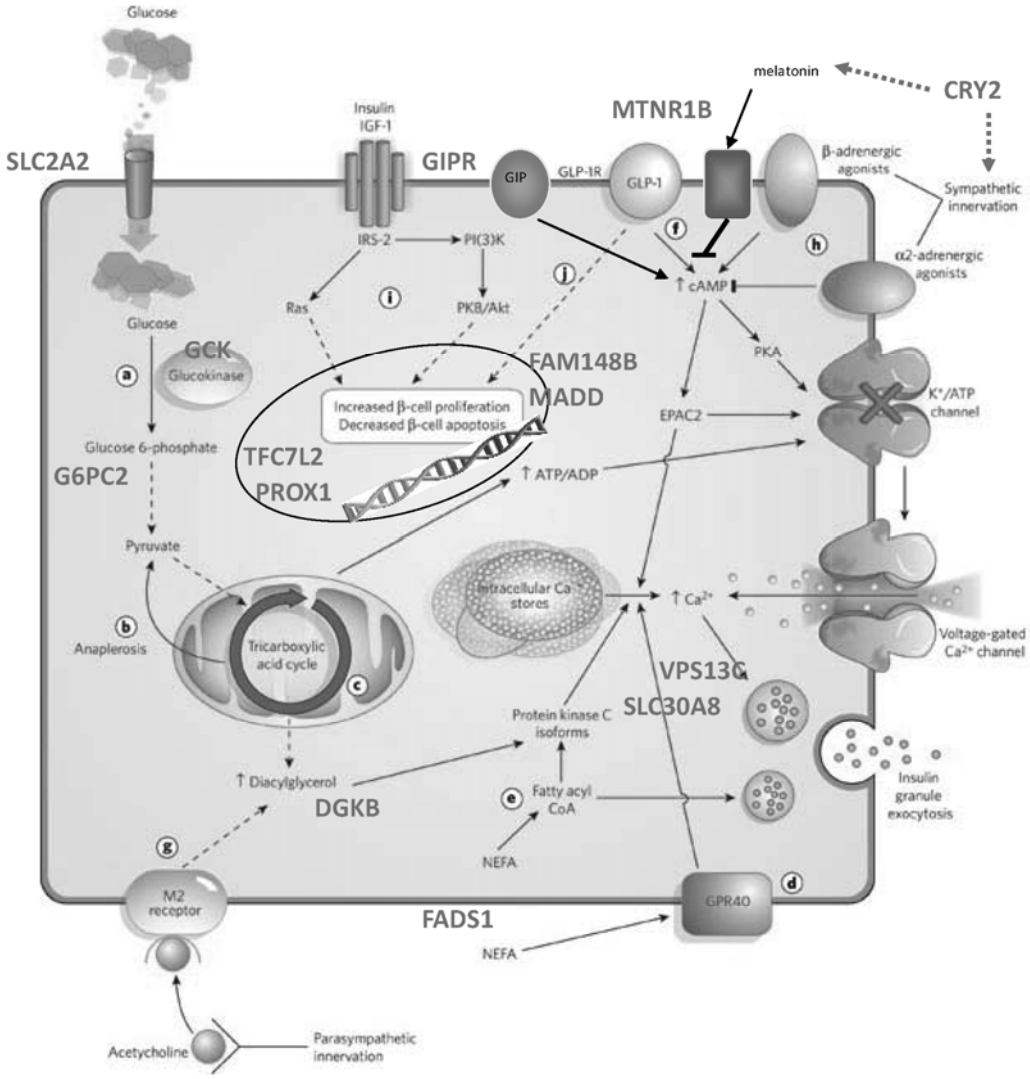
Supplementary Figure 1. Fasting proinsulin level (pmol/l) by MADD rs7944584 genotype adjusted for age, sex and fasting insulin in Framingham Heart Study* [uploaded separately]



Supplementary Figure 2. Forest plot for MADD rs7944584 associated with fasting proinsulin level (pmol/l) after adjustment for age, sex, study-specific covariates and fasting insulin. Individual studies (boxes) are plotted against the individual per-allele effect sizes. The size of the box is inversely proportional to the variance. Horizontal lines are the 95% confidence interval. The y-axis shows the value for no effect (beta coefficient=0) [uploaded separately]



Supplementary Figure 3. Suggestive mechanisms by which some of the examined genetic loci could influence glycemic regulation and thereby risk for type 2 diabetes [uploaded separately]



References

1. Dupuis, J., Langenberg, C., Prokopenko, I., Saxena, R., Soranzo, N., Jackson, A.U., Wheeler, E., and Glazer, N.L. 2009. Novel genetic loci implicated in fasting glucose homeostasis and their impact on related metabolic traits. *Submitted*.
2. Saxena, R., Hivert, M.-F., Langenberg, C., Tanaka, T., Pankow, J.S., Vollenweider, P., Lyssenko, V., and Bouatia-Naji, N. 2009. Genetic Variation in Gastric Inhibitory Polypeptide Receptor (GIPR) Impacts the Glucose and Insulin Responses to an Oral Glucose Challenge. *Submitted*.
3. Belfiore, F., Iannello, S., and Volpicelli, G. 1998. Insulin sensitivity indices calculated from basal and OGTT-induced insulin, glucose, and FFA levels. *Mol Genet Metab* 63:134-141.
4. Gutt, M., Davis, C.L., Spitzer, S.B., Llabre, M.M., Kumar, M., Czarnecki, E.M., Schneiderman, N., Skyler, J.S., and Marks, J.B. 2000. Validation of the insulin sensitivity index (ISI(0,120)): comparison with other measures. *Diabetes Res Clin Pract* 47:177-184.
5. Matsuda, M., and DeFronzo, R.A. 1999. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care* 22:1462-1470.
6. Stumvoll, M., Mitrakou, A., Pimenta, W., Jenssen, T., Yki-Jarvinen, H., Van Haeften, T., Renn, W., and Gerich, J. 2000. Use of the oral glucose tolerance test to assess insulin release and insulin sensitivity. *Diabetes Care* 23:295-301.

