Are centenarians genetically predisposed to lower disease risk?

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Received: 4 June 2011 / Accepted: 25 July 2011 / Published online: 6 September 2011
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Abstract Our study purpose was to compare a disease-related polygenic profile that combined a total of 62 genetic variants among (i) people reaching exceptional longevity, i.e., centenarians ($n=54$, 100–108 years, 48 women) and (ii) ethnically matched healthy controls ($n=87$, 19–43 years, 47 women). We computed a ‘global’ genotype score (GS) for 62 genetic variants (mutations/polymorphisms) related to cardiometabolic diseases, cancer or exceptional longevity, and also specific GS for main disease

categories (cardiometabolic risk and cancer risk, including 36 and 24 genetic variations, respectively) and for exceptional longevity (7 genetic variants). The ‘global’ GS was similar among groups (centenarians: 31.0 ± 0.6 ; controls 32.0 ± 0.5 , $P=0.263$). We observed that the GS for hypertension, cancer (global risk), and other types of cancer was lower in the centenarians group compared with the control group (all $P<0.05$), yet the difference became non significant after adjusting for sex. We observed significant between-group

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differences in the frequency of *GSTT1* and *GSTMI* (presence/absence) genotypes after adjusting for multiple comparisons. The likelihood of having the *GSTT1* low-risk (functional) allele was higher in centenarians (odds ratio [OR] 5.005; 95% confidence interval [CI], 1.810–13.839), whereas the likelihood of having the *GSTMI* low-risk (functional) allele was similar in both groups (OR 1.295; 95% CI, 0.868 – 1.931). In conclusion, we found preliminary evidence that Spanish centenarians have a lower genetic predisposition for cancer risk. The wild-type (i.e., functional) genotype of *GSTT1*, which is associated with lower cancer risk, might be associated with exceptional longevity, yet further studies with larger sample sizes must confirm these findings.

Keywords Centenarians · Genetics · Exceptional longevity · Ageing

Introduction

Centenarians are the survival tail of the population and a model of healthy aging. These people have postponed age-related diseases and their fatal consequences (Salvioli et al. 2008), and the onset of disability is generally delayed until they are well into their mid-90s (Terry et al. 2008; Christensen et al. 2008). Both environmental and genetic factors contribute to the complex phenotype of exceptional longevity.

One possible approach for identifying those gene variants that are associated with exceptional longevity is to compare the genotype of centenarians and ethnically matched controls (Martin et al. 2007). This approach lies on the hypothesis that exceptionally old individuals are carriers of multiple genetic variants that influence human lifespan (Hekimi 2006). Genetic association studies assessing the single and combined influence on exceptional longevity of the multiple gene variants that are potentially involved in the risk of having chronic diseases that are the most prominent causes of mortality in Western societies are of clinical and public health interest. A novel, simple algorithm to account for the putative combined influence of several gene variants on a given phenotype is the so-called genotype score (GS). For instance, Kathiresan et al. (2008) recently computed a GS of nine genotypes associated with modulation in

blood lipid levels; GS was shown to be an independent risk factor for incident cardiovascular disease. We recently used this method to predict other disease-related phenotype traits in healthy (Gomez-Gallego et al. 2010) and diseased people (Verde et al. 2010).

Our study purpose was to compare a polygenic profile (i.e., by calculating the GS) that combined a total of 62 mutations and polymorphisms among centenarians and ethnically matched healthy controls. The genetic variations we studied have previously been individually associated with the most prominent causes of disease mortality in western countries, i.e., cardiovascular and related ('cardiometabolic') disorders, and cancer, as well as with exceptional longevity (see Table 1). Thirty-three of the selected polymorphisms were previously chosen as candidate polymorphisms in a recent genetic association study from our group on longevity/chronic disease risk with a different Spanish cohort (Gomez-Gallego et al. 2010). The rest of polymorphisms were selected based on the literature search on genetics and longevity/chronic disease risk. We hypothesized that centenarians are endowed with a lower genetic predisposition to chronic diseases (cancer or cardiometabolic disorders).

Materials and methods

We studied 98 healthy adults (controls, age range: 19–43 years, 58 women) who were free of any diagnosed cardiometabolic disease or cancer and had no family history of exceptional longevity (≥ 90 years), and 65 centenarians (cases, aged 100–108 years, 58 women). It was a convenience sample. The most prevalent diseases in the centenarians' cohort were osteoarthritis (72%), hypertension (62.5%), dementia (50%) and coronary artery disease (30%). Three centenarians were free of any diagnosed disease. Written consent was obtained from each participant. The study protocol was approved by the institutional ethics committee (Universidad Europea de Madrid, Spain) and was in accordance with the Declaration of Helsinki for Human Research of 1974 (last modified in 2000). All study participants were of the same Caucasian (Spanish) descent for ≥ 3 generations. The majority (~90%) of them lived most of their lives and were born in the same area of Spain (Meseta Castellana, ~600 m altitude).

Table 1 List of study genes, their variants, genotypes, and association with increased disease risk or exceptional longevity (EL)

Symbol	Gene	Variant [rs]	Association with disease risk or likelihood of exceptional longevity	Genotypes (2 = highest disease risk or shorter life expectancy)
<i>ACE</i>	Angiotensin I converting enzyme	287 bp Ins(D)/Del(D) [rs1799752]	CAD (Zintzaras et al. 2008; Sancho et al. 1976), ischemic stroke (Slowik et al. 2007) type II diabetes (Zhou et al. 2009), EL (Fiuza-Luces et al. 2011)	0 = II, 1 = ID, 2 = DD
<i>ACTN3</i>	A-actinin-3	ACTN3 Arg(R)S577Ter(X) [rs1815739]		0 = XX, 1 = RX, 2 = RR
<i>ADBR1</i>	Adrenergic, beta-1-, receptor	Arg (C)389Gly(G) [rs1801253]	Cardiorespiratory fitness in CAD (Defoor et al. 2006; Wagener et al. 2002)	0 = GG, 1 = CG, 2 = CC
<i>ADRB2</i>	Adrenergic, beta-2-, receptor	Gly(G)16Arg(A) [rs1042713]	Metabolic syndrome (Dallongeville et al. 2003)	0 = AA, 1 = AG, 2 = GG
<i>ADRB2</i>	Adrenergic, beta-2-, receptor	Glu27Glu (M/N) [rs1042714]	Metabolic syndrome (Dallongeville et al. 2003)	0 = NN, 1 = NM, 2 = MM
<i>ADRB3</i>	Adrenergic, beta-3-, receptor	Trp(T)644Arg(C) [rs4994]	Obesity (Clement et al. 1995)	0 = TT, 1 = CT, 2 = CC
<i>AGT</i>	Angiotensinogen	Met(T)235Thr(C) [rs699]	Hypertension (Jeunemaitre et al. 1992) and CAD severity (Lanz et al. 2005)	0 = CC, 1 = CT, 2 = TT
<i>AGTR1</i>	Angiotensin II type I receptor	- A1166C [rs5186]	Hypertension (Niu and Qi 2010)	0 = AA, 1 = AC, 2 = CC
<i>APOA1</i>	Apolipoprotein A-I	75 G/A [rs5070]	Blood lipids (Ruano et al. 2006) and blood pressure (Sadaf et al. 2002)	0 = GG, 1 = GA, 2 = AA
<i>APOB</i>	Apolipoprotein B	Arg(G)3500Gln(A)	Hypercholesterolemia (Innerarity et al. 1990; Pullinger et al. 1995)	0 = GG, 1 = GA, 2 = AA
		Arg(C)3480Trp(T)		0 = CC, 1 = CT, 2 = TT
		Arg(C)3531Cys(T)		0 = CC, 1 = CA, 2 = TT
<i>APOE</i>	Apolipoprotein E	Arg(C)158Cys(T) [rs7412]	Hyperlipoproteinemia (Utermann 1987; Rall et al. 1989)	0 = CC, 1 = CT, 2 = TT
		Cys(T)112Arg(C) [rs429358]		0 = CC, 1 = CT, 2 = TT
<i>CETP</i>	Cholesteryl ester transfer protein	Arg (R)451Gly(Q) [rs1800777]	Lipid levels (Tsai et al. 2009) and EL related to lipid profile (Kolovou et al. 2010)	0 = TT, 1 = CT, 2 = CC
		TaqIB B1>B2 [rs708272]	Progression of coronary atherosclerosis (Kuivenhoven et al. 1998)	0 = RR, 1 = RQ, 2 = QQ
<i>COMT</i>	Catechol-O-methyltransferase	Val(G)158Met(A) [rs 4680]	Breast cancer risk (Miturunen et al. 2001)	0 = B1B1, 1 = B1B2 2 = B2B2,
<i>CYP17A1</i>	Cytochrome P450, family 17, subfamily A, polypeptide 1	-34 A(A1)>G(A2) [rs743572]	Breast cancer risk (Antognelli et al. 2009)	GG = 0, GA = 1, 2 = AA
<i>CYP19A1</i>	Cytochrome P450, family 19, subfamily A, polypeptide 1	1558C>T [rs10046]	Carcinogenesis and breast cancer prognosis (Fasching et al. 2008)	0 = A1A1, 1 = A1A2, 2 = A2A2
<i>CYP11A1</i>	Cytochrome P450, family 1, subfamily A, polypeptide 1	Ile462Val (A2455G) [rs1048943]	Breast cancer risk(Sergentanis and Economopoulos 2010)	0 = CC, 1 = CT, 2 = TT
<i>CYP11B1</i>	Cytochrome P450, family 1, subfamily B, polypeptide 1	CYP11B1*3 or Leu(G) 432Val (C)[rs1056836]	Lung (Chen et al. 2010) and ovarian cancer risk (Goodman et al. 2001)	0 = AA, 1 = AG, 2 = GG
		CYP11B1*4 or Asn(A)453Ser(G) [rs1800440]	Endometrial cancer risk (McGrath et al. 2004)	0 = GG, 1 = CG, 2 = CC
<i>DSG2</i>	Desmoglein 2	Arg45Gln	Arrhythmic right ventricular dysplasia (Awad et al. 2006)	0 = AA, 1 = AG, 2 = GG
		Arg48His		0 = Arg/Arg, 1 = Arg/Gln, 2 = Gln/Gln
		Trp305Ter (M/N)		0 = Arg/His, 1 = Arg/His, 2 = His/His
		Cys506Tyr		0 = MM, 1 = MN, 2 = NN
				0 = Cys/Cys, 1 = Cys/Tyr, 2 = Tyr/Tyr

Table 1 (continued)

Symbol	Gene	Variant [rs]	Association with disease risk or likelihood of exceptional longevity	Genotypes (2 = highest disease risk or shorter life expectancy)
		Gly811Cys		0 = Gly/Gly, 1 = Gly/Cys, 2 = Cys/Cys
<i>ESR1</i>	Estrogen receptor- α	PvuII (T397C) (PvuII) p>P [rs2234693]	EL (Corbo et al. 2011)	0 = CC, 1 = CT, 2 = TT
<i>F13A1</i>	Coagulation factor XIII, A1 polypeptide	Val(G)34Leu(T)	Prognosis of CAD (Satra et al. 2011)	0 = GG, 1 = GT, 2 = TT
<i>FII</i>	Factor II Prothrombin	G20210A [rs1799963]	Thrombosis (Margaglione et al. 2001)	0 = GG, 1 = AG, 2 = AA
<i>FX</i>	Factor V Leiden	Arg(G)506Gln(A) [rs6025]	Thrombosis (Bertina et al. 1994)	0 = GG, 1 = AG, 2 = AA
<i>FXB</i>	Fibrinogen-B beta	- 455G/A [rs1800790?]	Fibrinogen levels and ischemic stroke (Siegerink et al. 2009)	0 = GG, 1 = AG, 2 = AA
<i>GNAS1</i>	α -subunit of Gs proteins Gs	393T>C (Ile131Ile) [rs7121]	Tumor development and prognosis in several types of tumours (Alakus et al. 2009; Lehnerdt et al. 2008; Frey et al. 2005a, b, 2006)	0 = TT, 1 = CT, 2 = CC
<i>GNB3</i>	Guanine nucleotide binding protein beta	C825T Ser275Ser (M/N) [rs5443]	Hypertension (Siffert et al. 1998; Bagos et al. 2007)	0 = MM, 1 = MN, 2 = MM
<i>GSTM1</i>	Myelodysplastic syndrome (Chen et al. 1996)	presence/absence	EL (Christiansen et al. 2006)	0 = presence, 2 = absence
	Colorectal cancer (Liao et al. 2009)		Hypertension (Capoluongo et al. 2009)	
			Cervical cancer (Economopoulos et al. 2010)	
			Bladder cancer (Rothman et al. 2010)	
			Colorectal cancer (Economopoulos and Sergentanis 2010)	
			Breast cancer (Sergentanis and Economopoulos 2010)	
			Nasopharyngeal cancer risk (Zhuo et al. 2009)	
<i>GSTP1</i>	Glutathione S-transferase pi 1	Ile(A)105Val(G) [rs1695]	Lung Cancer (Ali-Osman et al. 1997)	0 = AA, 1 = AG, 2 = GG
		Ala(C)114Val(T) [rs1138272]	Lung Cancer (Wang et al. 2003)	0 = CC, 1 = CT, 2 = TT
<i>GSTT1</i>	Glutathione S-transferase theta 1	presence/absence	Myelodysplastic syndrome (Chen et al. 1996)	0 = presence, 2 = absence
			Colorectal cancer (Liao et al. 2009)	
			Breast cancer (Sergentanis and Economopoulos 2010)	
<i>IL6</i>	Interleukin 6	-174C/G [rs1800795]	Blood glucose (Huth et al. 2009)	0 = CC, 1 = GC, 2 = GG
<i>IL10</i>	Interleukin 10	-1082 A>G [rs1800896]	Longevity (Di Bona et al. 2009)	0 = GG, 1 = AG, 2 = AA
<i>ITGB3</i>	Integrin beta 3	Leu(T)33Pro (C) [rs5918]	Longevity (Lio et al. 2004)	0 = TT, 1 = CT, 2 = CC
<i>MMP3</i>	Matrix metalloproteinase 3	5A/6A [rs3025058]	Risk of atherosclerotic plaque rupture (Kucharska-Newton et al. 2011)	0 = 6A/6A, 1 = 5A/6A, 2 = 5A/5A
<i>MTHFR</i>	Methylenetetrahydrofolate reductase	Ala(C)222Val(T) [rs1801133]	Myocardial infarction (Abilleira et al. 2006)	0 = CC, 1 = CT, 2 = TT
<i>NAT2</i>	N-Acetyltransferase 2	Arg(R)64Gln(Q) [rs1801279]	Cardiovascular disease (through increase in homocysteine levels) (Varga et al. 2005)	0 = RR, 1 = RQ, 2 = QQ
		Tyr94Tyr (M/N) [rs1041983]	Bladder cancer (Garcia-Closas et al. 2005)	0 = MM, 1 = MN, 2 = NN
		Ile114Thr [rs1801280]		0 = II, 1 = IT, 2 = TT
		Leu161Leu (M/N) [rs1799929]		0 = MM, 1 = MN, 2 = NN
		Arg197Gln [rs1799930]		0 = AA, 1 = AG, 2 = GG
		Arg268Lys [rs1208]		0 = AA, 1 = AL, 2 = LL

<i>NOS3</i>	Nitric oxide synthase 3	Gly286Glu (M/N) [rs1799931] -786T/C [rs2070744]	CAD (Nakayama et al. 1999) CAD (Casas et al. 2006) and ischemic stroke risk (Berger et al. 2007) Cholesterol levels (Karvonen et al. 1998) and type II diabetes (Ukkola and Kesäniemi 2007) Lung cancer (Sugimura et al. 1999) CAD (Margaglione et al. 1998)	0 = MM, 1 = MN, 2 = NN 0 = TT, 1 = TC, 2 = CC 0 = GG, 1 = GT, 2 = TT 0 = AA, 1 = AG, 2 = GG 0 = CC, 1 = CG, 2 = GG 0 = SG5G, 1 = 4G5G, 2 = 4G4G
<i>NPY</i>	Neuropeptide Y	Leu(A)7Pro(G) [rs16139]		
<i>OGG1</i>	8-Oxoguanine DNA glycosylase	Cys(C)326Ser(G) [rs1052133]		
<i>PAI1</i>	Plasminogen activator inhibitor 1	4G/5G [rs1800629]		
<i>PGR</i>	Progesterone receptor	A331G [rs10895068]	Ovarian (Risch et al. 2006) and breast cancer risk (De Vivo et al. 2003)	0 = GG, 1 = AG, 2 = AA
<i>PON1</i>	Human paraoxonase 1	Gln(A)192Arg(G) [rs662]	EL (Lescat et al. 2009)	0 = AA, 1 = AG, 2 = GG
<i>SOD2</i>	Superoxide dismutase 2	Ala(C)16Val(T) [rs4880]	Pancreatic Cancer (Liu et al. 2004; Wheatley-Price et al. 2008)	0 = CC, 1 = CT, 2 = TT
<i>SRD5A2</i>	3-Oxo-5- α -steroid 4-dehydrogenase 2	Ala(G)49Thr(A) [rs523349]	Prostate cancer (Wang et al. 2010)	0 = GG, 1 = AG, 2 = AA
<i>SREBP2</i>	Sterol regulatory element-binding factor-2	Gly(G)595Ala (C) [rs2228314]	Myocardial infarction (Friedlander et al. 2008)	0 = GG, 1 = GC, 2 = CC
<i>SULT1A1</i>	Sulfotransferase	Arg(G)213His(A) [rs9282861]	Breast cancer (Jiang et al. 2010)	0 = GG, 1 = GA, 2 = AA
<i>VDR</i>	Vitamin D receptor	BsmI G>A Pos. +283 (b/B) [rs1544410]	Colon cancer (Jenab et al. 2009)	0 = BB, 1 = Bb, 2 = bb

CAD coronary artery disease

Genotyping

Table 1 shows the list of study genes by alphabetical order, their variants and genotypes. The variants were grouped into two main disease categories: (i) cardio-metabolic risk, and (ii) cancer. We also studied several variants previously related to exceptional longevity.

All genotyping was performed in the same laboratory (Progenika Biopharma, Parque Tecnológico de Zamudio, Derio-Vizcaya, Spain). We obtained DNA from saliva samples and amplified the study genes in six multiplex-polymerase chain reactions (PCRs). Genotyping was performed with a newly developed low-density DNA microarray based on allele-specific probes. The design, fabrication, validation and analysis of the arrays were performed following the procedure detailed elsewhere (Tejedor et al. 2005) with minor modifications. The PCR products were fluorescently labeled and hybridized to the DNA microarray in an automated platform (Tecan HS4800, Mannedorf, Switzerland). Finally, the microarrays were scanned (Innopsys S.A., Carbone, France) and we determined variants using a developed software that converts the intensity of the spots into the genotype of each variant (Tejedor et al. 2005). For genotyping control, sample analysis was made together with a DNA control processing with a known genotype for all the polymorphisms included in the study. DNA control genotypes were ensured to be correct before considering the analysis of the remaining samples included in the process.

Following recent recommendations for replicating human genotype–phenotype association studies (Chanock et al. 2007), the results of a subset of polymorphisms (rs1799752 and rs1815739) were corroborated in a second laboratory (Universidad Europea de Madrid, Spain) using a different methodology, i.e., PCR followed by genotyping of the resulting products with electrophoresis through agarose gel (rs1799752) and fragment length polymorphisms (rs1815739).

Disease risk genotype score and genotype score per disease category/subcategory

We assumed that an *individual* polymorphism would not have a major influence per se to the disease risk; therefore, we studied the *combined* influence of the 62 studied polymorphisms. First, we scored each

genotype within each polymorphism (Table 1). We assumed an additive model (equaling 0, 1 or 2), that is, on the basis of the number of alleles associated with higher disease risk (or lower exceptional longevity potential) that were carried by each subject for each polymorphism (Ruiz et al. 2009, 2010; Gomez-Gallego et al. 2010). Thus, we assigned a score of 0, 1 and 2 to each individual genotype associated with lower, medium and highest disease risk, respectively (longer, medium and shorter exceptional longevity potential). Second, we summed the score of each single genotype ($\text{Score}_{\text{ACEI/D}} + \dots + \text{Score}_{\text{VDR BsmI G} > \text{A Pos.} + 283(\text{b/B})}$). Third, the obtained sum of scores was ‘normalised’ (i.e., transformed to the scale of 0–100) for easier interpretation, as follows:

$$\text{GS} = (100/124) \times (\text{GS}_{\text{ACEI/D}} + \dots + \text{GS}_{\text{VDR BsmI G} > \text{A Pos.} + 283(\text{b/B})}),$$

where 124 is the result of multiplying 62 (number of studied polymorphisms, the so-called health-related global GS) by 2, which is the score given to the genotype associated with lowest disease risk (or highest exceptional longevity potential). For instance, a GS of 100 represents the theoretically less favorable profile for the polygenic profile of ‘health’, that is, that all individual GSs are 2; whereas a GS of 0 represents the theoretically most favorable profile for ‘health’.

Following the aforementioned methodology, we also computed specific GSs for the two main disease categories [*cardiometabolic risk* (36 genetic variants), and *cancer risk* (24 genetic variants)]. We also computed another GS for exceptional longevity (seven genetic variants)].

The genetic variants included in the specify phenotypes were as follows:

- (i) ‘*global cardiometabolic risk GS*’ (including *ACE* Ins/Del, *AGT* Met235Thr, *ADBR1* Arg389Gly, *ADRB2* Gly16Arg, *ADRB2* Gln27Glu, *ADRB3* Trp64Arg, *AGTR1* 1166C, *APOA1* –75G/A, *APOB* Arg3500Gln, *APOB* Arg3531Cys, *APOB* Arg3500Trp, *APOE* Arg158Cys, *APOE* Cys112Arg, *CETP* Arg451Gly, *CETP* TaqIB B1>B2, *DSG2* Arg45Gln, *DSG2* Arg48Gln, *DSG2* Trp305Ter, *DSG2* Cys506Tyr, *DSG2* Gly811Cys, *F13A1* Val34Leu, *FII* G20210A, *FV* Arg506Gln, *FGB* –455G/A, *GJ4A* Pro319Ser, *GNB3* 825C/T, *IL6* –174G/C, *GSTM1* ‘null allele’, *ITGB3* Leu33Pro, *MMP3* 5A/6A, *MTHFR* Ala222Val, *NOS3* –786T/C, *NOS3*

Glu298Asp, *NPY* Leu7Pro, *PAI1* 4G/5G, *SREBP* Gly595Ala)

- (ii) ‘*global cancer GS*’ (*COMT* Val158Met, *CYP17A1* –34 A>G, *CYP19A1* 11558C>T, *CYP1A1* Ile462Val, *CYP1B1* Leu432Val, *CYP1B1* Asn453Ser, *GNAS* Ile131Ile, *GSTM1* ‘null allele’, *GSTP1* Ile105Val, *GSTP1* Ala114Val, *GSTT1* ‘null’ allele, *NAT2* Arg64Gln, *NAT2* Tyr94Tyr, *NAT2* Ile114Thr, *NAT2* Leu161-Leu, *NAT2* Arg197Gln, *NAT2* Arg268Lys, *NAT2* Gly286Glu, *OGG1* Cys326Ser, *PGR* A331G, *SOD2* Ala16Val, *SRD5A2* Ala49Thr, *SULTA1* Arg213His, *VDR BsmI* G>A Pos. + 283 (b/B))
- (iii) ‘*Exceptional longevity GS*’ (*ACTN3* Arg577Ter, *CETP* Arg451Gly, *ESR1* PvuII (T397C), *GSTM1* ‘null allele’, *IL6* –174G/C, *IL10* –1082 A>G, *PON1* Gln192Arg)

We also computed GSs for each disease subcategory (≥ 3 variants each):

- (i) Cardiovascular disease (*ACE* Ins/Del, *ADBR1* Arg389Gly, *AGT* Met235Thr, *AGTR1* –A1166C, *CETP* TaqIB B1>B2, *F13A1* Val34Leu, *ITGB3* Leu33Pro, *MMP3* 5A/6A, *MTHFR* Ala222Val, *NOS3* –786T/C, *NOS3* Glu298Asp, *PAI1* 4G/5G, *SREBP* Gly595Ala)
- (ii) Hypertension (*AGT* Met235Thr, *AGTR1* 1166C, *APOA1* 75G/A and *GNB3* 825C/T, *GSTM1* ‘null allele’)
- (iii) Dyslipidemia (*APOA1* –75G/A, *APOB* Arg3500Gln, *APOB* Arg3531Cys, *APOB* Arg3500Trp, *APOE* Arg158Cys, *APOE* Cys112Arg, *CETP* Arg451Gly, and *NPY* Leu7Pro)
- (iv) Thrombosis/ischemic stroke (*ACE* Ins/Del, *FII* G20210A, *FV* Arg506Gln, *FGB* –455G/A, *GJ4A* Pro319Ser, *NOS3* Glu298Asp)
- (v) Insulin resistance (*ACE* I/D, *IL6* –174G/C and *NPY* Leu7Pro)
- (vi) Risk of sudden cardiac death (ARVD/C) [*DSG2* Arg45Gln, *DSG2* Arg48Gln, *DSG2* Trp305Ter (M/N), *DSG2* Cys506Tyr and *DSG2* Gly811Cys]
- (vii) Lung cancer (*CYP1B1* Leu432Val, *GSTP1* Ile105Val, *GSTP1* Ala114Val, *OGG1* Cys326Ser)
- (viii) Breast cancer (*COMT* Val158Met, *CYP17A1* –34 A>G, *CYP19A1* 11558C>T, *CYP1A1* Ile462Val, *GSTM1* ‘null allele’, *GSTT1* ‘null’ allele, *PGR* A331G, *SULTA1* Arg213His)
- (ix) Types of cancer other than breast and lung [*CYP1B1* Leu432Val, *GNAS* 393T>C

(Ile131Ile), *GSTM1* ‘null allele’, *GSTT1* ‘null allele’, *NAT2* Arg64Gln, *NAT2* Tyr94Tyr (M/N), *NAT2* Ile114Thr, *NAT2* Leu161Leu (M/N), *NAT2* Arg197Gln, *NAT2* Arg268Lys, *NAT2* Gly286Glu (M/N), *GSTT1* ‘null allele’, *PGR* A331G, *SOD2* Ala16Val, *VDR* BsmI G>A Pos. +283 (b/B))]

Statistical analysis

All analyses were performed with the Statistical Package for Social Sciences (SPSS, v. 18.0 for WINDOWS; SPSS Inc, Chicago). Two-sided Student’s *t*-test was conducted to compare the ‘health-related’ GS as well as the GS by disease categories/subcategories among groups (centenarians vs. controls). We repeated the analysis by further adjusting by age (analysis of covariance). We also compared the genotype frequency for each polymorphism between the groups using the χ^2 test. When between-group genotype differences were observed, we conducted binary logistic regression to examine the likelihood of having the low disease risk genotype (inserted in the model as independent variable, i.e., covariate) in centenarians (dependent variable) compared with the control group. Comparisons were adjusted for mass significance as described by Holm (Holm 1979; Gordi and Khamis 2004). The method described by Holm proceeds as follows: Sort the *P* values of the *k* tests in increasing order, $P_1, P_2, \dots, P_i, \dots, P_k$. If $P_1 > \alpha/k$, then none of the *k* tests are significant, and the test procedure is finished. If $P_1 \leq \alpha/k$, test 1 is significant, and now P_2 is examined. If $P_2 > \alpha/(k-1)$, none of the (*k*−1) remaining tests are significant, but if $P_2 \leq \alpha/(k-1)$, test 2 is significant and P_3 is examined. This procedure goes on until $P_i > \alpha/(k-i+1)$, and the procedure is interrupted. This method keeps family error rate to less than α . Family error rate is defined as the probability that one or more false significances out of *k* tests is less than or equal to α .

Results

Genotyping

There were no failures in sample collection, DNA acquisition or genotyping procedures, except for 11 centenarians and 11 controls, for which the amount of

DNA gathered from saliva was insufficient to allow genotype assessment. Parallel genotyping results showed 100% concordance between the two laboratories. Genotype distributions met Hardy–Weinberg equilibrium in the two study groups (all $P > 0.1$), except for the *CYP1A1* Ile462Val, *GSTM1* presence/absence, and *GSTT1* presence/absence genetic variants (all $P < 0.0001$).

Genotype frequencies in cases and controls

The genotype frequencies of the studied polymorphisms by group are depicted in Table 2. We observed significant differences in the *GSTT1* presence/absence and in the *GSTM1* presence/absence after correcting for multiple comparisons (Holm 1979) (both $P < 0.0001$). The likelihood of having the *GSTT1* low risk allele (functional, presence) was higher in the centenarians group (odds ratio [OR] 5.005; 95% confidence interval [CI], 1.810–13.839, $P = 0.002$), whereas the likelihood of having the *GSTM1* low risk allele (functional, presence) was similar between groups (OR 1.295; 95% CI, 0.868–1.931, $P = 0.206$) after controlling for sex. The between-group comparisons of allele frequencies confirmed the results observed with the between-group genotype comparisons (data not shown).

Disease risk genotype score

The computed ‘global’ GS was similar among groups (Table 3). We observed that the GS for hypertension, cancer (global risk), and other types of cancer was lower in the centenarians group compared with the control group (all $P < 0.05$), yet, once the analysis was adjusted by sex, the difference became non-significant (all $P > 0.05$). We repeated the analysis assuming the dominant and the recessive model and the findings remained the same (all $P > 0.1$, data not shown).

Discussion

The findings of the present study suggest that there is no evidence for a lower ‘overall’ disease risk in centenarians, yet we observed preliminary evidence that Spanish centenarians have a lower genetic predisposition for overall cancer risk. It is noteworthy, however, that statistical significance was not reached for any GS

Table 2 Genotype frequencies in centenarians and controls

Symbol	Variant [rs]	Genotype frequency		<i>P</i> dom	<i>P</i> rec	<i>P</i> overall
		No. of Centenarians/controls	Centenarians (0/1/2) ^a			
<i>ACE</i>	287 bp Ins(I)/Del(D) [rs1799752]	54/87	16.7, 31.4, 51.9	>0.1	>0.1	>0.1
<i>ACTN3</i>	ACTN3 Arg(R)577Tet(X) [rs1815739]	54/87	24.1, 50.0, 25.9	>0.1	>0.1	>0.1
<i>ADBR1</i>	Arg (C)389Gly(G) [rs1801253]	54/87	13.0, 44.4, 42.6	>0.1	>0.1	>0.1
<i>ADRB2</i>	Gly(G)16Arg(A) [rs1042713]	54/87	0, 42.6, 57.4	>0.1	>0.1	>0.1
<i>ADRB2</i>	Gln27Glu (M/N) [rs1042714]	54/87	38.9, 46.3, 14.8	>0.1	>0.1	>0.1
<i>ADRB3</i>	Trp(T)64Arg(C) [rs4994]	54/87	85.2, 14.8, 0	>0.1	>0.1	>0.1
<i>AGT</i>	Met(T)235Thr(C) [rs699]	53/87	37.7, 37.7, 24.5	>0.1	>0.1	>0.1
<i>AGTR1</i>	-A1166C [rs5186]	54/85	38.9, 55.6, 5.5	>0.1	>0.1	>0.1
<i>APOA1</i>	75 G/A [rs5070]	54/87	64.8, 31.5, 3.7	>0.1	>0.1	>0.1
<i>APOB</i>	Arg(G)3500Gln(A)	54/87	96.3, 3.7, 0	>0.1	>0.1	>0.1
	Arg(C)3480Trp(T)		98.1, 1.9, 0	>0.1	>0.1	>0.1
	Arg(C)3531Cys(T)		94.4, 5.6, 0	>0.1	>0.1	>0.1
<i>APOE</i>	Arg(C)158Cys(T) [rs7412]	54/87	87.0, 13.0, 0	>0.1	>0.1	>0.1
	Cys(T)112Arg(C) [rs429358]		81.5, 16.7, 1.9	>0.1	>0.1	>0.1
<i>CETP</i>	Arg (R)451Gly(Q) [rs1800777]	54/87	92.6, 7.4, 0	>0.1	>0.1	>0.1
	TaqIB B1>B2 [rs708272]		18.5, 40.7, 40.7	>0.1	>0.1	>0.1
<i>COMT</i>	Val(G)158Met(A) [rs 4680]	54/87	33.3, 37.1, 29.6	>0.1	>0.1	>0.1
<i>CYP17A1</i>	-34 A(A1)>G(A2) [rs743572]	54/85	46.3, 37.0, 16.7	0.04320	>0.1	0.04320
<i>CYP19A1</i>	1558C>T [rs10046]	54/87	27.8, 46.3, 25.9	>0.1	>0.1	>0.1
<i>CYP11A1</i>	Ile462Val (A2455G) [rs1048943]	54/87	96.3, 0, 3.7	>0.1	>0.1	0.04199
<i>CYP11B1</i>	CYP11B1*3 or Leu(G)432Val (C) [rs1056836]	54/87	29.6, 46.3, 24.1	>0.1	>0.1	>0.1
	CYP11B1*4 or Asn(A)453Ser(G) [rs1800440]		5.6, 35.2, 59.3	>0.1	>0.1	>0.1
<i>DSG2</i>	Arg45Gln	54/87	90.7, 9.3, 0	0.02038	>0.1	0.02038
	Arg48His		96.3, 3.7, 0	>0.1	>0.1	>0.1
	Trp305Ter (M/N)		94.4, 5.6, 0	>0.1	>0.1	>0.1
	Cys506Tyr		94.4, 5.6, 0	>0.1	>0.1	>0.1
	Gly811Cys		94.4, 5.6, 0	0.02626	>0.1	0.02626
<i>ESR1</i>	PvuII (T397C) (PvuII) p>P [rs2234693]	54/87	16.7, 53.7, 29.6	>0.1	0.04175	>0.1
<i>F13A1</i>	Val(G)34Leu(T)	54/87	53.7, 38.9, 7.4	>0.1	>0.1	>0.1
<i>FII</i>	G20210A [rs1799963]	54/87	98.1, 1.9, 0	>0.1	>0.1	>0.1

<i>FLV</i>	Arg(G)506Gln(A) [rs6025]	54/84	100, 0, 0	98.8, 1.2, 0	>0.1	>0.1	>0.1
<i>FGB</i>	−455G/A [rs1800790]	53/87	60.4, 30.2, 9.4	60.9, 32.2, 6.9	>0.1	>0.1	>0.1
<i>GJ44</i>	Pro319Ser (1019C>T) [rs1764391]	54/87	53.7, 38.9, 7.4	62.8, 22.1, 15.1	>0.1	>0.1	>0.1
<i>GN4S1</i>	393T>C (Ile131Ile) [rs7121]	54/87	35.8, 47.2, 17.0	33.3, 50.6, 16.1	>0.1	>0.1	>0.1
<i>GNB3</i>	C825T Ser275Ser (M/N) [rs5443]	54/86	55.6, 29.6, 14.8	39.5, 38.4, 22.1	>0.1	>0.1	>0.1
<i>GSTM1</i>	presence/absence	54/87	61.1, 0, 38.9	33.0, 0, 67.0	0.00149	>0.1	0.00005
<i>GSTP1</i>	Ile(A)105Val(G) [rs1695]	54/87	50.0, 37.0, 13.0	47.1, 37.9, 14.9	>0.1	>0.1	>0.1
	Ala(C)114Val(T) [rs1138272]		92.6, 7.4, 0	87.4, 11.5, 1.1	>0.1	>0.1	>0.1
<i>GSTT1</i>	presence/absence	53/86	84.9, 0, 15.1	40.7, 0, 59.3	0.00002	>0.1	<0.00001
<i>IL6</i>	−174C/G [rs1800795]	54/87	9.3, 46.3, 44.4	10.3, 47.1, 42.5	>0.1	>0.1	>0.1
<i>IL10</i>	−1082 A>G [rs1800896]	54/87	22.2, 31.5, 46.3	20.7, 37.9, 41.4	>0.1	>0.1	>0.1
<i>ITGB3</i>	Leu(T)33Pro (C) [rs5918]	54/87	72.2, 24.1, 3.7	70.1, 24.1, 5.7	>0.1	>0.1	>0.1
<i>MMP3</i>	5A/6A [rs3025058]	54/87	24.1, 48.1, 27.8	28.7, 43.7, 27.6	>0.1	>0.1	>0.1
<i>MTHFR</i>	Ala(C)222Val(T) [rs1801133]	54/87	44.4, 38.9, 16.7	36.8, 36.8, 26.4	>0.1	>0.1	>0.1
<i>NAT2</i>	Arg(R)64Gln(Q) [rs1801279]	54/87	0, 0, 100	0, 0, 100	>0.1	>0.1	>0.1
	Tyr94Tyr (M/N) [rs1041983]		57.4, 31.5, 11.1	55.2, 39.1, 5.7	>0.1	>0.1	>0.1
	Ile114Thr [rs1801280]		33.3, 33.3, 33.3	29.9, 48.3, 21.8	>0.1	>0.1	>0.1
	Leu161Leu (M/N) [rs1799929]		33.3, 40.7, 25.9	31.0, 48.3, 20.7	>0.1	>0.1	>0.1
	Arg197Gln [rs1799930]		11.1, 31.5, 57.4	5.7, 36.8, 57.5	>0.1	>0.1	>0.1
	Arg268Lys [rs1208]		33.3, 38.9, 27.8	27.6, 50.6, 21.8	>0.1	>0.1	>0.1
<i>NOS3</i>	Gly286Glu (M/N) [rs1799931]	54/87	100, 0, 0	94.3, 5.7, 0	>0.1	>0.1	>0.1
	−786 T/C [rs2070744]	54/87	42.6, 48.1, 9.3	31.0, 44.8, 24.2	>0.1	0.02678	>0.1
	Glu298Asp (G894T) [rs 1799983]		46.3, 38.9, 14.8	42.5, 49.4, 8.1	>0.1	>0.1	>0.1
<i>NPY</i>	Leu(A)7Pro(G) [rs16139]	54/87	90.7, 9.3	92.0, 8.0	>0.1	>0.1	>0.1
<i>OGG1</i>	Cys(C)326Ser(G) [rs1052133]	52/87	55.8, 34.6, 9.6	70.1, 24.1, 5.7	>0.1	>0.1	>0.1
<i>P4II</i>	4G/5G [rs1800629]	54/87	27.8, 40.7, 31.5	34.5, 37.9, 27.6	>0.1	>0.1	>0.1
<i>PGR</i>	A331G [rs10895068]	54/87	90.7, 9.3, 0	95.4, 4.6, 0	>0.1	>0.1	>0.1
<i>PON1</i>	Gln(A)192Arg(G) [rs662]	54/87	61.1, 25.9, 13.0	50.6, 35.6, 13.8	>0.1	>0.1	>0.1
<i>SOD2</i>	Ala(C)16Val(T) [rs4880]	54/87	23.1, 55.8, 21.2	34.5, 36.8, 28.7	>0.1	>0.1	>0.1
<i>SRD5A2</i>	Ala(G)49Thr(A) [rs523349]	54/87	96.3, 3.7, 0	100, 0, 0	>0.1	>0.1	>0.1
<i>SREBP2</i>	Gly(G)595Ala (C) [rs2228314]	54/87	66.7, 22.2, 11.1	60.9, 33.3, 5.8	>0.1	>0.1	>0.1
<i>SULT1A1</i>	Arg(G)213His(A) [rs9282861]	54/87	63.0, 25.9, 11.1	55.2, 37.9, 6.9	>0.1	>0.1	>0.1
<i>VDR</i>	BsmI G>A Pos. +283 (b/B) [rs1544410]	53/87	20.8, 43.4, 35.8	17.2, 40.2, 42.5	>0.1	>0.1	>0.1

^a 2 = highest disease risk or shorter life expectancy; 0 = lowest disease risk or longer exceptional longevity

P values before adjustment for multiple comparisons. *P* values that remained significant after adjusting for multiple comparisons are in *bold*

Table 3 Comparison of genotype scores per disease category and subcategory in centenarians ($n=54$, 6 men), and controls ($n=87$, 40 men)

Disease category/subcategory	No. of variants	Centenarians	Controls subjects	P^b
Health-related (global)	62	31.0±0.6	32.0±0.5	0.263
Cardiometabolic risk (global)	36	24.9±0.7	25.3±0.5	0.664
Cardiovascular disease	13	37.8±1.2	38.2±1.0	0.790
Hypertension	5	32.6±2.2	38.9±1.7	0.026
Thrombosis/ischemic stroke	6	25.9±1.8	24.2±1.4	0.445
Dyslipidemia	8	6.2±1.0	5.4±0.7	0.531
Insulin resistance	3	46.6±2.4	43.5±1.9	0.306
Cancer (global)	24	37.8±1.1	40.6±0.8	0.048
Lung cancer	4	27.1±2.7	26.4±2.0	0.830
Breast cancer ^a	8	28.0±2.1	28.2±2.2	0.963
Other types	14	43.0±1.6	47.7±1.2	0.020
Exceptional longevity	7	51.8±2.1	53.8±1.6	0.457

Values are means ± standard error

^a Analysis conducted on 47 centenarians and 43 control women

^b P values from unpaired Student's t test. All P values >0.05 after adjusting for sex

disease category once sex was taken into account. Overall, we found no association for those genetic variants that we included in our model based on their potential association with the most common causes of disease-related mortality in western countries, that is, 'cardiometabolic' disorders. With regard to individual genetic variations, preliminary evidence for an association with exceptional longevity was found for the deletions in the glutathione S -transferase superfamily genes *GSTT1* and *GSTM1*. Indeed, the likelihood of having the *GSTT1* low risk (functional, presence) allele or the *GSTM1* low risk (functional, presence) was ~5 and 1.3 times higher, respectively, among centenarians compared with controls. Our findings on *GSTT1* and *GSTM1* corroborate and extend previous research in the field. To our knowledge, this is the first attempt to determine the genetic influence on exceptional longevity using a simple algorithm model that accounts for the polygenic nature of disease phenotypes.

The *GSTM1* and *GSTT1* enzymes mediate exposure to various cytotoxic and genotoxic agents, and their metabolic role becomes more important with ageing, owing to a decline in toxic defense mechanisms (Pesch et al. 2004). Carriage of the 'null' variant allele (in which the entire gene is absent) in *GSTM1* or *GSTT1* genes increases the risk of having several types of cancer: cervical cancer (*GSTM1*) (Economopoulos et al. 2010), colorectal cancer,

which is one of the most common types of cancer in westerners [*GSTM1* (Economopoulos and Sergentanis 2010) and *GSTT1* (Liao et al. 2009)], bladder cancer (*GSTM1*) (Rothman et al. 2010), breast cancer, which is the most frequent cancer among women (*GSTT1*) (Sergentanis and Economopoulos 2010), and myelodysplastic syndrome (*GSTT1*) (Chen et al. 1996). In addition, carriage of the null *GSTM1* or *GSTT1* genotype, especially of the latter, (i) has been negatively associated with exceptional longevity in Danish women (Christiansen et al. 2006), (ii) shows an decreasing prevalence when comparing old Italians (80–100 years) with younger controls (Santovito et al. 2008), or (iii) is less prevalent in German octogenarians with cancer than in their age-matched peers without cancer (Pesch et al. 2004). Furthermore, *GSTM1* and *GSTT1* gene variants are also associated with oxidative stress related diseases, whereas inflammation and oxidative stress are strong determinants of the ageing process (Onder et al. 2011). In relation to this, the *GSTM1* wild-type (i.e., functional) genotype has been recently associated with decreased mortality among older people with high levels of inflammation (Onder et al. 2011). The present findings together with those published previously support a beneficial role in terms of longevity for the functional allele of the *GSTM* gene variant and, especially, the *GSTT* gene variant.

We believe the results of our study are overall valid, as all the following criteria were met (Attia et al. 2009): the phenotype ‘exceptional longevity’ was properly defined by using a cohort of centenarians, both groups of centenarians and controls were ethnically matched, genetic assessment was accurate and unbiased, the great majority of genotype distributions (59 out of 62 polymorphisms) were in the Hardy–Weinberg equilibrium, we adjusted our statistical analyses for multiple comparisons, and our results (particularly on *GSTM1* and *GSTT1* gene) are in overall agreement with previous findings. However, the low sample size of our cohorts and the lack of data from a ‘replication’ cohort of a different ethnic background does considerably limit the ‘external validity’ (and therefore generalizability) of our results. More research is needed using our model on larger groups of different ethnic backgrounds.

Our study is limited by the fact that we only analyzed a snapshot of the numerous polymorphisms and mutations that can potentially affect disease risk and exceptional longevity. For instance, we did not include in our analyses two polymorphisms that have been linked with exceptional longevity in other Caucasian (Italian) cohorts, i.e., the +896 A→G (Asp299Gly) polymorphism in the Toll-like receptor 4 (*TLR4*) gene (Balistreri et al. 2004) or the rs1333049 (C/G) polymorphism on chromosome 9p2 (Emanuele et al. 2010). Our findings also are limited by the fact that we did not study genetic susceptibility to other types of diseases, notably infectious diseases. This is an important consideration because centenarians have escaped diseases of the pre-antibiotic era (Salvioli et al. 2008). Nevertheless, it must be kept in mind that it is difficult to account for all the candidate genetic polymorphisms that can be potentially associated with disease risk and thus survival. We assumed that the effect of the genotype and the disease risk was additive. With regard to this potential limitation, the results remained the same after repeating the analysis using the dominant or recessive model (data not shown). All gene variants were given the same weight in the total score, but whether the selected genes and the gene variants explain the same proportion of the variance in the disease risks is not known. On the other hand, while keeping in mind the low number of centenarians we studied (which we believe is justifiable given the uniqueness of this type of population) and the difficulty of finding suitable

controls for any centenarians’ cohort, we believe the major strength in our study stems in the novel algorithm approach we used.

In summary, we found preliminary evidence that Spanish centenarians might have a lower genetic predisposition for overall cancer risk, yet not for cardiometabolic disorders. With regard to individual genetic variations, the wild-type (i.e., functional) genotype of *GSTT1*, which is associated with lower cancer risk, could be associated with exceptional longevity. Further studies with larger sample sizes and cohorts of different ethnicities must confirm these findings.

Acknowledgements This study was supported by a grant from Fondo de Investigaciones Sanitarias (FIS, grant #PI09-00194), and by the Spanish Ministry of Science and Innovation (RYC-2010-05957).

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