

# Population studies of polymorphism, paucimorphism and private mutation in human growth pathway genes.

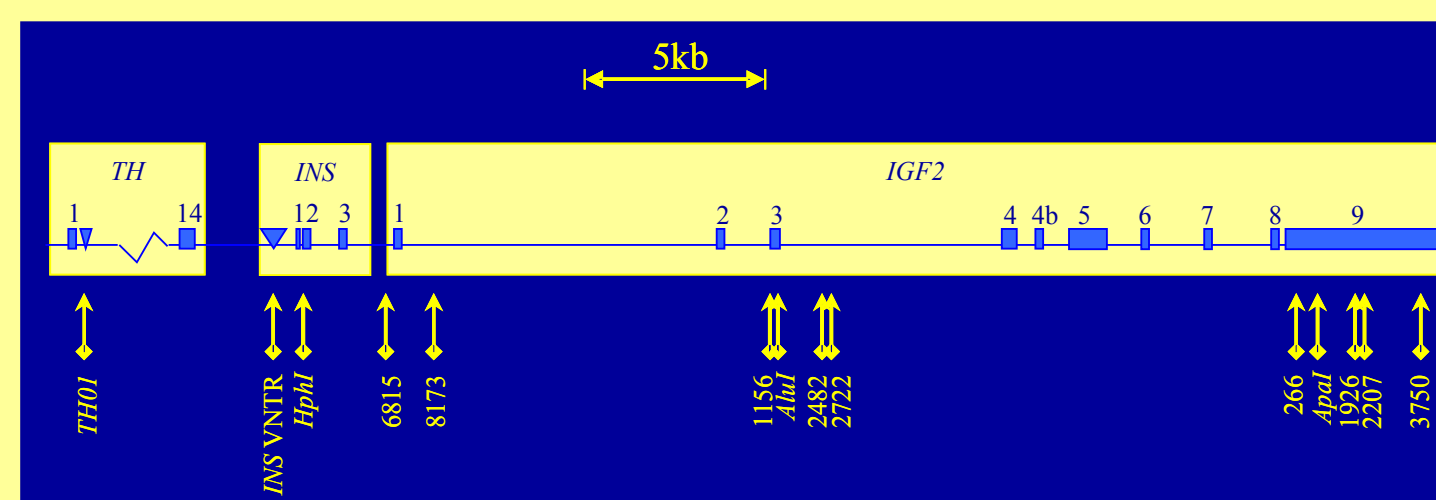
<sup>1</sup>T.R. Gaunt, <sup>1</sup>X-he Chen, <sup>1</sup>S. Huang, <sup>1</sup>T.H.T. King, <sup>1</sup>H. Patel, <sup>1</sup>M.J. Kiessling, <sup>1</sup>P.J. Briggs, <sup>1</sup>A. Voropanov, <sup>1</sup>S. Ye, <sup>1</sup>A.J. Lotery, <sup>1</sup>N.C.P. Cross, <sup>1</sup>D.M. Eccles, <sup>1</sup>S. Rodriguez, <sup>1</sup>I.N.M. Day, <sup>2</sup>E. Dennison, <sup>2</sup>A. Aihie Sayer, <sup>2</sup>H.E. Syddall, <sup>2</sup>F. Tabassum, <sup>2</sup>D.J.P. Barker, <sup>2</sup>D.I.W. Phillips, <sup>2</sup>C. Cooper, <sup>3</sup>N.J. Timpson, <sup>3</sup>G. Davey Smith, <sup>3</sup>S. Ebrahim, <sup>3</sup>S.E. Humphries, <sup>3</sup>R.A. Whittall, <sup>3</sup>G.J. Miller, <sup>4</sup>I. Simpson, <sup>5</sup>H. Rassoulain, <sup>6</sup>J. Gilg, <sup>6</sup>P. Whincup, <sup>6</sup>D.G. Cook

1. Human Genetics Division, University of Southampton, Duthie Building, Southampton General Hospital, Tremona Road, Southampton SO16 6YD, UK, 2. MRC Epidemiology Resource Centre, Developmental Origins of Health and Disease Division, University of Southampton, Southampton General Hospital, Tremona Road, Southampton SO16 6YD, UK, 3. Department of Social Medicine, University of Bristol, Canynge Hall, Whiteladies Road, Bristol BS8 2PR, UK, 4. Division of Cardiovascular Genetics, Department of Medicine, University College London, British Heart Foundation Laboratories, Rayne Institute, 5. University Street, London WC1E 6JJ, UK, 5. Wessex Cardiothoracic Centre, Southampton University Hospitals NHS Trust, Southampton General Hospital, Tremona Road, Southampton SO16 6YD, UK, 6. Medical Engineering, Southampton University Hospitals NHS Trust, Southampton General Hospital, Tremona Road, Southampton SO16 6YD, UK, 7. Department of Epidemiology, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE, UK

## ABSTRACT

There are two classical models for the impact of genetic variation in human disease. Firstly, rare but severe single gene mutations can cause a phenotype which shows strong familial clustering. Secondly, common polymorphisms (or haplotypes) may exert small individual effects but with significant population attributable risk - for example we have shown that haplotypes of IGF2-INS-TH influence a variety of cardiovascular risk traits (Rodriguez et al 2004, Human Molecular Genetics 13; 15-25) and that SNP and microsatellite markers across the GH-CSH gene cluster associate both with early growth, and traits of ageing including bone loss and metabolic syndrome traits (Dennison et al 2004, J Clin End Metab 89 (10) 4898-903 and Day et al 2004, J Clin End Metab 89 (11) 5569-5576). We previously estimated that 4/5 of all codon mutations in LDLR do not come to clinical attention as familial hypercholesterolemia (Day et al 1997, Human Mutation 10; 116-127), raising the question whether they cause moderate or mild hypercholesterolemia, have no effect or might be protective; and we have shown that these arguments pertain to growth genes too. We have also considered the theoretical case for an intermediate model in which 'paucimorphism' (arbitrarily 0.05<q<0.0005) of moderate effect could be an important genetic contribution to diseases (Day et al 2004, Current Genomics 5; 431-438). We have recently developed technologies for economical high throughput identification of unknown mutations in large population DNA banks and applied these to several genes including GHR and MC4R, scoping the full mutational spectrum in up to 10,000 unselected growth phenotyped subjects. These studies illustrate a holistic approach to examining the contribution of the complete mutational spectrum of growth pathway genes to human disease traits.

## Haplotypes of IGF2-INS-TH and cardiovascular risk traits\*



Tyrosine hydroxylase (*TH*), insulin (*INS*) and insulin-like growth factor II (*IGF2*) on chromosome 11 (11p15.5)

\*Rodriguez et al 2004, Human Molecular Genetics 13; 15-25

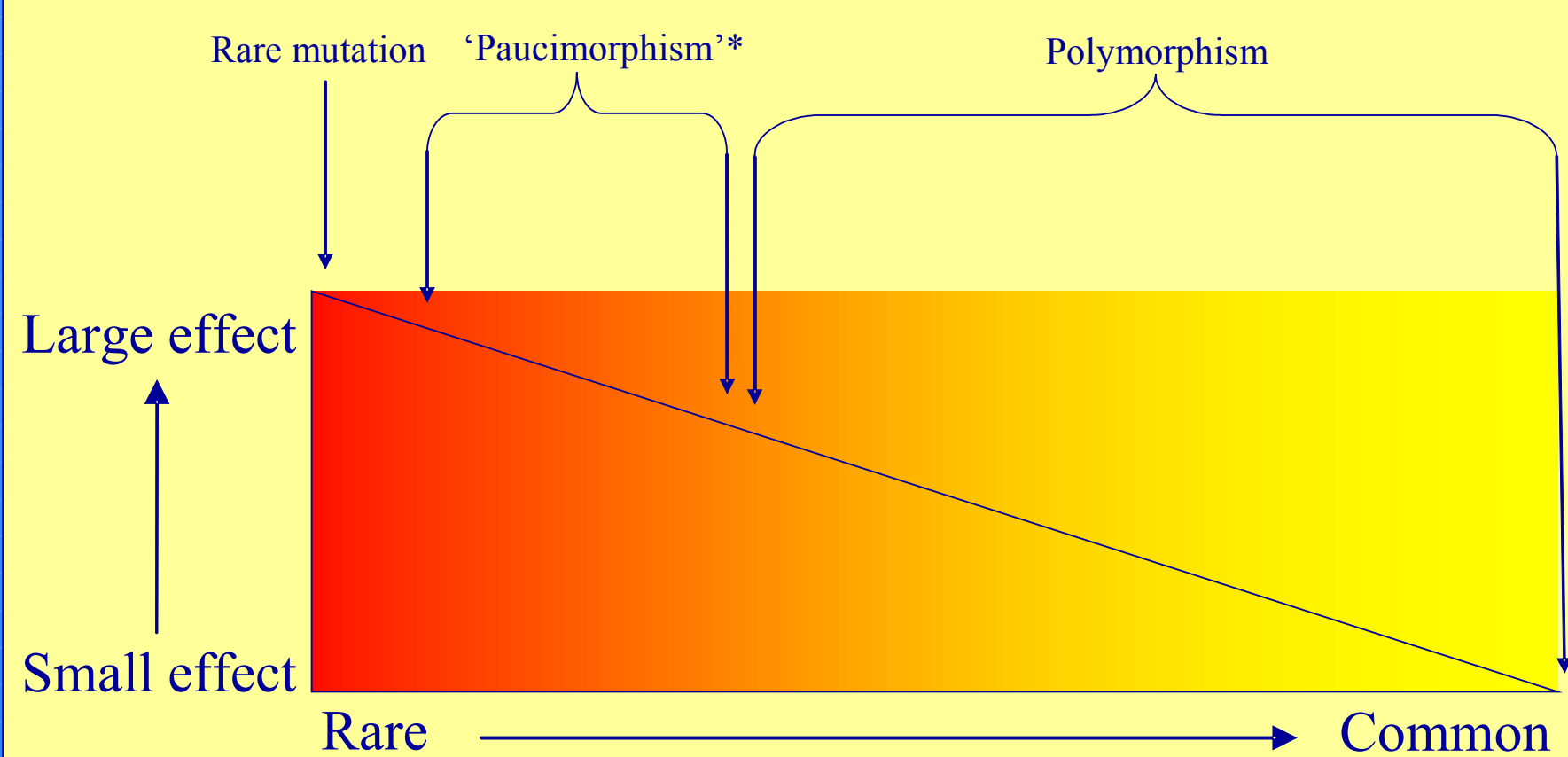
## Summary of TH-INS-IGF2 effects

- Haplotype 3: Increased weight and height
- Haplotype 4: Increased fat (% and FM) and diastolic BP
- Haplotype 5: Decreased weight, BMI, LBM and plasma TG
- Haplotype 6: Increased plasma TG
- Haplotype 8: Decreased height
- Haplotype 10: Decreased fat mass

INS VNTR class III (low insulin production) in haplotypes 4, 6 and 8  
Higher BP in haplotype 4 equates to about 6-8% increment in stroke risk and 4-5% in CHD risk<sup>1</sup>  
Lower BMI (1 unit) in haplotype 5 equates to approx 10% reduced rate of coronary events<sup>2</sup>  
Functional element of haplotypes could be in any of the 3 genes

<sup>1</sup> MacMahon et al., Lancet 335:765-774  
<sup>2</sup> Shaper et al., BMJ 314:1311-1317

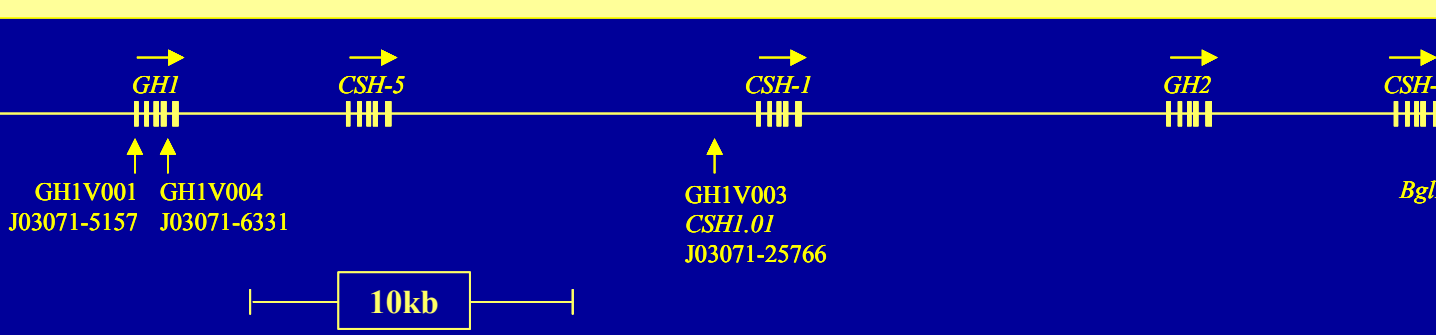
## Polymorphism, paucimorphism and mutation



\*Paucimorphism\* - arbitrarily rare allele freq. 0.05 to 0.0005 (Day et al 2004, Current Genomics 5; 431-438)

## Variation in the GH-CSH gene cluster, early growth and metabolic syndrome traits\*

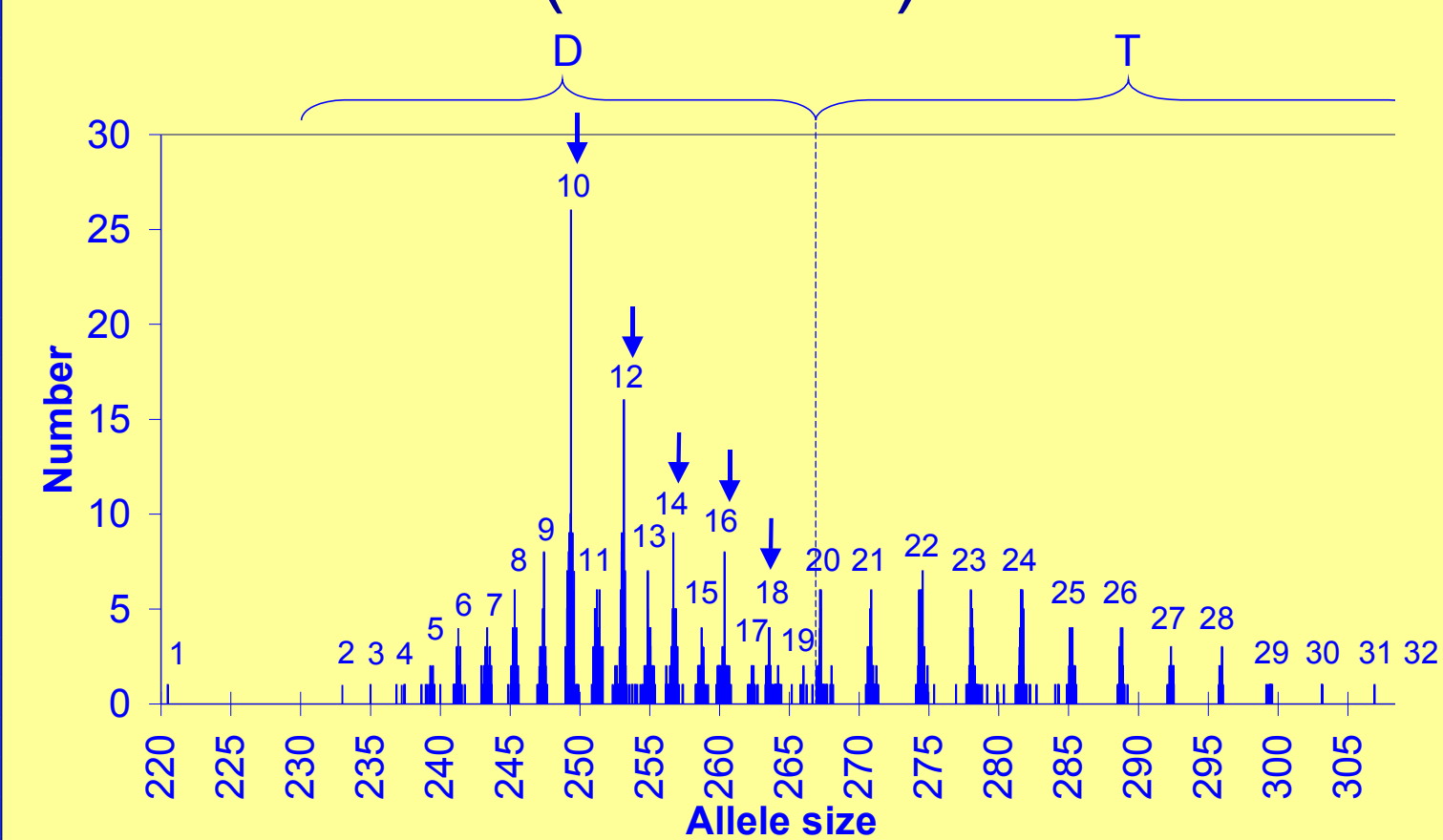
Growth Hormone (*GH1*), Chorionic Somatomammotropin Hormones (*CSH1* and *CSH2* plus *CSHL1/CSH5*) and Growth Hormone Variant (*GH2*) on chromosome 17 (17q24.2)  
Hertfordshire cohort, Herts, UK - 1108 men and women 61-73yo.



GH1V001 and GH1V004 - SNPs in *GH1* gene  
CSH1.01 (GH1V003) - microsatellite upstream of *CSH1*  
BglII-B - restriction site polymorphism downstream of *CSH2*

\*Day et al 2004, J Clin End Metab 89 (11) 5569-5576

## Allele binning in the GH microsatellite (CSH1.01)



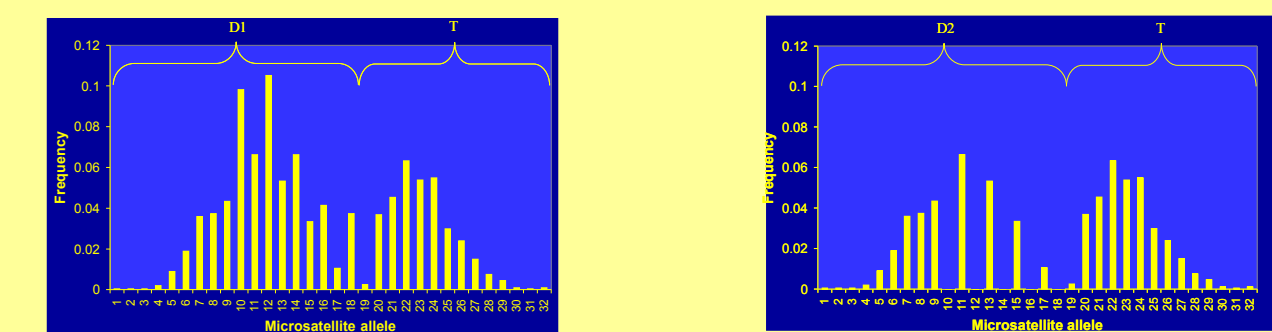
Data from Tabitha King and Xiao-he Chen

## Variation in the GH-CSH gene cluster, early growth and adult bone mass\*

- 205 men and 132 women aged 61-73yo from Hertfordshire, UK
- Bone density change over 4 years:
  - GH1V001 SNP rare allele associated with significantly greater bone loss at spine (p = 0.03) and hip (p = 0.04)
  - GH1V004 SNP rare allele associated with significantly greater bone loss at spine (p = 0.008)
- Trend (non-significant) for association between GH1V001 rare allele and lower weight at one year old in men only (consistent with significant findings from the microsatellite association with 1yr weight in the full cohort)

\*Dennison et al 2004, J Clin End Metab 89 (10) 4898-903

## Phenotypic associations with GH microsatellite I



	T/T	D1/T	D1/D1	N	p	T/T	D2/T	D2/D2	N	p
Systolic blood pressure	171	162.7	163.2	214	0.28	171	162.8	153.4	94	0.004
Diastolic blood pressure	93.1	90.1	90.2	214	0.44	93.1	89.4	85.6	94	0.03
Pulse pressure	77.8	72.6	72.9	214	0.35	77.8	73.4	67.8	94	0.02
Pulse rate (per min.)	74.1	70	69.6	214	0.47	74.1	67.1	71.9	94	0.04*
Ln (ins0)	4.06	3.72	3.56	206	0.001	4.06	3.86	3.55	92	0.009
Ln (ins30)	5.82	5.65	5.47	202	0.005	5.83	5.67	5.44	89	0.03
Ln (ins120)	5.21	4.97	4.90	197	0.18	5.21	5.04	4.97	86	0.37
Ln (glu0)	1.8	1.8	1.77	212	0.23	1.8	1.83	1.75	94	0.49
Ln (glu30)	2.3	2.26	2.22	209	0.05	2.3	2.28	2.16	92	0.04
Ln (glu120)	1.91	1.87	1.86	204	0.49	1.91	1.92	1.84	88	0.57
Ln (fasting triglycerides)	0.586	0.355	0.318	212	0.06	0.586	0.426	0.298	94	0.08
Birthweight	121.3	126.7	125	593	0.4	121.3	126	121.9	270	0.74
One year weight	354.9	363.6	363	593	0.24	354.9	362.3	374.2	270	0.008
Height	1.708	1.727	1.718	590	0.04*	1.708	1.723	1.73	270	0.03
Weight	78.76	80.61	79.76	590	0.85	78.76	79.94	80.9	270	0.23
Body mass index	26.98	26.96	26.97	590	0.99	26.98	26.94	26.99	270	0.97
Waist-hip ratio	0.941	0.937	0.935	589	0.34	0.941	0.934	0.934	270	0.46

P values - regression on allele (1 d.f.) except \* for genotype (2 d.f.)

Men only

## Technologies for high-throughput mutation detection

- Detection of paucimorphisms (rare allele freq. 0.05 to 0.0005) in populations of several thousand
- Conventional approaches (sequencing, SSCP, DGGE etc) time-consuming and expensive for scanning many amplicons in thousands of subjects
- Melt-MADGE\*
- microplate array diagonal gel electrophoresis with a thermal ramp in time
- Each gel carries 96 samples in a microplate compatible format (8x12)
- Up to 10 gels in one temperature-controlled electrophoresis tank
- Temperature ramp over 1 to 2 hours for 960 samples per tank

\*Day et al 1998, Trends Biotechnol. 16(7):287-90

## Technologies for high-throughput mutation detection II

### Endo-VII MADGE\*

- Combination of T4 Endonuclease VII and MADGE - fluorescently labelled heteroduplexes are cleaved using the enzyme and resolved by high-throughput gel electrophoresis
- Ideal for many amplicon/many sample situations
- Constant temperature electrophoresis, up to ten 96-well MADGE gels per tank

\*Day et al 2004, Current Genomics 5; 431-438

## Summary

- Associations between SNPs and haplotypes in the *TH-INS-IGF2* region and BMI, triglycerides and blood pressure
- Associations between a complex microsatellite in the Growth Hormone gene region and both early growth and metabolic and cardiovascular risk traits
- Associations between SNPs in the Growth Hormone gene region and both early growth and adult bone mass
- Development of new technologies for mutation and "paucimorphism" screening in large populations

## Acknowledgements

### Contributors

- X Chen, S Huang, THT King, H Patel, MJ Kiessling, PJ Briggs, A Voropanov, S Ye, AJ Lotery, NCP Cross, DM Eccles, S Rodriguez, INM Day
- EM Dennison, A Aihie Sayer, HE Syddall, F Tabassum, DJP Barker, DIW Phillips, C Cooper
- NJ Timpson, G Davey Smith, S Ebrahim
- SE Humphries, RA Whittall, G J Miller
- I Simpson
- H Rassoulain
- J Gilg, P Whincup, DG Cook

### Cohort studies

- UK Northwick Park Heart Study
- Southampton Atherosclerosis Study
- UK Hertfordshire Studies
- UK School Heart and Health Study
- British Women's Heart and Health Study

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- British Heart Foundation
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- US National Institutes of Health
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- DuPont Pharma

### References

- Rodriguez et al 2004, Human Molecular Genetics 13; 15-25
- Dennison et al 2004, J Clin End Metab 89 (10) 4898-903
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