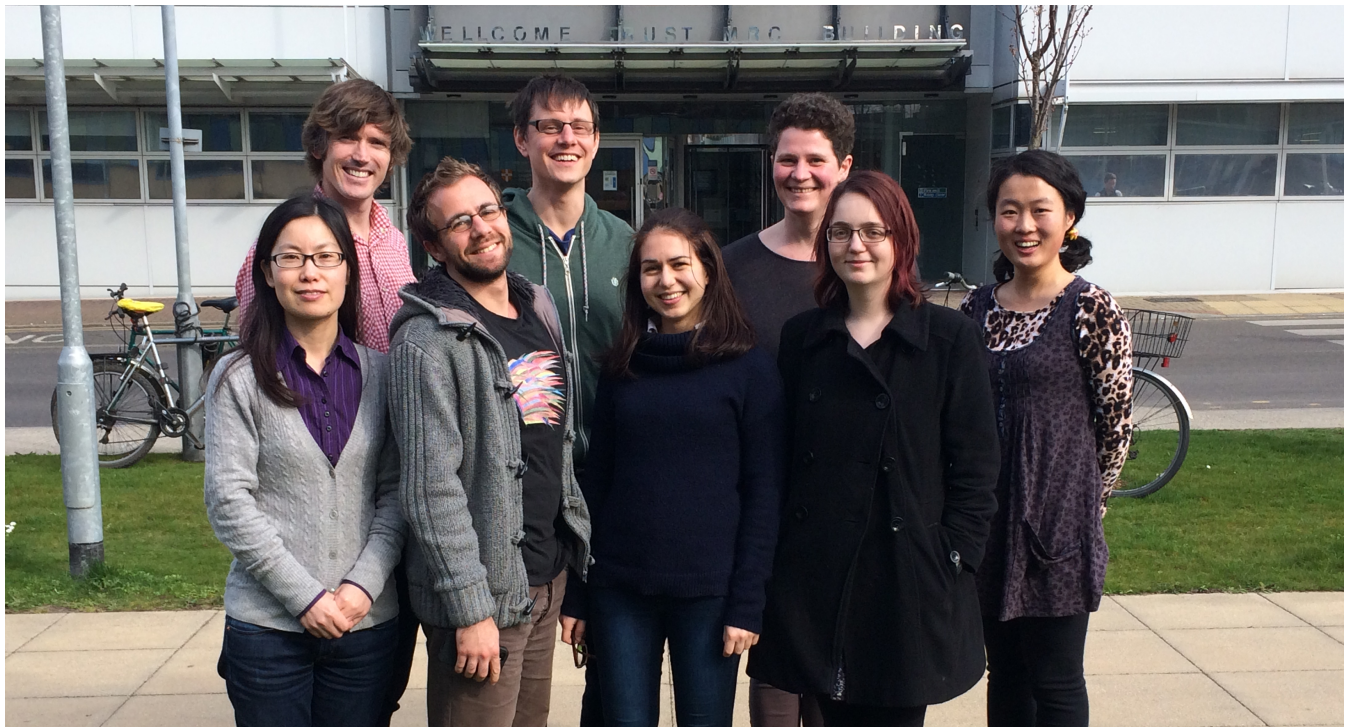
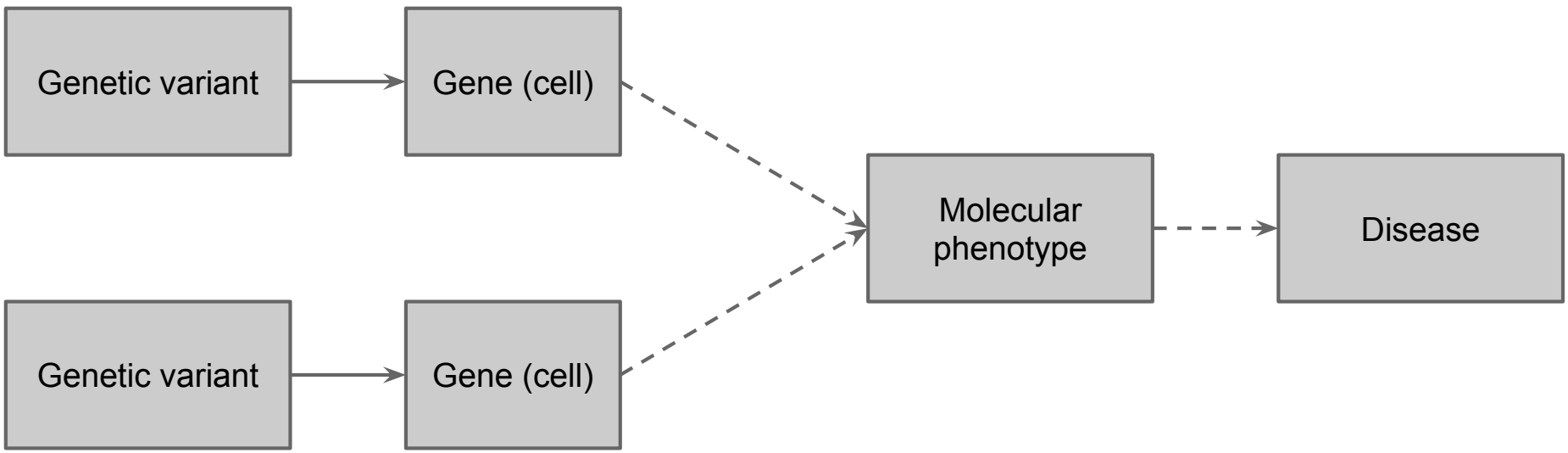
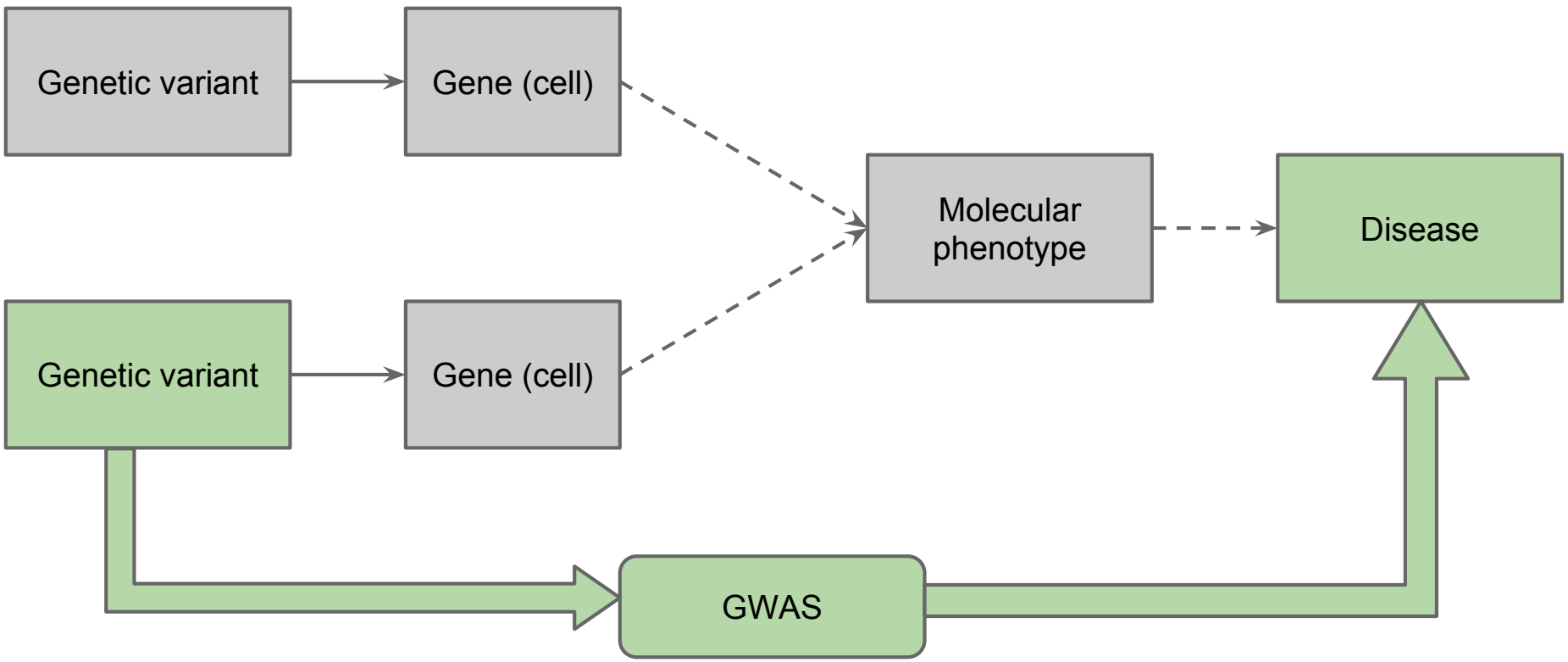
Integrating genetic association and gene expression to identify causal genes in type 1 diabetes

Chris Wallace




Genetic variant
Gene (cell)
Genetic variant
Gene (cell)
Molecular phenotype
Disease


Genetic variant
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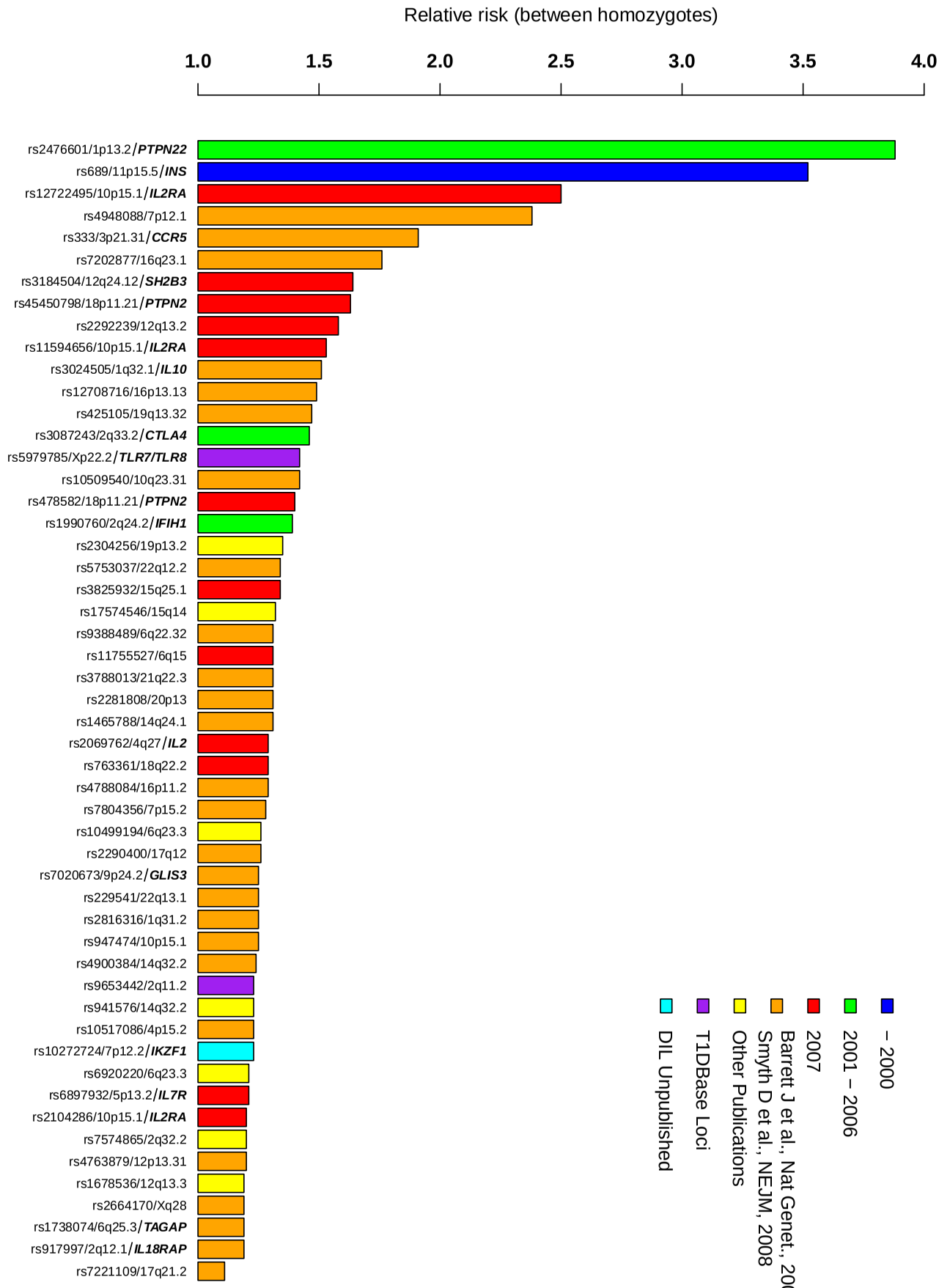
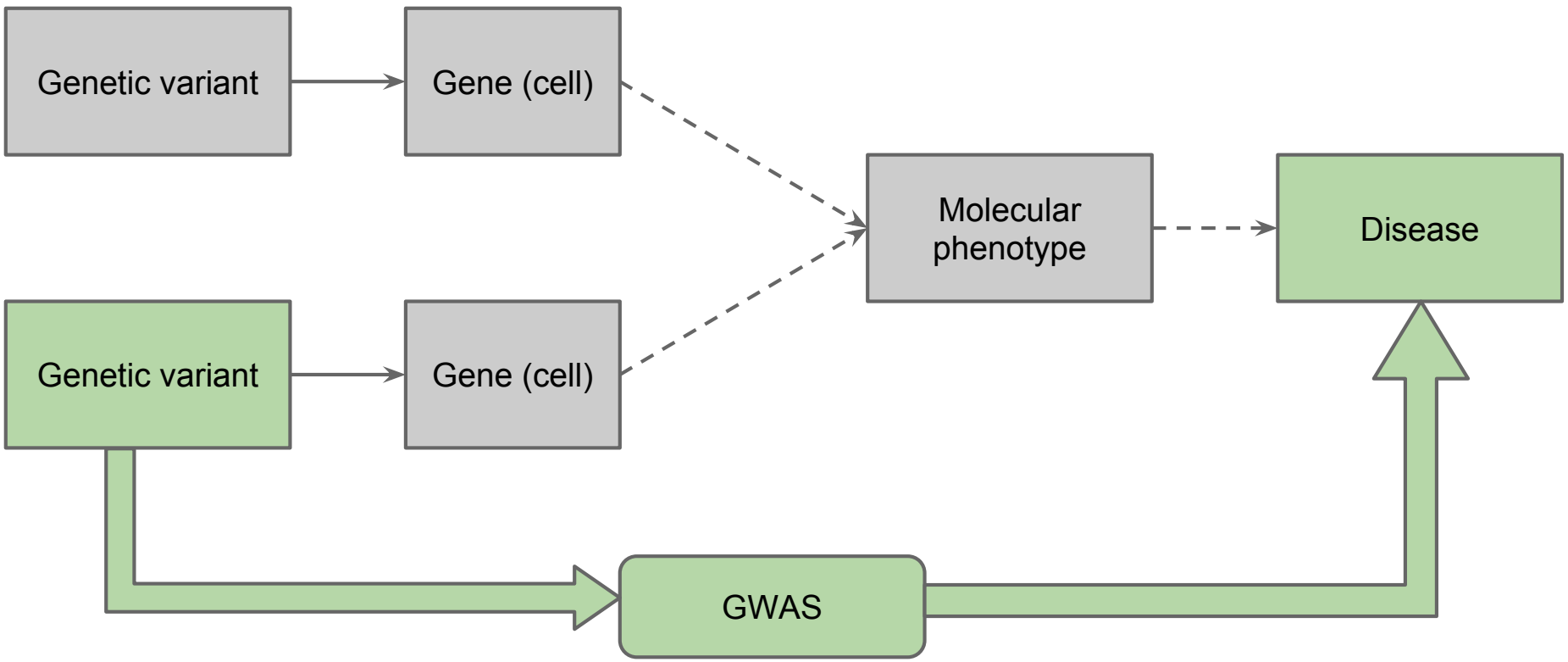
GWAS


Relative risk (between homozygotes)
1.0
1.5
2.0
2.5
3.0
3.5
4.0
rs2476601/1p13.2/PTPN22
rs689/11p15.5/INS
rs12722495/10p15.1/IL2RA
rs4948088/7p12.1
rs333/3p21.31/CCR5
rs7202877/16q23.1
rs3184504/12q24.12/SH2B3
rs45450798/18p11.21/PTPN2
rs2292239/12q13.2
rs11594656/10p15.1/IL2RA
rs3024505/1q32.1/IL10
rs12708716/16p13.13
rs425105/19q13.32
rs3087243/2q33.2/CTLA4
rs5979785/Xp22.2/TLR7/TLR8
rs10509540/10q23.31
rs478582/18p11.21/PTPN2
rs1990760/2q24.2/IFIH1
rs2304256/19p13.2
rs5753037/22q12.2
rs3825932/15q25.1
rs17574546/15q14
rs9388489/6q22.32
rs11755527/6q15
rs3788013/21q22.3
rs2281808/20p13
rs1465788/14q24.1
rs2069762/4q27/IL2
rs763361/18q22.2
rs4788084/16p11.2
rs7804356/7p15.2
rs10499194/6q23.3
rs2290400/17q12
rs7020673/9p24.2/GLIS3
rs229541/22q13.1
rs2816316/1q31.2
rs947474/10p15.1
rs4900384/14q32.2
rs9653442/2q11.2
rs941576/14q32.2
rs10517086/4p15.2
rs10272724/7p12.2/IKZF1
rs6920220/6q23.3
rs6897932/5p13.2/IL7R
rs2104286/10p15.1/IL2RA
rs7574865/2q32.2
rs4763879/12p13.31
rs1678536/12q13.3
rs2664170/Xq28
rs1738074/6q25.3/TAGAP
rs917997/2q12.1/IL18RAP
rs7221109/17q21.2
– 2000
2001 – 2006
2007
Barrett J et al., Nat Genet., 2009;
Smyth D et al., NEJM, 2008
Other Publications
T1DBase Loci
DIL Unpublished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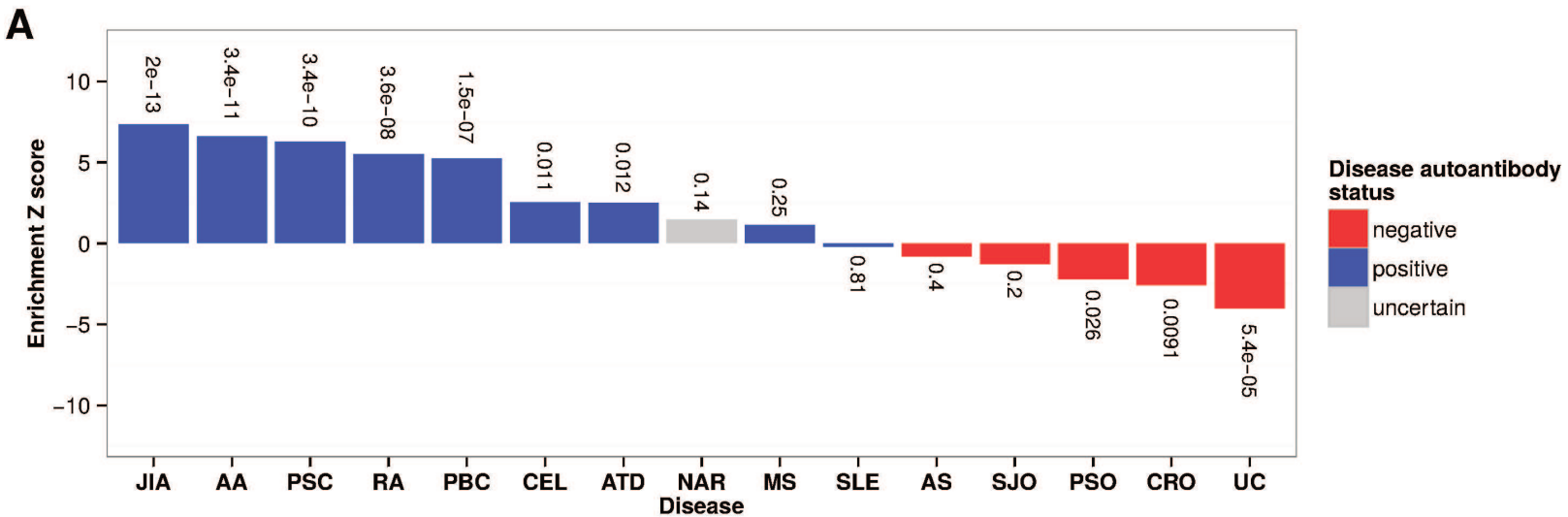
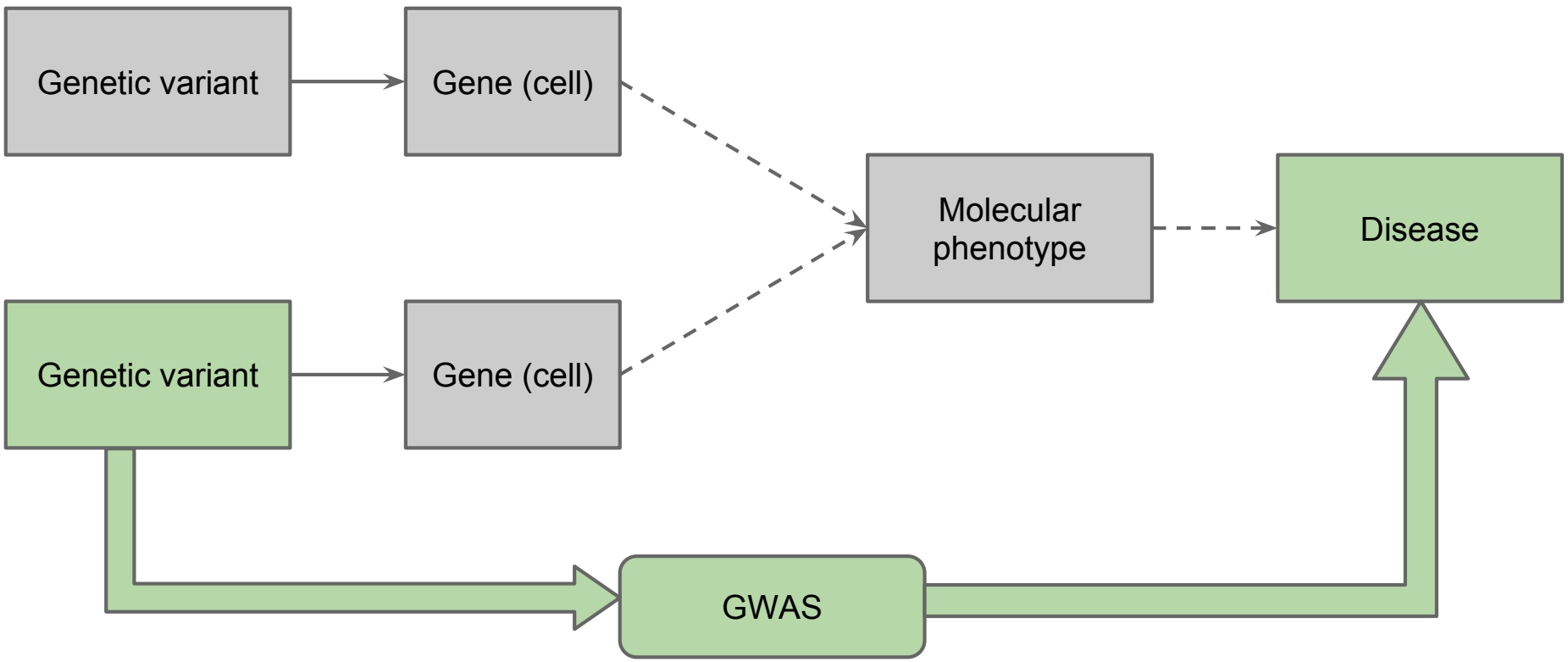
Gene (cell)
Molecular phenotype
Disease
Genetic variant
Gene (cell)
GWAS

ImmunoChip: dense genotyping of immune disease associated genetic regions

- Four new T1D associated regions
- Associated SNPs localized to enhancer sequences active in thymus, T and B cells
- Collation of results using same chip enabled comparative analyses with other autoimmune diseases



Onengut-Gumuscu et al., Nat Genet, 2015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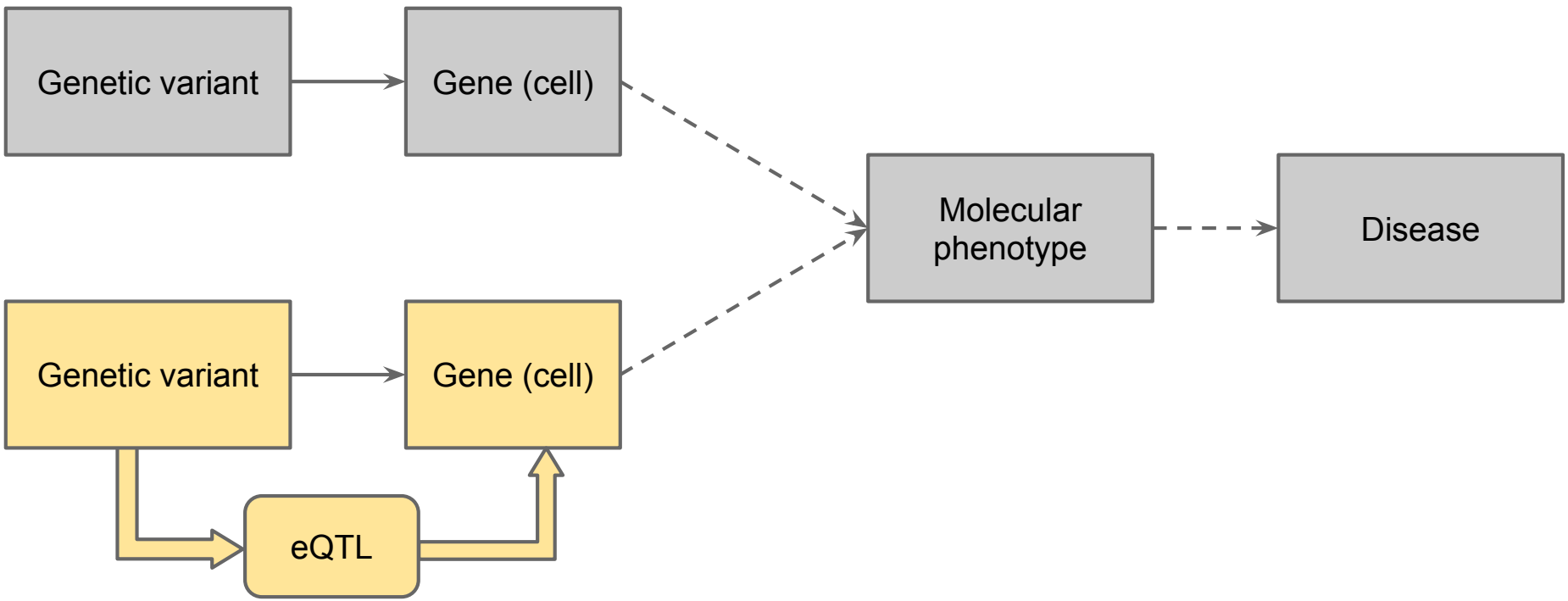
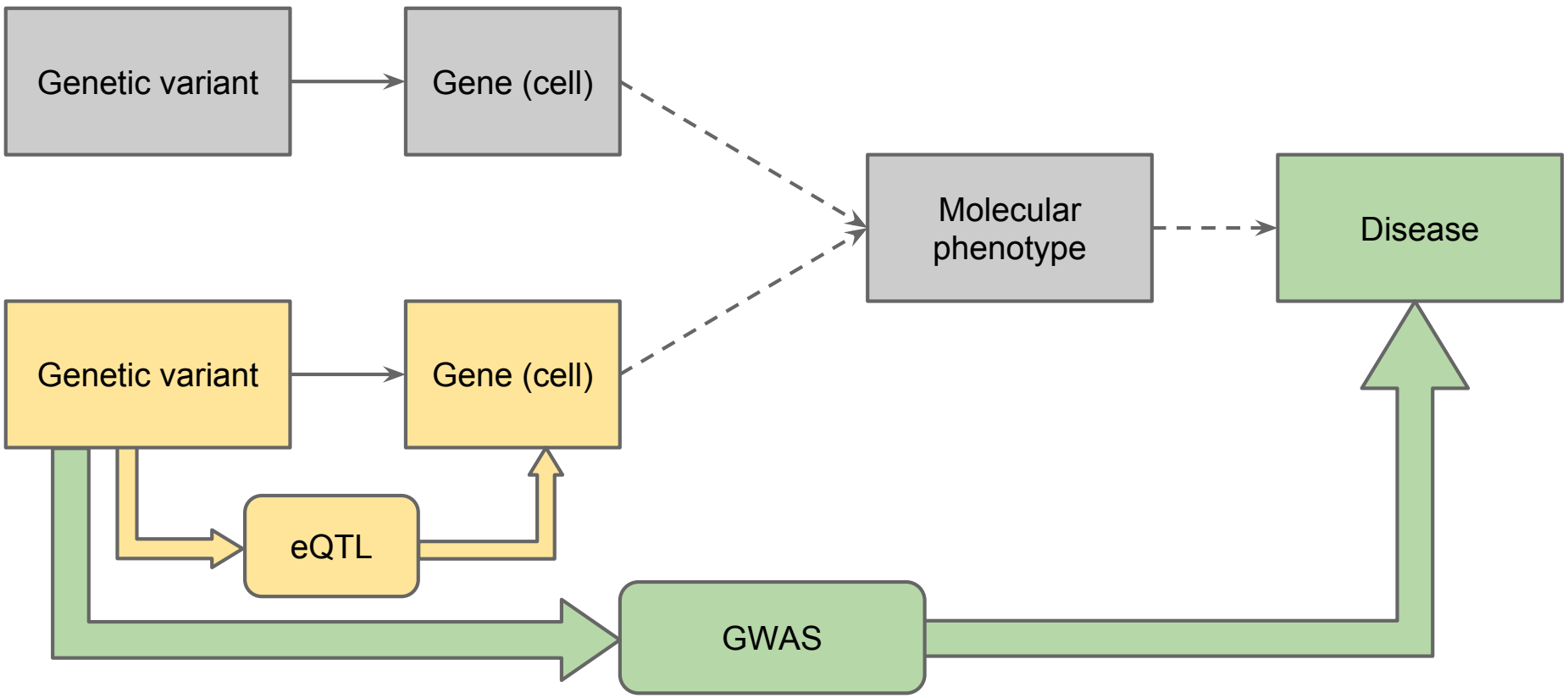

Genetic variant
Gene (cell)
Molecular phenotype
Disease
Genetic variant
Gene (cell)
eQTL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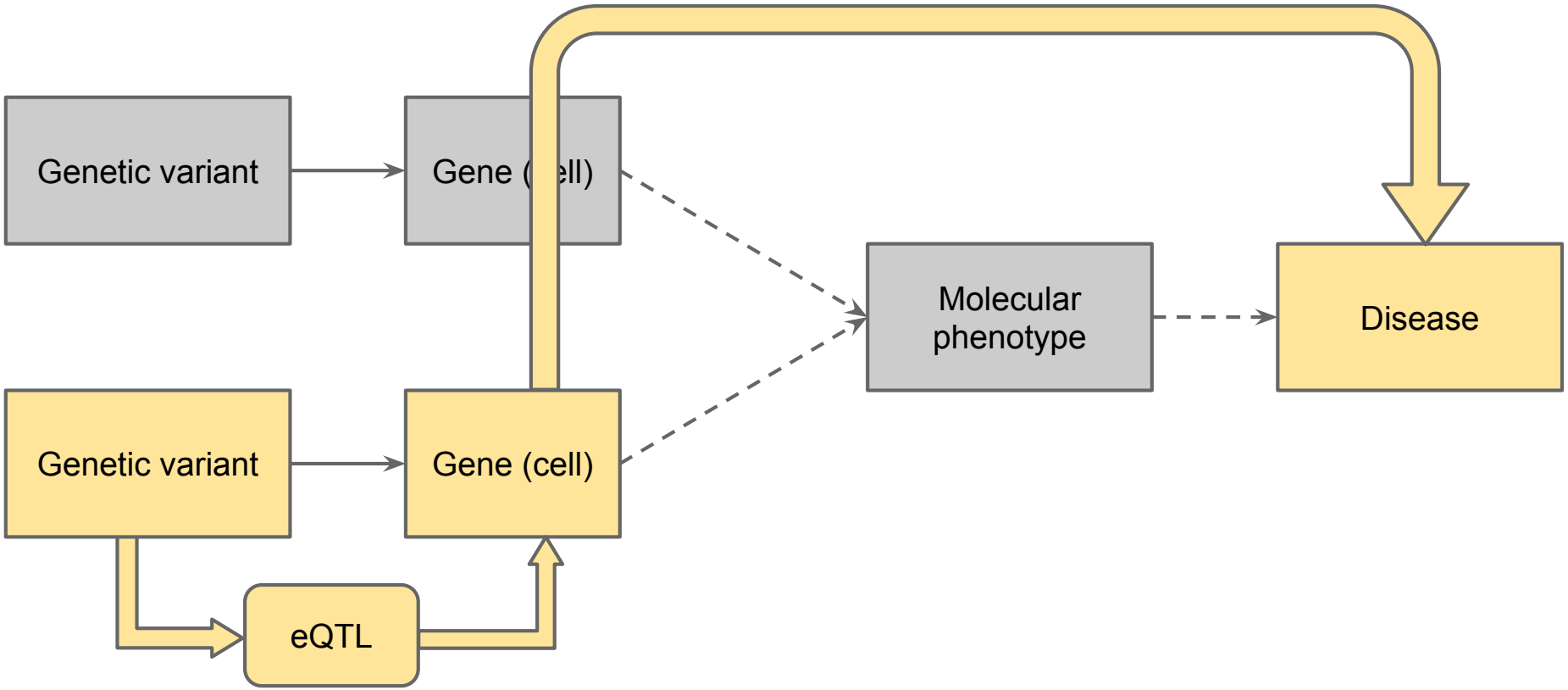
Genetic variant
Gene (cell)
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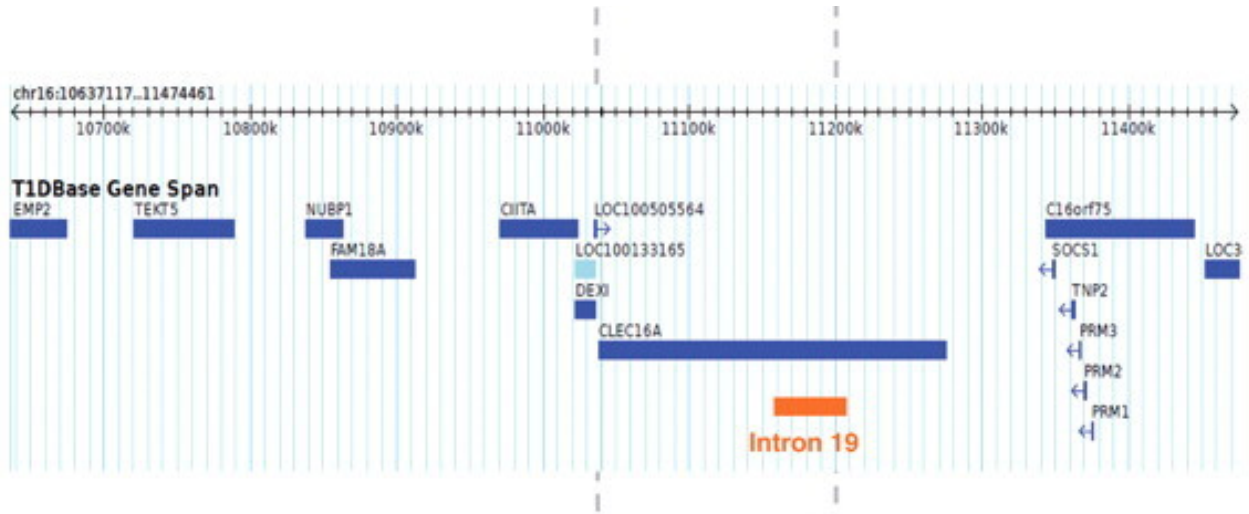
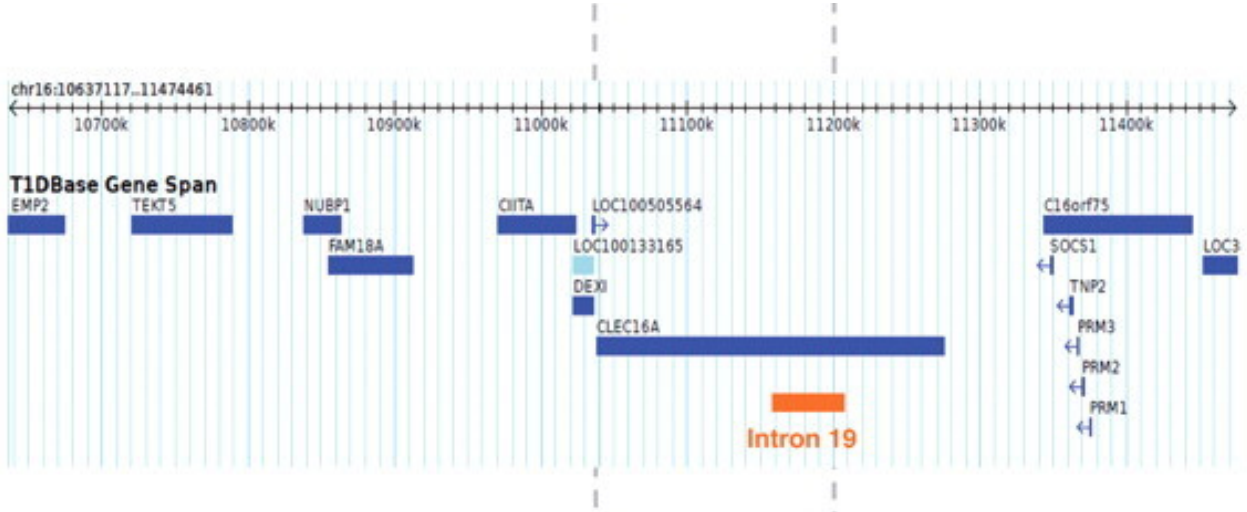
T1D / monocyte eQTL study identified *DEXI* as a candidate causal T1D gene

- 1,400 eQTL monocyte samples (Gutenberg Heart Study)
- highlighted 21 genes in 14/49 distinct T1D regions
- including *DEXI* on chromosome 16p13.3

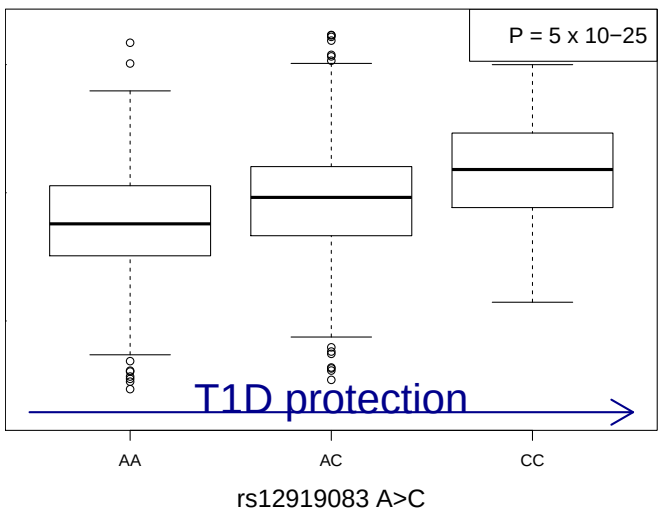


Wallace et al., Hum Mol Genet 2012, Davison et al., Hum Mol Genet 2012

T1D / monocyte eQTL study identified *DEXI* as a candidate causal T1D gene

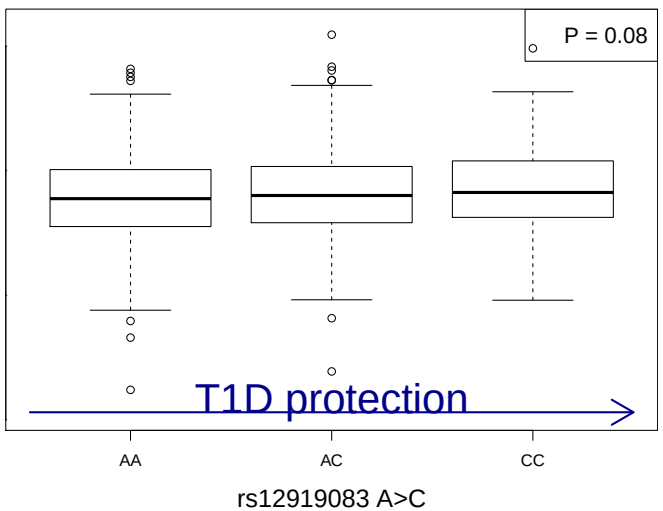


DEXI expression



rs12919083 A>C

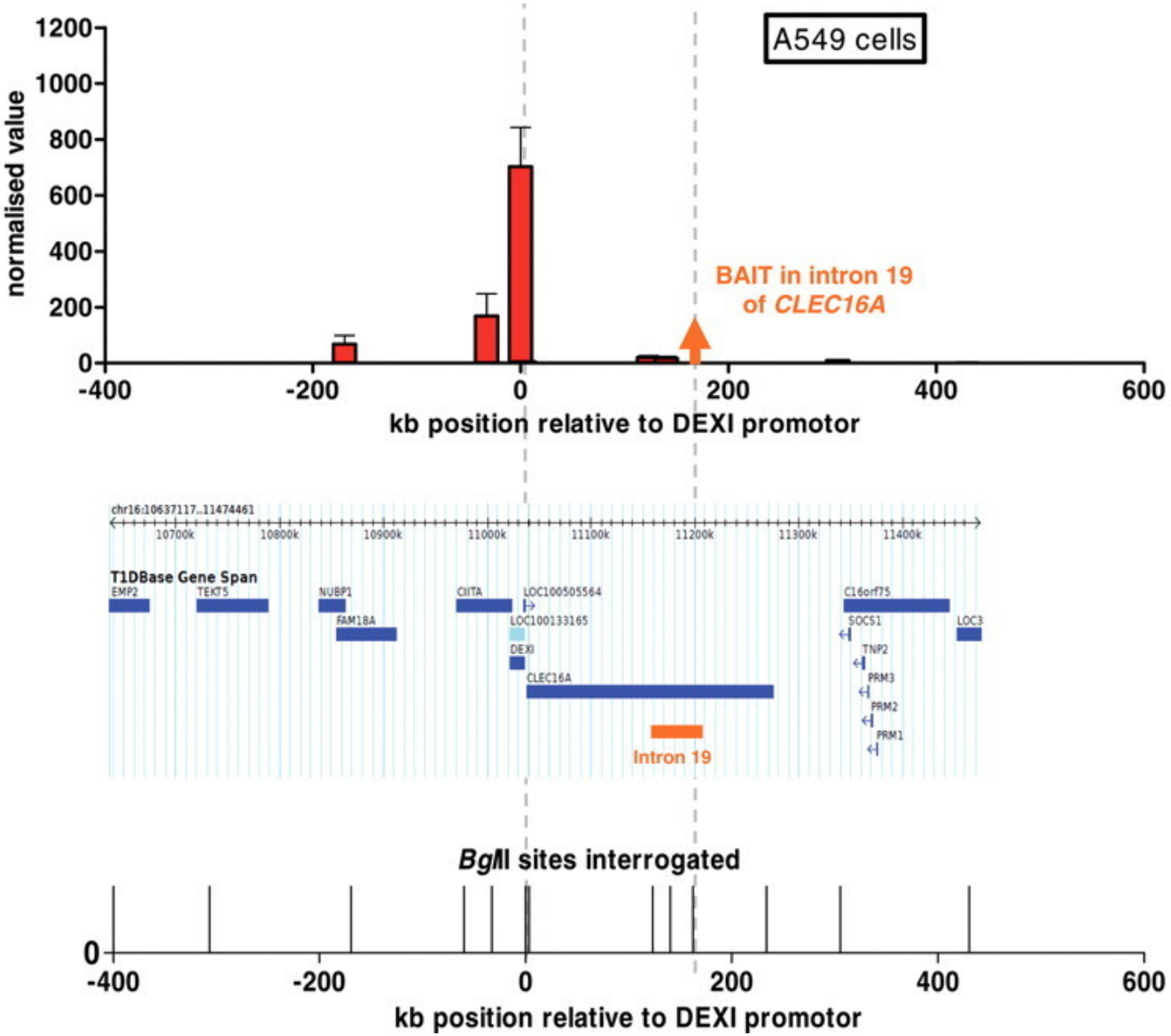
CLEC16A expression



rs12919083 A>C

Wallace et al., Hum Mol Genet 2012, Davison et al., Hum Mol Genet 2012

Chromosome conformation capture (3C) supported *DEXI*



Davison et al., Hum Mol Genet 2012

Colocalisation anaysis of ten autoimmune diseases with B cell, monocyte, stimulated monocyte eQTLs

+

B cells, monocytes (∼ 300 samples)

Genetics of gene expression in primary immune cells identifies cell type–specific master regulators and roles of HLA alleles

Benjamin P Fairfax[1], Seiko Makino[1], Jayachandran Radhakrishnan[1], Katharine Plant[1], Stephen Leslie[2], Alexander Dilthey[3], Peter Ellis[4], Cordelia Langford[4], Fredrik O Vannberg[1,5] & Julian C Knight[1]

Trans-acting genetic vari
expression. Using paired
trans expression quantita
B cells, respectively. Addi
disease locus, with *IP6K*
suggesting roles for cell
pathogenesis. We also fir
AOAH and *ARHGAP24* i
cell populations identifie
disease susceptibility.

Defining the genetic deter
understanding the biologic
iation. This is particularly
variants underlying obser
wide association studies (
functional activity of many
the context of relevant cell
state[2–4]. This context-dep

Innate Immune Activity Conditions the Effect of Regulatory Variants upon Monocyte Gene Expression

Benjamin P. Fairfax,* Peter Humburg, Seiko Makino, Vivek Naranbhai, Daniel Wong, Evelyn Lau, Luke Jostins, Katharine Plant, Robert Andrews, Chris McGee, Julian C. Knight*

Introduction: Many genetic variants associated with common disease susceptibility occur close to immune-related genes in noncoding DNA, suggestive of a regulatory function. The definition of functional variants and the specific genes that they regulate remains challenging and in many cases is unresolved. We hypothesized that a significant proportion of variants, including those implicated in disease, may show activity in a context-specific manner and therefore only be identifiable upon triggering of immune responses.

Methods: We mapped interindividual variation in gene expression as a quantitative trait, defining expression quantitative trait loci (eQTLs). To investigate the effect of innate immune stimuli on eQTLs, we exposed primary CD14⁺ human monocytes from 432 European volunteers to the inflammatory proxies interferon-γ (IFN-γ) or differing durations (2 or 24 hours) of lipopolysaccharide (LPS). eQTL mapping was performed on a genome-wide basis with an additive linear model. A subset of 228 individuals with expression data available for all experimental conditions enabled cross-treatment comparisons.

Results: Stimulation with LPS or IFN-γ resulted in profound effects across monocyte eQTLs, with hundreds of genes and associated pathways demonstrating context-specific eQTLs dependent on the type and duration of stimulus. Context-specific eQTLs frequently intersected established canonical pathways of monocyte signaling and included key nodal genes and effector molecules. These eQTLs are typically more distal to the transcriptional start site and, in some cases, showed reversal of

on context, paralleling the expression of class II genes. Induced eQTLs were enriched for disease-risk loci with context-specific associations to many putative causal genes including at *ATM*, *IRF8*,

READ THE FULL ARTICLE ONLINE
http://dx.doi.org/10.1126/science.12469

Cite this article as B. P. Fairfax *et al.*, *Science* **343**, 1246949 (2014). DOI: 10.1126/science.1246949

FIGURES IN THE FULL ARTICLE

Fig. 1. Genotype modulates the gene expression response to innate immune stimuli in monocytes.

Fig. 2. Trans-eQTL demonstrate context specificity and identify master regulatory loci after treatment.

Fig. 3. Temporal effects for a stimulus-specific trans-eQTL.

Fig. 4. Cis regulation of IRF2 at rs13149
has profound transcriptional consequenc
in trans.

Fig. 5. Stimulus-specific eQTL and GWAS.

Fig. 6. Examples of context-specific eQTL informative for disease risk.

SUPPLEMENTARY MATERIALS

Materials and Methods
Figs. S1 to S12

sociation with di
FN-β signaling.
th *IFNB1* expressio
a single–nucleotide polymorphism (rs2275
revealed after 2 hours of LPS stimulation of m

Activated monocytes (∼ 300 samples)

=

?

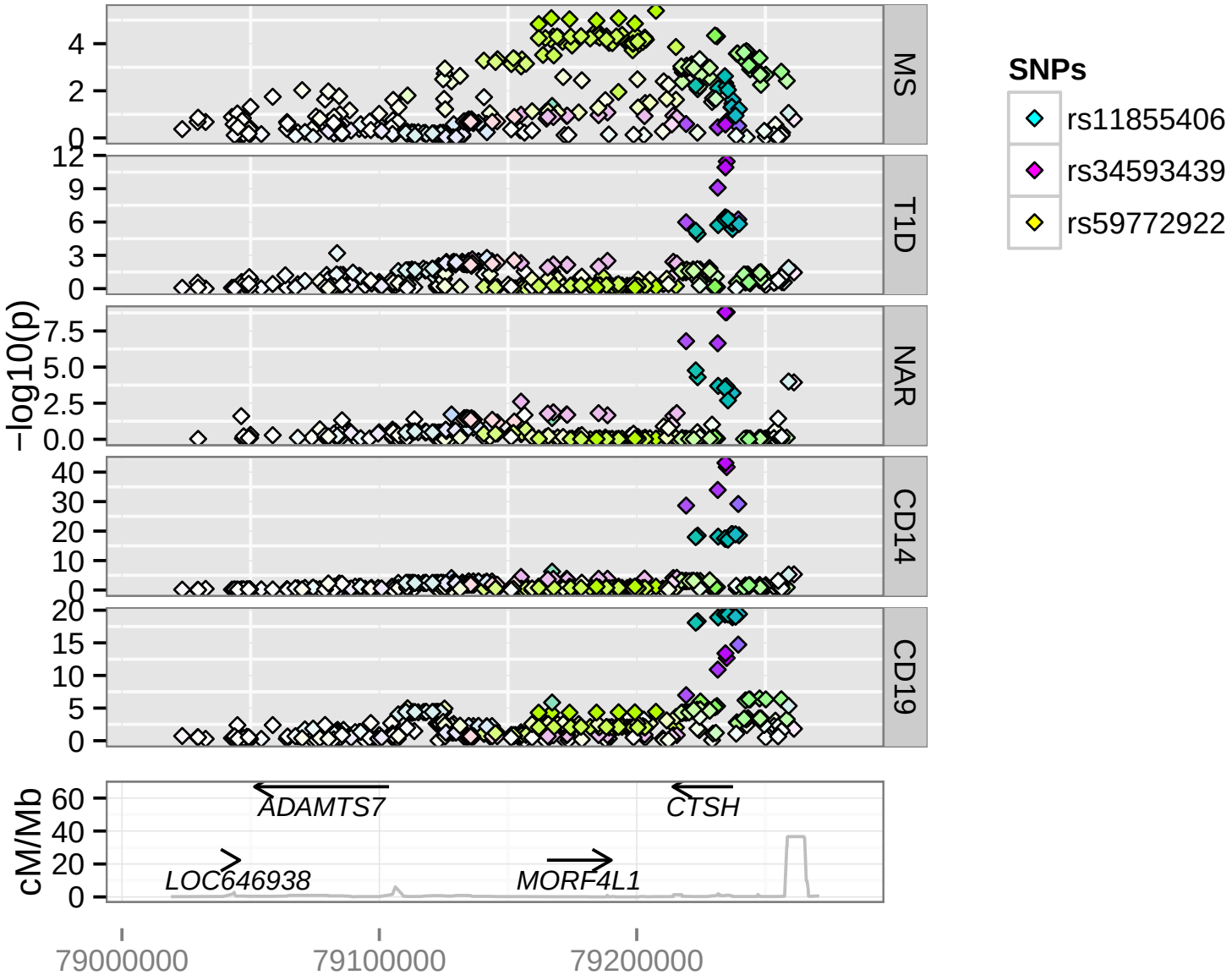
candidate causal autoimmune genes

Guo et al., Hum Mol Genet, published online

Six candidate causal autoimmune disease genes

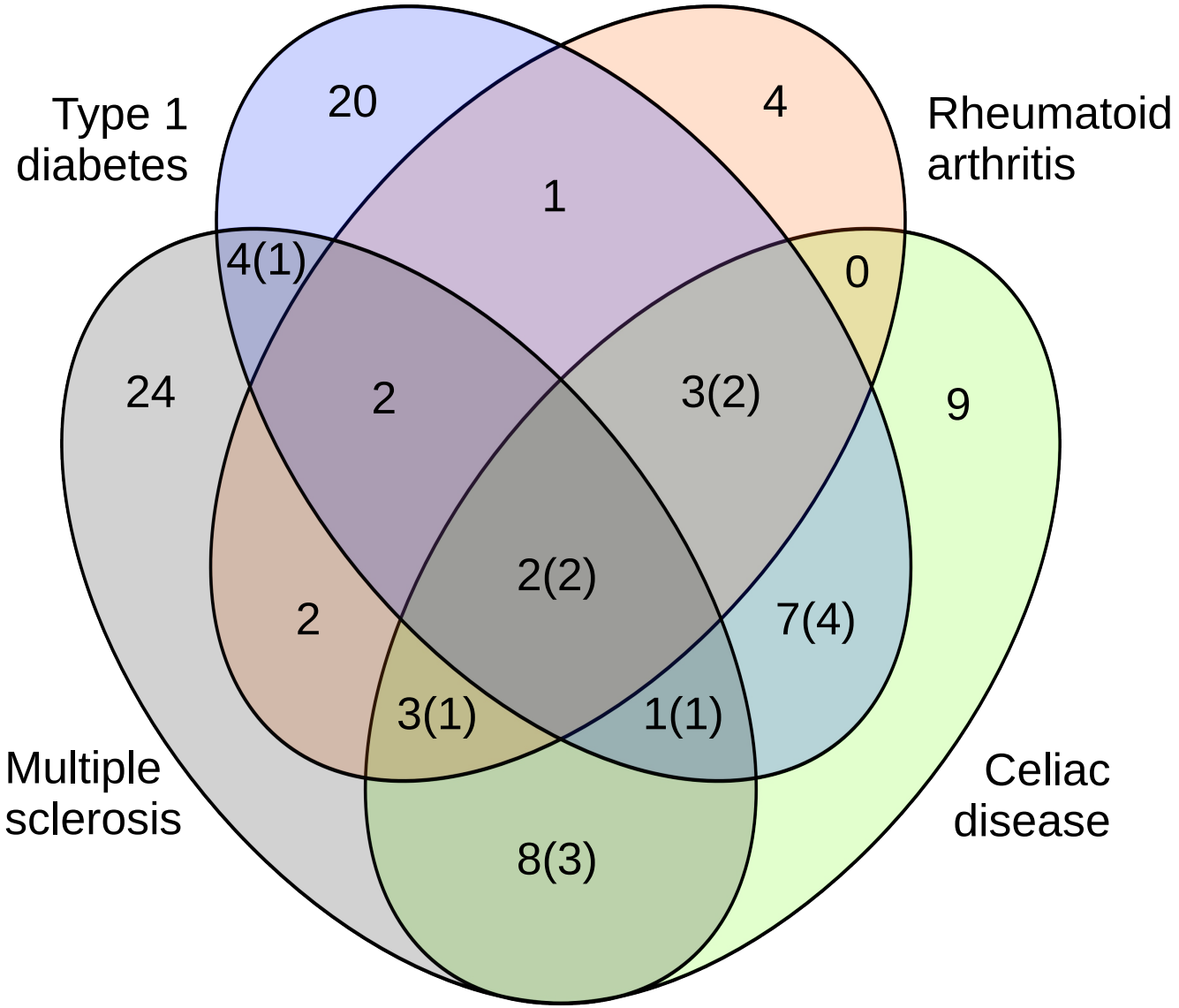
Gene	Disease(s)	Direction
Resting B cells + monocytes		
RGS1	Celiac, MS	-
SYNGR1	Primary Billiary cirrhosis	+
Resting + activated monocytes		
ADAM15	Crohn's	?
CARD19	Crohn's, ulcerative colitis	+
LTBR	Primary Billiary cirrhosis	+
CTSH	T1D, narcolepsy	-

CTSH monocyte expression linked to T1D and narcolepsy



Guo et al., Hum Mol Genet, published online

Colocalisation applied to four autoimmune diseases



Fortune et al, Nat Genet, in press

Leveraging information across diseases identified 11 novel associations

Four new T1D associations

- *FASLG* (celiac disease)
- *ANKRD55* (rheumatoid arthritis, multiple sclerosis)
- *TNFAIP3* (rheumatoid arthritis, celiac disease)
- *IKZF1* 5' region (multiple sclerosis)

Two new associations with T1D regions

- *AFF3* (rheumatoid arthritis, **celiac disease**)
- *CTSH* (**celiac disease**)

Fortune et al, Nat Genet, in press

3/9 T1D unique regions overlap type 2 diabetes associations

Region	Candidate Gene	T1D association OR	T1D association P	T2D association OR	T2D association P	Concordance
6q22.32	–	1.108	6.76×10^{-8}	1.06	7.9×10^{-5}	↑
9p24.2	*GLIS3*	1.092	2.97×10^{-6}	1.05	7.3×10^{-5}	↑
16q23.1	*BCAR1*	1.266	3.47×10^{-14}	1.12	2.0×10^{-5}	↓

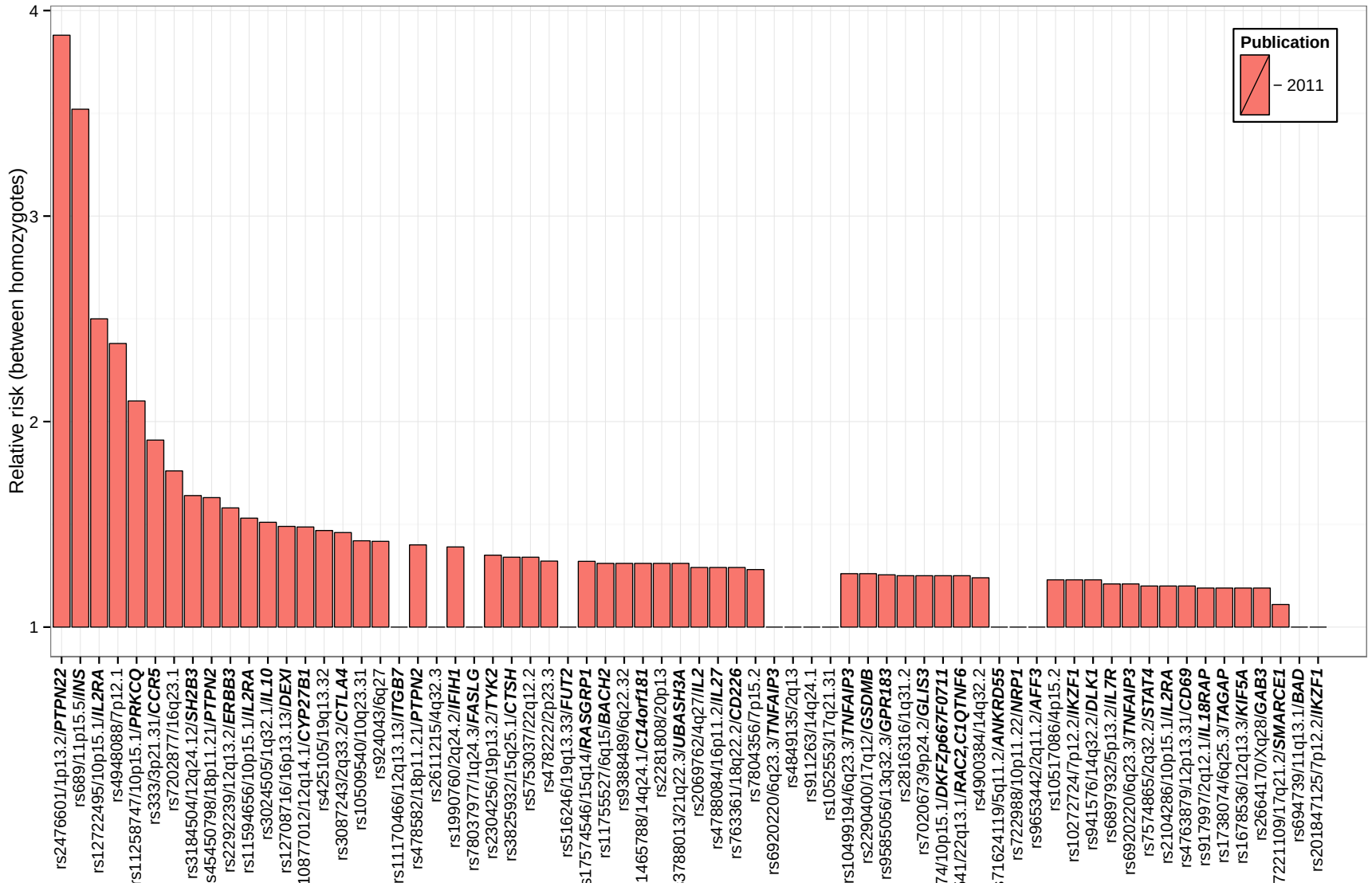
Disease unique regions more likely to relate to target of autoimmune destruction

Fortune et al, Nat Genet, in press

Systematic integration of GWAS, eQTL and other data for interpretation of GWAS results

- Identification of candidate causal genes through eQTL/disease colocalisation analyses
  - We need a wider variety of cells/states in eQTL studies (T cells, NK cells recently published)
  - We need larger eQTL studies (eg large multiethnic study of PBMCs recently published)
- Cross disease colocalisation can identify novel associations, disease-specific (autoimmune target specific?) associations
- Large scale application of chromosome conformation capture ("Hi-C") for gene regulatory regions

Where are we now?



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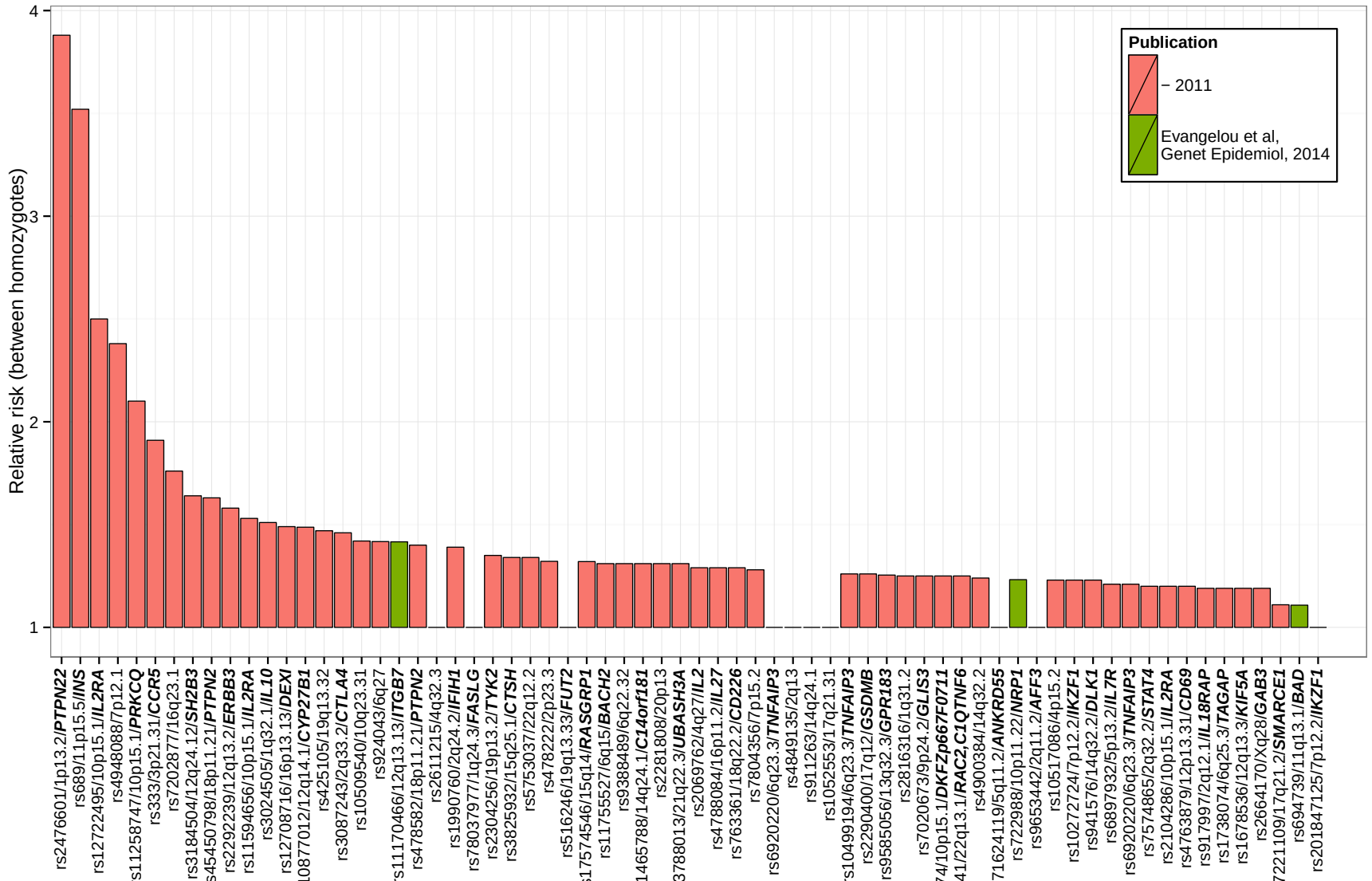

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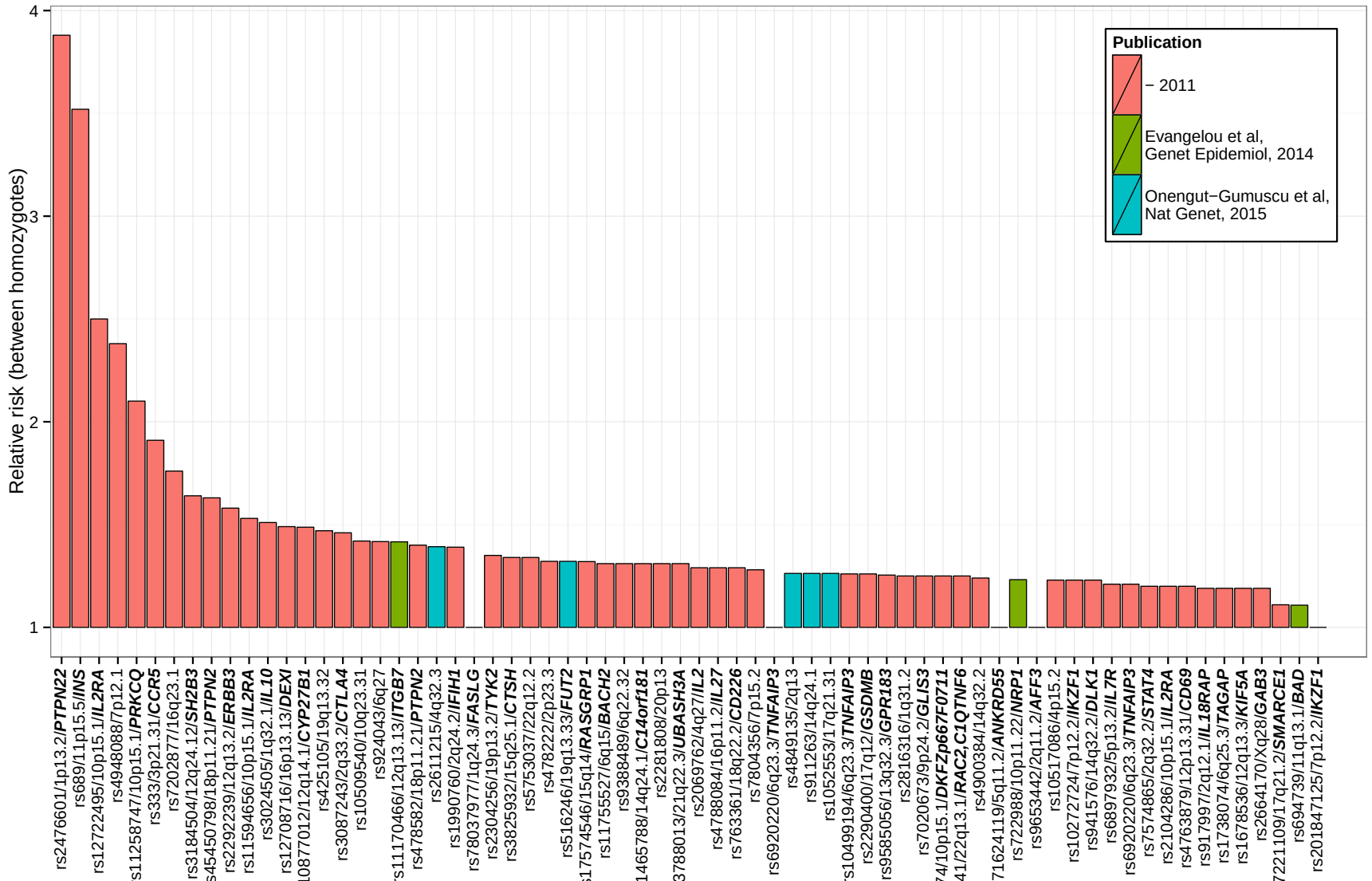

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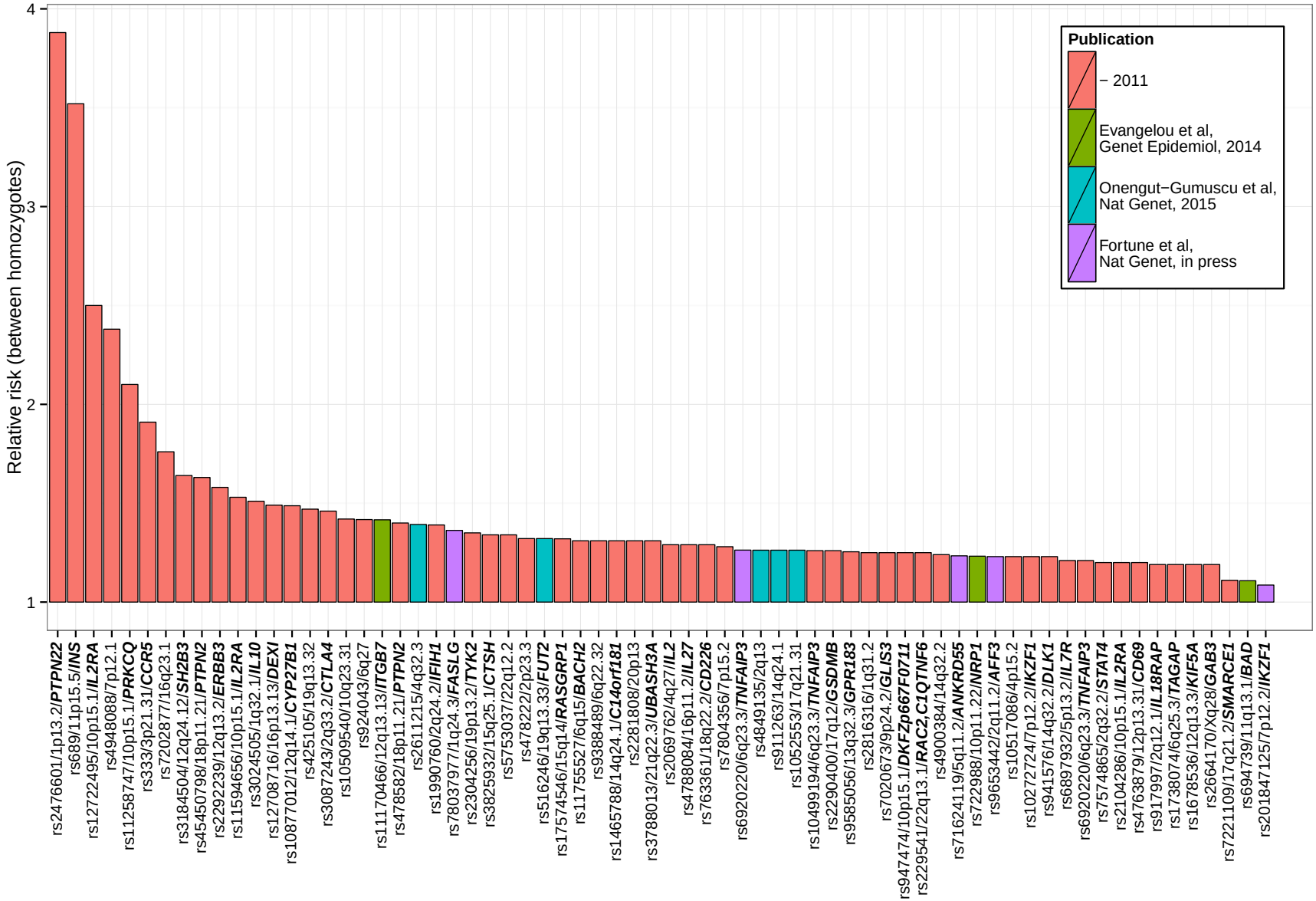

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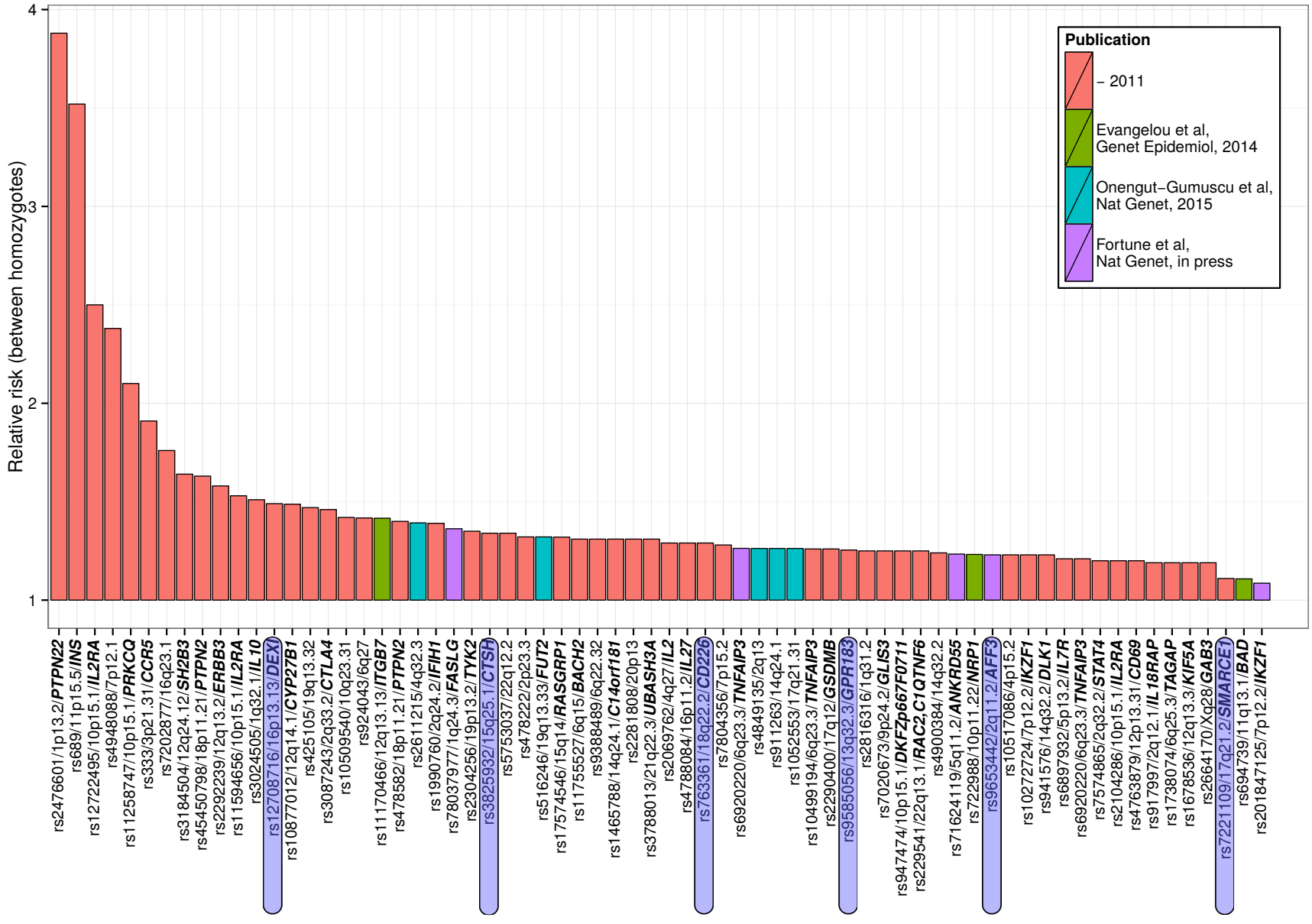
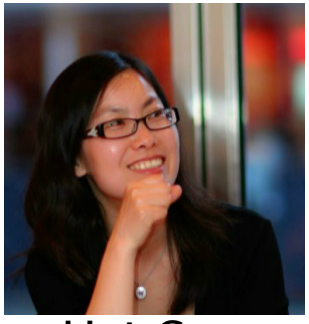
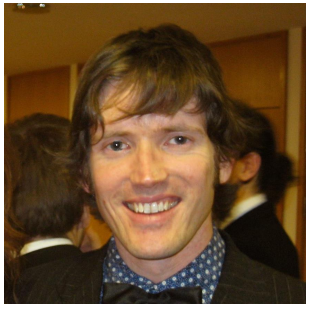


Thanks to


Mary Fortune


Hui Guo


Olly Burren


Nick Cooper


John Todd

Stephen Sawcer (MS), Steve Eyre (RA), Steve Rich (T1D),
David van Heel (celiac disease)