

Genetic Variation in the *CYP2C* Monooxygenase Enzyme Subfamily Shows No Association With Longevity in a German Population

Friederike Flachsbart,¹ Mike Ufer,^{2,3} Rabea Kleindorp,¹ Susanna Nikolaus,^{4,5} Stefan Schreiber,^{1,4} and Almut Nebel¹

¹Institute of Clinical Molecular Biology, Christian-Albrechts-University Kiel, Germany.

²Institute of Experimental and Clinical Pharmacology, University Hospital Schleswig-Holstein, Kiel, Germany.

³Novartis Pharma AG, Novartis Institutes for Biomedical Research, Basel, Switzerland.

⁴Clinic for Internal Medicine I, University Hospital Schleswig-Holstein, Kiel, Germany.

⁵Popgen Biobank, Christian-Albrechts-University, Kiel, Germany.

Address correspondence to Almut Nebel, PhD, Institute of Clinical Molecular Biology, Christian-Albrechts-University Kiel, Schittenhelmstraße 12, 24105 Kiel, Germany. Email: a.nebel@mucosa.de

Cytochrome P450 enzymes, especially the *CYP2C* subfamily, are involved in the generation of reactive oxygen species and are regarded as susceptibility factors for age-related diseases. Furthermore, the *CYP2C*-encoding genes are known to be highly polymorphic, with a number of variants leading to changes in enzyme activity. These observations prompted us to investigate whether allelic variation in the *CYP2C*-encoding genes was associated with human longevity. In a comprehensive haplotype tagging approach, we genotyped 56 single nucleotide polymorphisms located in the *CYP2C* gene family (*CYP2C8*, *CYP2C9*, *CYP2C18*, and *CYP2C19*) in our extensive collection of 1,384 long-lived individuals (centenarians and nonagenarians) and 945 younger controls. None of the tested single nucleotide polymorphisms showed a significant association with the longevity phenotype at the allele, genotype, or haplotype level. These results suggest that there is no notable influence of sequence variation in the *CYP2C* genes on longevity in the examined German population.

Key Words: Genetic association—Centenarians—Ageing—Cytochrome P450—*CYP2C* enzymes.

Received May 16, 2011; Accepted June 14, 2011

Decision Editor: Placido Navas, PhD

LONGEVITY in humans is considered a multifactorial trait to which various genetic and environmental factors are likely to contribute. About 30% of the variation in adult life span is attributable to genetic parameters that show their strongest effect later in life (>60 years of age) (1–6). Epidemiological studies have revealed that people who survive to an exceptional old age (ie, ≥95 years) have often avoided or survived age-associated diseases. Hence, these long-lived individuals (LLI) show a favorable course of the ageing process and offer the unique opportunity to explore the genetic basis of the “healthy ageing” phenotype (7,8). It has been suggested that the genetic composition of the LLI differs from that of average-lived individuals in the following regards: (i) LLI are enriched for advantageous variants in so-called “longevity-enabling genes” and/or (ii) their genetic constitution shows a depletion of risk alleles for age-related diseases (9,10).

Cytochrome P450 enzymes (CYPs) are monooxygenases that are commonly known as important drug-metabolizing enzymes (11). They are also regarded as susceptibility factors for age-related cardiovascular diseases that represent

the leading cause of death worldwide (12–14). Furthermore, CYP enzymes, particularly the *CYP2C* isoenzyme subfamily, are involved in the generation of reactive oxygen species (ROS) (15). Already more than 50 years ago, the accumulation of ROS was suggested to cause changes in physical or cognitive functions with ageing (16). To date, findings in the area of longevity research support a role of ROS and oxidative damage in age-related cellular decline ((17) and reviewed in (18)) and the development of age-related diseases (19).

In humans, the *CYP2* enzyme subfamily C consists of four genes (*CYP2C8*, *CYP2C9*, *CYP2C18*, and *CYP2C19*) that are located next to each other on chromosome 10q (Figure 1). These enzymes are mainly expressed in human liver (20) but are also expressed in various other tissues, including the cardiovascular system, where they are involved in the modulation of vascular homeostasis by metabolizing endogenous regulators of vascular tone (21). Consequently, *CYP2C* inhibition has been reported to reduce ischemia–reperfusion injury in myocardial tissue (22–24). Furthermore, the *CYP2C*-encoding genes are also known to be highly polymorphic. Some of these variants

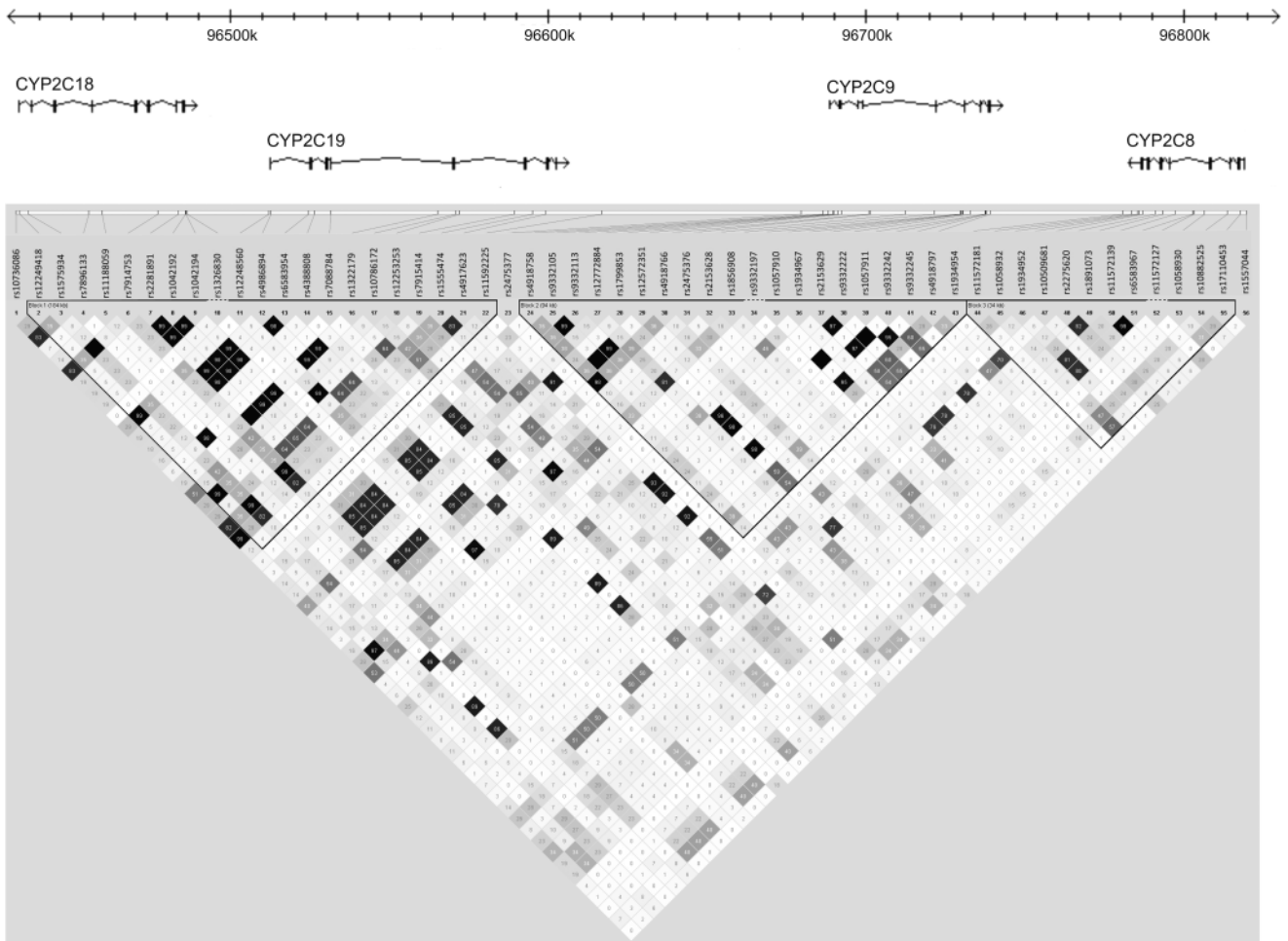


Figure 1. *CYP2C* gene region on chromosome 10. The physical position (in kilobases) of all 56 genotyped single nucleotide polymorphisms refers to the Genome Reference Consortium Human genome build 37. A schematic representation of the gene structures for *CYP2C18*, *CYP2C19*, *CYP2C9*, and *CYP2C8* is shown. The linkage disequilibrium (LD) plot of the locus is based on the measure r^2 and was generated with Haploview 3.32 using the data of the whole German case-control sample.

lead to a markedly reduced or no enzyme activity, whereas other alleles induce an increased activity or expression. In the context of drug metabolism, the variants *CYP2C9**2 (rs1799853) and *2C9**3 (rs1057910) associated with reduced enzyme activity are both known to be of particular clinical relevance. Recently, the U.S. federal drug agency (Food and Drug Administration) has encouraged prospective *CYP2C9* genotyping as a clinical tool to allow for individualized dose adjustment of the oral anticoagulant warfarin that is metabolized by *CYP2C9* (25).

As *CYP2C* enzymes also play an important role in the generation of ROS and are regarded as susceptibility factors for age-related diseases (15), they appear to be attractive candidates to be studied in the context of human longevity. Here, we performed a comprehensive fine mapping of the four *CYP2C* genes by testing altogether 56 single nucleotide polymorphisms (SNPs) in our extensive collection of

1,384 LLI (centenarians and nonagenarians) and 945 appropriately matched younger controls.

METHODS

Participants

The LLI sample comprised 1,384 unrelated German study participants of exceptional age (age range: 95–109 years, mean: 98.8 years), including 616 centenarians (mean age: 101.3 years). The gender ratio was 73% females versus 27% males. The 945 German control participants were between 60 and 75 years of age (mean age: 66.9 years) and matched the LLI by ancestry, gender, and geographical origin within the country. A detailed description of the samples and the recruitment procedure is given elsewhere (26). All participants gave informed written consent prior to participation. The study was approved by the Ethics Committee of the University Hospital Schleswig-Holstein in Kiel.

SNP Genotyping

DNA samples from LLI and control participants were analyzed for 56 SNPs in the *CYP2C* gene region (Figure 1, Table 1) by using the SNPlex Genotyping System (Applied Biosystems, Foster City, CA) (27). The complete marker set consists of (i) a maximally informative panel of SNPs, selected through a haplotype tagging approach (based on the HapMap genotypes of Europeans with the pairwise tagging option; pairwise $r^2 \geq .8$; $p_{\text{HWE}} > .001$) to ensure that most of the allelic variation of the genomic regions was captured and the common haplotypes ($\geq 2\%$) were represented; (ii) potentially functional SNPs that are located in exons, exon–intron boundaries, promoter regions, and 5' and 3' untranslated regions; and (iii) polymorphisms that have already been proven to be functionally relevant in the context of *CYP2C* enzyme activity (28–30). Of the 56 analyzed markers, 14 are located in the *CYP2C8* gene, 20 SNPs in *CYP2C9*, 10 SNPs in *CYP2C18*, and 12 SNPs in *CYP2C19* (Figure 1, Table 1).

Statistical Analysis

Allele-based single marker case–control analyses (CCA) were performed with χ^2 statistics and the appropriate degrees of freedom using the open-source analysis toolset PLINK v.1.07 (<http://pngu.mgh.harvard.edu/~purcell/plink/>). p values smaller than .05 were considered nominally statistically significant, and Bonferroni correction for 56 tests was applied to the single-point results: Of the 56 tested markers, 28 were in strong linkage disequilibrium with each other (pairwise $r^2 > .8$; calculated with the Haploview tagger pairwise option) so that the number of markers considered for the Bonferroni correction could be reduced to 28. As two case–control analyses (whole sample and centenarians) were performed, altogether 56 tests need to be taken into account. The software programme Haploview version 4.1 (<http://www.broad.mit.edu/mpg/haploview/>) was used to assess all polymorphisms for significant deviation from the Hardy–Weinberg equilibrium (HWE), to calculate linkage disequilibrium (r^2) between each marker pair, and to conduct haplotype association analyses in blocks (31).

RESULTS

The whole sample of 1,384 German LLI, a subset of 616 centenarians, and a control group of 945 younger individuals were subjected to a gender-matched case–control analysis of 56 SNPs located in the *CYP2C* genes (*CYP2C8*, *CYP2C9*, *CYP2C18*, and *CYP2C19*). All SNPs were found to be in HWE ($p > .001$). Only one nominally significant association signal (rs11188059; $p_{\text{CCA}} = .04$) was observed in the analysis of the centenarian sample (Table 1) that did not pass correction for multiple testing (Bonferroni-adjusted $p_{\text{CCA}} = 1$, assuming 56 tests; see “PARTICIPANTS and METHODS” and “Statistical Analysis”). In the entire longevity sample (1,384 LLI and 945 controls), none of the tested SNPs showed a

significant association, even without consideration of multiple testing (data not shown).

The 56 SNPs form three haplotype blocks (Figure 1). Block 1 comprises 20 markers, Block 2 comprises 19 markers, and Block 3 comprises 11 markers (Figure 1), which define eight common haplotypes (each present at a frequency of at least 2% in the population) for each block. None of the observed haplotypes differed significantly in frequency between cases and controls (data not shown).

DISCUSSION

Cytochrome P450 enzymes, especially the *CYP2C* isoforms, are involved in the generation of ROS (15). They are expressed in tissues of the cardiovascular system and are considered susceptibility factors for age-related diseases (15). Furthermore, the *CYP2C*-encoding genes are known to be highly polymorphic, with a number of variants leading to changes in enzyme activity.

These observations prompted us to investigate whether allelic variation in the *CYP2C*-encoding genes was associated with human longevity. Altogether, we genotyped 56 markers in our extensive DNA collection of more than 2,300 LLI and controls. None of the tested SNPs or haplotypes showed a statistically significant association with longevity, neither in the whole sample nor in the centenarian subset.

Candidate gene association studies have emerged as powerful tools in longevity research (32–47). So far, two longevity relevant genes (*APOE* and *FOXO3A*) have been confirmed in many different populations (32,35–37,39,48–55). Because *APOE* and *FOXO3A* have been identified by candidate gene association studies, it seems that this method is still relevant for human longevity research, even in the era of genome-wide association studies. Although genome-wide association studies offer the advantage of detecting new longevity genes without a priori hypothesis, the power of hypothesis-driven candidate approaches is much higher than that of genome-wide association studies where millions of SNPs are tested, and multiple comparisons have to be taken into consideration as an essential part of determining statistical significance (56). Hence, so far it has been difficult to detect new longevity variants by genome-wide studies, and apart from the *APOE* locus, none of the reported genome-wide association studies signals achieved conventional levels of statistical significance (51,57,58). Altogether, candidate gene association studies still play an important role for the identification of longevity loci.

With the applied approach, we are likely to have captured all common variation present in the analyzed samples for the *CYP2C* gene region. Acknowledging that the selected markers might be insufficient to tag the genetic variation comprehensively, we cannot rule out the presence of rare polymorphisms that could influence longevity. However, with the consideration of haplotype differences, if present,

Table 1. Association Statistics for 56 SNPs Located in the *CYP2C* Gene Region (for the centenarian subset)

Gene	SNP	Position on CHR 10 (GRCh37/hg19)	Min AF Cases	Min AF Controls	p_{CCA}	Bonferroni-Adjusted p_{CCA}	
CYP2C18	rs10736086	96441650	.489	.503	.45	1	
	rs12249418	96442917	.242	.215	.08	1	
	rs1575934	96445609	.464	.448	.36	1	
	rs7896133	96464730	.059	.069	.24	1	
	rs11188059	96468899	.106	.131	.04	1	
	rs7914753	96486504	.464	.448	.36	1	
	rs2281891	96493058	.164	.164	.99	1	
	rs1042192	96495284	.166	.167	.97	1	
	rs1042194	96495484	.164	.163	.92	1	
	rs1326830	96495793	.008	.012	.28	1	
	CYP2C19	rs12248560	96521657	.241	.216	.11	1
rs4986894		96522365	.164	.165	.95	1	
rs6583954		96534263	.166	.166	.99	1	
rs4388808		96536056	.158	.168	.47	1	
rs7088784		96541373	.059	.068	.31	1	
rs1322179		96575242	.164	.164	.99	1	
rs10786172		96581094	.334	.344	.54	1	
rs12253253		96582156	.241	.213	.07	1	
rs7915414		96599510	.223	.232	.54	1	
rs1555474		96605327	.464	.450	.44	1	
rs4917623		96609568	.491	.502	.55	1	
rs11592225		96627191	.143	.136	.59	1	
CYP2C9		rs2475377	96690371	.044	.049	.53	1
		rs4918758	96697252	.379	.381	.93	1
	rs9332105	96698925	.186	.181	.71	1	
	rs9332113	96700402	.187	.181	.68	1	
	rs12772884	96700630	.386	.416	.10	1	
	rs1799853	96702047	.122	.125	.82	1	
	rs12572351	96703220	.186	.180	.65	1	
	rs4918766	96711884	.380	.377	.87	1	
	rs2475376	96712400	.148	.149	.92	1	
	rs2153628	96723424	.236	.212	.11	1	
	rs1856908	96732731	.339	.362	.20	1	
	rs9332197	96740908	.038	.049	.14	1	
	rs1057910	96741053	.058	.065	.44	1	
	rs1934967	96741426	.191	.211	.17	1	
	rs2153629	96741795	.135	.129	.66	1	
	rs9332222	96744064	.134	.125	.46	1	
	rs1057911	96748737	.058	.066	.42	1	
	rs9332242	96748893	.134	.125	.46	1	
	rs9332245	96749181	.063	.068	.52	1	
	rs4918797	96750251	.196	.196	.98	1	
	CYP2C8	rs1934954	96792202	.090	.079	.27	1
rs11572181		96795046	.061	.052	.29	1	
rs1058932		96796861	.201	.197	.81	1	
rs1934952		96797500	.344	.346	.92	1	
rs10509681		96798749	.118	.107	.36	1	
rs2275620		96802598	.345	.372	.12	1	
rs1891073		96804911	.305	.327	.19	1	
rs11572139		96808886	.302	.296	.70	1	
rs6583967		96814475	.301	.294	.70	1	
rs11572127		96814689	.043	.049	.45	1	
rs1058930		96818119	.052	.059	.41	1	
rs10882525		96825332	.353	.333	.24	1	
rs17110453		96829529	.146	.150	.73	1	
rs1557044		96831389	.136	.138	.88	1	

Notes: Centenarian subset: 616 German centenarians = 100–109 years; 945 younger controls = 60–75 years; CHR 10 = chromosome 10; GRCh37 = Genome Reference Consortium Human genome build 37; hg19 = release of the February 2009 human genome browser, UCSC version hg19; Min AF = minor allele frequency; p_{CCA} = p value obtained from an allele-based case-control comparison using a χ^2 test with 1 df ; SNP = single nucleotide polymorphism. Bold indicates SNP showed a nominally significant association signal in the analysis of the centenarian sample but did not pass correction for multiple testing.

the effect of rare variants should be statistically detectable as these effects ought to be carried by one of the common background haplotypes (59). Furthermore, common variants can act as significant modifiers of the effects of rare variants (60). Thus, rare variants that are functionally relevant are often identified by common variant associations (61–64). Altogether, it seems unlikely that we have missed such rare variant effects in our comprehensive approach. The possibility that the negative association finding is due to population stratification in our samples is also rather improbable because the validity and efficacy of our large and well-characterized study population for genetic longevity research have already been shown with the identification and validation of previous association findings (26,32–34). Overall, our results suggest that there is no noteworthy influence of sequence variation in the *CYP2C* genes on human longevity in Germans.

FUNDING

This study was supported by the Faculty of Medicine (intramural research support), Christian-Albrechts-University Kiel; and the DFG Excellence Cluster “Inflammation at Interfaces.”

ACKNOWLEDGMENTS

We thank all study participants for their cooperation. For excellent technical assistance, we wish to acknowledge the laboratory personnel of the Institute of Clinical Molecular Biology and the members of the Popgen Biobank.

REFERENCES

- Herskind AM, McGue M, Holm NV, Sorensen TI, Harvald B, Vaupel JW. The heritability of human longevity: a population-based study of 2872 Danish twin pairs born 1870–1900. *Hum Genet.* 1996;97:319–323.
- Finch CE, Tanzi RE. Genetics of aging. *Science.* 1997;278:407–411.
- Ljungquist B, Berg S, Lanke J, McClearn GE, Pedersen NL. The effect of genetic factors for longevity: a comparison of identical and fraternal twins in the Swedish Twin Registry. *J Gerontol A Med Sci.* 1998;53:M441–M446.
- Skytthe A, Pedersen NL, Kaprio J, et al. Longevity studies in GenomEUtwin. *Twin Res.* 2003;6:448–454.
- Gogele M, Pattaro C, Fuchsberger C, Minelli C, Pramstaller PP, Wjst M. Heritability analysis of life span in a semi-isolated population followed across four centuries reveals the presence of pleiotropy between life span and reproduction. *J Gerontol A Biol Sci Med Sci.* 2011;66:26–37.
- Hjelmborg JvB, Iachine I, Skytthe A, et al. Genetic influence on human lifespan and longevity. *Hum Genet.* 2006;119:312–321.
- Franceschi C, Bonafé M. Centenarians as a model for healthy aging. *Biochem Soc Trans.* 2003;31:457–461.
- Perls TT. The different paths to 100. *Am J Clin Nutr.* 2006;83:484S–487S.
- Perls T, Kunkel LM, Puca AA. The genetics of exceptional human longevity. *J Am Geriatr Soc.* 2002;50:359–368.
- Johnson TE. Genes, phenes, and dreams of immortality: the 2003 Kleemeier Award lecture. *J Gerontol A Biol Sci Med Sci.* 2005;60:680–687.
- Guengerich FP. Cytochrome P450s and other enzymes in drug metabolism and toxicity. *AAPS J.* 2006;8:E101–E111.
- Murray CJ, Lopez AD. Evidence-based health policy—lessons from the Global Burden of Disease Study. *Science.* 1996;274:740–743.
- Guilbert JJ. The world health report 2002—reducing risks, promoting healthy life. *Educ Health (Abingdon).* 2003;16:230.
- Reddy KS, Yusuf S. Emerging epidemic of cardiovascular disease in developing countries. *Circulation.* 1998;97:596–601.
- Chehal MK, Granville DJ. Cytochrome p450 2C (CYP2C) in ischemic heart injury and vascular dysfunction. *Can J Physiol Pharmacol.* 2006;84:15–20.
- Harman D. Aging: a theory based on free radical and radiation chemistry. *J Gerontol.* 1956;11:298–300.
- Ungvari Z, Ridgway I, Philipp EER, et al. Extreme longevity is associated with increased resistance to oxidative stress in *Arctica islandica*, the longest-living non-colonial animal. *J Gerontol A Biol Sci Med Sci.* 2011;66:741–750.
- Bokov A, Chaudhuri A, Richardson A. The role of oxidative damage and stress in aging. *Mech Ageing Dev.* 2004;125:811–826.
- Morris BJ. A forkhead in the road to longevity: the molecular basis of lifespan becomes clearer. *J Hypertens.* 2005;23:1285–1309.
- Evans WE, Relling MV. Pharmacogenomics: translating functional genomics into rational therapeutics. *Science.* 1999;286:487–491.
- Fleming I. Cytochrome p450 and vascular homeostasis. *Circ Res.* 2001;89:753–762.
- Khan M, Iyyapu KM, Kutala V, et al. Sulfaphenazole protects heart against ischemia-reperfusion injury and cardiac dysfunction by overexpression of iNOS leading to enhancement of nitric-oxide bioavailability and tissue oxygenation. *Antioxid Redox Signal.* 2008;11:725–738.
- Hunter AL, Kerjner A, Mueller KJ, McManus BM, Granville DJ. Cytochrome P450 2C enzymes contribute to peritransplant ischemic injury and cardiac allograft vasculopathy. *Am J Transplant.* 2008;8:1631–1638.
- Granville DJ, Tashakkor B, Takeuchi C, et al. Reduction of ischemia and reperfusion-induced myocardial damage by cytochrome P450 inhibitors. *Proc Natl Acad Sci U S A.* 2004;101:1321–1326.
- Gage BF, Lesko LJ. Pharmacogenetics of warfarin: regulatory, scientific, and clinical issues. *J Thromb Thrombolysis.* 2008;25:45–51.
- Nebel A, Croucher PJ, Stiegeler R, Nikolaus S, Krawczak M, Schreiber S. No association between microsomal triglyceride transfer protein (MTP) haplotype and longevity in humans. *Proc Natl Acad Sci U S A.* 2005;102:7906–7909.
- De la Vega FM, Lazaruk KD, Rhodes MD, Wenz MH. Assessment of two flexible and compatible SNP genotyping platforms: TaqMan SNP genotyping assays and the SNPlex genotyping system. *Mutat Res.* 2005;573:111–135.
- Dai D, Zeldin DC, Blaisdell JA, et al. Polymorphisms in human CYP2C8 decrease metabolism of the anticancer drug paclitaxel and arachidonic acid. *Pharmacogenetics.* 2001;11:597–607.
- Haining RL, Hunter AP, Veronese ME, Trager WF, Rettie AE. Allelic variants of human cytochrome P450 2C9: baculovirus-mediated expression, purification, structural characterization, substrate stereoselectivity, and prochiral selectivity of the wild-type and I359L mutant forms. *Arch Biochem Biophys.* 1996;333:447–458.
- Rettie AE, Wienkers LC, Gonzalez FJ, Trager WF, Korzekwa KR. Impaired (S)-warfarin metabolism catalysed by the R144C allelic variant of CYP2C9. *Pharmacogenetics.* 1994;4:39–42.
- Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics.* 2005;21:263–265.
- Flachsbart F, Caliebe A, Kleindorp R, et al. Association of FOXO3A variation with human longevity confirmed in German centenarians. *Proc Natl Acad Sci U S A.* 2009;106:2700–2705.
- Flachsbart F, Caliebe A, Nothnagel M, et al. Depletion of potential A2M risk haplotype for Alzheimer’s disease in long-lived individuals. *Eur J Hum Genet.* 2009;18:59–61.
- Nebel A, Flachsbart F, Till A, et al. A functional EXO1 promoter variant is associated with prolonged life expectancy in centenarians. *Mech Ageing Dev.* 2009;130:691–699.
- Pawlikowska L, Hu D, Huntsman S, et al. Association of common genetic variation in the insulin/IGF1 signaling pathway with human longevity. *Aging Cell.* 2009;8:460–472.

36. Willcox BJ, Donlon TA, He Q, et al. FOXO3A genotype is strongly associated with human longevity. *Proc Natl Acad Sci U S A*. 2008;105:13987–13992.
37. Schächter F, Faure-Delanef L, Guenot F, et al. Genetic associations with human longevity at the APOE and ACE loci. *Nat Genet*. 1994;6:29–32.
38. Caliebe A, Kleindorp R, Blanche H, et al. No or only population-specific effect of PON1 on human longevity: a comprehensive meta-analysis. *Ageing Res Rev*. 2010;9:238–244.
39. Christensen K, Johnson TE, Vaupel JW. The quest for genetic determinants of human longevity: challenges and insights. *Nat Rev Genet*. 2006;7:436–448.
40. Flachsbart F, Croucher PJ, Nikolaus S, et al. Sirtuin 1 (SIRT1) sequence variation is not associated with exceptional human longevity. *Exp Gerontol*. 2006;41:98–102.
41. Flachsbart F, Franke A, Kleindorp R, et al. Investigation of genetic susceptibility factors for human longevity—a targeted nonsynonymous SNP study. *Mutat Res*. 2010;694:13–19.
42. Kleindorp R, Flachsbart F, Puca AA, Malovini A, Schreiber S, Nebel A. Candidate gene study of FOXO1, FOXO4 and FOXO6 reveals no association with human longevity in Germans. *Ageing Cell*. 2011. doi:10.1111/j.1474-9726.2011.00698.x.
43. Nebel A, Flachsbart F, Schafer A, et al. Role of the toll-like receptor 4 polymorphism Asp299Gly in longevity and myocardial infarction in German men. *Mech Ageing Dev*. 2007;128:409–411.
44. von Wurmb-Schwark N, Caliebe A, Schwark T, et al. Association of TH01 with human longevity revisited. *Eur J Hum Genet*. 2011. doi:10.1038/ejhg.2011.43.
45. Wang XY, Hurme M, Jylha M, Hervonen A. Lack of association between human longevity and polymorphisms of IL-1 cluster, IL-6, IL-10 and TNF-alpha genes in Finnish nonagenarians. *Mech Ageing Dev*. 2001;123:29–38.
46. Corbo RM, Ulizzi L, Positano L, Scacchi R. Association of CYP19 and ESR1 pleiotropic genes with human longevity. *J Gerontol A Biol Sci Med Sci*. 2011;66:51–55.
47. Barbieri M, Rizzo MR, Papa M, et al. The IRS2 Gly1057Asp variant is associated with human longevity. *J Gerontol A Biol Sci Med Sci*. 2010;65:282–286.
48. Anselmi CV, Malovini A, Roncarati R, et al. Association of the FOXO3A locus with extreme longevity in a Southern Italian Centenarian Study. *Rejuvenation Res*. 2009;12:95–104.
49. Li Y, Wang WJ, Cao H, et al. Genetic association of FOXO1A and FOXO3A with longevity trait in Han Chinese populations. *Hum Mol Genet*. 2009;18:4897–4904.
50. Soerensen M, Dato S, Christensen K, et al. Replication of an association of variation in the FOXO3A gene with human longevity using both case-control and longitudinal data. *Ageing Cell*. 2010;9:1010–1017.
51. Deelen J, Beekman M, Uh HW, et al. Genome-wide association study identifies a single major locus contributing to survival into old age; the APOE locus revisited. *Ageing Cell*. 2011. doi:10.1111/j.1474-9726.2011.00705.x.
52. Novelli V, Viviani Anselmi C, Roncarati R, et al. Lack of replication of genetic associations with human longevity. *Biogerontology*. 2008;9:85–92.
53. Kervinen K, Savolainen MJ, Salokannel J, et al. Apolipoprotein E and B polymorphisms—longevity factors assessed in nonagenarians. *Atherosclerosis*. 1994;105:89–95.
54. Seripa D, Franceschi M, Matera MG, et al. Sex differences in the association of apolipoprotein E and angiotensin-converting enzyme gene polymorphisms with healthy aging and longevity: a population-based study from southern Italy. *J Gerontol A Biol Sci Med Sci*. 2006;61:918–923.
55. Frisoni GB, Louhija J, Geroldi C, Trabucchi M. Longevity and the epsilon 2 allele of apolipoprotein E: the Finnish Centenarians Study. *J Gerontol A Med Sci*. 2001;56:M75–M78.
56. Johnson RC, Nelson GW, Troyer JL, et al. Accounting for multiple comparisons in a genome-wide association study (GWAS). *BMC Genomics*. 2010;11:724.
57. Newman AB, Walter S, Lunetta KL, et al. A meta-analysis of four genome-wide association studies of survival to age 90 years or older: the Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium. *J Gerontol A Biol Sci Med Sci*. 2010;65:478–487.
58. Lunetta KL, D'Agostino RB Sr, Karasik D, et al. Genetic correlates of longevity and selected age-related phenotypes: a genome-wide association study in the Framingham Study. *BMC Med Genet*. 2007;8(suppl 1):S13.
59. Lin S, Chakravarti A, Cutler DJ. Exhaustive allelic transmission disequilibrium tests as a new approach to genome-wide association studies. *Nat Genet*. 2004;36:1181–1188.
60. Felix R, Bodmer W, Fearnhead NS, van der Merwe L, Goldberg P, Ramesar RS. GSTM1 and GSTT1 polymorphisms as modifiers of age at diagnosis of hereditary nonpolyposis colorectal cancer (HNPCC) in a homogeneous cohort of individuals carrying a single predisposing mutation. *Mutat Res*. 2006;602:175–181.
61. Bodmer W, Bonilla C. Common and rare variants in multifactorial susceptibility to common diseases. *Nat Genet*. 2008;40:695–701.
62. Kaser A, Lee AH, Franke A, et al. XBP1 links ER stress to intestinal inflammation and confers genetic risk for human inflammatory bowel disease. *Cell*. 2008;134:743–756.
63. Nejentsev S, Walker N, Riches D, Egholm M, Todd JA. Rare variants of IFIH1, a gene implicated in antiviral responses, protect against type 1 diabetes. *Science*. 2009;324:387–389.
64. Johansen CT, Wang JA, Lanktree MB, et al. Excess of rare variants in genes identified by genome-wide association study of hypertriglyceridemia. *Nat Genet*. 2010;42:684–687.