European Journal of Human Genetics (2012) 20, 696–700

www.nature.com/ejhg

ARTICLE

Codon 72 polymorphism (rs1042522) of *TP53* is associated with changes in diastolic blood pressure over time

Erwin Reiling*[,1], Valeriya Lyssenko[2], Jolanda MA Boer[1], Sandra Imholz[1], W Monique M Verschuren[1], Bo Isomaa[3,4], Tiinamaija Tuomi[3,5], Leif Groop[2,5] and Martijn ET Dollé[1]

p53 is involved in stress response, metabolism and cardiovascular functioning. The C-allele of rs1042522 in the gene encoding for p53 is associated with longevity and cancer. In this study, we aimed to investigate the association of rs1042522 with changes in blood pressure, BMI and waist circumference using a longitudinal approach. Rs1042522 was analyzed in two longitudinal studies; the Doetinchem Cohort Study (DCS) and the Botnia Prospective Study (BPS). Changes in quantitative traits over time were investigated according to rs1042522 genotypes. An association between rs1042522 and changes in diastolic blood pressure (DBP) in the DCS over time was observed (P=0.004). Furthermore, a borderline significant association was detected with changes in waist circumference over time (P=0.03). These findings were also observed in the BPS (P=0.02 and P=0.05). The C/C-genotype (Pro/Pro) showed the most moderate time-related increase for the studied endpoints. Furthermore, data from the BPS suggested that the C/C-genotype protects against increases in glucose levels over time at 30 and 60 min during oral glucose tolerance test (P=0.01 and P=0.02). In conclusion, we found an association between the C/C-genotype of rs1042522 and changes in DBP and waist circumference over time. This might contribute to the longevity phenotype observed for the same genotype by others.

European Journal of Human Genetics (2012) **20,** 696–700; doi:10.1038/ejhg.2011.240; published online 21 December 2011

Keywords: p53; blood pressure; metabolism; longitudinal study

INTRODUCTION

The tumor protein p53 (encoded by *TP53*), as described by many studies, suppresses the cell cycle and induces apoptosis on activation after DNA damage. These processes are important for the protection against cancer and *TP53* mutations and polymorphisms have been widely associated with cancer.[1,2]

A common *TP53* polymorphism is at codon 72 (rs1042522), which changes an arginine into a proline. Allele distribution depends on ethnic background. Although the Pro-allele is most common in African regions, the Arg-allele is more common in Caucasians. The Pro-allele of rs1042522 has been associated with several forms of cancer. However, next to increased cancer susceptibility, the Pro-allele has also been associated with longevity,[3–5] raising the hypothesis that this allele increases longevity at the cost of increased cancer susceptibility.

The main causes of death in developed countries are cancer and cardiovascular diseases. Important risk factors for cardio vascular diseases are body composition, type 2 diabetes, dyslipidemia and blood pressure. Evidence is emerging that the p53 protein mediates metabolism. It has been shown that p53 expression in adipose tissue is associated with insulin resistance and subsequently with age-related cardiovascular disorders.[6,7] Therefore, we hypothesized that rs1042522 might have an effect on maintenance of metabolism, body composition and blood pressure during ageing.

In this study, we aimed to investigate the relation of rs1042522 with blood pressure and several anthropometric (BMI and waist circumference) and biochemical parameters as measures of successful ageing. Using a longitudinal design, we are able to monitor changes over time, which reflect the effects of ageing. As rs1042522 is the most frequent non-synonymous SNP in *TP53* and the only SNP known in this locus to be involved in longevity, we focused only on this variant.

MATERIALS AND METHODS

Study populations

For this study two longitudinal populations were used. The first sample is from the Dutch Doetinchem Cohort Study (DCS), which has been described in detail previously.[8] In short, this population-based study aims to identify the impact of lifestyle factors and biological risk factors on aspects of health, such as the incidence of chronic diseases, physical and cognitive functioning and quality of life. Participants were randomly selected from the municipal population register from the city of Doetinchem, The Netherlands. A random subset of participants was re-invited with intervals of approximately 5 years. Inclusion criteria for genetic studies were stable smoking behavior and absence of pregnancy at time of measurement as described previously.[9] Data from four visit rounds are now available. However, waist circumference was not assessed

[1]National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands; [2]Department of Clinical Sciences/Diabetes and Endocrinology and Lund University Diabetes Centre, Lund. University Malmö Hospital, Malmö, Sweden; [3]Folkhälsan Research Center, Helsinki, Finland; [4]Department of Social Services and Health Care, Jakobstad, Finland; [5]Helsinki University Central Hospital, Department of Medicine, and University of Helsinki, Research Program for Molecular Medicine, Helsinki, Finland
*Correspondence: Dr E Reiling, National Institute for Public Health and the Environment (RIVM), PO Box 1, 3720 BA Bilthoven, The Netherlands. Tel: +31302744150; Fax: +31302744446; E-mail: Erwin.reiling@rivm.nl
Received 4 August 2011; revised 17 October 2011; accepted 23 November 2011; published online 21 December 2011

Table 1 Characteristics of study populations

	r1	*r2*	*r3*	*r4*
Doetinchem Cohort Study (N=2679)				
Genotype (CC/CG/GG) (% male)		*209/1018/1390 (47/50/48)*		
Age – years (SD)	39.8 (9.8)	46.3 (9.8)	51.3 (9.7)	56.3 (9.7)
DBP – mm Hg (SD)	77.0 (10.1)	79.6 (10.5)	81.0 (10.7)	NA
SBP – mm Hg (SD)	120.7 (14.4)	124.6 (16.0)	128.5 (17.5)	NA
Waist – cm (SD)	NA	89.9 (11.0)	92.7 (11.3)	94.7 (11.5)
BMI – kg/m^2 (SD)	24.4 (3.2)	25.3 (3.5)	26.0 (3.8)	26.4 (3.9)
Follow-up – years (SD)[a]	NA	6.0 (0.1)	5.0 (0.2)	5.0 (0.2)
Botnia Prospective Study (N=2338)				
Genotype (CC/CG/GG) (% male)		*201/955/1152 (47/46/46)*		
Age – years (SD)	45.3 (3)	53.0 (14.0)	NA	NA
DBP – mm Hg (SD)	78.1 (10.6)	80.2 (9.7)	NA	NA
SBP – mm Hg (SD)	127.0 (17.4)	132.0 (19.0)	NA	NA
Waist – cm (SD)	86.4 (11.8)	90.5 (12.4)	NA	NA
BMI – kg/m^2 (SD)	25.4 (4.0)	26.4 (4.3)	NA	NA
Follow-up – years (SD)[a]	NA	8.8 (4.2)	NA	NA

Abbreviation: NA, not available.
[a]Follow-up time since the preceding round. The Doetinchem Cohort Study included four visit rounds, the Botnia Prospective Study included two visit rounds. First visit round is the baseline measurement and therefore no follow-up time can be presented in the first cell.
Quantitative traits showed for available visit rounds (r1–r4).

in the first measurement round. Furthermore, no compatible data for blood pressure were available from the fourth visit round. In total, data were available from 2679 participants (49% male). Participants were not fasted before investigation.

The second sample was the Botnia Prospective Study (BPS). This is a family-based study aiming to identify genes increasing susceptibility to type 2 diabetes. Details of the study cohort and sampling strategy have been presented earlier.[10] In brief, individuals with type 2 diabetes from the area of five health-care centers in western Finland were invited to participate, together with their family members.[11] An oral glucose tolerance test (OGTT) was performed for all participants aged 18–70 years who had fasting plasma glucose concentration <11 mmol/l. Glucose tolerance was defined according to the current World Health Organization criteria.[12] Data at baseline and after follow-up were available from 2338 participants (46% male). Mean follow-up time was 8.8 years ($\pm$4.2 years).

Characteristics are summarized in Table 1.

All participants gave written informed consent. Both studies were approved by the local ethics committees and were in accordance with the principles of the Declaration of Helsinki.

Genotyping and quality control

For genotyping of rs1042522 in DCS, KASPar SNP genotyping Chemistry from KBioscience (KBioscience, Hoddesdon, UK) was used. SNPviewer (version 1.93, KBioscience, Hoddesdon, UK) was used for automated genotype calling. Genotype clusters were visually observed for validation of automated genotype calling. The success rate was 98%. Genotype distribution obeyed HWE (P=0.24).

The BPS was genotyped using an allelic discrimination assay-by-design method on ABI 7900 (Applied Biosystems, Foster City, CA, USA). We obtained a genotyping success rate of 99%. Genotypes were in HWE (P=0.73).

Statistics

To test for differences in changes in the outcome variable over time according to rs1042522 genotype, we used linear mixed models (PROC MIXED), with adjustment for age, gender and follow-up time with a random slope and intercept. Differences in changes over time were assessed by including the interaction between SNP and time in the model. Alpha was set at 0.05.

For analysis of blood pressure, participants who used anti-hypertensive medication were excluded (n=274 for DCS and n=335 for BPS). For the analysis of OGTT measures (BPS only), participants who converted to type 2 diabetes were excluded. As the BPS is a family-based study, we adjusted for family relation by clustering for family number in the mixed model. All analysis were performed using SAS 9.2 (SAS Institute Inc., Cary, NC, USA).

Power calculation

Power calculations were performed using G*Power 3.1.[13] The required effect size was calculated using MANOVA for between factors (SNP effect), within factors (time effect) and within-between interactions (SNP*time interaction). The correlation between visit rounds was set at 0.5, which was observed in the DCS and the required power was set at 80%. The estimated minimal effect sizes were 0.05% SD for a SNP effect, 0.02% SD for a time effect and 0.05% SD for a SNP*time interaction.

RESULTS

First, the DCS was genotyped and analyzed for associations with changes in blood pressure. Blood pressure was available from the first three visit rounds. Rs1042522 was significantly associated with changes in both systolic blood pressure (SBP) and diastolic blood pressure (DBP) over time. The C/C genotype (Pro/Pro) showed a blunted increase in SBP and DBP over time (P=0.008 and 0.004, respectively, Figure 1). SBP increased 1.4 mm Hg in C/C carriers, 5.1 mm Hg in C/G carriers and 5.0 Hg in G/G carriers over time. DBP increased 0.8 mm Hg in C/C carriers, 3.4 mm Hg in C/G carriers and 3 mm Hg in G/G carriers between the third and first visit round (Supplementary Table 1). SBP was also significantly lower in C/C carriers compared with C/G and G/G carriers in visit round 3 according to a general linear model (P=0.002).

As changes in blood pressure are age dependent,[14] participants were divided in those below and above the median age at baseline (40 years). Although the trend was the same, the association with changes in SBP over time was stronger in the subgroup above the median age (P_{below}=0.18, P_{above}=0.04, data not shown). For DBP, this was the other way around, the association was the strongest in the subgroup below the median age (P_{below}=0.009, P_{above}=0.29).

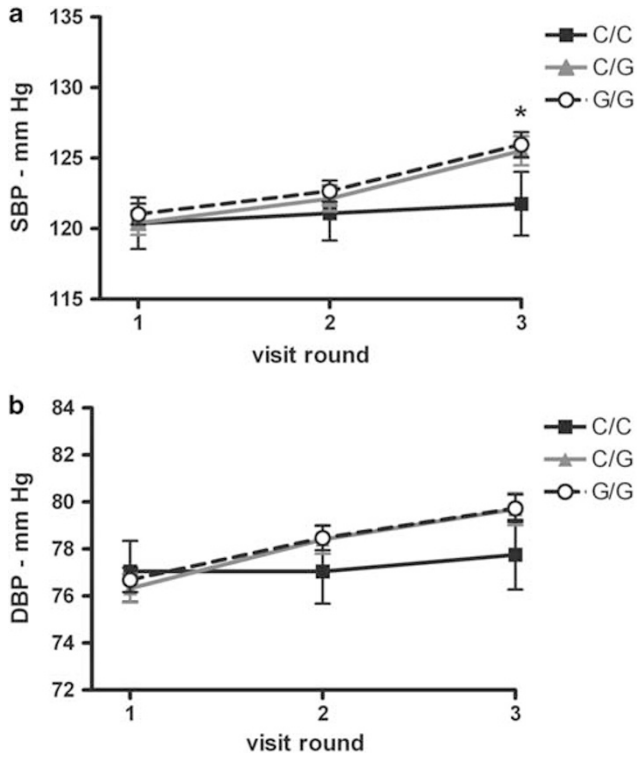

Figure 1 Time-dependent changes in blood pressure in the DCS according to rs1042522 genotype. (**a**) SBP, P=0.008. (**b**) DBP, P=0.004. Error bars represent 95% confidence intervals. *P=0.002.

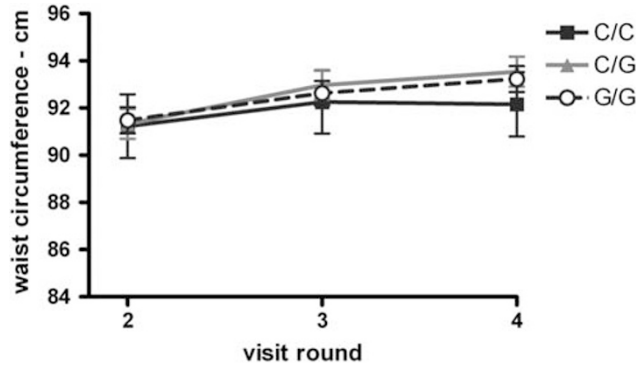

Figure 2 Time-dependent changes in waist circumference in the DCS according to rs1042522 genotype, P=0.03. Error bars represent 95% confidence intervals.

Next, we analyzed changes in BMI and waist circumference over time. BMI data were available from all four visit rounds. Waist circumference data were available from visit round 2 to 4. Although no association was observed with changes in BMI over time (P=0.69, Supplementary Table 1), the rs1042522 variant showed a borderline significant association with changes in waist circumference over time (P=0.03, Figure 2). Carriers of the C/C-genotype (Pro/Pro) showed a blunted increase, compared with G/G and C/G carriers. Waist circumference increased 1.1 cm in C/C carriers, 2.2 cm in C/G carriers and 1.7 cm in G/G carriers over time (Supplementary Table 1). No associations were observed with changes in non-fasting HDL and total cholesterol in relation to time (data not shown).

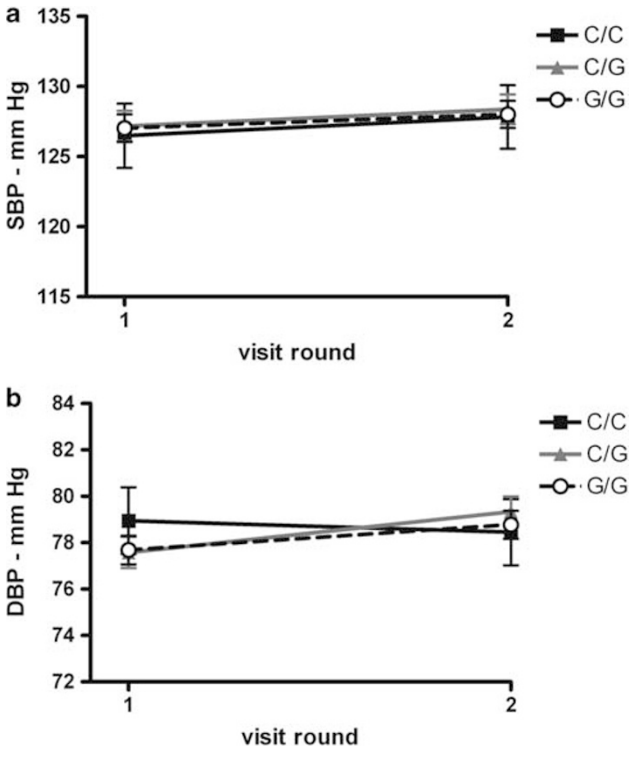

Figure 3 Time-dependent changes in blood pressure in the BPS according to rs1042522 genotype. (**a**) SBP, P=0.64. (**b**) DBP, P=0.02. Error bars represent 95% confidence intervals.

In order to replicate our findings, we analyzed changes in blood pressure and waist circumference over time in the BPS. Although our findings for SBP were not confirmed, an association with changes in DBP over time was observed (P=0.64 and 0.02, respectively, Figure 3), where the C/C genotype (Pro/Pro) showed a blunted increase in DBP. DBP decreased 0.4 mm Hg in C/C carriers while it increased 1.7 mm Hg in C/G carriers and 1.1 in G/G carriers between follow-up and baseline measurement (Supplementary Table 1).

Stratification according to the median age at baseline (43.7 years) showed also no evidence for an association with SBP changes over time. However, the association with DBP was the strongest in the subgroup below the median age (P_{below}=0.04, P_{above}=0.53), which is consistent with data from DCS. Adjustment for family relation in the BPS resulted in identical outcome (data not shown).

Next, we analyzed changes in waist circumference over time for the different genotypes. Corresponding to the DCS data, we observed a borderline significant association with changes in waist circumference (P=0.05, Figure 4), where C/C carriers (Pro/Pro) showed a blunted increase in waist circumference over time. Waist circumference increased 1.6 cm in C/C carriers, 2.6 cm in C/G carriers and 2.8 cm in G/G carriers (Supplementary Table 1). Correction for family relation did not affect the outcome (data not shown). Adjustment of the association between rs1042522 and changes in DBP for waist circumference showed that the association could not be explained by waist circumference (data not shown).

Finally, we also analyzed the association of rs1042522 with changes in OGTT measures. Although no association with changes in fasting glucose and 2-h glucose levels over time were observed, there were associations with changes in glucose levels at 30 and 60 min after glucose load between follow-up and baseline measurement (P=0.01

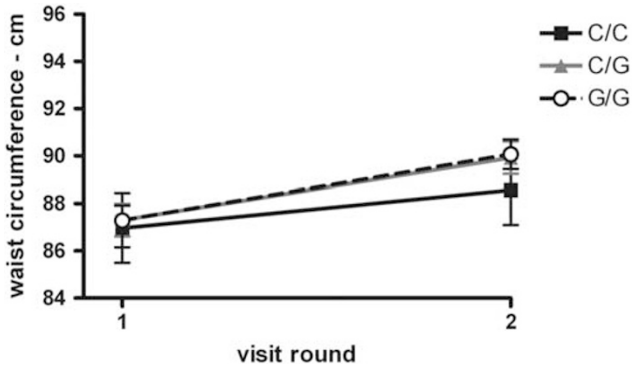

Figure 4 Time-dependent changes in waist circumference in the BPS according to rs1042522 genotype, P=0.05. Error bars represent 95% confidence intervals.

and 0.02, respectively, Supplementary Figure 1, Supplementary Table 2). Again, the C/C genotype showed a blunted increase. Furthermore, testing glucose levels independent of time at baseline and follow-up measurement revealed significant differences between genotypes at 30 and 60 min, only at follow-up measurement (P=0.005 and 0.01, respectively). Adjustment for family relation did not affect the results (data not shown).

DISCUSSION

Here, we show evidence in two independent longitudinal studies that the C/C genotype (Pro/Pro) of the rs1042522 variant might be associated with the most modest increases in DBP over time as compared with the other genotypes. The differences in changes in DBP over time are most pronounced in younger subjects (age below the median age). This corresponds with the observation that DBP increases predominantly below 60 years of age.[14] The association with changes in SBP observed in the DCS could not be replicated in the BPS. Furthermore, weak evidence was observed that the same genotype is also associated with the most modest increase in waist circumference in both studies. Our findings implicate that sequence variation in the *TP53* gene might be associated with changes in traits of metabolism and cardiovascular function during ageing.

In this study, we have only analyzed rs1042522 located in the *TP53* locus. In this locus, several other SNPs are present. However, according to the HapMap database, rs1042522 is the only non-synonymous SNP with a minor allele frequency (MAF) above 5%. Other SNPs with a MAF above 5% are all located in non-coding parts of the gene and not known to be associated with longevity. Therefore, we exclusively focused this research on rs1042522.

Although adjustment of the association between rs1042522 and changes in DBP for waist circumference was not possible in the DCS, the BPS showed that the association with changes in DBP was not explained by waist circumference.

As only one candidate SNP was studied and a limited number of selected quantitative traits a stringent correction for multiple testing is not applicable. In order to decrease the risk of chance findings, results were tested in a second cohort (BPS).

However, there are some differences between the two studies used for this research. The follow-up time between visit rounds and the number of visit rounds differs. Furthermore, the DCS is a population-based study whereas the BPS is a family-based study. This might introduce heterogeneity. Selection of more homogeneous cohorts has proven difficult. Most available population studies are of a cross-sectional design. Other longitudinal studies often include largely elder subjects, which might have complicated analyses for DBP, as the effects were mainly observed in younger subjects. Within our criteria the selected studies were the best choice. The differences between the studies might explain the lack of replication for SBP. Therefore, we cannot rule out an association between rs1042522 and changes in SBP. The possibility of chance findings can, however, also not be excluded.

Genome-wide association studies, which investigated the effect of SNPs on blood pressure did not observe p53 SNPs among their top hits.[15–23] As these studies have a cross-sectional design, it is not possible to examine the effect of a SNP on development of a trait. We suggest that rs1042522 has a role in the development of DBP and waist circumference during ageing and therefore, a SNP effect alone will be less informative compared with a SNP*time interaction in this case. This is highlighted by our findings, showing only a SNP*time interaction but no independent SNP effect.

Data from the BPS, suggest that rs1042522 is also associated with changes in 30- and 60-min glucose levels during OGTT. It can be speculated that rs1042522 affects insulin sensitivity and subsequently cardiovascular functioning and metabolism. This corresponds with previous observations that p53 is involved in insulin resistance and subsequently cardiovascular and metabolic diseases, although there is currently no strong evidence that rs1042522 leads to increased susceptibility for these diseases.[7,24] Our results are based on only a single study, because no OGTT was performed in the DCS. Therefore, this observation merits replication.

Previously, it has been shown that the Pro-allele of rs1042522 is associated with longevity.[3,4] Other studies showed that the Pro-variant is less efficient in inducing apoptosis compared with the Arg-variant.[25,26] This difference seems to become more pronounced with advancing age.[27] One hypothesis that has been suggested is that individuals homozygous for the Pro-allele preserve their stem cell stock as a result of decreased apoptosis and therefore maintain better cell renewal and homeostasis compared with Arg-carriers.[4] If decreased apoptosis does not lead to mortality caused by cancer, preserved regenerative capacity could increase longevity. Another study suggests that the longevity phenotype is caused by increased survival after diagnosis of cancer in C/C carriers.[3,24] Our data support an additional, not mutually exclusive hypothesis. We suggest that the C/C genotype is associated with blunted increases in blood pressure, waist circumference and OGTT measures in our study, which are hallmarks of human ageing. It is therefore tempting to speculate that the association with longevity may be also caused by these observed blunted increases, possibly initiated by enhanced maintenance of insulin sensitivity. However, since we are the first to observe associations with changes over time and to the best of our knowledge the rs1042522 polymorphism has not been associated with blood pressure, waist circumference or glucose metabolism before, future research is necessary to elaborate on our findings and elucidate the mechanism of p53 in metabolic development and ageing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We acknowledge all participants for their cooperation. Epidemiologists and fieldworkers of the Municipal Health Service in Doetinchem are acknowledged for their contribution to the data collection for this study. Logistic management was provided by J Steenbrink and P Vissink, and administrative support by EP van der Wolf. Data management was provided by A Blokstra, AWD van Kessel and PE Steinberger. This article was funded by a grant from the National

Institute for Public Health and the Environment and the Ministry of Health, Welfare and Sport of The Netherlands (S/340020) and the National Institutes of Health/National Institute on Ageing (3PO1 AG017242). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the paper.

1 Ko LJ: Prives C: p53: puzzle and paradigm. *Genes Dev* 1996; **10**: 1054–1072.
2 Lozano G, Elledge SJ: p53 sends nucleotides to repair DNA. *Nature* 2000; **404**: 24–25.
3 Orsted DD, Bojesen SE, Tybjaerg-Hansen A, Nordestgaard BG: Tumor suppressor p53 Arg72Pro polymorphism and longevity, cancer survival, and risk of cancer in the general population. *J Exp Med* 2007; **204**: 1295–1301.
4 van Heemst D, Mooijaart SP, Beekman M *et al*: Variation in the human TP53 gene affects old age survival and cancer mortality. *Exp Gerontol* 2005; **40**: 11–15.
5 Whibley C, Pharoah PD, Hollstein M: p53 polymorphisms: cancer implications. *Nat Rev Cancer* 2009; **9**: 95–107.
6 Ahima RS: Connecting obesity, ageing and diabetes. *Nat Med* 2009; **15**: 996–997.
7 Minamino T, Orimo M, Shimizu I *et al*: A crucial role for adipose tissue p53 in the regulation of insulin resistance. *Nat Med* 2009; **15**: 1082–1087.
8 Verschuren WM, Blokstra A, Picavet HS, Smit HA: Cohort profile: the Doetinchem Cohort Study. *Int J Epidemiol* 2008; **37**: 1236–1241.
9 van den Berg SW, Dolle ME, Imholz S *et al*: Genetic variations in regulatory pathways of fatty acid and glucose metabolism are associated with obesity phenotypes: a population-based cohort study. *Int J Obes (Lond)* 2009; **33**: 1143–1152.
10 Groop L, Forsblom C, Lehtovirta M *et al*: Metabolic consequences of a family history of NIDDM (the Botnia study): evidence for sex-specific parental effects. *Diabetes* 1996; **45**: 1585–1593.
11 Lyssenko V, Almgren P, Anevski D *et al*: Predictors of and longitudinal changes in insulin sensitivity and secretion preceding onset of type 2 diabetes. *Diabetes* 2005; **54**: 166–174.
12 World Health Organization: *Definition, Diagnosis and Classification of Diabetes Mellitus, Report of a WHO Consultation, Part 1: Diagnosis and Classification of Diabetes Mellitus*. Geneva: WHO/NCD/NCS/99.2, 1999.
13 Faul F, Erdfelder E, Lang AG, Buchner A: G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods* 2007; **39**: 175–191.
14 Franklin SS, Gustin Wt, Wong ND *et al*: Hemodynamic patterns of age-related changes in blood pressure. The Framingham Heart Study. *Circulation* 1997; **96**: 308–315.
15 Diabetes Genetics Initiative of Broad Institute of Harvard and MIT, Lund University, and Novartis Institutes of BioMedical ResearchSaxena R, Voight BF, Lyssenko V *et al*: Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. *Science* 2007; **316**: 1331–1336.
16 Kato N, Miyata T, Tabara Y *et al*: High-density association study and nomination of susceptibility genes for hypertension in the Japanese National Project. *Hum Mol Genet* 2008; **17**: 617–627.
17 Levy D, Ehret GB, Rice K *et al*: Genome-wide association study of blood pressure and hypertension. *Nat Genet* 2009; **41**: 677–687.
18 Levy D, Larson MG, Benjamin EJ *et al*: Framingham Heart Study 100K Project: genome-wide associations for blood pressure and arterial stiffness. *BMC Med Genet* 2007; **8** (Suppl 1): S3.
19 Newton-Cheh C, Johnson T, Gateva V *et al*: Genome-wide association study identifies eight loci associated with blood pressure. *Nat Genet* 2009; **41**: 666–676.
20 Org E, Eyheramendy S, Juhanson P *et al*: Genome-wide scan identifies CDH13 as a novel susceptibility locus contributing to blood pressure determination in two European populations. *Hum Mol Genet* 2009; **18**: 2288–2296.
21 Sabatti C, Service SK, Hartikainen AL *et al*: Genome-wide association analysis of metabolic traits in a birth cohort from a founder population. *Nat Genet* 2009; **41**: 35–46.
22 Wang Y, O'Connell JR, McArdle PF *et al*: From the Cover: whole-genome association study identifies STK39 as a hypertension susceptibility gene. *Proc Natl Acad Sci USA* 2009; **106**: 226–231.
23 Wellcome Trust Case Control C: Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 2007; **447**: 661–678.
24 Bojesen SE, Nordestgaard BG: The common germline Arg72Pro polymorphism of p53 and increased longevity in humans. *Cell Cycle* 2008; **7**: 158–163.
25 Thomas M, Kalita A, Labrecque S, Pim D, Banks L, Matlashewski G: Two polymorphic variants of wild-type p53 differ biochemically and biologically. *Mol Cell Biol* 1999; **19**: 1092–1100.
26 Zhu F, Doll ME, Berton TR *et al*: Mouse models for the p53 R72P polymorphism mimic human phenotypes. *Cancer Res* 2010; **70**: 5851–5859.
27 Bonafe M, Salvioli S, Barbi C *et al*: The different apoptotic potential of the p53 codon 72 alleles increases with age and modulates *in vivo* ischaemia-induced cell death. *Cell Death Differ* 2004; **11**: 962–973.

Supplementary Information accompanies the paper on European Journal of Human Genetics website (http://www.nature.com/ejhg)