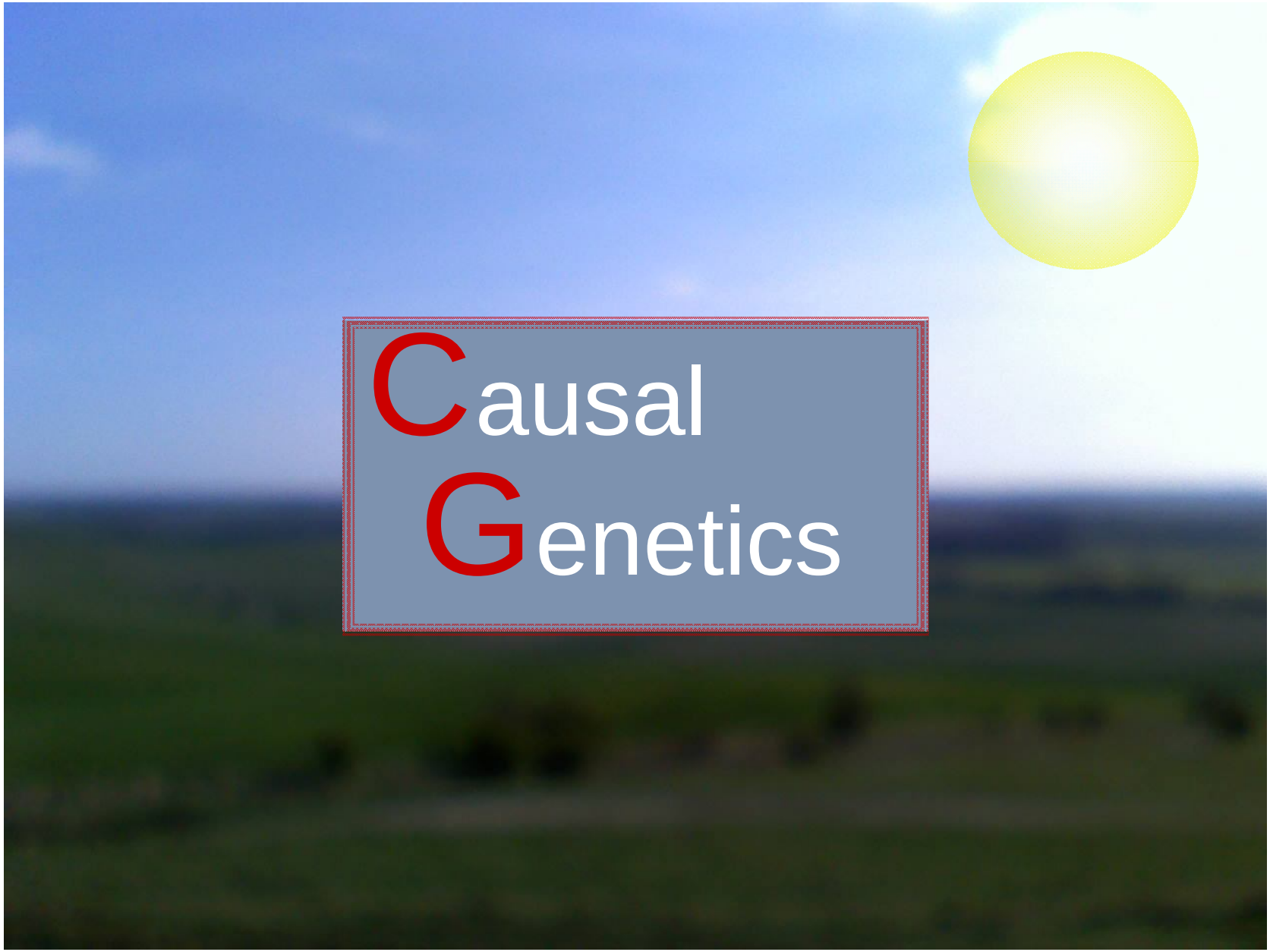
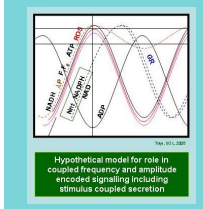


Elucidating The Causal Basis Of Human Metabolic Diseases Through Genetics Led Systematic Studies In Mouse Models

Ayo A. Toye (PhD.)

Principal Fellow of The Causal Genetics Institute (CGenI), UK
CEO of Causal Genetics Limited, UK

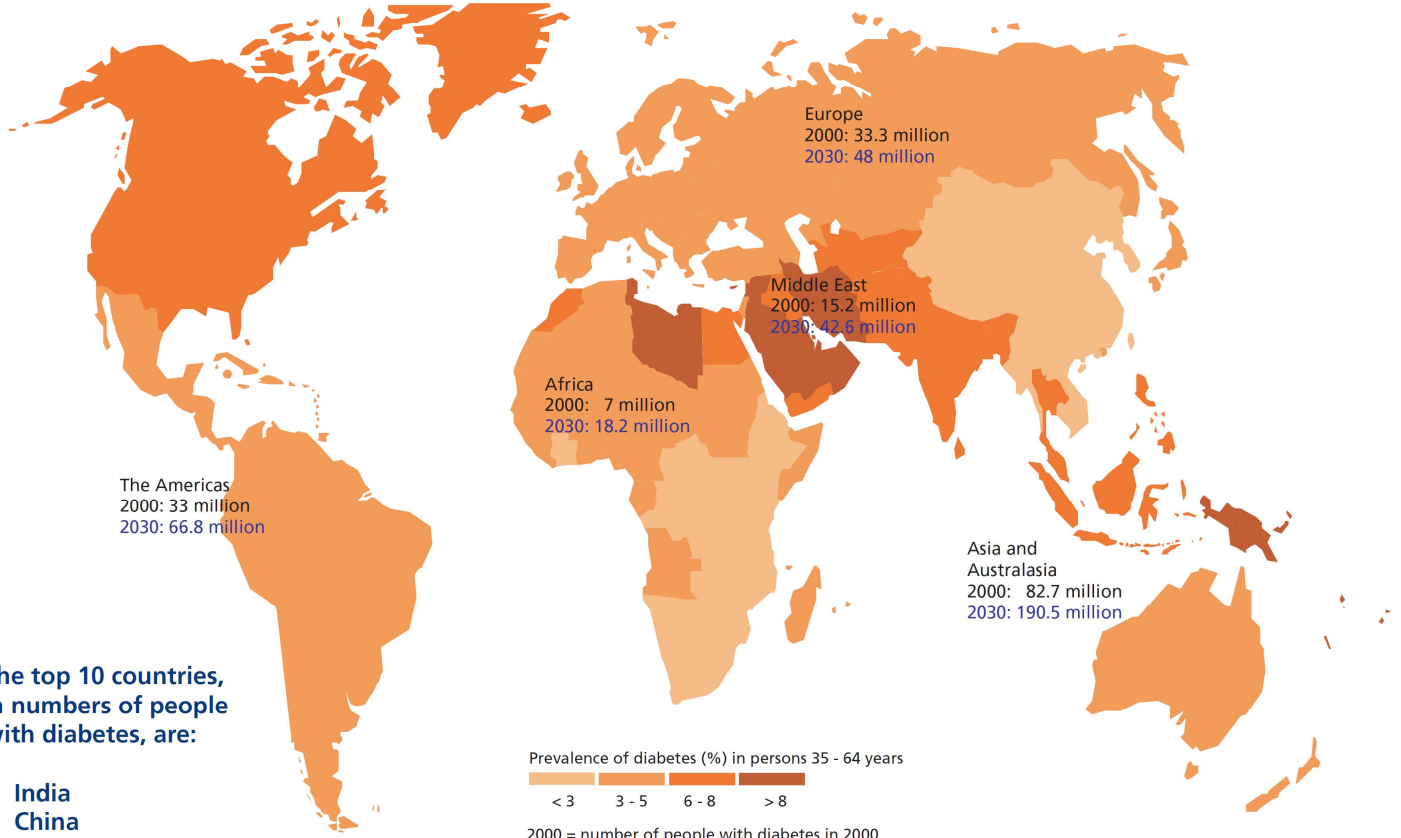
NSHBT 37TH ANNUAL SCIENTIFIC PRE CONFERENCE WORKSHOP, AUGUST 18 – 19, 2009: IGCLM, IBADAN, NIGERIA.



Outline

- Introduction
- Use of reverse genetics to define causal basis of disease
  - A multimodal sequential nesting approach
  - A multimodal systems based approach
- Conclusion and Perspectives

Prevalence of diabetes: 170 million affected in AD 2000. Expected to double by AD 2030.



Source: Wild et al, 2004

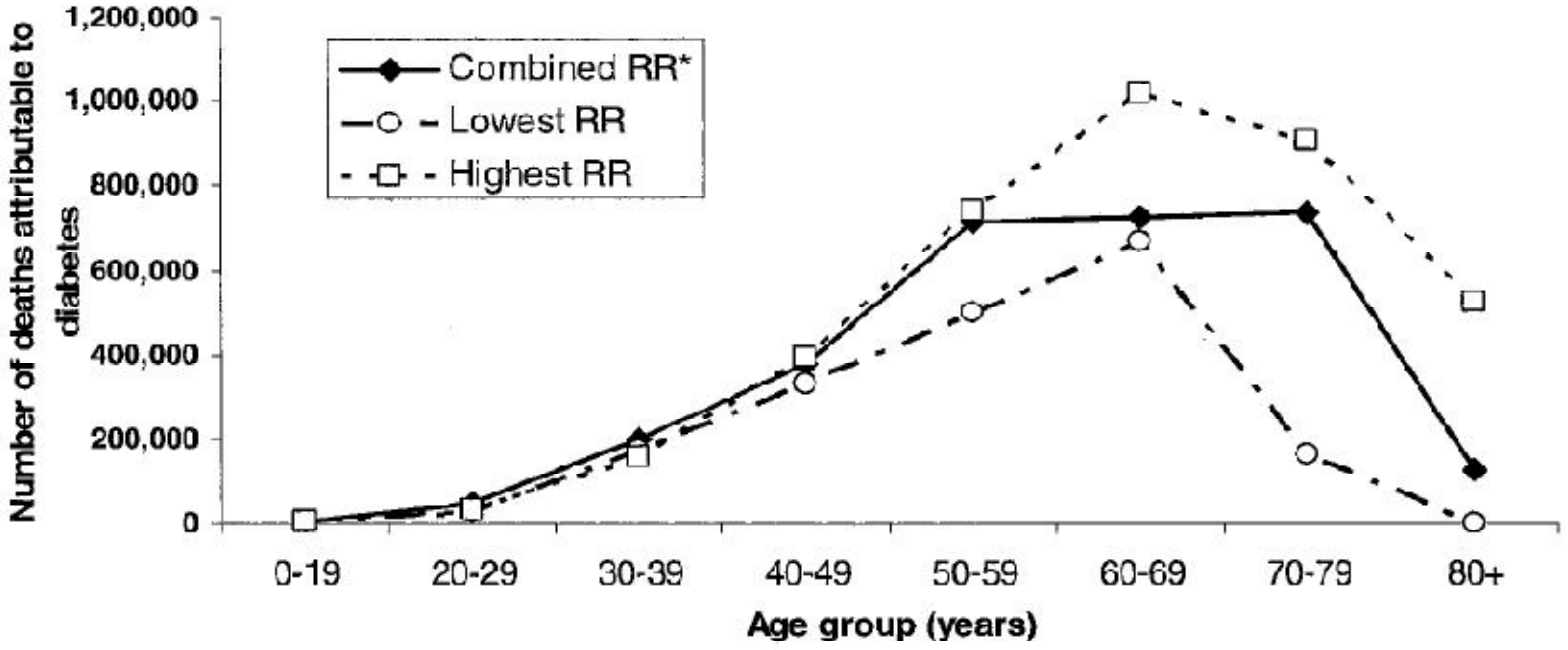
The top 10 countries, in numbers of people with diabetes, are:

India
China
USA
Indonesia
Japan
Pakistan
Russia
Brazil
Italy
Bangladesh

Year		2000	2030
Ranking	*Country*	*People with diabetes (millions)*	
1	India	31.7	79.4
2	China	20.8	42.3
3	United States of America	17.7	30.3

Diabetes Facts:

- 5th leading cause of death globally
- 5.2% of all deaths globally

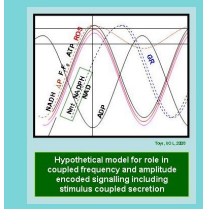


Roglic *et al*., (2005). Diabetes Care 28: 2130 -2135.

Why mice?

- Use of the mouse as a model organism in systematic screens to define gene function is well established, and the value is proven through insights into human disease

- The success of the mouse derives from its:
  - molecular, cellular anatomical and evolutionary similarity to humans
  - amenability to requirements for straightforward deductive inference; they are docile, permit control of breeding and experimental conditions, and scale in replicates required for robust inference within superior cost and space efficiency relative to other mammalian models
  - The mouse also offers a superior spectrum of existing genetic variants required for studies of the role of DNA variation in disease and, the required infrastructure and know-how for their production.



Use of reverse genetics to define causal basis of disease

A multimodal sequential nesting approach

- Identifying variants
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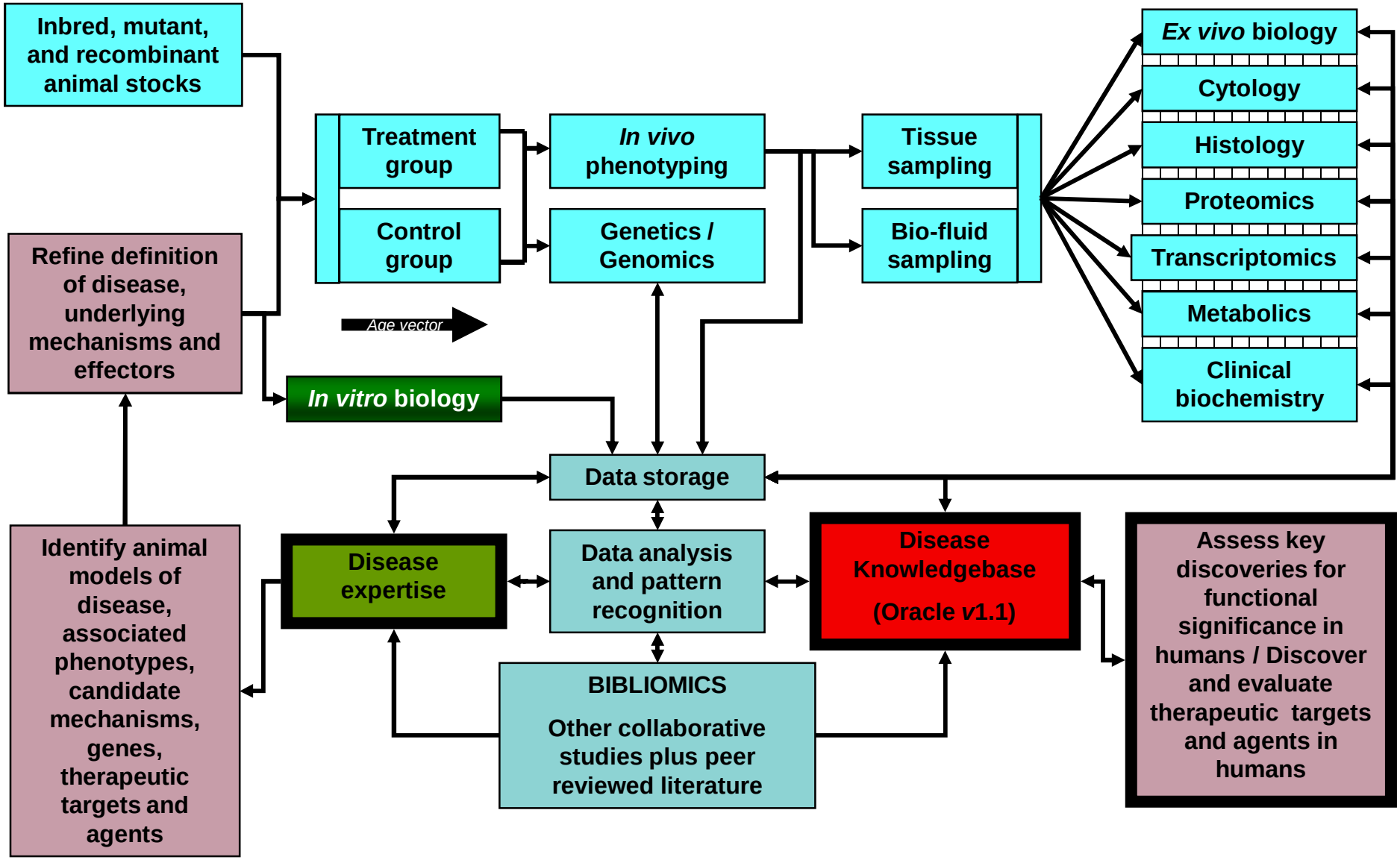
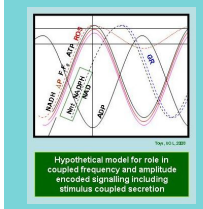
- Candidate gene studies in humans

Mouse model pipeline: An integrated system for dissection of genetic and environmental determinants of disease susceptibility



Discovery pipeline: Ayo A. Toye



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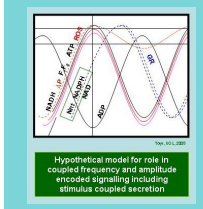
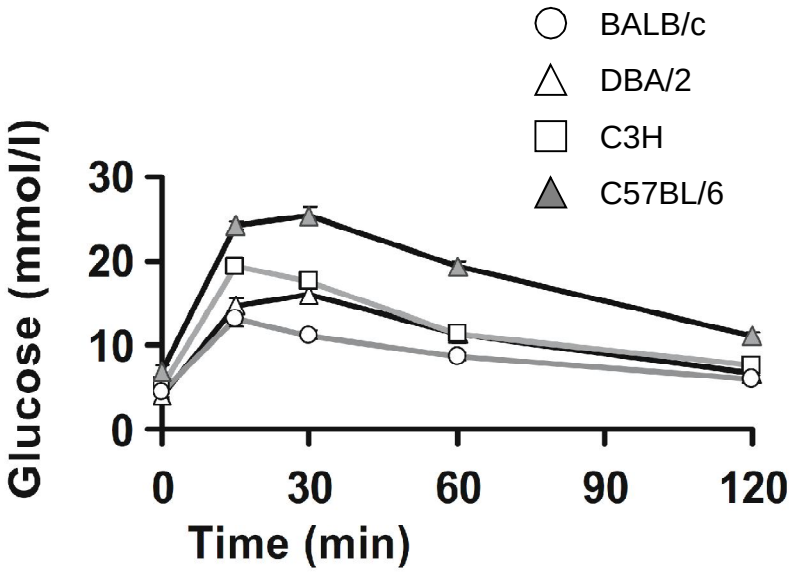
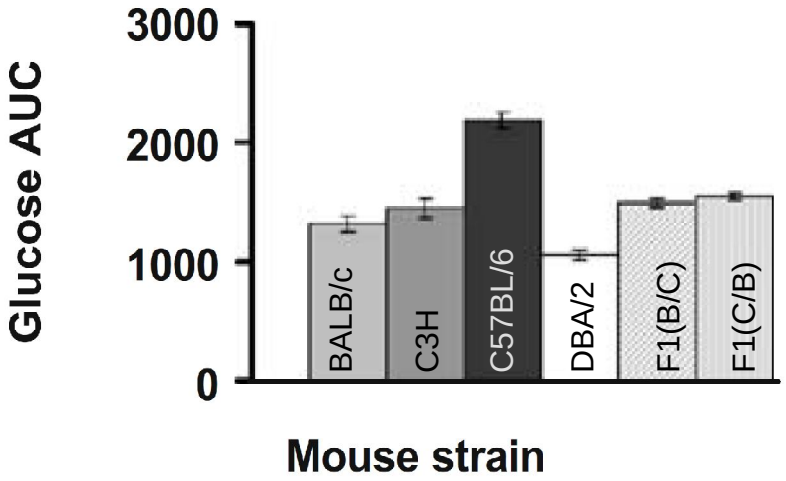
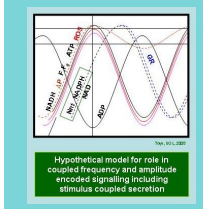


IPGTT on male mice at age 12 weeks







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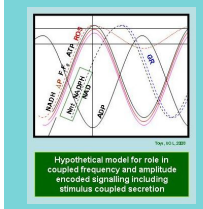
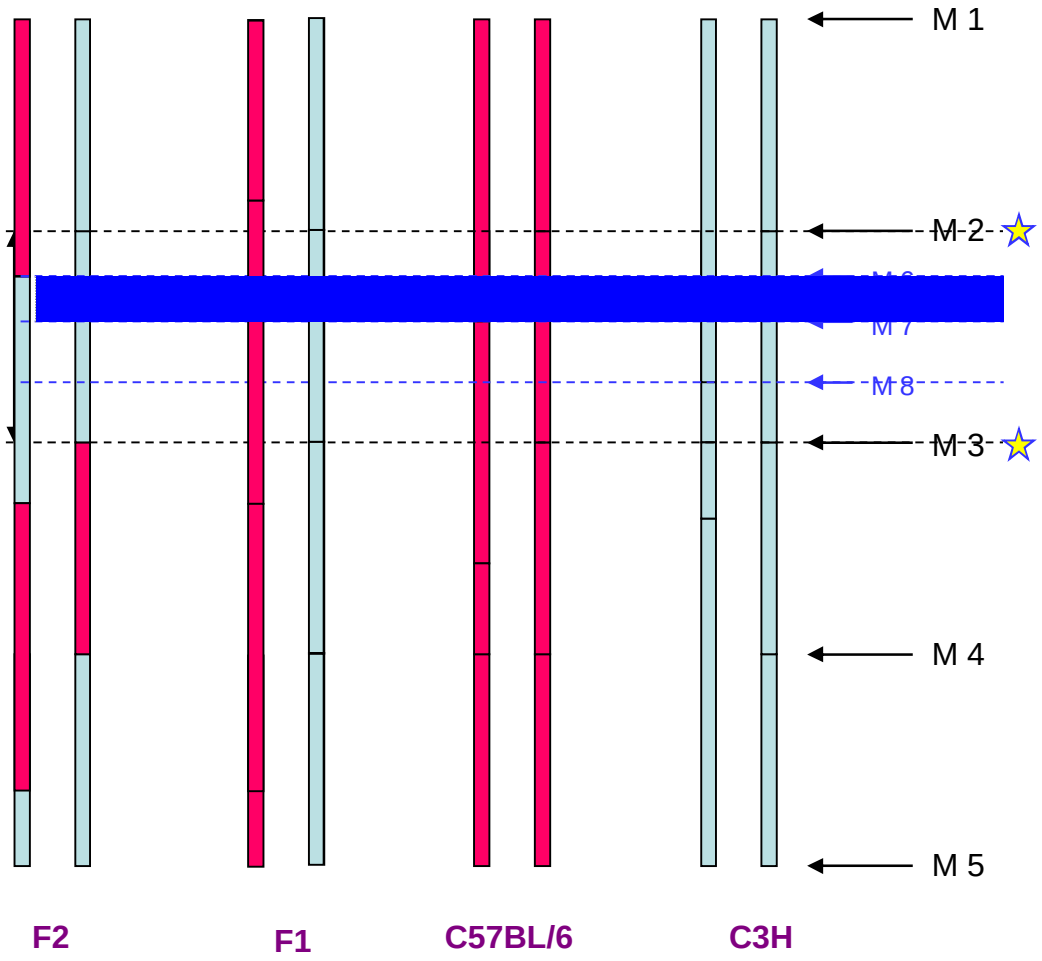
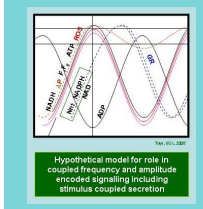
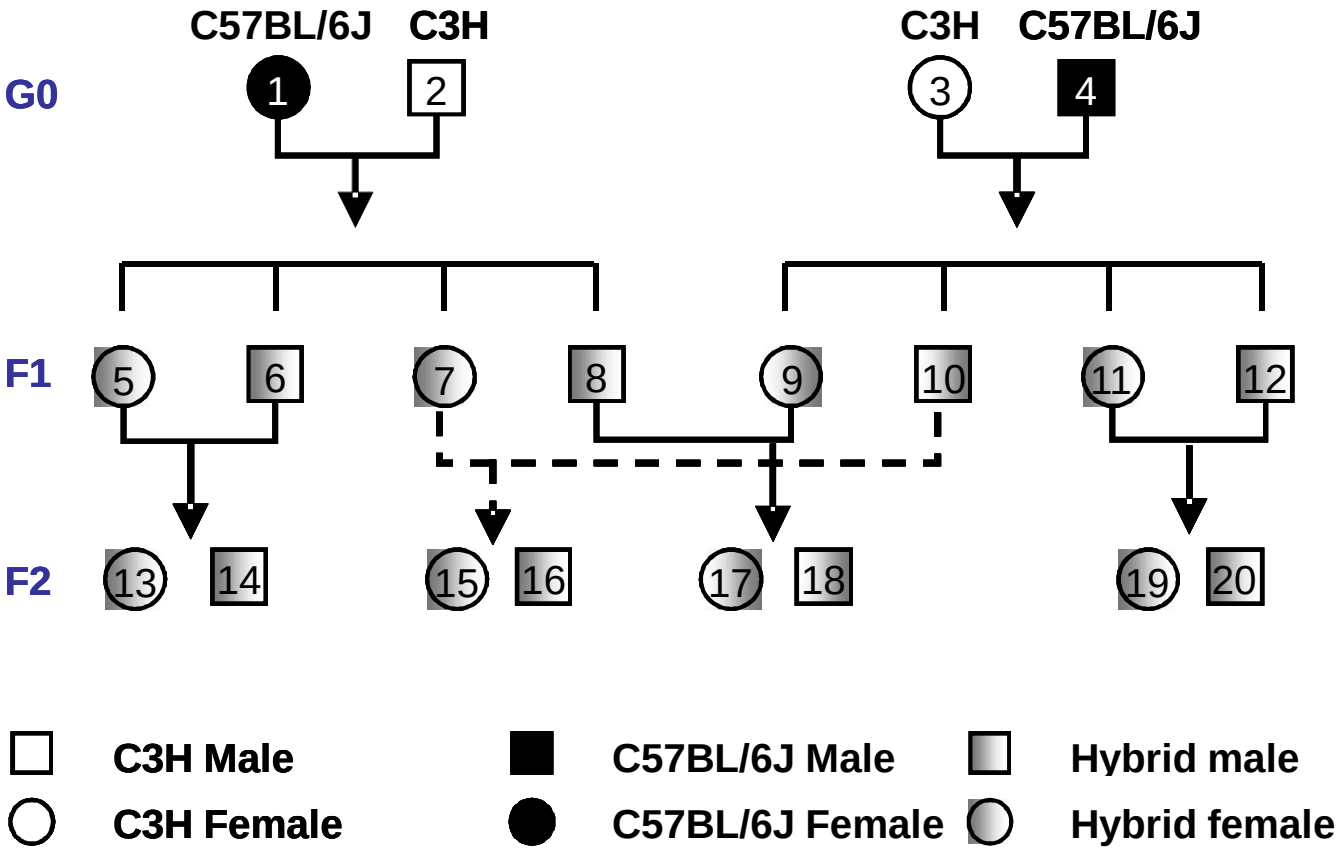


Segregation of parental chromosomes (C3H and C57BL/6) in F2 male mice

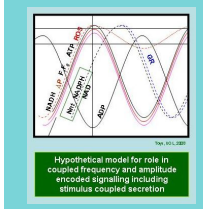




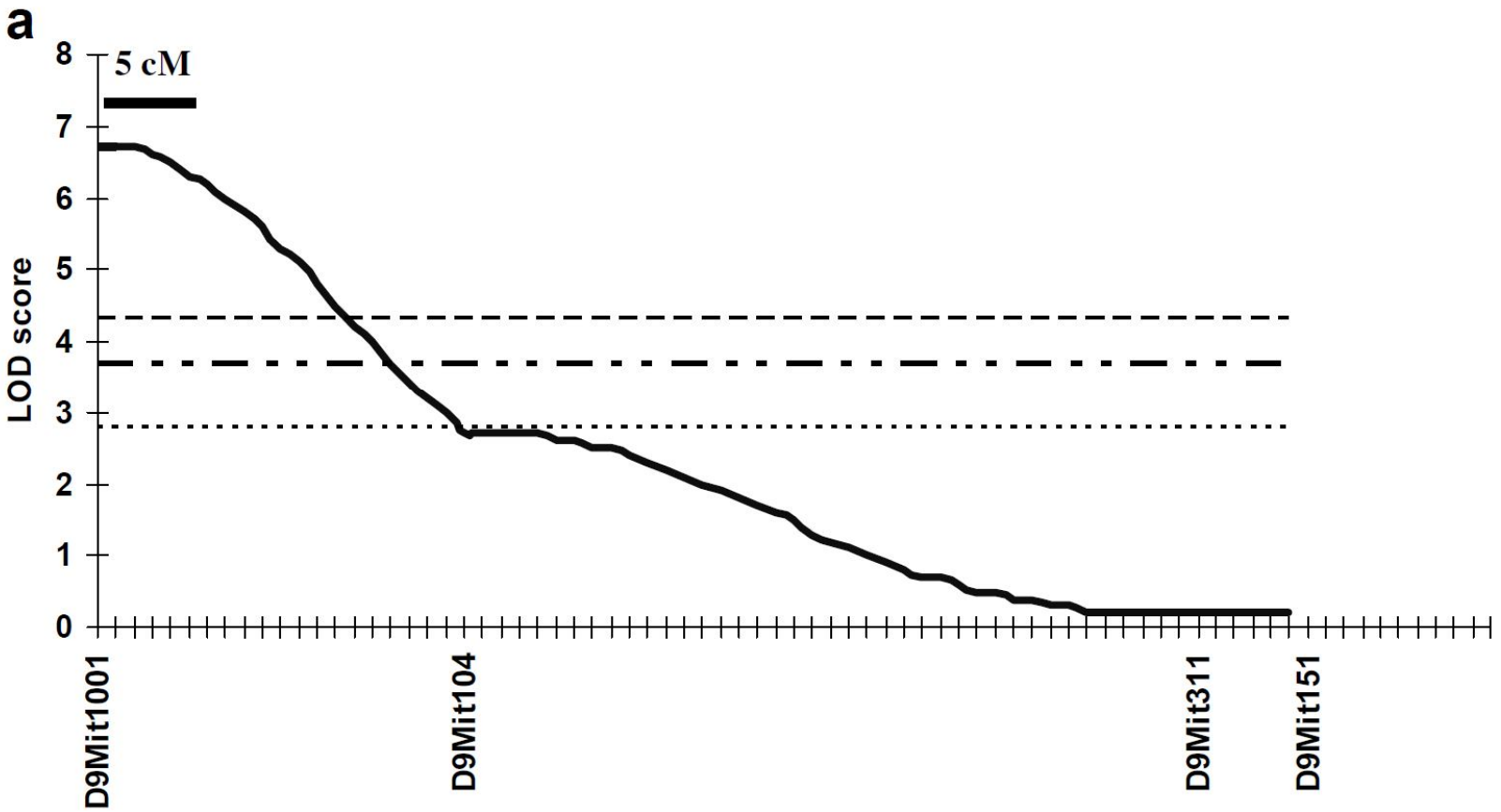
Genetic mapping of C57BL/6J insulin insufficiency glucose intolerance

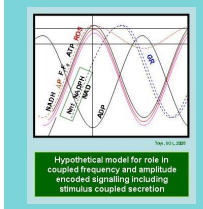


* Male map used for positional cloning

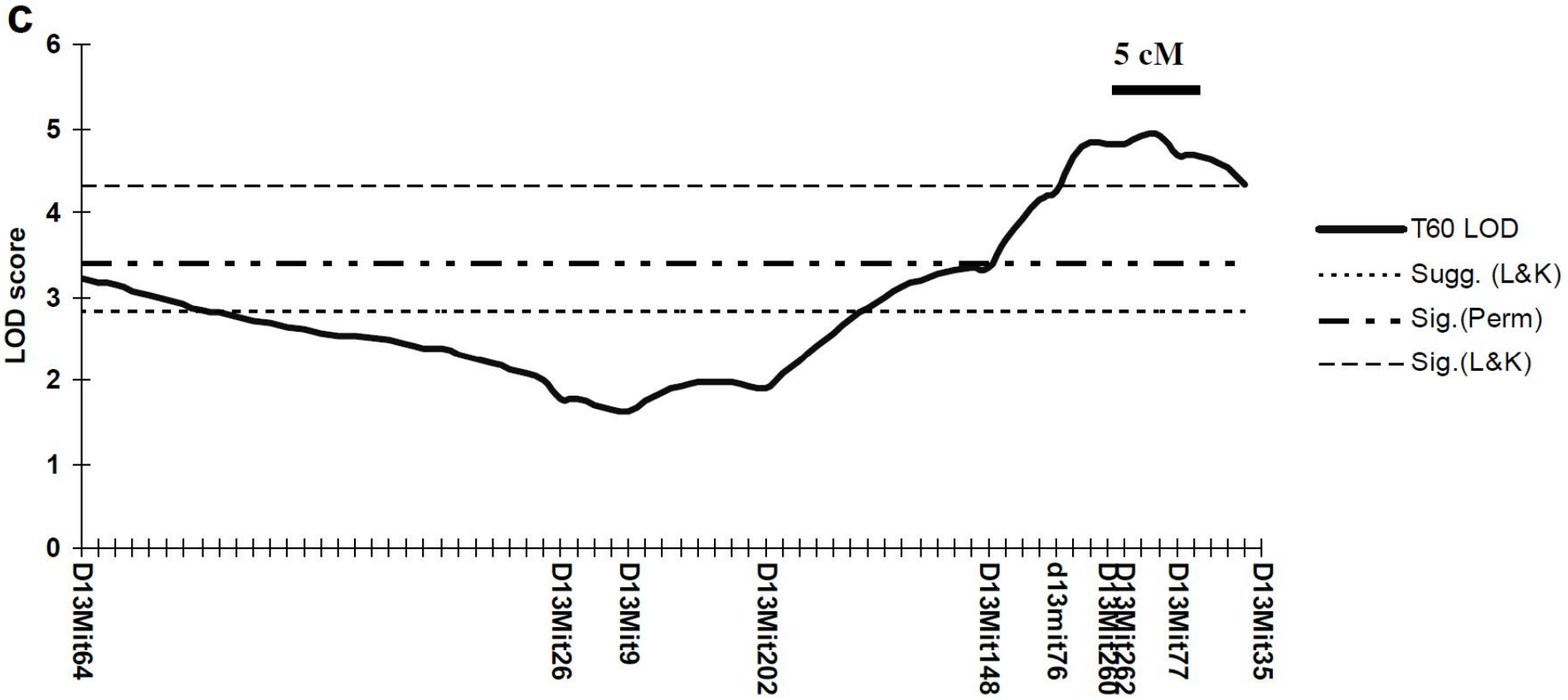


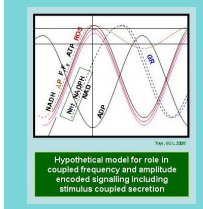
A QTL determinant of glucose tolerance on mouse chromosome 19



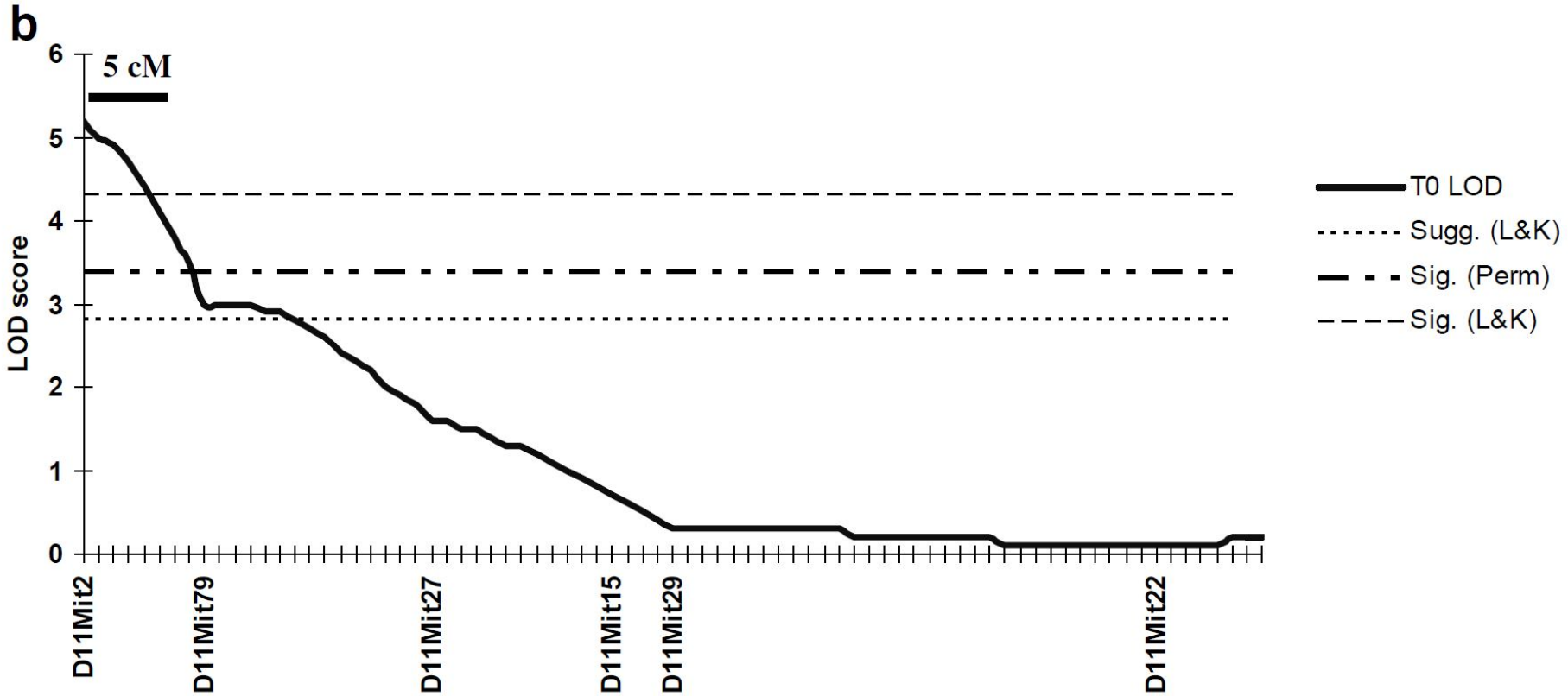


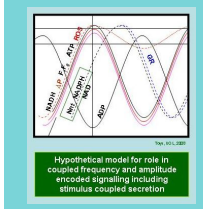
A QTL determinant of glucose tolerance on mouse chromosome 13





A QTL determinant of glucose tolerance on mouse chromosome 11





QTLs identified in a genome scan for genetic determinants of glucose tolerance and insulin secretion

Plasma trait[a]	Chromosome (locus)	LRS (LOD)[b]	Percentage	*p* value	CI[c]	Add[d]	Dom[e]	Phenotype at closest marker[f]		
								C3H (*n*)	F1 (*n*)	C57BL/6J (*n*)
T0 glucose (mmol/l)	11 (D11Mit2)	23.75 (52)	9	1E−05	23	−0.78	−0.32	4.91±1.87 (66)	5.4±1.89 (139)	6.44±1.81(68)
T30 glucose (mmol/l)	13 (D13Mit77)	22.8 (5)	8	1E−05	24	−2.16	−0.11	17.05±5.14 (71)	18.96±5.16 (126)	21.39±5.2(64)
T60 glucose (mmol/l)	13 (D13Mit262)	22.1 (4.8)	8	2E−05	25	−1.9	−0.5	13.6±4.75 (72)	14.97±4.71 (132)	17.36±4.71 (66)
AUC (min·mmol^{-1} l^{-1})	13 (D13Mit77)	21.3 (4.6)	8	2E−05	26	−180	−19	1,535.76±444 (70)	1,685.45±444 (125)	1,898.31±444 (64)
T30 insulin (ng/ml)	9 (D9Mit1001)	30.9 (6.7)	24	2E−07	20	0.19	−0.15	0.59±0.24(23)	0.3±0.23(61)	0.26±0.27(29)

Free model LOD score profile across autosomes for T0, T30, T60, AUC glucose and T30 insulin measurements. *n*=280 random mice for all glucose traits. *n*=120 mice, 60 each from opposite extremes of AUC distribution, for T30 insulin. Only significant (Lander and Kruglyak [23]) linkages are shown

Percentage % of variance explained by this locus (at D*n*Mit*n* microsatellite marker name)

[a]Measure of blood plasma trait at time indicated or AUC

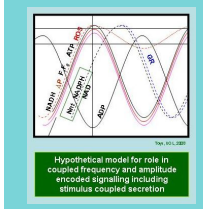
[b]Likelihood ratio statistic (LOD score)

[c]The interval around the marker, in which the QTL will localise in 95% of attempts to map it

[d]Additive regression coefficient

[e]Dominance regression coefficient

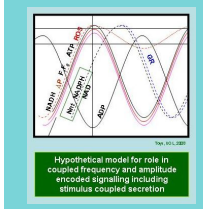
[f]Average±SD phenotype values for the indicated locus marker in the three possible genotype states: C3H homozygous, F1 heterozygous, C57BL/6J homozygous (*n* number of animals)



ANOVA Table for regression of Multiple QTLs on plasma glucose tolerance (AUC)

Factor	Type III SS	Degrees of freedom	Mean square	*F* statistic[a]	*p* value[b]	Exp (%)[c]
Corrected model	22,412,056.41	24	933,835.68	6.642044	7.56141E−16	41.36
Intercept	427,405,402.26	1	427,405,402.26	30.39.984	4.6828E−133	
D13Mit77	3,394,911.79	2	1,697,455.90	12.07341	1.04285E−05	9.65
D13Mit64	492,705.72	2	246,352.86	1.752221	0.175735623	1.53
D19Mit41	1,032,581.65	2	516,290.82	3.672195	0.026949558	3.15
D13Mit64 * D19Mit41	4,354,787.68	4	1,088,696.92	7.743517	7.2731E−06	12.05
D2Mit200	101,147.70	2	50,573.85	0.359714	0.698274735	0.32
D9Mit311	578,235.94	2	289,117.97	2.056394	0.130301167	1.79
D2Mit200 * D9Mit311	2,895,102.84	4	723,775.71	5.147961	0.000548529	8.35
D7Mit91	2,844,846.90	2	1,422,423.45	10.1172	6.19226E−05	8.22
D6Mit268	3,263,969.15	2	1,631,984.57	11.60773	1.58957E−05	9.32
D16Mit146	1,839,186.94	2	919,593.47	6.540744	0.001732053	5.47
Error	31,774,385.23	226	140,594.62			
Total	781,242,405.41	251				
Corrected total	54,186,441.64	250				
r^2=0.414						
(Adjusted r^2=0.351)						

Factor is source of plasma glucose AUC variation; SS is type III sum of squares adjusting for all other terms in the model
[a]Based on adjusted sum of squares
[b]Based on the *F* distribution
[c]Estimate of variance explained by a factor or 'eta square', expressed in percentage terms (Exp%=100×SSfactor/[SSfactor+SSerror])



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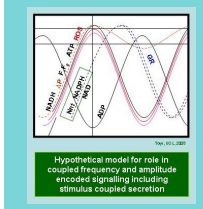

A 5 exon deletion in C57BL/6J Nnt determines insulin insufficiency glucose intolerance (GlucHos1)


```
                261       270       280       290       300       310       320       330       340       350       360       370       380       390
                |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
      129retro  AALEQFKSLGAEPLEVDLKESGEGQGGYAKEMSKEFIEAEMKLFAQQCKEVDILISTALIPGKKAPVLFSKEMIESMKEGSVVVDLAAEAGGNFETTKPGELYVHKGITHIGYTDLPSRMATQASTLYSN
        nod_retro  AALEQFKSLGAEPLEVDLKESGEGQGGYAKEMSKEFIEAEMKLFAQQCKEVDILISTALIPGKKAPVLFSKEMIESMKEGSVVVDLAAEAGGNFETTKPGELYVHKGITHIGYTDLPSRMATQASTLYSN
        fvb_retro  AALEQFKSLGAEPLEVDLKESGEGQGGYAKEMSKEFIEAEMKLFAQQCKEVDILISTALIPGKKAPVLFSKEMIESMKEGSVVVDLAAEAGGNFETTKPGELYVHKGITHIGYTDLPSRMATQASTLYSN
retro_B6xCBAJ_F!  AALEQFKSLGAEPLEVDLKESGEGQGGYAKEMSKEFIEAEMKLFAQQCKEVDILISTALIPGKKAPVLFSKEMIESMKEGSVVVDLAAEAGGNFETTKPGELYVHKGITHIGYTDLPSRMATQASTLYSN
        b6j_retro  AALEQFKSLGAEPLEVDLKESGEGQGGYAKEMSKEFIEAEMKLFAQQCKEVDILISTALIPG----------------------------------------------------------------
        Consensus  AALEQFKSLGAEPLEVDLKESGEGQGGYAKEMSKEFIEAEMKLFAQQCKEVDILISTALIPGkkapvlfskemiesmkegsvvvdlaaeaggnfettkpgelyvhkgithigytdlpsrmatqastlysn

                391       400       410       420       430       440       450       460       470       480       490       500       510       520
                |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
      129retro  NITKLLKAISPDKDNFHFEVKDDFDFGTMSHVIRGTVVMKDGKVIFPAPTPKNIPEEAPVKPKTVAELEAEKAGTVSMYTKTLTTASVYSAGLTGMLGLGIVAPNVAFSQMVTTFGLAGIIGYHTVWGVT
        nod_retro  NITKLLKAISPDKDNFHFEVKDDFDFGTMSHVIRGTVVMKDGKVIFPAPTPKNIPEEAPVKPKTVAELEAEKAGTVSMYTKTLTTASVYSAGLTGMLGLGIVAPNVAFSQMVTTFGLASIIGYHTVWGVT
        fvb_retro  NITKLLKAISPDKDNFHFEVKDDFDFGTMSHVIRGTVVMKDGKVIFPAPTPKNIPEEAPVKPKTVAELEAEKAGTVSMYTKTLTTASVYSAGLTGMLGLGIVAPNVAFSQMVTTFGLASIIGYHTVWGVT
retro_B6xCBAJ_F!  NITKLLKAISPDKDNFHFEVKDDFDFGTMSHVIRGTVVMKDGKVIFPAPTPKNIPEEAPVKPKTVAELEAEKAGTVSMYTKTLTTASVYSAGLTGMLGLGIVAPNVAFSQMVTTFGLAGIIGYHTVWGVT
        b6j_retro  ----------------------------------------------------------------------------------------------------------------------------
        Consensus  nitkllkaispdkdnfhfevkddfdfgtmshvirgtvvmkdgkvifpaptpknipeeapvkpktvaeleaekagtvsmytktlttasvysagltgmlglgivapnvafsqmvttfgla.iigyhtvwgvt

                521       530       540       550       560       570       580       590       600       610       620       630       640       650
                |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
      129retro  PALHSPLMSVTNAISGLTAVGGLALMGGHFYPSTTSQSLAALATFISSVNIAGGFLVTQRMLDMFKRPTDPPEYNYLYLLPGGTFVGGYLAALYGGYNIEEIMYLGSGLCCVGALGGLSTQGTARLGNAL
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        fvb_retro  PALHSPLMSVTNAISGLTAVGGLALMGGHFYPSTTSQSLAALATFISSVNIAGGFLVTQRMLDMFKRPTDPPEYNYLYLLPGGTFVGGYLAALYGGYNIEEIMYLGSGLCCVGALGGLSTQGTARLGNAL
retro_B6xCBAJ_F!  PALHSPLMSVTNAISGLTAVGGLALMGGHFYPSTTSQSLAALATFISSVNIAGGFLVTQRMLDMFKRPTDPPEYNYLYLLPGGTFVGGYLAALYGGYNIEEIMYLGSGLCCVGALGGLSTQGTARLGNAL
        b6j_retro  ----------------------------------------------------GFLVTQRMLDMFKRPTDPPEYNYLYLLPGGTFVGGYLAALYGGYNIEEIMYLGSGLCCVGALGGLSTQGTARLGNAL
        Consensus  palhsplmsvtnaisgltavgglalmgghfypsttsqslaalatfissvniagGFLVTQRMLDMFKRPTDPPEYNYLYLLPGGTFVGGYLAALYGGYNIEEIMYLGSGLCCVGALGGLSTQGTARLGNAL

                651       660       670       680       690       700       710       720       730       740       750       760       770       780
                |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
      129retro  GMIGVAGGLAATLGGLKPDPQLLAQMSGAMAMGGTIGLTIAKRIQISDLPQLVAAFHSLVGLAAVLTCMAEYIVEYPHFAMDATSNFTKIVAYLGTYIGGVTFSGSLVAYGKLQGILKSAPLLLPGRHAL
        nod_retro  GMIGVAGGLAATLGGLKPDPQLLAQMSGAMAMGGTIGLTIAKRIQISDLPQLVAAFHSLVGLAAVLTCMAEYIVEYPHFAMDATSNFTKIVAYLGTYIGGVTFSGSLVAYGKLQGILKSAPLLLPGRHAL
        fvb_retro  GMIGVAGGLAATLGGLKPDPQLLAQMSGAMAMGGTIGLTIAKRIQISDLPQLVAAFHSLVGLAAVLTCMAEYIVEYPHFAMDATSNFTKIVAYLGTYIGGVTFSGSLVAYGKLQGILKSAPLLLPGRHAL
retro_B6xCBAJ_F!  GMIGVAGGLAATLGGLKPDPQLLAQMSGAMAMGGTIGLTIAKRIQISDLPQLVAAFHSLVGLAAVLTCMAEYIVEYPHFAMDATSNFTKIVAYLGTYIGGVTFSGSLVAYGKLQGILKSAPLLLLGRHAL
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        Consensus  GMIGVAGGLAATLGGLKPDPQLLAQMSGAMAMGGTIGLTIAKRIQISDLPQLVAAFHSLVGLAAVLTCMAEYIVEYPHFAMDATSNFTKIVAYLGTYIGGVTFSGSLVAYGKLQGILKSAPLLLpGRHAL

                781       790       800       810       820       830       840       850       860       870       880       890       900       910
                |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
      129retro  NAGLLAASVGGIIPFMADPSFTTGITCLGSVSGLSTLMGVTLTAAIGGADMPVVITVLNSYSGWALCAEGFLLNNNLLTIVGALIGSSGAILSYIMCVAMNRSLANVILGGYGTTSTAGGKPMEISGTHT
        nod_retro  NAGLLAASVGGIIPFMADPSFTTGITCLGSVSALSTLMGVTLTAAIGGADMPVVITVLNSYSGWALCAEGFLLNNNLLTIVGALIGSSGAILSYIMCVAMNRSLANVILGGYGTTSTAGGKPMEISGTHT
        fvb_retro  NAGLLAASVGGIIPFMADPSFTTGITCLGSVSALSTLMGVTLTAAIGGADMPVVITVLNSYSGWALCAEGFLLNNNLLTIVGALIGSSGAILSYIMCVAMNRSLANVILGGYGTTSTAGGKPMEISGTHT
retro_B6xCBAJ_F!  NAGLLAASVGGIIPFMADPSFTTGITCLGSVSALSTLMGVTLTAAIGGADMPVVITVLNSYSGWALCAEGFLLNNNLLTIVGALIGSSGAILSYIMCVAMNRSLANVILGGYGTTSTAGGKPMEISGTHT
        b6j_retro  NAGLLAASVGGIIPFMADPSFTTGITCLGSVSALSTLMGVTLTAAIGGADMPVVITVLNSYSGWALCAEGFLLNNNLLTIVGALIGSSGAILSYIMCVAMNRSLANVILGGYGTTSTAGGKPMEISGTHT
        Consensus  NAGLLAASVGGIIPFMADPSFTTGITCLGSVSaLSTLMGVTLTAAIGGADMPVVITVLNSYSGWALCAEGFLLNNNLLTIVGALIGSSGAILSYIMCVAMNRSLANVILGGYGTTSTAGGKPMEISGTHT
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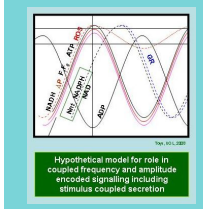
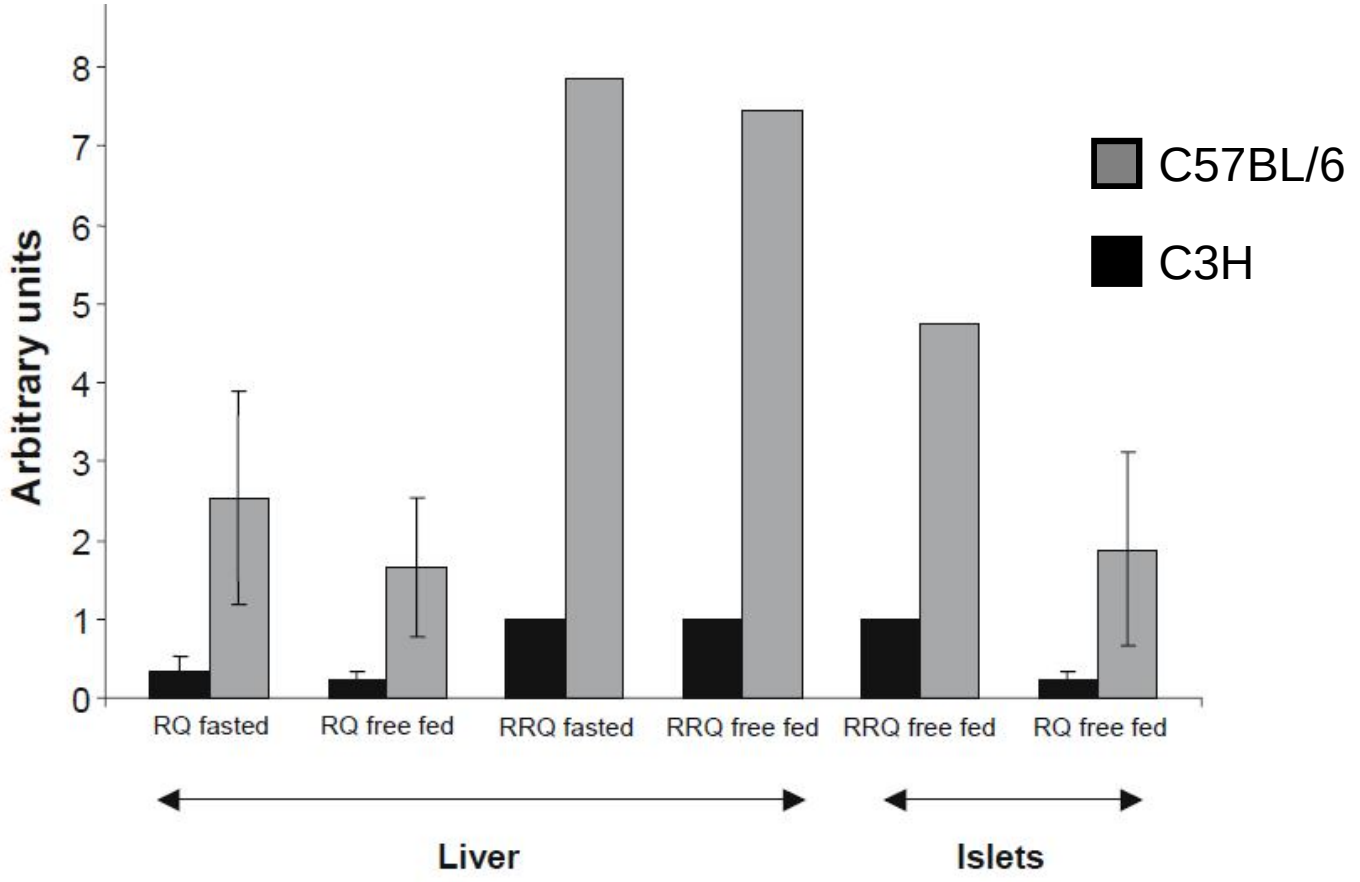
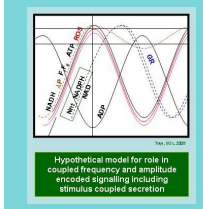
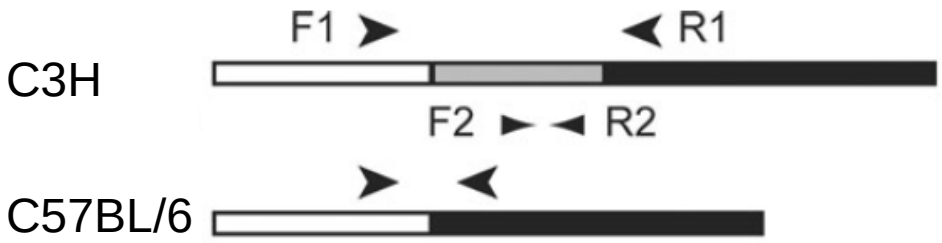


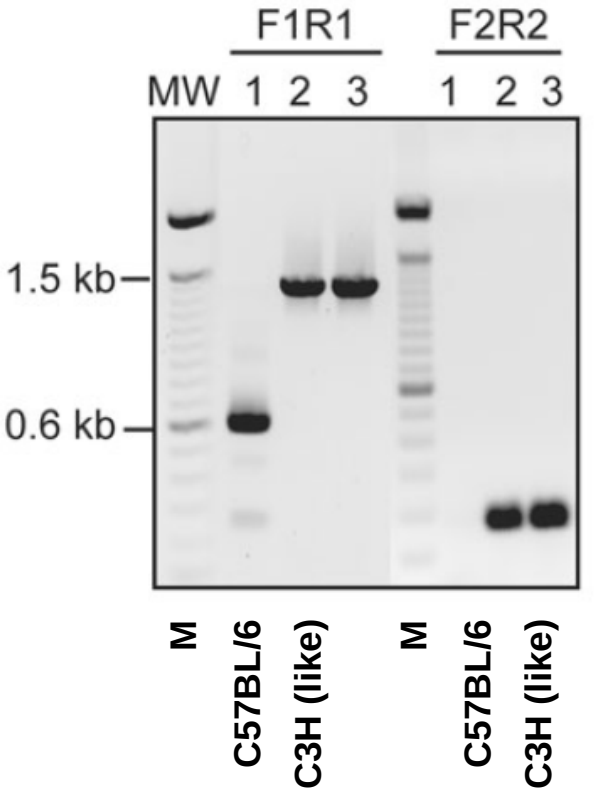
Quantitative RT-PCR assessment of Nnt gene expression in liver and islets

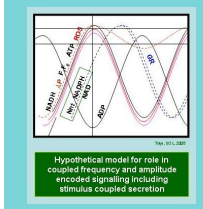




C57BL/6 Nnt transcript lacks exons 7 - 11







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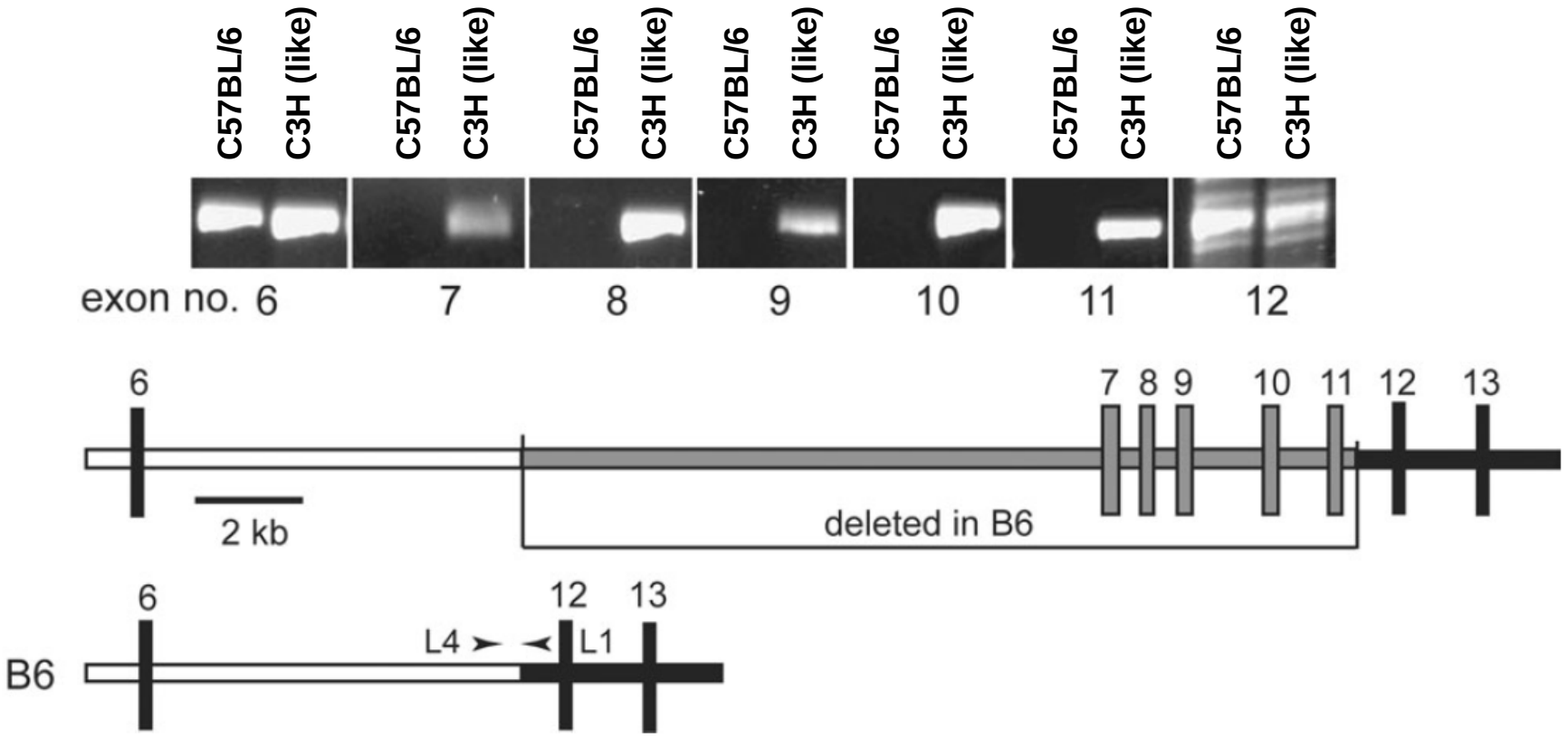
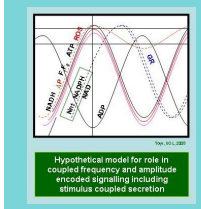
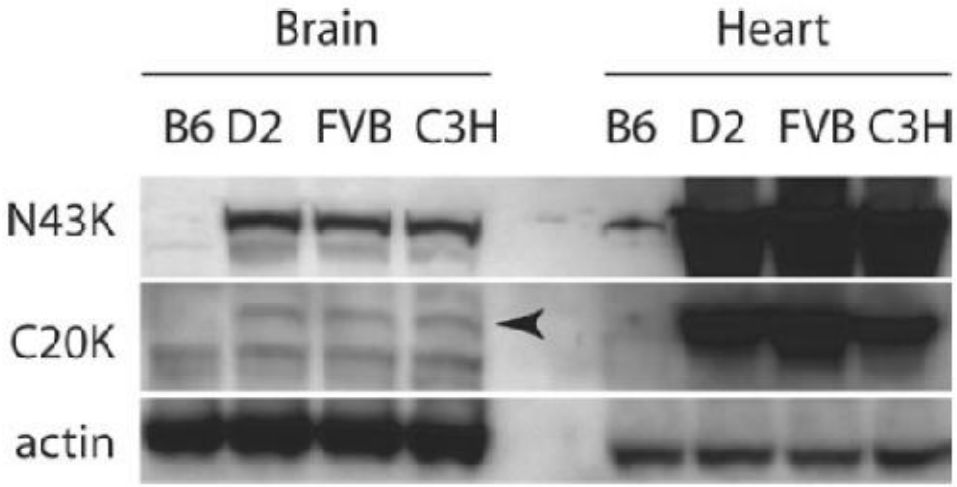


T.T. Huang et al, (2006)



NNT Protein is absent in C57BL/6 mice homozygouse for the Nnt gene exon 7 – 11 deletion

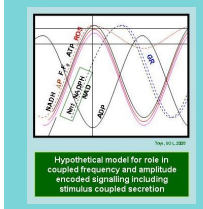


N-Terminal 43K peptide

C-Terminal 20K peptide

Control

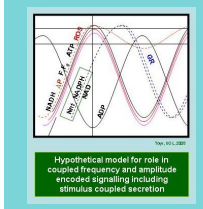
T.T. Huang et al, (2006)



Use of reverse genetics to define causal basis of disease

A multimodal sequential nesting approach

- Identifying variants
- Genetic mapping of gene determinants
- Positional cloning of causal mutations
- Elucidation of functional mechanism underlying gene action in disease
- Candidate gene studies in humans



Probing the steps leading from glucose sensing to insulin action

Mitochondrial ATP synthesis/transport to cytoplasm

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Rise in ATP/ADP ratio

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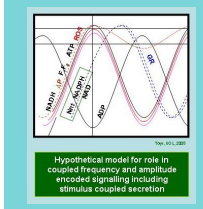
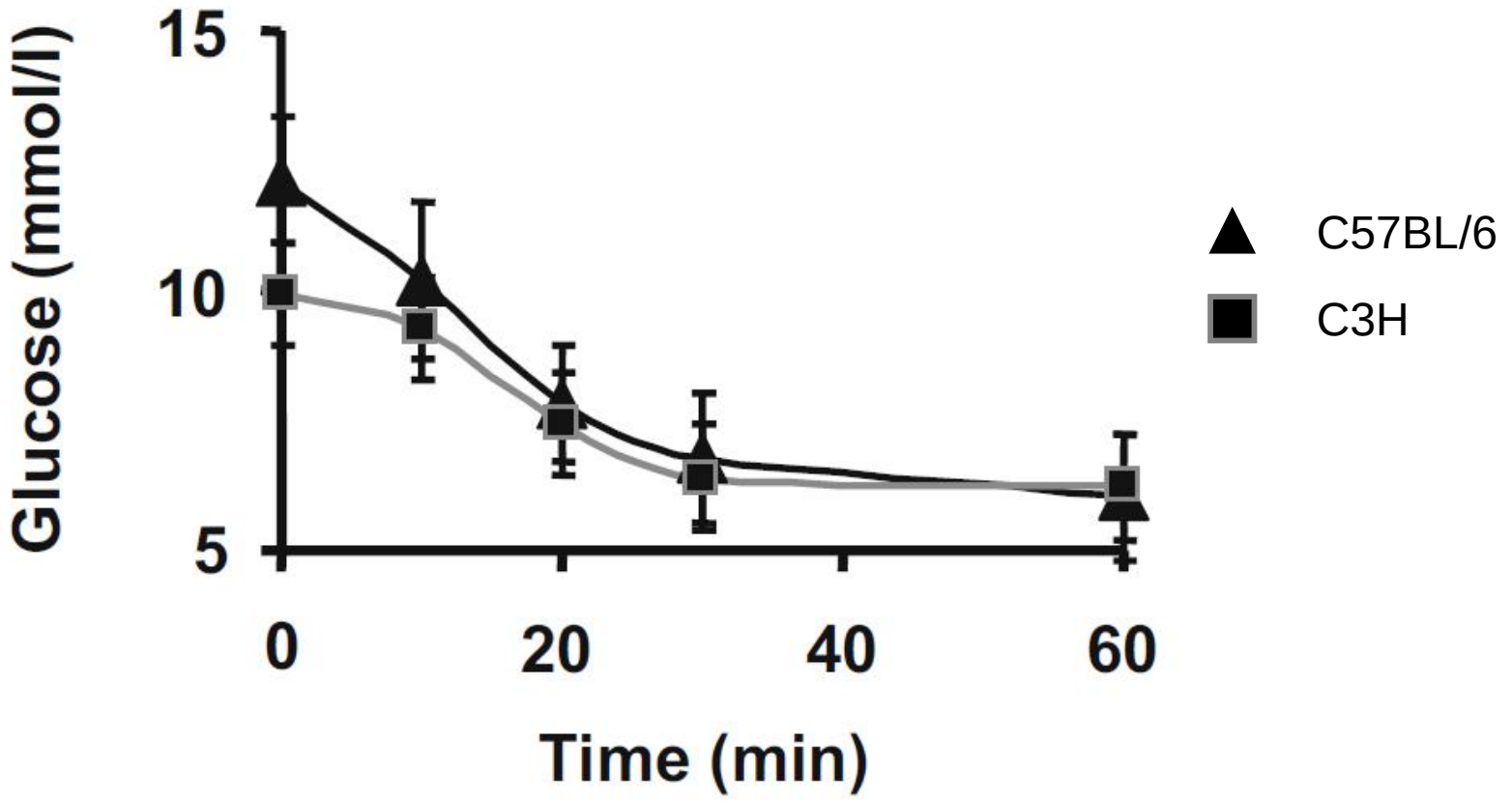
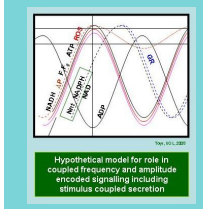
Insulin release into circulation

→ Insulin binding to receptor at target tissue and activation of signalling cascade to drive glucose uptake



Insulin tolerance test





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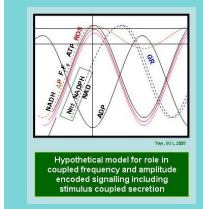


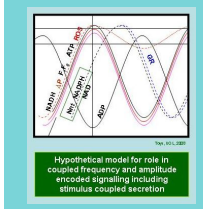
Insulin secretion in response to glucose in an IPGTT

Table 3 Male C57BL/6J mice secrete less insulin than C3H/HeH mice in response to a glucose challenge in an IPGTT

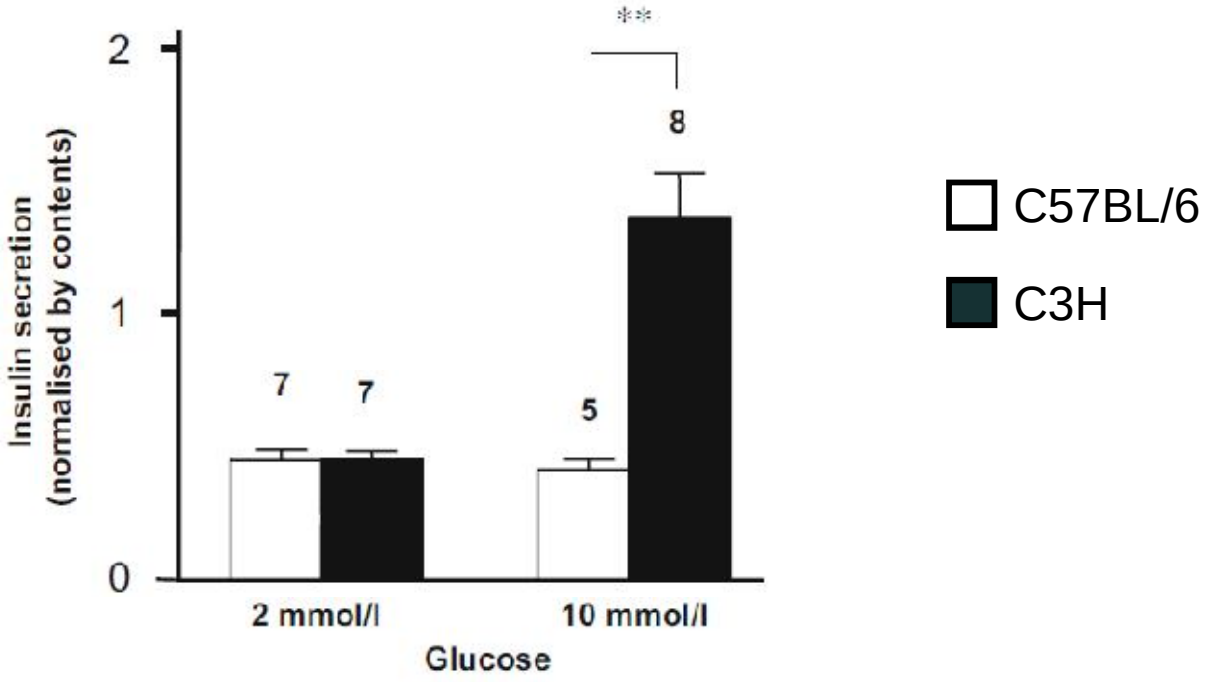
Strain	Body weight, g±SD***	Plasma insulin at timepoints in an IPGTT±SD (in ng/ml)			
		0 min**	10 min**	20 min**	30 min*
C3H/HeH (n=8)	31.77±0.99	0.46±0.22		1.00±0.57	0.63±0.44
C57BL/6J (n=8)	25.45±1.18	0.19±0.00		0.32±0.32	0.23±0.06

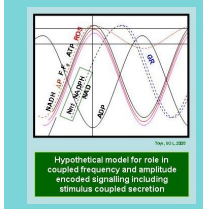
Mice were approximately 11.5 to 13 weeks old. The lower sensitivity of the insulin ELISA assays is 0.19 ng/ml and thus the levels of insulin in C57BL/6J mice at 0 and 10 min may actually be lower than shown. Differences are significant at the values of *≤0.05, **≤0.01 and ***≤0.001

*** Male C57BL/6 mice secrete significantly less insulin in response to glucose**



Pancreatic islet insulin secretion in response to glucose





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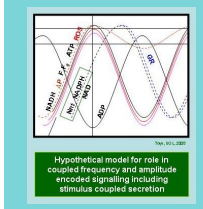
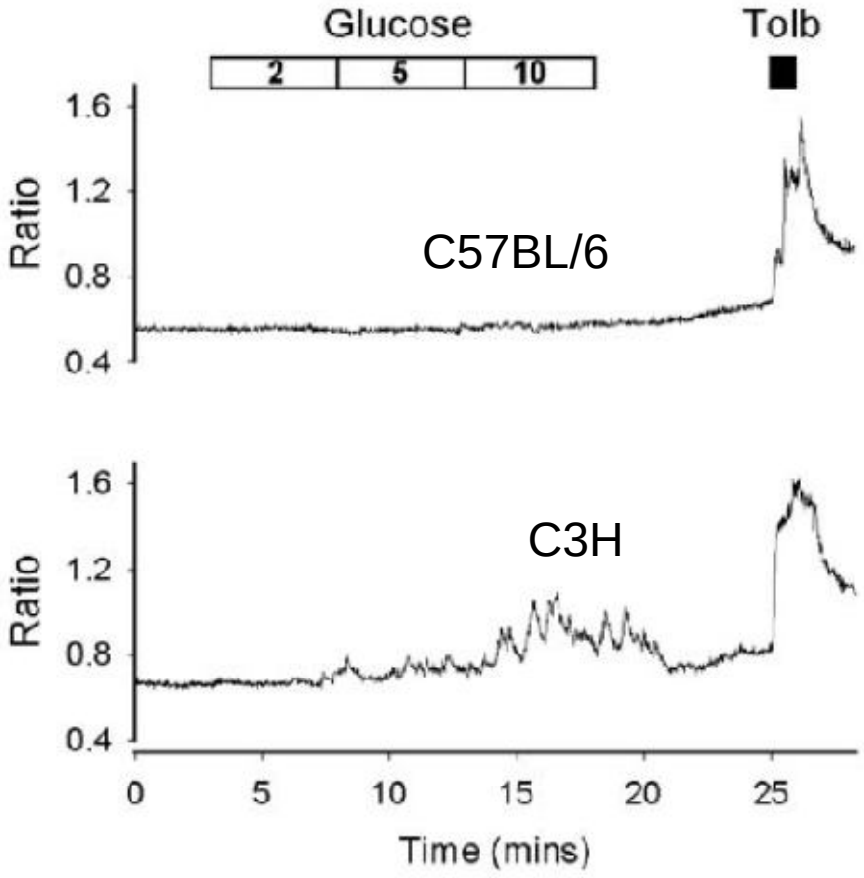
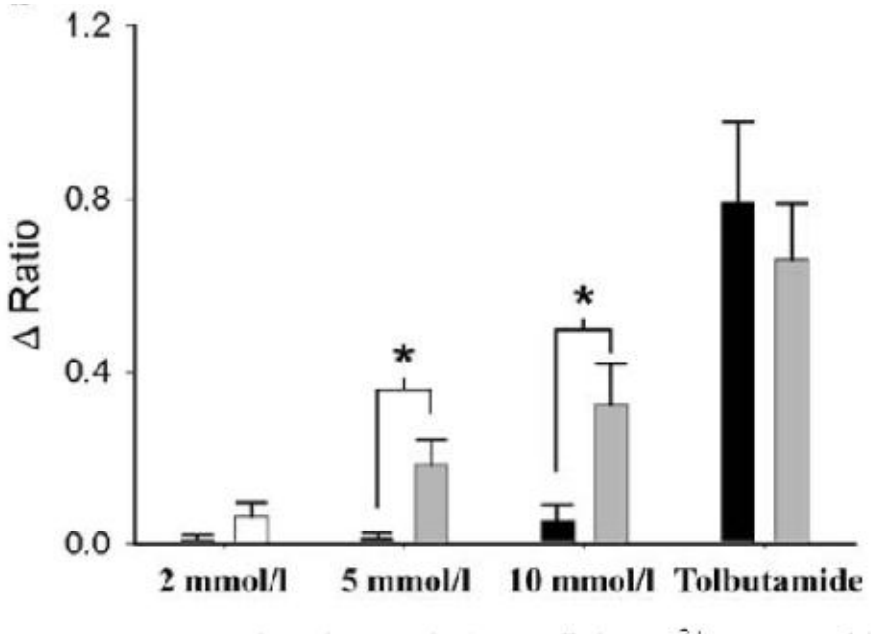
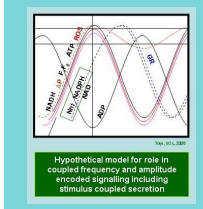


Changes in intracellular Ca^{2+} in single islets (Fura-2 fluorescence)





*C57BL/6 calcium channels are less sensitive to glucose as measured by changes in intracellular calcium



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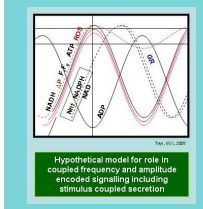
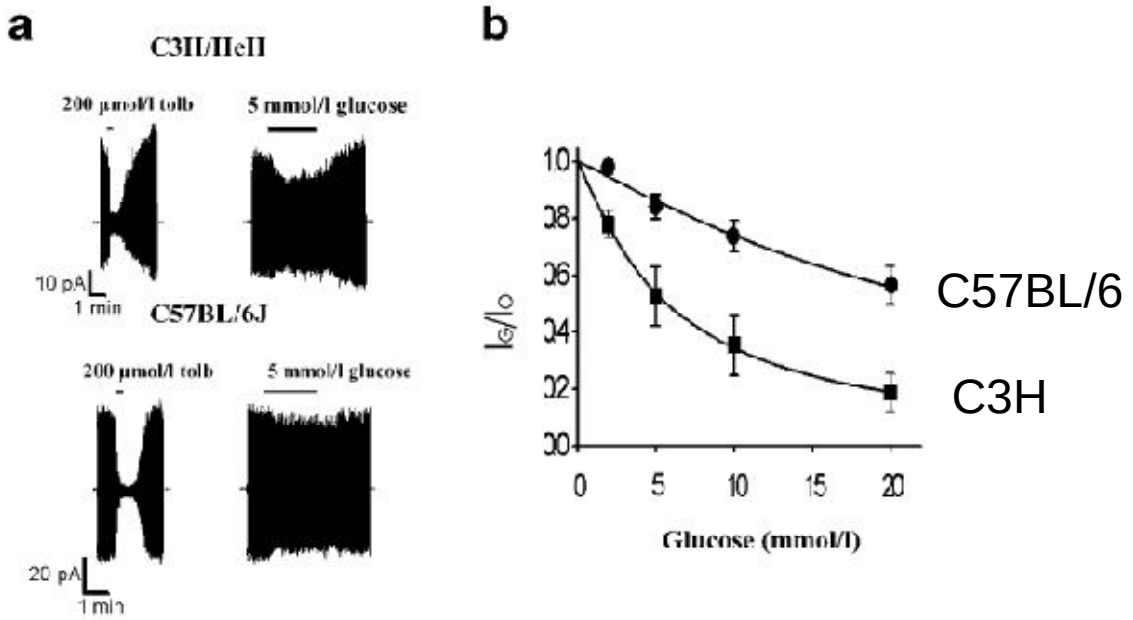
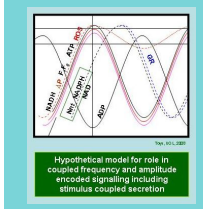


Effects of Glucose on K_{ATP} currents



Whole cell perforated patch recording from beta cells in response to ±20 mV pulses from -70 mV.

*C57BL/6 is less sensitive to glucose as measured by K_{ATP} channel closure



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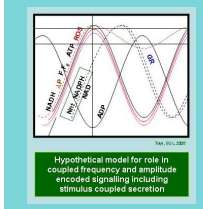
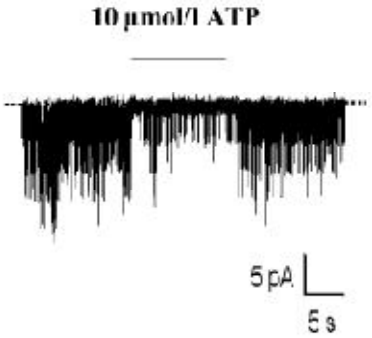
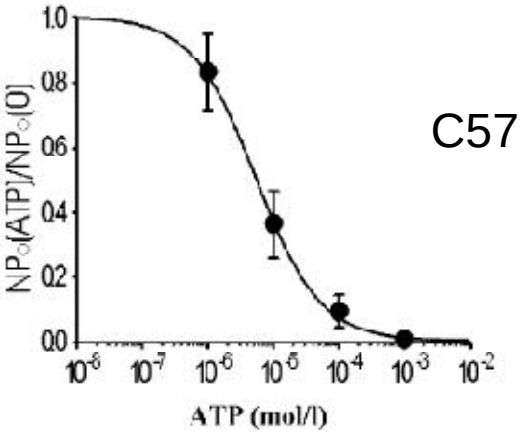


Mean ATP concentration (K_{ATP} currents) response relationship

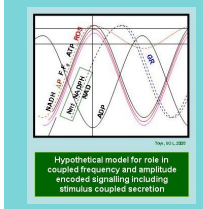




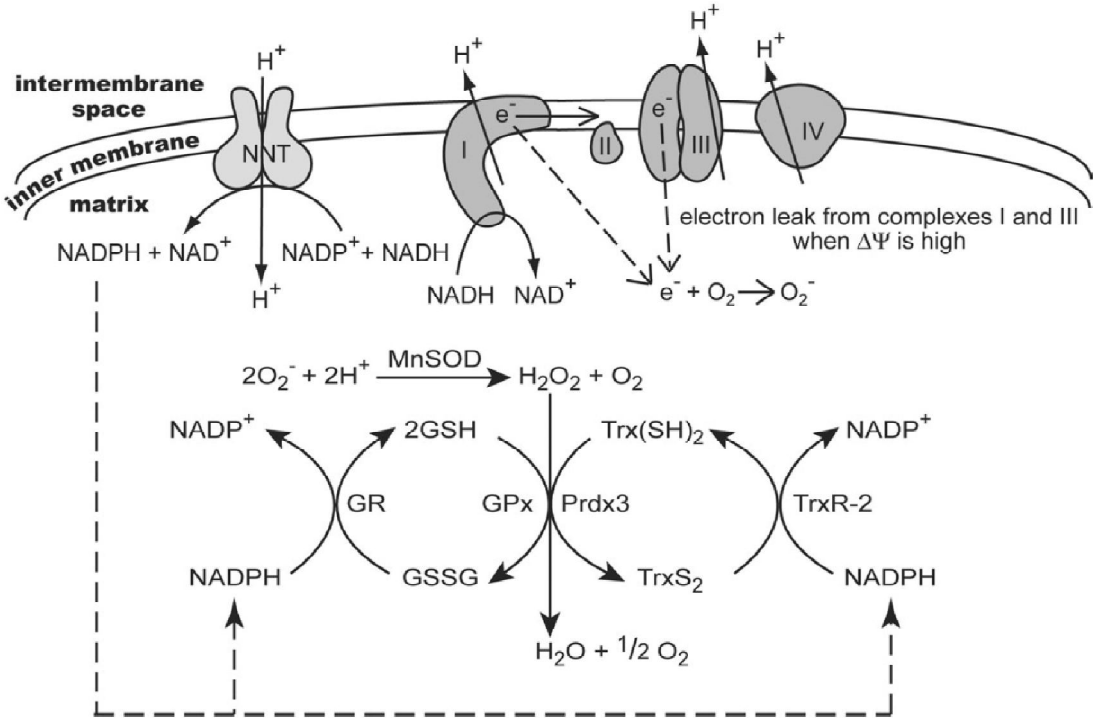
C57BL/6 = C3H

Single channel currents recorded at -60 mV from inside-out patches excised from beta cells.

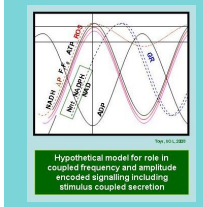
*No difference between C57BL/6 and C3H sensitivity to ATP as measured by K_{ATP} channel closure



Nnt function in mitochondria



Huang et al (2006)



Energy anatomy

Extracellular space

Cytoplasm

Mitochondrion

HEAT

UCP

↑ **Energy** for:

↑ATP/ADP ratio→ metabolism coupled secretion (e.g. insulin); Growth; Maintenance; Proliferation; Apoptosis

ATP

ADP

O_2

ETC

ΔP

ATP synthase

Other mitochondrial enzymes e.g.

Idh2 isocitrate dehydrogenase, Me3 malate dehydrogenase, Glud1 glutamate dehydrogenase

Krebs cycle

NADH

NADP+

Nnt

ROS

PLA?

PGE?

Glutamate / Amino acid metabolism (cue responsive)

NADPH

GSH/ TRXred

GR/ TRXR

GPX/PXR

GSSG/ TRXox

Messenger: Signalling; Transcription factor activation; Inflammation; Proliferation; Apoptosis

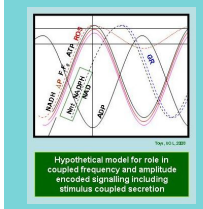
Steroid hormone biosynthesis (sex and stress hormone spatiotemporal and cue-responsive secretion and action)

Urea cycle

Lipid synthesis (mitochondrial pathway)

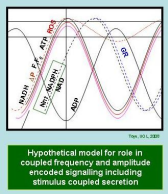
ROS

Genetic- and environmental-cause extrinsic diffusible ROS (oxidative stress syndromes) – pharmacological agents, chronic infection, high fat diet, alcoholism, nuclear/mitochondrial genomic defects in ROS regulation, ageing



Conclusions

- C57BL/6 is glucose intolerant relative to other strains and serves as a model of human disease
- C57BL/6 Glucose intolerance results from insulin insufficiency
- At least three genetic loci determine glucose intolerance in C57BL/6
- The chromosome 13 QTL effect is the result of a 5-exon deletion in the transmembrane region of the Nnt gene in C57BL/6
- Nnt action in the insulin secretion cascade occurs prior to K_{ATP} channel action
- Loss of Nnt limits glucose stimulated ATP synthesis and consequently insulin secretion, as a result, glucose intolerance develops
- Based on our mouse studies, Nnt is a strong candidate gene for type 2 diabetes causation in humans
- These studies highlight the utility of mouse based reverse genetics for defining the causal defects in heritable forms of metabolic disease as an adjunct to human studies



Perspectives

Nnt is the dynamic regulator of acute ROS and UCP is the regulator of chronic ROS in mitochondria and both molecules work together to set limits of homeostatic mitochondrial ROS and membrane potential.

Loss of Nnt drives compensatory UCP expression and function thus inhibiting appropriate stimulus coupled ROS and membrane potential homeostasis

Loss of Nnt effects are subtle under normal conditions (at least in the example of C57BL/6J laboratory mouse which lacks Nnt). Administration of stress, acutely (insulin secretion in response to elevated glycaemia) however reveals critical Nnt function. Nnt may modulate response to additional stresses, for example, we and others have shown that mice that have genetic loss or impairment of Nnt function (C57BL/6J and 129) are more sensitive to insulin resistance and fatty liver in response to high fat diet stress (Toye *et al.*, 2007) [18]. Further, such mice exhibit the broad hallmarks of oxidative stress syndromic conditions.

Current evidence suggests that Nnt is a flexible supermodifier of acute and chronic stress across a broad range of (non-) communicable diseases and pharmacological modulation of its action may constitute a very productive target for drug development.

Need: Epidemiology – various populations, various diseases e.g. oxidative stress, insulin insufficiency, and inflammatory syndromic conditions.

Need: commercial, reliable, rapid and sensitive cheap enzyme function assay.

Need: specific druggable agonist, antagonist and modulator – good candidates will be cue responsive and trifunctional, integrating ROS, ΔP and NADPH regulation.

Need: cell and animal models of gene function – control of spatiotemporal action important for elucidating function e.g. inducible and tissue specific knockout models.

Need: definition of regulome – metabolic, transcriptomic and proteomic genes, pathways and networks.

Need: further experimental paradigms for testing effect of tissue specific and or inducible knockout models.

Diabetes, fatty liver and ageing currently exist. Infection underway. Cancer studies exploration

Need: further mouse variomics.

Application: mitochondrial ageing modulator.

Application: oxidative stress, insulin insufficiency, and inflammatory syndrome modulator.

Application: chemotherapy / photodynamic therapy/ toxicology modulator.

Application: ischemia / reperfusion injury modulator

Application: Nanomachines – Nnt provides exquisite model of molecular linear motors incorporating vectorial and scalar properties. May aid development of cue sensitive nanomachines for smart drug delivery.

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ARTICLE

A. A. Toye · J. D. Lippiat · P. Proks · K. Shimomura · L.Bentley · A. Hugill · V. Mijat · M. Goldsworthy · L.Moir · A. Haynes · J. Quarterman · H. C. Freeman · F. M. Ashcroft · R. D. Cox

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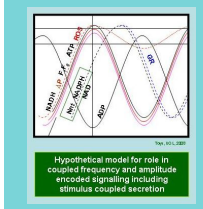

Use of reverse genetics to define causal basis of disease

A multimodal systems biology approach

- Defining the systems approach
- Collection of data
- Unimodal and multimodal analysis
- Deduction
- Candidate gene studies in humans

Acknowledgements

- University of Leicester, Leicester, UK
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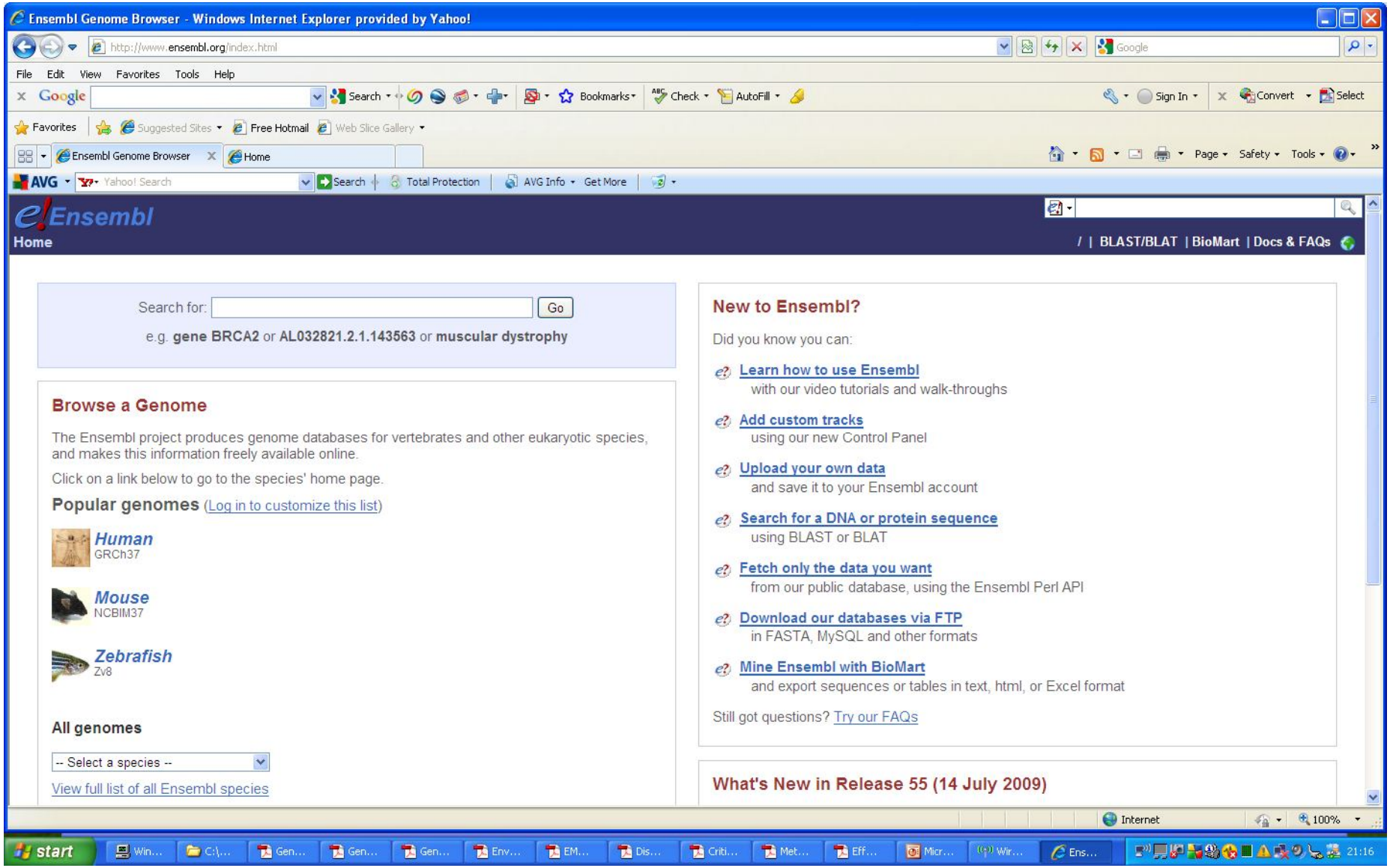
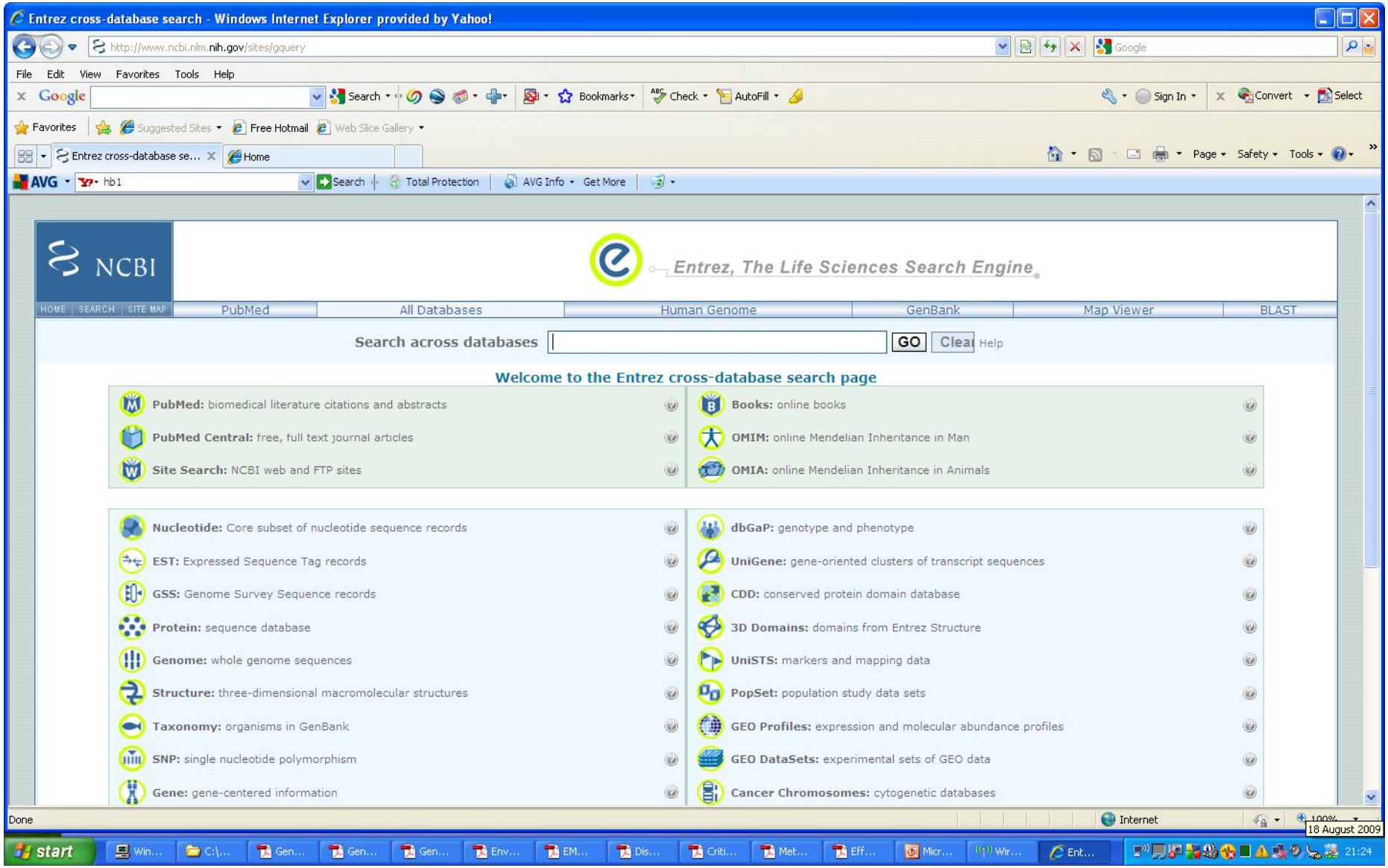
- University of Ilorin, Ilorin, Nigeria
- BAIR: Imperial College and Cambridge University

A useful site for bioinformatics



http://www.ensembl.org/

A useful site for bioinformatics



http://www.ncbi.nlm.nih.gov/sites/gquery

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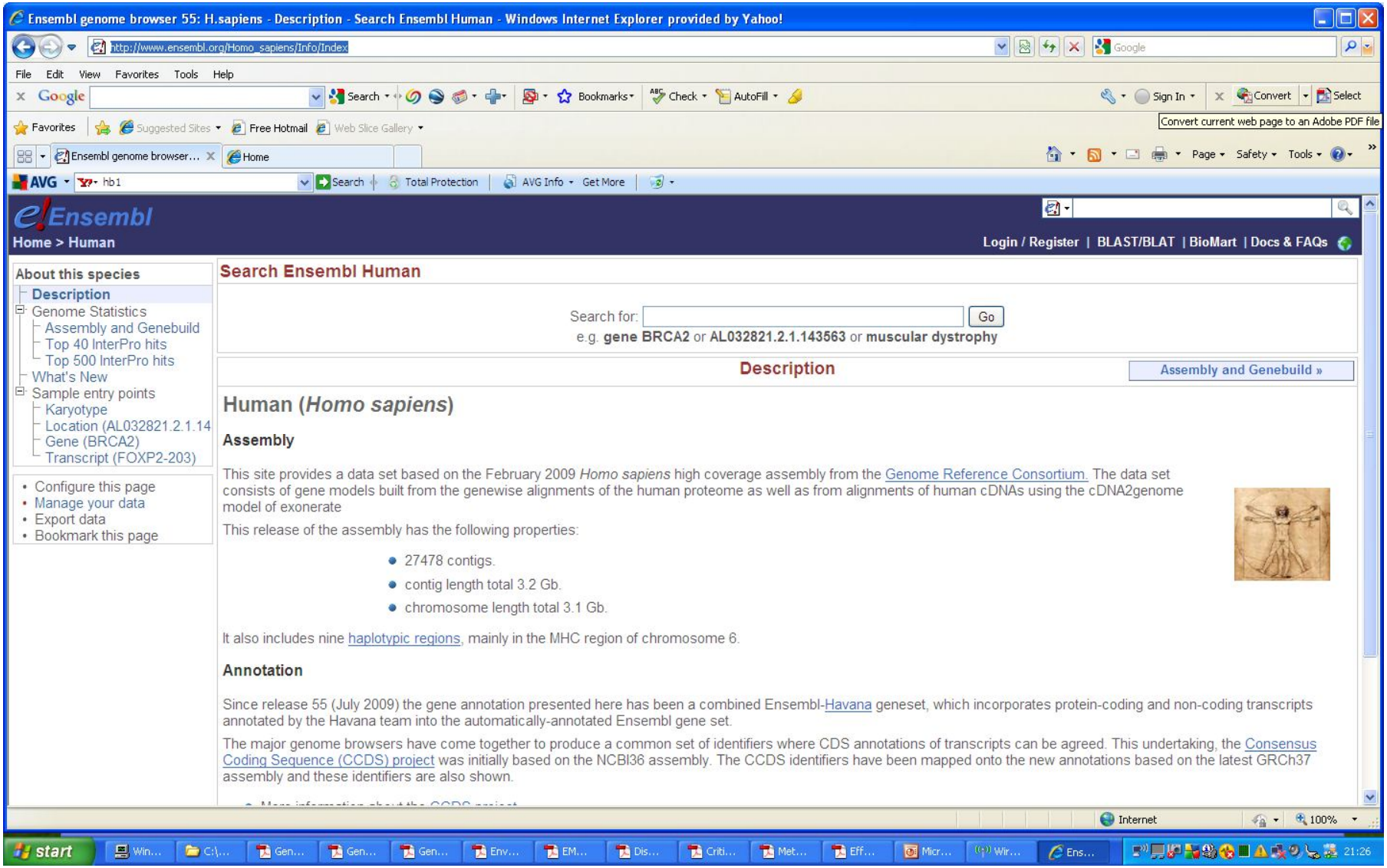

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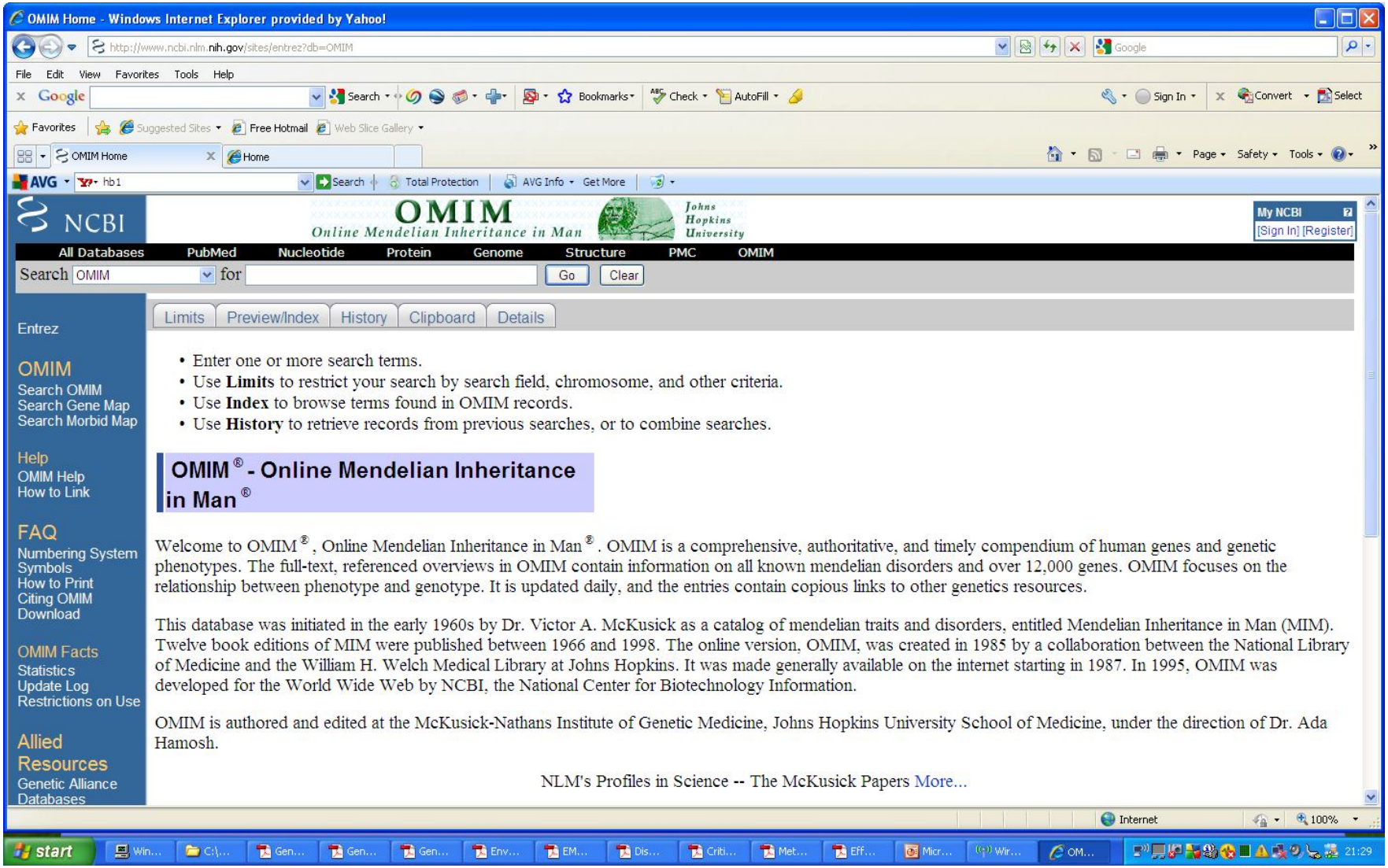

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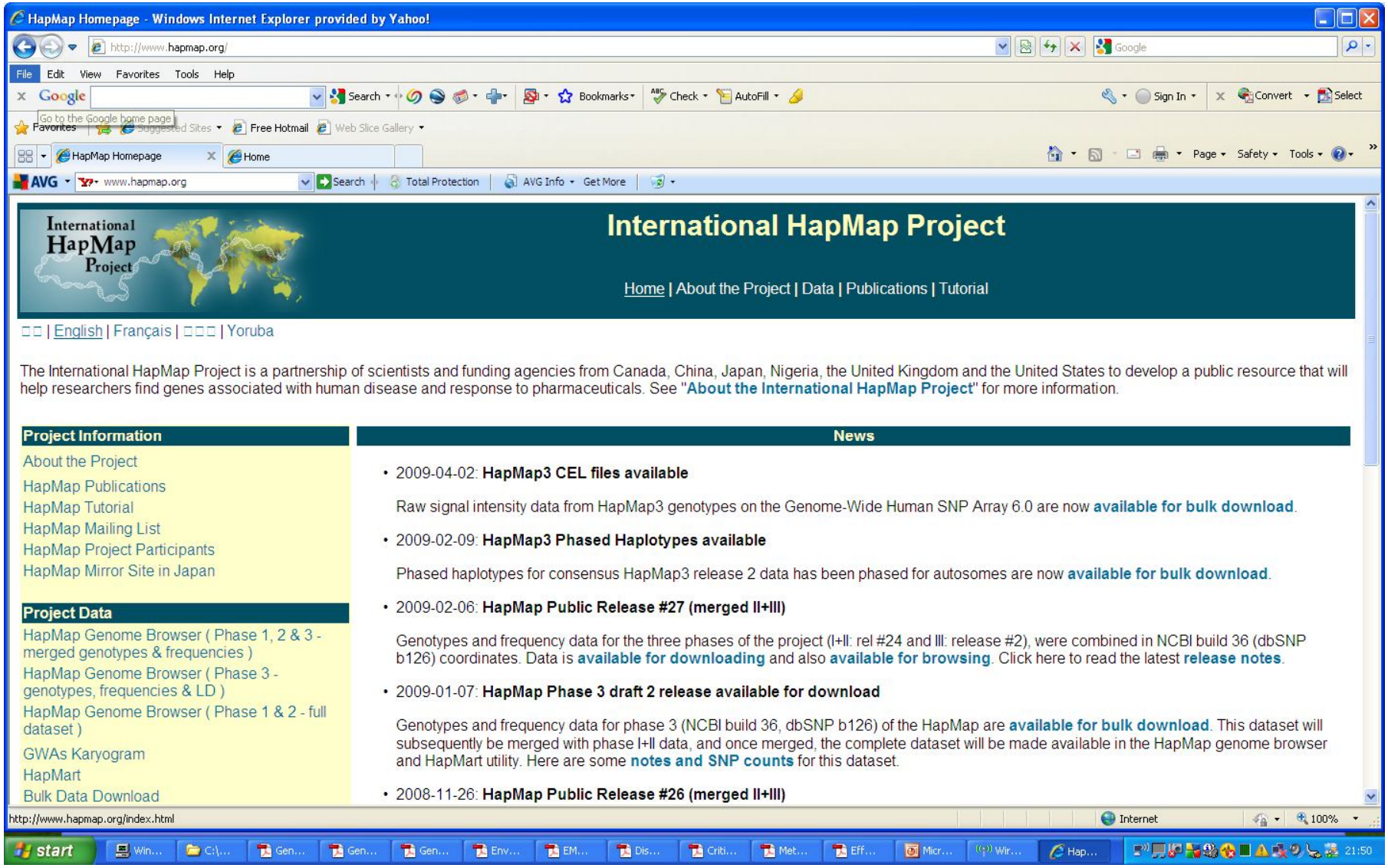

http://www.hapmap.org/