A study into genes encoding longevity in humans

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CHAPTER 3

The Liver X Receptor Alpha Associates with Human Lifespan

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Abstract

In the nematode *Caenorhabditis elegans*, nuclear hormone receptor DAF-12 regulates the decision to go into a resistant dauer diapause, in which the worm exhibits a decreased rate of aging. Using sequence similarity searches, we previously identified the liver X receptor alpha (LXRA) as one of the human nuclear hormone receptors the protein sequence of which is most similar to *C. elegans* DAF-12. Here, we studied whether variation in the gene encoding LXRA associates with human life span. In the Leiden 85-plus Study, a population-based prospective follow-up study, we genotyped four polymorphisms spanning the gene coding for *LXRA* (NR1H3) and tagged four common haplotypes. Among 563 participants, haplotype 2 associated with reduced mortality during the 7-year follow-up (hazard ratio 0.78; $p=0.015$), predominantly caused by reduced mortality from infectious disease (hazard ratio 0.31; $p=0.023$). Haplotype 2 also associated with higher levels of plasma apolipoprotein E, a target gene of the LXRA ($p=0.018$), and higher levels of triglycerides ($p=0.041$). Genetic variation in the gene coding for the LXRA (NR1H3) associates with human lifespan.

Introduction

Human lifespan is under genetic control (Schoenmaker et al., 2006; vB et al., 2006), but only few specific genes modulating lifespan have been identified. In the nematode worm *Caenorhabditis* elegans, DAF-12 is a nuclear hormone receptor (NHR) that in response to environmental cues regulates the entry into dauer diapause (Antebi et al., 1998). Under adverse environmental conditions, unliganded DAF-12 coordinately turns down essential traits—such as metabolism, feeding, and reproduction— making the worm more stress resistant and extending larval survival up to 5-fold (Klass and Hirsh, 1976), which suggests that during the diapause the worm ages at a lower rate. Genetic mutations in *daf-12* can be either dauer defective or dauer constitutive (Antebi et al., 2000) and, in parallel, can decrease or increase adult life span of *C. elegans* (Fisher and Lithgow, 2006). Using sequence similarity searches, we previously identified the liver X receptor alpha (LXRA) as one of the human NHRs the protein sequence of which is most similar to *C. elegans* DAF-12 (Mooijaart et al., 2005). However, nothing is known about the association of genetic variants in the gene coding for the LXRA (NR1H3) with human lifespan.

In humans, the *LXRA* is expressed in the liver, kidney, macrophages, astrocytes, and other tissues (Peet et al., 1998). Oxysterols are breakdown products of cholesterol and serve as ligands for the LXRA (Janowski et al., 1999). Binding of ligands leads to the transcription of target genes that coordinately regulate various processes that together result in increased catabolism and excretion of cholesterol from the body (Lu et al., 2001; Repa et al., 2000). In humans, cholesterol is a major determinant of mortality in old age, especially from infectious disease (Weverling-Rijnsburger et al., 1997).The LXRA is also involved in innate immunity, as activation of human macrophages that produce cytokines is dependent on LXRA (Joseph et al., 2003). In humans, cytokine production is a highly heritable characteristic (de Craen et al., 2005) and associates with diseases and mortality up to the highest age category (van den Biggelaar et al., 2004a). These observations make the *LXRA* a candidate to affect human life span.

To test the hypothesis that the *LXRA* is involved in modulating human lifespan, we made use of genetic variation in the gene coding for the LXRA (NR1H3). Out of the data that recently came available from the HapMap Project, we selected four evenly spaced haplotype-tagging single nucleotide polymorphisms (SNPs) spanning the NR1H3 gene. In the Leiden 85-plus Study, a prospective population-based follow-up study of 563 elderly persons aged 85 years or older and onwards, we studied the association of the common haplotypes with survival during a mean follow-up period of almost 5 years. To further explore a potential role of the LXRA in biological mechanisms associated with modulation of human lifespan, we associated the genetic variation to mortality-related phenotypic markers.

Participants and methods

Participants

The Leiden 85-Plus Study is a prospective population-based study, in which inhabitants of Leiden, The Netherlands, aged 85 years, were invited to take part. There were no selection criteria related to health or demographic characteristics. The study population consists of 599 individuals (all members of the 1912–1914 birth cohort) who were enrolled in the month of their 85th birthday between 1997 and 1999 (der Wiel et al., 2002). DNA was available for 563 people. The Medical Ethical Committee of the Leiden University Medical Center approved the study, and written informed consent was obtained from all participants.

Causes of death

All participants in the Leiden 85-plus Study were followed for mortality until August 1, 2005. Primary causes of death were obtained from death certificates registered at the Dutch Central Bureau of Statistics and categorized according to the 10th International Classification of Diseases (ICD). Specific causes of death were categorized into cardiovascular disease (ICD codes I00–I99), infectious disease (ICD codes A00–B99 or J11–J18), cancer (ICD codes C00–D48), or other causes (all other ICD codes).

Plasma measurements

At baseline, participants were visited twice at their place of residence within 1 month after their 85th birthday. All blood samples were collected early in the morning, but fasting was not required.

Plasma levels of total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, and C-reactive protein (CRP) were analyzed on fully automated computerized analyzers (Hitachi 747 and 911; Hitachi, Ltd, Tokyo, Japan). The level of low-density lipoprotein (LDL) cholesterol was estimated by the Friedewald equation (LDL cholesterol [mmol/L] ¼ total cholesterol – HDL cholesterol – [triglycerides/2.2]), whereby participants with a triglyceride concentration higher than 443 mg/dL (5 mmol/L) were excluded (n=5).

Apolipoprotein E (ApoE) levels were determined in 2005 in one batch of plasma samples that were collected at age 85 years at study baseline and stored frozen. Plasma ApoE levels were determined using a human ApoE-specific sandwich enzyme-linked immunosorbent assay (ELISA) essentially as described (van Vlijmen et al., 1994). The detailed procedure is described in (Mooijaart et al., 2006).

Cytokine production capacity of the innate immune system

The cytokine production capacity of the innate immune system was assessed by stimulating *ex vivo* whole-blood samples with lipopolysaccharide (LPS) as described elsewhere (van der Linden et al., 1998). In short, all venous blood samples were drawn in the morning before 11 AM to exclude circadian variation, diluted 2-fold with RPMI-1640, and stimulated with *Escherichia coli*–derived LPS (10 ng/mL; Difco Laboratories, Detroit, MI). After 4 hours and after 24 hours of incubation at 378 C^0 and 5 % CO_2, supernatants were collected and stored at -808 C^0 to measure tumor necrosis factor-alpha (TNF-a), interleukin-1 beta (IL-1b), IL-6, IL-10, IL-12, IL-1 receptor antagonist (IL-1Ra), and interferon-gamma (IFN-c), respectively. Standard ELISA techniques were performed according to the manufacturer's guidelines (Central Laboratory of the Blood Transfusion Service, Amsterdam, The Netherlands). Because of a possible distortion by frailty (van den Biggelaar et al., 2004b), we restricted these analyses to those participants who survived for at least 2 years (n=463).

SNP selection

Four SNPs in the NR1H3 gene were selected using the HapMap database (*http://www.hapmap.org*; version June 2005). Only validated SNPs were selected, and calculations on linkage disequilibrium (LD) and frequencies were performed using data from the European Centre d'Etude du Polymorphisme Humain (CEPH) population. As boundaries, the first SNP upstream of the ATG start site (LXRA5 untranslated region [UTR], rs11039149) and the first SNP downstream of the stop codon (LXRA3UTR, rs1449627) were selected. The expected D' between these two SNPs was 1, indicating that the entire region is in strong LD. We additionally selected one SNP in exon 3 (LXRAex3, rs227923) and one in intron 6 (LXRAint6, rs712011), resulting in a set of four evenly spaced SNPs, separated by 5 kb.

Genotyping

The polymorphisms were genotyped using either an Assay-by-Design or an Assay-on-Demand (Applied Biosystems, Nieuwerkerk aan den IJssel, The Netherlands), consisting of PCR primers and TaqMan Major Groove Binding (MGB) probes. For LXRA5UTR an Assay-by-Design was used with forward primer GAGCATCTGCAGGGTTCTCA, reverse primer GCCAGTGAAGTGCTGTAATGGAA, one probe CCCCTGTAGCCCACC labeled with VIC, and one probe CCCTGTGGCCCACC labeled with FAM. For the LXRAex3 SNP an Assay-on-Demand was used with identification number C_15967384_10. For LXRAint6 an Assay-on-Demand was used with identification number C_1301060_20. For LXRA3UTR, an Assay-by-Design was used with forward primer CCTCACGTGCATGTGTAGCAT, reverse primer AGGTCTTTCAGGTTGTGCCTTTT, one probe CCTTGGTTTTTCC labeled with VIC, and one probe CCTTGGGTTTTCC labeled with FAM. Amplification reactions were performed at standard conditions except for the following modifications. A qPCR core kit was used (Eurogentec, Maastricht, The Netherlands) with half of the amount of primers and probes. Real-time PCR was performed on an ABI 7900 HT (Applied Biosystems), and genotypes were called using the Sequence Detection System 2.1 (Applied Biosystems). A random 10 % of all genotypes were performed in duplicate, and genotyping errors were < 2 % for all assays.

Statistical analysis

The program Haploview (Barrett et al., 2005) was used to estimate allele frequencies, test the consistency of genotype frequencies at each SNP locus with Hardy–Weinberg equilibrium, and estimate and plot pairwise LD between the SNPs examined. LD was estimated for all two-way comparisons of individual SNPs using two common measures: the r2 (the square of the standardized correlation coefficient) and the Lewontin D' (D'=D/Dmax if D > 0 or D'=D/Dmin if D < 0). Haplotypes and haplotype frequencies were estimated using the SNPHAP software (*http://www-gene.cimr.cam.ac.uk/clayton/software*). The posterior probabilities of pairs of haplotypes per subject as estimated by SNPHAP, were used as weights in the following analyses.

Continuous variables were normally distributed, except for plasma ApoE levels, triglycerides, CRP levels, and cytokines, which therefore were ln-transformed. All analyses were sex adjusted, using homozygosity for the most common haplotype as the referent group. Associations between haplotypes and metabolic profile were analyzed using linear regression. Mortality risks and 95 % confidence intervals (CI) were calculated with the Cox proportional hazard model. These analyses included all the estimated haplotypes in the model weighted for probability, except the reference haplotype. Clustered robust standard errors were calculated using individuals as clustering variable. These models assume an additive effect of the haplotypes. Haplotypes with low frequencies (< 5 %) fully participated in these analyses, but results on these haplotypes are not reported as their accuracy is low due to small numbers. The analyses were performed using STATA statistical software, version 9.0 (STATA Corp., College Station, TX).

Results

The baseline characteristics of the study populations are listed in Table 1. All participants were aged 85 years, and 67 % were female.

The position of the selected SNPs relative to the gene structure is shown in Figure 1a. The SNPs were in strong LD (D' > 0.97) and constituted one haplotype block (Figure 1b) with seven haplotypes, of which the predicted frequencies are listed in Table 2. For the present analyses

Table 1. Baseline characteristics of the study population

Characteristic	Value
Total number	563
Female (n, %)	375 (67 %)
Age (mean, SD)*	85.0 (-)
Lipid and lipoprotein plasma level	
Total cholesterol, mean mmol/L (SD)	5.71 (1.13)
LDL cholesterol, mean mmol/L (SD)	3.68 (0.97)
HDL cholesterol, mean mmol/L (SD)	1.31 (0.40)
Triglycerides, median mmol/L (IQR)	1.34 (1.01–1.95)
CRP, median mg/L (IQR)	4.00 (1.00–8.00)
LPS-stimulated cytokines	
IL-1b, median ng/mL (IQR)	3.50 (2.10–6.50)
IL-1RA, median ng/mL (IQR)	30.8 (28.3–46.0)
IL-6, median ng/mL (IQR)	60.7 (43.2–82.9)
IL-10, median pg/mL (IQR)	762 (487–1089)
IL-12, median ng/mL (IQR)	6.70 (4.30–10.20)
IFN-c, median ng/mL (IQR)	139 (43.0–448)
TNF-a, median ng/mL (IQR)	10.3 (7.40–13.3)

*All participants were enrolled within the month of their 85th birthday; SD=standard deviation; LDL=low-density lipoprotein; HDL=high-density lipoprotein; IQR=interquartile range; CRP=C-reactive protein; LPS=lipopolysaccharide; IL=interleukin; IFN=interferon; TNF=tumor necrosis factor; RA=receptor antagonist

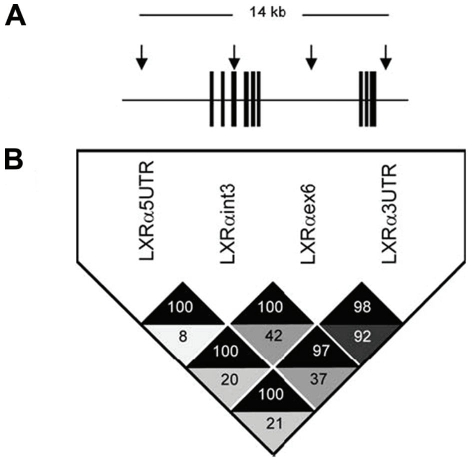

Figure 1. Haplotype structure of the NR1H3 gene in the Leiden 85-plus Study population. (A) Relative position of the selected single nucleotide polymorphisms (SNPs) in the NR1H3 gene. Vertical bars: exons 1–9. Arrows: positions of SNPs. (B) Visual representation of linkage disequilibrium within the gene. Based on standard confidence interval criteria, all four SNPs constitute one haplotype block. Top triangles (pointing upwards, all black) indicate strong linkage disequilibrium of all four SNPs. Pairwise linkage disequilibrium is indicated by the numbers in the top triangles. Pairwise R^2 values are indicated by the numbers in the bottom triangles (pointing downwards), in which light gray triangles indicate low R^2, and dark gray triangles indicate high R^2.

Table 2. Haplotype structures and frequencies

	SNP allele				
Haplotype	LXRa5UTR	LXRaex3	LXRaint6	LXRa3UTR	Frequency
1	A	C	T	T	0.367
2	G	C	T	T	0.273
3	A	T	C	G	0.176
4	A	C	C	G	0.164
5	A	C	T	G	0.016
6	A	T	C	T	0.004
7	A	C	C	T	0.003

Minor alleles are depicted in bold. Minor allele frequencies of the four polymorphisms were: LXRa5UTR, 0.27 (G); LXRaex3, 0.18 (T); LXRaint6, 0.35 (C); LXRa3UTR, 0.36 (G). All genotype distributions were in Hardy–Weinberg equilibrium, SNP=single nucleotide polymorphism; LXR=liver X receptor; UTR=untranslated region; ex = exon; int=intron

we report the results of the four most common haplotypes (frequency > 5 %) that cumulatively account for > 97% of the haplotypes.

During a mean follow-up time of 4.9 years, 320 participants (57 %) had died. We compared the mortality risk associated with the various haplotypes, using the most common haplotype 1 as the reference category (Figure 2). The mortality risk was lower for haplotype 2 compared to haplotype 1 (hazard ratio [HR] = 0.78; 95 % CI, 0.64–0.95; *p=0.015*), whereas other haplotypes were not significantly associated with a higher or lower mortality risk. When assessing specific causes of death, the lower mortality risk that was associated with haplotype 2 was mainly caused by a lower mortality risk from infectious disease (HR=0.31, 95 % CI, 0.12–0.85; *p=0.023*) and from mortality in the category "other causes" (HR= 0.71, 95 % CI, 0.50–1.00; *p=0.052*).

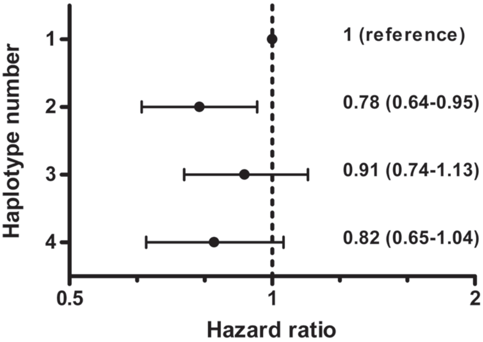

Figure 2. Dots represent hazard ratios calculated using a Cox proportional model adjusting for gender; bars represent 95 % confidence intervals. Mean follow-up time was 4.9 years, in which time a total number of 320 participants (57 %) had died.

The relationship between the four common haplotypes and variables in lipid metabolism is shown in Table 3. Haplotype 2 associated with significantly higher plasma ApoE levels (+0.48 mg/dL, *p=0.018*) and triglyceride levels (+0.098 mmol/dL, *p=0.041*) compared to haplotype 1. Haplotype 4 also associated with higher plasma ApoE levels compared to haplotype 1, although the association was borderline statistically significant (+0.45 mg/dL, *p=0.057*), possibly due to the lower haplotype frequency.

To explore the association of NR1H3 haplotypes with innate immune function, we assessed cytokine production capacity by *ex vivo* whole-blood LPS-stimulated cytokine levels (Table 4). We found no association of any haplotype with cytokine production capacity. Finally, to investigate the possibility that the LXRA regulates inflammation through alternative mechanisms, we associated the haplotypes with circulating CRP level, a plasma marker of systemic inflammation. We found no association for any haplotype with circulating levels of CRP (Table 4).

Discussion

In *C. elegans*, the NHR DAF-12 has been shown to be one of the key components that modulate lifespan in response to environmental cues. Based on protein sequence comparisons, we recently identified the LXRA as one of the human NHRs most similar to *C. elegans* DAF-12 (Mooijaart et al., 2005). Here we report that genetic variation in the gene coding for the LXRA (NR1H3) associates with human life span.

We found that a common haplotype of the NR1H3 gene associated with lifespan extension, predominantly attributable to decreased death from infectious disease. The LXRA is involved in various processes that contribute to infectious disease. The LXRA regulates specific processes that increase resistance to pathogens. For instance, LXRA regulates the expression of APa, a scavenging receptor that inhibits macrophage apoptosis and promotes the killing of the bacteria (Joseph et al., 2004). Although LXR agonists reduce inflammatory gene expression in models

Table 3. Association of NR1H3 haplotypes with parameters of lipid metabolism

	Haplotype 1	Haplotype 2		Haplotype 3		Haplotype 4	
Plasma Component	Mean (95 % CI)	Difference (95 % CI)[1]	p-value[1]	Difference (95 % CI)[1]	p-value[1]	Difference (95 % CI)[1]	p-value[1]
Apolipoprotein							
ApoE (mg/dL)[2]	4.95 (4.52–5.42)	0.48 (0.08-0.91)	0.018	0.30 (-0.13-0.79)	0.188	0.45 (-0.01-0.96)	0.057
Lipids							
Total cholesterol, mmol/L	5.93 (5.74–6.13)	0.06 (-0.10-0.21)	0.473	-0.021 (-0.18-0.14)	0.802	-0.10 (-0.29-0.09)	0.294
LDL cholesterol, mmol/L	3.84 (3.67–4.02)	0.02 (-0.12- 0.15)	0.806	-0.072 (-0.21-0.07)	0.311	-0.10 (-0.27-0.06)	0.226
HDL cholesterol, mmol/L	1.39 (1.32–1.46)	-0.01 (-0.07-0.20)	0.813	-0.004 (-0.07-0.06)	0.890	-0.01 (-0.08-0.06)	0.813
Triglycerides, mmol/L[2]	1.38 (1.28–1.49)	0.10 (0.00-0.20)	0.041	0.07 (-0.04-0.19)	0.195	0.02 (-0.08-0.14)	0.662

[1] Compared to Haplotype 1; [2] Geometric mean; Freq= frequency; ApoE=apolipoprotein E; LDL=low-density lipoprotein; HDL=high-density lipoprotein. Data represent sex-adjusted means and 95% confidence intervals (CI). All participants were aged 85 years

Table 4. Association of NR1H3 haplotypes with whole-blood lipopolysaccharide (LPS)-stimulated cytokine levels at baseline

	Haplotype 1	Haplotype 2		Haplotype 3		Haplotype 4	
	Mean (95 % CI)	Difference (95 % CI)[1]	p-value[1]	Difference (95 % CI)[1]	p-value[1]	Difference (95 % CI)[1]	p-value[1]
Innate immunity[2]							
IL-1b, ng/mL	3.30 (2.80-3.80)	0.10 (-0.30-0.60)	0.680	-0.20 (-0.60-0.30)	0.440	0.40 (-0.10-1.00)	0.141
IL1-RA, ng/mL	35.4 (32.4-38.6)	-1.20 (-3.70-1.40)	0.340	0.10 (-2.80-3.20)	0.972	-0.20 (-3.30-3.30)	0.859
IL-6, ng/mL	57.4 (51.8-63.4)	-0.90 (-5.20-3.70)	0.682	-1.40 (-6.20-3.90)	0.592	0.60 (-4.30-6.10)	0.794
IL-10, pg/mL	709 (623-807)	5.00 (-64.0-82.0)	0.888	-6.00 (-85.0-83.0)	0.892	-19.0 (-101.0-75.0)	0.680
IL-12, ng/mL	6.00 (5.20-6.90)	0.20 (-0.40-1.00)	0.494	-0.60 (-1.30-0.01)	0.054	0.50 (-0.30-1.30)	0.249
IFN-c, ng/mL	151 (108-209)	10.0 (-27.0-59.0)	0.617	-35.0 (-66.0-8.0)	0.100	15.0 (-32.0-82.0)	0.575
TNF-a, ng/mL	9.80 (9.00-10.7)	0.40 (-1.10-0.30)	0.240	-0.80 (-1.60-0.10)	0.035	0.30 (-0.50-1.30)	0.432
Chronic inflammation[2]							
CRP, mg/L	2.00 (1.50-2.70)	0.20 (-0.30-0.80)	0.440	0.40 (-0.20-1.10)	0.224	0.20 (-0.40-0.90)	0.599

[1] Compared to Haplotype 1; [2] Geometrical means; Freq=frequency; IL=interleukin; RA=receptor antagonist; IFN=interferon; TNF=tumor necrosis factor; CRP=C-reactive protein. Data represent sex-adjusted means and 95% confidence intervals (CI). All participants were aged 85 years

of dermatitis and atherosclerosis (Joseph et al., 2003), LXRA$^{-}/^{-}$ knockout mice are more susceptible to infection with *Listeria monocytogenes* (Joseph et al., 2004).

In the search for an intermediate phenotype, we associated genetic variation in the NR1H3 gene with mortality-related markers in lipid metabolism and immunity. We selected these markers because these phenotypes are known to associate with mortality and a functional relationship with the LXRA protein was plausible. Cholesterol metabolism is related to various causes of death (Weverling-Rijnsburger et al., 1997), and the LXRA is involved in various components of lipid metabolism, such as reverse cholesterol transport, cholesterol excretion, and fatty acid synthesis (Lu et al., 2001). We observed an association of haplotype 2 of the NR1H3 gene with increased plasma ApoE and triglyceride levels. ApoE is a component of triglyceride-rich lipoproteins, such as very low- density lipoprotein (VLDL), which may explain why haplotype 2 associates with plasma levels of both ApoE and triglycerides. Furthermore, LXRA agonists have been suggested for therapeutic use against cardiovascular disease, but a serious side effect of the use of LXR agonists as therapeutic agents is the concomitant increase in liver and serum triglycerides (Grefhorst et al., 2002). These effects are caused by a strong induction of lipogenic genes in the liver and an increased VLDL production (Grefhorst et al., 2002). In line with these animal data, we found that haplotype 2 also associated with higher ApoE levels. Thus, the associations with increased levels of triglycerides and ApoE presented here suggest that haplotype 2 associates with increased LXRA activity in the liver. It is interesting that triglyceride-rich lipoproteins (of which ApoE is a component) act as agents of the innate immune system (Barcia and Harris, 2005), for example, by binding and neutralizing bacterial components. ApoE redirects lipopolysaccharides (bacterial components) in the liver from Kupffer cells to hepatocytes and protects against endotoxemia in rats (Rensen et al., 1997). Recently, it was discovered that ApoE is also involved in lipid antigen presentation (van den et al., 2005) and that high plasma ApoE levels associate with increased systemic inflammation (Mooijaart et al., 2006).

LPS-stimulated cytokine production levels are highly heritable (de Craen et al., 2005), and cytokine production profiles associate with patterns of old age mortality (van den Biggelaar et al., 2004a). However, genetic variation in the genes coding for the cytokines has so far been insufficient to explain the heritable component (Haukim et al., 2002). In the present study, variation in the NR1H3 gene did not associate with *ex vivo* LPS-stimulated whole-blood cytokine levels or with circulating CRP levels. Others have demonstrated an association of the LXRA with inflammation in macrophage and monocyte cell cultures (Joseph et al., 2003; Landis et al., 2002). However, inflammatory cytokines and other serum mediators were not different between LXRA and LXRB knockout mice and wild types (Joseph et al., 2004). We interpret that NR1H3 may not be a major determinant of cytokine production capacity in blood upon stimulation by LPS. This interpretation does not, however, exclude the possibility that in other cell types NR1H3 haplotypes may influence the local production of cytokines.

We did not observe a beneficial effect of haplotype 2 on death from cardiovascular causes. In mouse models, LXR agonists reduce the formation of atherosclerotic lesions (Joseph et al., 2002) whereas macrophage-specific LXRA knockout aggravates atherosclerosis development (Tangirala et al., 2002). To date, the function of the LXRA in lipid and cholesterol metabolism has been studied in mouse models and in human cultured cell lines, mostly macrophages. However, caution must be taken in extrapolating these results based on cultured cells and mouse models of atherosclerosis to humans (Repa and Mangelsdorf, 2002). Whereas macrophage LXR has been shown to be antiatherogenic (Levin et al., 2005; Tangirala et al., 2002), this beneficial effect on cardiovascular disease may be balanced by proatherogenic effect of liver LXR activation.

Genetic variation in both *C. elegans* daf-12 and the human NR1H3 gene associates with differences in life span, suggesting that the two genes may, at least to some extent, be functionally conserved. Other evolutionarily conserved pathways have previously been implicated in life-span regulation. For example, it was first discovered that the *C. elegans* daf-2 mutant was long-lived (Kenyon et al., 1993). Later it was discovered that the daf-2 gene shows homology to the mammalian genes encoding the insulin receptor (IR) and insulin-like growth factor 1 receptor (IGF-1R) (Kimura et al., 1997), which are conserved throughout evolution. Extended life span was then also demonstrated in IR mutants in *Drosophila melanogaster* (Tatar et al., 2001) and in IR and IGF-1R mutants in mice (Bluher et al., 2003; Holzenberger et al., 2003). Recently we showed that reduced insulin signaling in humans also associates with longevity (van Heemst et al., 2005). These observations suggest that the approach of studying evolutionarily conserved pathways is fruitful in identifying genes that regulate human life span.

Very recently, several articles report on the biological function of daf-12 in *C. elegans*. Two hormones were identified that function as DAF-12 ligands (Motola et al., 2006), and the biosynthetic pathway of production of these ligands was described in more detail (Rottiers et al., 2006). Furthermore, cholestenoic acid was found to rescue the worm from dauer diapause in a DAF-12–dependent manner (Held et al., 2006). These studies provide important new hints to investigate the functional conservation of life-span regulation throughout evolution and the biological function of the human LXRs or other NHRs.

A limitation of our study is that it does not include analyses of the gene encoding the LXRB (NR1H2). The LXRA and LXRB are highly similar proteins, as their amino acid sequences are very alike and the proteins have similar functions in lipid metabolism and inflammation. It could therefore be hypothesized that a loss of function of one of the genes will be compensated for by the function of the other receptor and will therefore not have dramatic effects. The LXRA and LXRB are encoded by different genes and have different expression patterns. Whereas the LXRA is expressed only in a limited number of tissues, the LXRB is expressed ubiquitously. Indeed, in activated macrophages the inhibiting effect of LXR ligands on cytokine expression is completely abrogated in double knockout macrophages (nr1h3^{-}/$^{-}$ nr1h2^{-}/$^{-}$). However, there was also a partial reduction of this effect in nr1h3^{-}/$^{-}$ macrophages (Joseph et al., 2003). Furthermore, whereas LXRA^{-}/$^{-}$ knockout increases susceptibility to bacterial infection, additional knockout of LXRB does not increase this susceptibility (Jonsson et al., 2003). These observations suggest that genetic variation in LXRA may have functional significance independent of LXRB. A second limitation is that it is unclear which genetic variant within the haplotype is responsible for the observed functional variation. The SNP that tags haplotype 2 is located in the 5` UTR of the gene. It is, however, unknown whether this SNP itself has functional significance—for instance, by affecting promoter function or interaction domains. Alternatively, the 5` UTR SNP may be in LD with an SNP elsewhere in the genome, for instance in the coding region of the gene, which leads to an alteration with functional significance.

The strong point of our study is that we selected genetic variants tagging all common haplotypes of the NR1H3 gene and associated them with a range of variables in inflammation and lipid metabolism. Furthermore, the prospective nature of the mortality analyses and the relatively large number of deaths yield a powerful tool to study mortality risk.

As this is the first report on the effect of common genetic variants in the human NR1H3 gene on human life span, the results of our observational study need further replication. Furthermore, more research is warranted to confirm which specific genetic variation on the haplotypes actually changes the function of the gene or protein.

Acknowledgments

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