Ageing Research Reviews 41 (2018) 82–97

Contents lists available at ScienceDirect

Ageing Research Reviews

journal homepage: www.elsevier.com/locate/arr

Review

Werner syndrome (*WRN*) gene variants and their association with altered function and age-associated diseases

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ARTICLE INFO

Keywords:
Werner syndrome
Single nucleotide polymorphisms
Age-related phenotypes
Longevity
Cancer

ABSTRACT

Werner syndrome (WS) is a heritable autosomal recessive human disorder characterized by the premature onset of several age-associated pathologies including cancer. The protein defective in WS patients, WRN, is encoded by a member of the human *RECQ* gene family that contains both a DNA exonuclease and a helicase domain. WRN has been shown to participate in several DNA metabolic pathways including DNA replication, recombination and repair, as well as telomere maintenance and transcription modulation. Here we review base pair-level genetic variation that has been documented in *WRN*, with an emphasis on non-synonymous coding single nucleotide polymorphisms (SNPs) and their associations with anthropomorphic features, longevity and disease risk. These associations have been challenging to identify, as many reported *WRN* SNP associations appear to be further conditioned upon ethnic, age, gender or other environmental co-variables. The *WRN* variant phenotypic associations identified to date are intriguing, and several are of clear clinical import. Consequently, it will be important to extend these initial associations and to identify the mechanisms and conditions under which specific *WRN* variants may compromise WRN function to drive cellular and organismal phenotypes as well as disease risk.

1. Introduction

Werner Syndrome (WS; MIM# 277700) is an autosomal recessive disorder characterized by genomic instability and the premature onset of a number of age-related diseases including ocular cataracts, dyslipidemia, diabetes mellitus, osteoporosis, atherosclerosis, and cancer (Epstein et al., 1966; Lauper et al., 2013; Oshima et al., 2017). Additional clinical features include short stature, a characteristic “birdlike” facies, premature hair graying with alopecia, scleroderma-like changes (pigment alterations, atrophic skin, refractory skin ulcers), soft-tissue calcification, and musculoskeletal manifestation (David et al., 2016; Epstein et al., 1966; Oshima et al., 2017; Takemoto et al., 2013). Premature atherosclerosis and malignant tumors are the most common cause of death (Goto, 1997; Goto et al., 2013; Oshima et al., 2017). WS patients may develop a diversity of neoplasia. However, there is an excess of soft tissue sarcomas, osteosarcomas, myeloid disorders, meningiomas, malignant melanomas, and thyroid carcinomas associated with WS (Goto et al., 2013; Goto et al., 1996; Lauper et al., 2013).

The gene responsible for WS (*WRN*) was identified by positional cloning (Yu et al., 1996) and its product contains an evolutionary conserved RECQ DNA helicase consensus domain (Gray et al., 1997; Yu et al., 1996). The protein also has a 3′to-5′ exonuclease activity unlike the other RECQ type DNA helicases (Croteau et al., 2014). Accumulating evidence indicates that WRN is involved in DNA replication, recombination and repair, telomere maintenance and transcription (Oshima et al., 2017; Rossi et al., 2010; Tang et al., 2016). Many of these functional associations are further supported by WRN protein interactions with proteins important for homologous recombination, non-homologous recombination, and long-patch base excision repair pathways (Croteau et al., 2014; Rossi et al., 2010; Shamanna et al., 2017). In addition to defects in DNA replication and repair, WS cells display alterations of gene expression that implicate WRN in transcription regulation as well (Rossi et al., 2010; Tang et al., 2016). More than ninety distinct mutations potentially inactivating the WRN protein have been described in WS patients to date based on The International Registry of Werner Syndrome (Department of Pathology, University of Washington, Seattle, WA, USA (www.wernersyndrome.org) and additional case reports (Fu et al., 2017; Yokote et al., 2017). These mutations include base substitutions, insertions, deletions and more complex mutations that lead to a disrupted *WRN* open reading frame. Many of these mutations lead to loss of function by destabilizing the protein, or a failure to localize to the nucleus where WRN resides and acts (Yokote

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https://doi.org/10.1016/j.arr.2017.11.003
Received 5 October 2017; Received in revised form 7 November 2017; Accepted 9 November 2017
Available online 14 November 2017
1568-1637/

et al., 2017). Many less clearly pathogenic variants of *WRN* exist in the human population, involve the *WRN* coding region, and thus may affect WRN function and disease risk. This review focuses on the subset of *WRN* variants that lead to non-synonymous amino acid substitutions within the WRN protein. These non-synonymous *WRN* coding region single nucleotide polymorphisms (SNPs) have been associated with physiological traits or age-related pathologies in different ethnic populations. We discuss genetic variation in the *WRN* locus first, then focus on these non-synonymous coding SNP variants and their phenotypic associations. Finally, we speculate on the mechanisms by which genetic variants in *WRN* may affect WRN protein structure, function and interactions with cellular protein partners.

2. Genetic variation in the *WRN* gene

The human *WRN* gene is located on the proximal short arm of chromosome 8 and encompasses nucleotides 31,033,801 to 31,173,769 in the GRCh38/hg38 human reference genome assembly (http://genome.ucsc.edu/). *WRN* was recognized early as a human gene with considerable genetic variation (Passarino et al., 2001). Since this initial report there has been an explosion of information on human genetic variation coming from genome and genome diversity projects (e.g., 1000 Genomes Projects (1KGP), Exome Sequencing project (ESP), and the Exome Aggregation Consortium (ExAC) Projects (Auton et al., 2015; Fu et al., 2013; Lek et al., 2016) together with a rapidly expanding set of data derived from somatic genetic variants identified in human tumor specimens (e.g., COSMIC (Forbes et al., 2010)). In a recent analysis, Fu et al. (2017) assembled a new database of clinically-ascertained mutations (hereafter referred to as RECQMutdb), together with human genetic variation data for *WRN* obtained from the ESP and 1KGP data archives, supplemented with non-TCGA data from ExAC (Fu et al., 2017). This analysis identified 91 clinically ascertained pathogenic variants in *WRN*, and an additional 759 variants in the population data sources outlined above. Among the known pathogenic variants, 25 (or 27%) were also present in these sources (1000 Genomes, Exome Sequencing Project and ExAC). An additional analysis to define the frequency of pathogenic alleles in population data identified *WRN* as the *RECQ* helicase with the highest frequency of known deleterious alleles in the population samples, 2.12% among 9019 individuals included in ESP/1KGP data. In contrast, the frequencies of known deleterious variants for the other two *RECQ* helicase deficiency syndrome genes, *BLM* or *RECQL4* where loss of function leads, respectively, to Bloom and Rothmund-Thomson syndromes, were an order of magnitude lower (0.06% and 0.11% respectively). This high frequency of *WRN* variants appears to reflect the contribution of population-specific high frequency alleles for *WRN* alone, in contrast to *BLM* or *RECQL4* (Fu et al., 2017). In contrast to these known pathogenic variants, many *WRN* variants segregating in the human population are of uncertain functional significance or ability to promote the development of age-related diseases in the general population.

3. Linking WRN genetic variation to phenotype

In order to link WRN genetic variation to specific phenotypes, we used PubMed searches performed with the key words 'WRN'; 'polymorphisms'; and 'genome-wide association study (GWAS)' to identify analyses that were *WRN* gene-focused or that included *WRN* variants as part of genome-wide association studies (GWAS). GWAS that include SNPs in the *WRN* gene were also identified via the GRASP web site (Genome-wide Repository of Associations between SNPs and Phenotypes). The full GRASP 2.0.0.0 catalog covers 2082 GWASs (https://grasp.nhlbi.nih.gov/Overview.aspx) (Leslie et al., 2014) as of September 2017. Based on these PubMed and GRASP searches; five non-synonymous SNPs with minor allele frequencies ≥ 0.01 were associated with a physiological or disease phenotype conditioned on the ethnicity of the populations under study. Fig. 1 shows the position of these five SNPs in the WRN protein. For clarity in the following discussion we have indicated specific WRN SNP variants by the codon/residue number in the WRN protein; followed by the single letter code for the specific amino acid residue encoded by each SNP variant in the WRN open reading frame; e.g.; 1367R. The frequencies of the five non-synonymous SNPs in population data are indicated in Table 1. We review the different SNP analyses showing specific phenotype associations in detail below.

4. WRN variants linked to anthropomorphic and developmental phenotypes

4.1. WRN polymorphisms and short stature

WS patients fail to undergo an adolescent growth spurt (Epstein et al., 1966; Oshima et al., 2017). A meta-analysis of GWAS data from 46 studies, comprising 133,653 individuals of recent European ancestry, was performed to identify common genetic variation associated with adult height (Lango Allen et al., 2010). This analysis identified a modest association for WRN SNP 387I with height ($P = 0.022$), though no other association of WRN non-synonymous coding SNPs with height.

4.2. Head circumference in infant

Head circumference in infancy is often used as a surrogate measurement to estimate brain size and development. Larger head circumference in infancy is associated with higher IQ scores in childhood, though the underlying mechanisms are poorly understood (Taal et al., 2012). Taal et al. (2012) performed a meta-analysis on seven GWAS (N = 10,768 individuals of European ancestry enrolled in pregnancy and/or birth cohorts). They found that the WRN SNP 114I was positively associated with head circumference ($P = 0.0037$), though this association was not replicated in a follow up study that included six replication cohorts (N = 19,089 European individuals).

4.3. Educational attainment

Twin and family studies suggest that a broad range of psychological traits, economic preferences, and social and economic outcomes are moderately heritable. For example, educational attainment is strongly associated with social outcomes, and there is a well-documented health-education gradient (Benjamin et al., 2012). Nonetheless, estimates suggest that around 40% of the variance in educational attainment is explained by genetic factors (Rietveld et al., 2013). Furthermore, educational attainment is moderately correlated with other heritable characteristics, including cognitive function and personality traits related to persistence and self-discipline (Krapohl et al., 2014). Using the International Standard Classification of Education (ISCED 1997) scale, Rietveld et al. (2013) conducted a GWAS of educational attainment in a discovery sample of 101,069 individuals and a replication sample of 25,490 subjects (Rietveld et al., 2013). They found that the WRN SNP 1367R was positively associated with education attainment (years of education) with a $P = 0.032$ in their discovery sample. However, this association was not confirmed in their replication cohort (Rietveld et al., 2013).

4.4. Cardiovascular malformation syndromes

Congenital heart disease affects ~1% of live births and is a major source of morbidity and mortality in childhood. Approximately 20% of congenital heart disease occurs in the setting of chromosomal conditions or multisystem malformation syndromes. Family studies in the remaining 80% of sporadic cases indicate a significant complex genetic component to the disease (Oyen et al., 2009). Tetralogy of Fallot (ToF) is the commonest form of cyanotic congenital heart disease, affecting ~3 per 10,000 newborns (Botto et al., 2001). Although ToF is usually

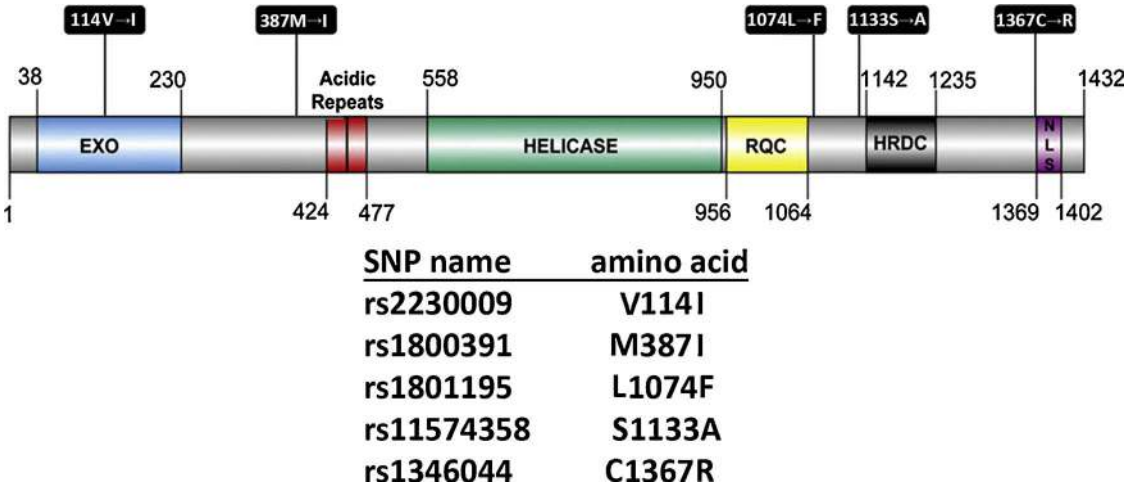

Fig. 1. Schematic representation of the human WRN protein with the different polymorphic residues reviewed in this report. A = alanine; C = cysteine; F = phenylalanine; I = isoleucine; L = leucine; M = methionine; R = arginine; S = serine; V = valine. Domain boundaries were drawn according to Uniprot (UniProtKB − Q14191) and (Kitano, 2014).

Table 1
Single nucleotide polymorphism (SNP) nomenclature used in this review.

SNP name	Codon Change	Amino acid change	Amino acid position	Major allele frequency*	Minor allele frequency*
rs2230009	GTT > ATT	V > I	114	V: 0.934	I: 0.006
Nomenclature used in the text					
WRN V114I = SNP at position 114 of the WRN protein			114I = I allele	V/I = heterozygote; I/I = homozygote genotype	
SNP name	Codon Change	Amino acid change	Amino acid position	Major allele frequency*	Minor allele frequency*
rs1800391	ATG > ATA	M > I	387	M: 0.929	I: 0.071
Nomenclature used in the text					
WRN M387I = SNP at position 387 of the WRN protein			387I = I allele	M/I = heterozygote; I/I = homozygote genotype	
SNP name	Codon Change	Amino acid change	Amino acid position	Major allele frequency*	Minor allele frequency*
s1801195	TTG > TTT	L > F	1074	L: 0.558	F: 0.442
Nomenclature used in the text					
WRN L1074F = SNP at position 1074 of the WRN protein			1074L = L allele	L/F = heterozygote; F/F = homozygote genotype	
SNP name	Codon Change	Amino acid change	Amino acid position	Major allele frequency*	Minor allele frequency*
rs11574358	TCA > GCA	S > A	1133	S: 0.992	A: 0.008
Nomenclature used in the text					
WRN S1133A = SNP at position 1133 of the WRN protein			1133A = A allele	S/A = heterozygote; A/A = homozygote genotype	
SNP name	Codon Change	Amino acid change	Amino acid position	Major allele frequency*	Minor allele frequency*
rs1346044	TGT > CGT	C > R	1367	C: 0.704	R: 0.296
Nomenclature used in the text					
WRN C1367R = SNP at position 1367 of the WRN protein			1367R = R allele	C/R = heterozygote; R/R = homozygote genotype	

* Allele frequencies based on HapMap-Ceu (https://www.ncbi.nlm.nih.gov/snp).

repaired in infancy with low mortality, there is substantial late morbidity, in particular from pulmonary valvular insufficiency and atrial or ventricular arrhythmias. An elevated risk of ToF was recently identified in carriers of the WRN SNP 1367R in a northern European discovery cohort ($P = 0.03$) (Cordell et al., 2013). However, this finding was not replicated in a second independent European cohort.

5. WRN polymorphisms and longevity

It is important to bear in mind that though living to very old age may run strongly in families (Perls et al., 2000; Schoenmaker et al., 2006), longevity itself is a complex phenotype that includes not only overall disease-free survival but also survival with various age-related diseases. Given this complexity, it is unlikely that a single gene or a few genes would alone confer this survival advantage (Sebastiani et al., 2012). Hence, it is perhaps not surprising that few studies have reported significant associations between different *WRN* polymorphisms and longevity.

The most common non-synonymous coding SNPs in *WRN,* with minor allele frequencies of > 0.05 include WRN SNP variants 1074F and 1367R. Castro et al. (1999) found an age-dependent decline of the F/F homozygous 1074F SNP variant in Finnish and Mexican populations. They found, however, no significant association between the WRN SNP 1367R and longevity in these populations (Castro et al., 1999). Other studies on Brazilian, Polish, and Leiden-base (Netherlands) populations did not show significant association between WRN SNPs 1367R or 1074F and longevity (Kuningas et al., 2006; Polosak et al., 2011; Smith et al., 2005). In one study, WRN SNPs were analyzed as part of a meta-analysis of nonagenarian cases from the Rotterdam Study, Leiden 85-plus study, and Danish 1905 cohort. This meta-analysis included 4149 nonagenarian cases and 7582 younger controls. It was initially found that homozygous subjects with the minor R allele at WRN SNP 1367 were associated with decreased longevity ($P = 0.034$). However, this association was lost after correcting for multiple testing (Deelen et al., 2011).

Using a GWAS approach, Kulminski and Culminskaya reported that subjects with one A allele at the WRN SNP 1133A was associated with an earlier onset of cardiovascular diseases and cancer (and thus decreased longevity) when compared with individuals homozygous for a serine residue (S/S) at this SNP in the Framingham Heart Study (Kulminski and Culminskaya, 2013). These results have not been replicated in prior or subsequent studies, in part due to a failure to

include the comparatively rare WRN SNP 1133A (minor allele frequency of 0.008 in HapMap-CEU and of 0 in the Asian and African populations; https://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=11574358) in many GWAS studies. Most GWAS usually involve the sequencing of SNPs with a minor allele frequency greater than 0.05. The WRN SNP 1133A, in contrast, was included in the Framingham Heart Study cohort despite a minor allele frequency of 0.04 in the (Kulminski and Culminskaya, 2013).

Altogether, these results indicate only one significant association between longevity and the WRN SNP 1133A. This association, however, will need to be replicated in other studies.

6. WRN polymorphism-disease associations

In contrast to longevity, specific *WRN* SNPs may make more readily identifiable contributions to disease risk by altering one or more disease-specific molecular mechanisms. We review current associations for non-neoplastic as well as neoplastic disease below.

6.1. Cardiovascular disease

Associations between *WRN* polymorphisms and cardiovascular diseases were first reported in the late 1990's. In an initial report, 149 cases of myocardial infarction were compared to 198 age-matched Japanese controls. This study indicated that homozygosity for the C allele of the WRN SNP 1367R predicted a 2.78-fold greater risk of myocardial infarction (Ye et al., 1997). In a second study involving 218 Japanese control subjects, 166 subjects with myocardial infarction, and 201 subjects with ischemic stroke, the frequency of the C allele of the same SNP was also significantly higher in patients with myocardial infarction than in control subjects ($P = 0.0047$). In a multiple logistic regression analysis, this association was independent of other risk factors (age, hypertension, diabetes mellitus, smoking, and lipid profile). Conversely, this polymorphism did not associate with ischemic strokes in this Japanese cohort (Morita et al., 1999). The authors of these studies suggested that the R allele at WRN residue 1367 may actively protect against myocardial infarction. However, subsequent analyses on cohorts of European ancestry were not able to confirm this. For example, the Finnish population is known to have a very high rate of coronary atherosclerosis. If the WRN 1367R SNP was protective, one would have suspected the R allele to be enriched among centenarians. However an analysis of this question revealed the same distribution of WRN 1367 R and C alleles in both centenarians and newborns, suggesting no protective effect in this Finnish population (Castro et al., 1999). Similarly, an analysis of 88 controls and 53 subjects with coronary artery disease from the Baltimore Longitudinal Study failed to identify an association between the WRN SNP 1367R and coronary artery disease in Caucasians (Bohr et al., 2004). Note that a major limitation of these studies is the small size of the Caucasian cohorts.

Subsequent larger and multiple case-control studies were performed to assess the most intriguing findings above. Luke et al. (2007) identified a significant association of the WRN SNP 387I with epicardial coronary stenosis in case-control studies of U.S. subjects from European ancestry (781 cases and 603 controls, $P = 0.0062$), though they could not find an association of the WRN SNP 1367R with coronary artery disease (Luke et al., 2007). However, the WRN 387I SNP-epicardial coronary stenosis association could not be confirmed in two other U.S. case-control studies involving 471 cases versus 298 controls and 554 cases versus 373 controls. A limitation of these analyses was that angiography alone may not have been sufficiently sensitive to identify circumferential disease and thus may have led to an under-estimation of the extent of coronary artery disease in control subjects (Luke et al., 2007).

Thanassoulis et al. (2013) published a GWAS involving 6942 participants presenting an aortic valve calcification and 3795 participants presenting mitral annular calcification as detected by computed tomography scanning. The discovery cohort in this study consisted of persons of white European ancestry from three independent cohorts participating in the Cohorts for Heart and Aging Research in Genomic Epidemiology consortium. Their findings were tested in an independent replication cohort of patients with either valvular calcification or clinical aortic stenosis detected by the same imaging methodology (Thanassoulis et al., 2013). Although the WRN SNP 1367R was specifically associated with mitral annular calcification in the discovery cohort ($P = 0.019$), this finding could not be confirmed in the replication cohort.

In an attempt to better power WRN SNP association studies, a large meta-analysis was recently reported that tested the hypothesis that *WRN* genetic variation was associated with risk of ischemic vascular disease in the general population (Christoffersen et al., 2017). This meta-analysis included 58,284 participants from two general population cohorts, the Copenhagen City Heart Study (CCHS) and the Copenhagen General Population Study (CGPS). Of these participants, 6312 developed ischemic vascular disease during follow-up. In the CCHS study (N = 10,250), all non-synonymous variants in *WRN* with reported minor allele frequencies ≥ 0.5% in Caucasians were genotyped. Variants that were associated with ischemic vascular disease in the CCHS cohort were then genotyped in the CGPS cohort (N = 48,034). The authors found that in a combined study of the CCHS and CGPS cohorts, the C/C homozygous genotype at WRN residue 1367 was associated with an increased risk of ischemic stroke ($P = 0.008$). The 1367C/C genotype, however, was trending though not significantly associated with ischemic heart disease, myocardial infarction, or all-cause mortality. The F/F homozygous genotype at position 1074 of the WRN protein was associated with an increased risk for ischemic stroke in the CCSH cohort ($P = 0.04$), but was not significant in the combined meta-analysis. Overall, this report concluded that WRN SNP 1367C conferred a 14% increased risk, whereas the 1367R allele conversely conferred a protective effect against ischemic stroke (Christoffersen et al., 2017). Of note, these associations did not extend to other types of ischemic cardiovascular disease: the 1376R allele did not confer a significant protection specifically against myocardial infarction in these European populations, in contrast to what was found in the Japanese cohorts (Morita et al., 1999; Ye et al., 1997).

6.2. Endocrine/metabolic phenotypes: dyslipidemia and diabetes

Body mass index (BMI) is a simple, widely used and non-invasive measure of obesity that predicts the risk for multiple disorders, including Type 2 diabetes mellitus (T2DM) and cardiovascular disease. A meta-analysis of GWAS from the GIANT (Genetic Investigation of ANtropometric Traits) consortium comprising 123,865 individuals of European ancestry indicated that the WRN SNP 1367R was associated with BMI with a nominal P-value = 0.035 (Speliotes et al., 2010). However, a follow-up analysis of 125,931 additional individuals of European ancestry failed to replicate this association (Speliotes et al., 2010).

Serum concentrations of total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides are important risk factors for coronary artery disease, and are often targeted for therapeutic intervention. One GWAS reported that the WRN SNP 114I was associated with HDL cholesterol levels in 19,840 individuals with a nominal P-value = 0.045 (Kathiresan et al., 2009). However, a follow-up on 20,623 additional individuals did not replicate this association. A year later, a meta-analysis of GWAS that included > 100,000 individuals of European ancestry indicated that the WRN SNP 114I was nominally associated with total cholesterol level (P-value = 0.04) and LDL cholesterol (P-value = 0.0026) (Teslovich et al., 2010). Interestingly, a WRN cDNA expression vector encoding the 114 V variant significantly affected cholesterol efflux in WS fibroblasts (Bérubé et al., 2013). These results implicate a functional effect of this WRN polymorphism on cholesterol metabolism at least in a cell culture

system (Bérubé et al., 2013). In addition to WRN SNP 114I, the WRN SNP 1133A was also found to be associated with total cholesterol in the Framingham Heart Study ($P = 1.90 \times 10^{-11}$) (Kulminski and Culminskaya, 2013). However, since the WRN SNP 1133A has a very low minor allele frequency (< 0.05), this SNP was not included in other studies.

Statins are the most widely prescribed drug class for the prevention of cardiovascular disease, and act primarily by lowering plasma cholesterol. Differences in statin-induced reductions of cholesterol among individuals may reflect genetic differences. A combined GWAS from three clinical trials using simvastatin, pravastatin, or atorvastatin, found that the WRN SNP 114I was significantly associated with the magnitude of HDL cholesterol change upon statin treatments in 3928 individuals of European ancestry (Barber et al., 2010).

Blood levels of lipoprotein-associated phospholipase A_2 (Lp-PLA_2) show a strong association with atherosclerosis in humans. Lp-PLA_2, also known as platelet-activating factor acetylhydrolase, is produced by cells of hematopoietic lineages (monocytes/macrophages, T-lymphocytes, mast cells, megakaryocytes and platelets). The enzyme reacts with oxidized phospholipids to generate the pro-inflammatory lipids lysophosphatidylcholine and oxidized free fatty acids. The activity of Lp-PLA_2 is a heritable trait, and a GWAS of Lp-PLA_2 activity in 6668 Caucasian subjects included in the population-based Framingham Heart Study indicated that the WRN SNP 114I was associated with Lp-PLA_2 activity ($P = 0.0398$) (Suchindran et al., 2010). These reports collectively suggest that the WRN V114I SNP may be modifying cholesterol, statins, and Lp-PLA_2 levels in populations of European ancestry.

Diabetes (T2DM) is another important risk factor for cardiovascular disease. One report on 272 randomly recruited T2DM subjects at the Tohoku University Hospital in Japan indicated that the age at diagnosis of T2DM was greater in patients heterozygous for the WRN SNP 1367R than in 1367C/C homozygous individuals ($P = 0.011$). Diabetes-free survival rate over the age range of 30–70, analyzed by Kaplan-Meier method, differed significantly between these two genotype-defined groups ($P = 0.0125$). The authors of this study suggested that WRN SNP 1367R protects against the development of T2DM in Japanese subjects (Hirai et al., 2005). In contrast, there is no reported association between WRN SNP 1367R and T2DM in populations of European ancestry. These differences between European and Japanese populations may be explained by a combination of ethnic genetic differences and marked differences in lifestyle and exposure histories. Interestingly, it has been reported that the WRN SNP 1367R is three times more frequent in Mexicans, Finns, and North Americans than in Japanese individuals (Castro et al., 1999). It has been further suggested that in the Japanese population the WRN SNP 1367R may be in linkage disequilibrium with another, so far unidentified diabetogenic polymorphism, that is not present in Caucasians (Kuningas et al., 2006).

6.3. Renal disease including hypertension

Hypertension is an important risk factor not only for coronary heart disease and ischemic stroke but also for chronic kidney disease. Although hypertension is not a common feature of WS (Oshima et al., 2017), vascular disorders including myocardial infarction are a cause of early death in WS. An intriguing though small Japanese study found that the prognosis of long-term hemodialysis was strongly influenced by WRN SNP status. This retrospective study included 230 healthy controls, 60 patients who had been on hemodialysis for < 6 months (short term hemodialysis), and 50 patients who had been on hemodialysis therapy for > 15 years (long term hemodialysis). Among long-term hemodialysis patients, the WRN SNP 1367R was almost seven fold more prevalent than in the short-term hemodialysis group (Yamada et al., 2000). These results suggest the 1367R/R genotype may confer a survival benefit in Japanese individuals undergoing long-term dialysis (Yamada et al., 2000).

In a second Japanese study, Yoshida et al. (2009) asked whether genetic variants in the *WRN* gene might heighten the risk of chronic kidney disease among individuals with hypertension (Yoshida et al., 2009). The study, which included 3696 Japanese individuals with hypertension, 1257 individuals with chronic kidney disease and 2439 controls, found that the WRN SNP 1367R was significantly associated with the prevalence of chronic kidney disease in hypertensive individuals. The authors concluded that the WRN 1367R allele was protective against chronic kidney disease in hypertensive Japanese subjects (Yoshida et al., 2009).

In a more recent study, Kottgen et al. (2010) performed a meta-analysis of GWAS encompassing 67,093 individuals of European ancestry from 20 predominantly population-based studies with the aim of identifying susceptibility SNPs (including WRN SNPs) for reduced renal function as estimated by serum creatinine, serum cystatin c, and glomerular dysfunction. The analysis was followed by an independent replication cohort study in 22,982 Caucasian individuals. The authors found that the WRN SNPs 1133A and 387I were associated with serum cytostatin C levels in patients with chronic kidney disease ($P = 0.02$ and $P = 0.033$, respectively), though they could not replicate this association in an independent replication cohort that included 22,982 Caucasian individuals (Kottgen et al., 2010). In light of the substantial differences in Japanese and European cohort study designs, it is unclear whether the suggested WRN SNP associations with renal disease might be more general, or have an ethnic population-specific component.

6.4. Osteoporosis

Osteoporosis is clinically characterized by reduced bone mass and compromised bone strength, leading to an increased risk of fracture. WS patients show osteoporosis (Murata and Nakashima, 1982; Walton et al., 2000) with possible impaired osteoblastic bone formation (Shiraki et al., 1998), but normal osteoclastic bone resorption (Laroche et al., 1997; Rubin et al., 1992). Ogata et al. (2001) were the first to report a significant association of the WRN SNP 1367R with bone density in 377 unrelated Japanese postmenopausal women living in Japan. They found significantly lower bone densities in the lumbar spines (L2-4) of women carrying the minor 1367R allele versus non-carriers ($P = 0.037$), and lower total body bony density in 1376R allele carriers after adjusting for age and weight ($P = 0.015$ and 0.042, respectively) (Ogata et al., 2001). In contrast, Zhou et al. (2016) did not find an association between this WRN SNP 1367R and femoral fractures in 924 consecutive autopsies of Japanese men (Zhou et al., 2016). They did, however, identify a significant association of WRN SNP 114I with the prevalence of femoral fracture ($P < 0.05$) and with fracture morbidity. This result was replicated in a study of 251 unrelated postmenopausal Japanese women with osteoporosis and 269 non-institutionalized, community-dwelling Japanese adults (Zhou et al., 2015). Discrepant results in the studies of Ogata et al. and Zhou et al. may in part reflect different methods used to evaluate bone fragility (bone density measures versus calculated fracture incidence) (Mori and Zhou, 2016). Additional WRN SNP association analyses, performed using a study cohort of 1632 consecutive Japanese autopsies at the Tokyo Metropolitan Geriatric Hospital between 1995 and 2011, in which 140 patients had experienced a bone fracture during their lifetime (Mori and Zhou, 2016), identified a statistically significant association between the WRN SNP 114I and fracture risk. Moreover, fracture incidence scaled with the number of I alleles ($P = 0.0120$). All these studies suggest that Japanese subjects bearing at least one WRN 114I allele are at significantly higher risk of femoral fracture than those lacking a 114I allele (Mori and Zhou, 2016). In contrast, Mori and Zhou (2016) noted that a meta-analysis of at least 17 GWAS studies on individuals of Northern European descent did not identify *WRN* as a major candidate gene for osteoporosis. Such findings raised the possibility again of ethnic differences as one explanation for discrepant results when Japanese study populations were compared with those of Northern European descent (Mori and Zhou, 2016).

6.5. Ocular cataracts

Age-related cataract is one of the most common causes of severe visual impairment among the elderly worldwide, rendering this disease a major public health issue (Di Stefano, 2001). Age-related cataract is a progressive opacification of the ocular lens, leading to visual impairment and blindness, and can be further classified as lens nuclear (N), cortical (C), posterior sub capsular (PSC), or mixed type (M) (Klein et al., 1992). Although 99% of WS patients develop ocular cataracts (Oshima et al., 2017), only one epidemiological study has indicated an association between different WRN SNPs and cataract. This study, with a Chinese Han population (504 cases and 244 controls), found a significant difference in the frequency of the WRN SNP 1367R with an odds ratio of 0.66 in R allele carriers. This reduction in risk was cataract type-specific and best fit a dominant genetic model with individuals carrying at least one R allele having a reduced risk of developing cortical age-related cataract ($P < 0.001$). An association between posterior sub capsular cataract and the WRN SNP 114I was also identified before Bonferroni (multiple-testing) correction (Jiang et al., 2013).

Refractive errors, the most common human eye disorder, is often divided into three major subtypes: myopia, astigmatism, and hyperopia (Pizzarello et al., 2004). Refractive error is usually expressed as a measure of the lens diopter value required to achieve proper distance correction. Myopia ('near-sightedness') is by convention represented by negative refractive error values, and affects more than one in four individuals over age 40 in the United States and Western Europe. Hyperopia (positive refractive error values) is present in about 10% of individuals in the same age group. In Asia, the prevalence of myopia is even higher, exceeding 70% in some Asian countries, which makes it an even stronger public health concern (He et al., 2004; Lin et al., 2004). Worldwide, more than 150 million people are estimated to be visually impaired because of uncorrected refractive error, of whom eight million are functionally blind (Holden et al., 2008). Both environmental and genetic factors are known to affect the development of refractive error (Stambolian et al., 2013). A GWAS performed on 7280 samples from five cohorts found that the WRN SNP 114I had the strongest impact on refractive error ($P = 0.0076$). The cohorts included in this analysis included: 1) the Age-Related Eye Disease Study (AREDS); 2) the KORA study (Cooperative Health Research in the Region of Augsburg); 3) the Framingham Eye Study (FES); 4) the Ogliastra Genetic Park-Talana (OGP-Talana) Study; and 5) the Multiethnic Study of Atherosclerosis (MESA) (Stambolian et al., 2013).

6.6. Central nervous system disease

WS was long believed to spare the central nervous system. However, advances in brain imaging have documented brain atrophy in 40% of WS patients (Goto et al., 2013). Alzheimer dementia has not been reported for WS, but 10% of WS patients showed clinical signs of schizophrenia (Goto et al., 2013). No association between non-synonymous WRN polymorphisms and Alzheimer disease or multiple sclerosis has been observed (Briggs et al., 2010; Payao et al., 2004). The WRN SNP 1367R was reported to be associated with bipolar disorder in a meta-analysis on a combined sample of 5568 European cases versus 7187 European controls and 1000 Taiwanese cases versus 1000 Taiwanese controls ($P = 0.015$) (Chen et al., 2013a). This association was replicated in a second analysis composed of 1115 European cases and 2728 European controls (Chen et al., 2013a). In contrast, an association between the WRN SNP 1367R and schizophrenia was reported in a European cohort composed of 871 cases and 863 controls ($P = 0.03$), though could not be replicated in four independent cohorts comprising 1460 patients and 12,995 controls of European origin (Need et al., 2009).

Prion diseases are fatal neurodegenerative diseases of humans and animals caused by the misfolding and aggregation of prion protein (PrP). The most common human prion disease is sporadic or classical Creutzfeldt–Jakob disease (CJD), which like other sporadic neurodegenerative disorders occurs with increasing incidence in older adults. Human prion diseases comprise three etiologies: acquired, inherited, and sporadic (Mead et al., 2012). The heritable forms are under strong genetic control, most notably at the PrP-coding *PRNP* gene itself, but few other risk factors are known. While less common, acquired prion diseases are important because of public health concerns, such as those following the transmission of the cattle prion disease, bovine spongiform encephalopathy, to predominantly young British adults as variant Creutzfeldt–Jakob disease (vCJD). Mead et al. (2012) conducted a GWAS of sporadic CJD (sCJD), variant CJD (vCJD), iatrogenic CJD, inherited prion disease, kuru and kuru resistance (kuru transmission failure despite attendance at mortuary feasts) (Mead et al., 2012). In this analysis the WRN SNPs 1367R and 1074F were associated with sCJD but not with other variants of the disease in the UK ($P = 0.033$ and $P = 0.047$, respectively). This association was not identified either in Germany or in Papua New Guinea. The authors concluded that UK subjects with the minor WRN SNP 1367R allele were at highest risk of developing sCJD, while UK subjects with the minor WRN SNP 1074F allele had reduced risk (Mead et al., 2012). A haplotype analysis of these SNPs was not reported, however.

6.7. WRN polymorphism-cancer associations

WS patients are at elevated risk of developing soft-tissue sarcomas, osteosarcoma, malignant melanoma, meningioma, thyroid epithelial neoplasms and myeloid proliferative disease. The elevated risk of specific tumor types in WS patients ranged from 9-fold to nearly 54-fold higher versus relevant population controls (Goto et al., 1996; Lauper et al., 2013). The ratio of epithelial to non-epithelial neoplasms is nearly equivalent in WS patients, in contrast to the strong bias in favor of epithelial cancers in the adult general population (Goto et al., 2013). The reason for this high proportion of mesenchymal as opposed to epithelial neoplasms in WS patients is still unclear. Common epithelial malignancies such as lung, gastro-intestinal, bladder, prostate, and breast carcinomas do occur in WS patients, thought are not elevated in frequency above general population controls (Lauper et al., 2013). Nevertheless, several association studies have been conducted to identify associations between specific cancers and different non-synonymous WRN SNP variants.

6.7.1. Bone and soft tissue sarcomas

Nakayama et al. (2008) examined potential associations of WRN SNPs with bone and soft tissue sarcomas in two Japanese cohorts. The analysis of each cohort, and of the combined cohorts (544 cases and 1378 controls), indicated that the WRN SNP 1367R was associated with bone and soft tissue sarcomas ($P = 0.005$), with the R allele having a protective effect (Nakayama et al., 2008). Further subgroup analysis suggested that the WRN SNP 1367R was associated with soft tissue sarcomas, sarcomas with reciprocal chromosomal translocations and malignant fibrous histiocytoma, though these analyses did not include a multiple testing correction (Nakayama et al., 2008).

Skull base chordoma is a rare tumor with unknown risk factors. Wang et al. (2014) analyzed a series of 65 patients with pathologically confirmed skull base chordoma, and 65 control subjects who were part of a case-control study from the Han Chinese population. Comparisons of genotype distributions and allele frequencies of WRN SNP 1367R did not reveal a significant difference between the groups. However, when they analyzed their cohort based on gender, they found that men though not women exhibited a protective effect for the R allele ($P = 0.009$) (Wang et al., 2014).

6.7.2. Meningiomas

Meningiomas account for up to 37% of all primary brain tumors in the general population (Bethke et al., 2008). Genetic susceptibility to meningioma is well established, with the risk among relatives of

meningioma patients being approximately three-fold higher than that in the general population (Hemminki and Li, 2003). This led Bethke et al. (2008) to ask whether variants in the DNA repair genes including *WRN* could contribute to disease susceptibility (Bethke et al., 2008) The study was performed using participants from four countries in Europe that included 631 patients and 637 control subjects. This study only showed a trend ($P = 0.051$, calculated by logistic regression and adjusted for study center) for association of the WRN SNP 1367R with meningiomas (Bethke et al., 2008).

6.7.3. Hematologic neoplasia: Non-Hodgkin lymphomas

The non-Hodgkin lymphomas (NHL) are a heterogeneous group of B and T cell malignancies characterized by clonal expansion in peripheral lymphoid tissues. NHL subtypes vary in presentation, survival expectation, morbidity, and responses to treatment. B-cell lymphomas make up the majority of cases, and of these, diffuse large B-cell lymphoma and follicular lymphoma are the two major subtypes. Besides well-known risk factors, including family history, immune dysfunction (e.g., autoimmune diseases, immune deficiency syndromes, and iatrogenic immune suppression after organ transplant), immune stimulation, and infections (e.g., human T-lymphotrophic virus type I, human immunodeficiency virus), a number of additional occupational and environmental exposures have been proposed as NHL risk factors (Hill et al., 2006). Although no major susceptibility genes have been identified, NHL risk is elevated among individuals with a family history of hematopoietic malignancy, and migrants tend to retain the NHL incidence rates and patterns of their country of origin together with common risk variants (Au et al., 2005).

Chromosomal translocations, insertions, and deletions are common early events in non-Hodgkin lymphoma (NHL) carcinogenesis, and implicated in their formation are the V(D)J recombination processes involved in antigen-receptor diversification. DNA repair genes including the *WRN* gene product respond to the double- and single-strand breaks induced by these processes and may influence NHL etiology (Hill et al., 2006). Hill et al. (2006) studied the potential association of different WRN SNPs with NHL in a cohort composed of 1172 cases and 982 matched controls who participated in a population-based NHL study in Los Angeles, Seattle, Detroit, and Iowa (Hill et al., 2006). In this study, the WRN SNP 114I was less common among cases than controls, and NHL risk decreased with an increasing number of the I alleles ($P = 0.04$) across NHL subtypes. In another case-control study, this time among women in Connecticut (518 NHL cases and 597 controls), Shen et al. (2006b) found the WRN SNP 1367R was associated with both a general decreased risk of NHL ($P = 0.007$) together with a decreased risk of diffuse large B-cell lymphoma or follicular lymphoma ($P < 0.024$) (Shen et al., 2006b). A subsequent meta-analysis addressing associations between different variants in several DNA repair genes (including the *WRN* gene) and different types of cancer indicated that WRN SNP 1367R was nominally associated ($P < 0.05$) with lymphoma (the R allele being protective) in at least four different studied cohorts composed of both women and men (Vineis et al., 2009).

Jiao et al. (2012) further explored the relationship between WRN SNP variants and occupational solvent exposure-associated risk of NHL. The authors interviewed 518 histologically confirmed NHL cases and 597 control women from Connecticut for occupational exposure to organic solvents including benzene, formaldehyde or any chlorinated solvent including chloroform, carbon tetrachloride, dichloromethane, dichloroethane, methyl chloride, and trichloroethylene (Jiao et al., 2012). They found that WRN SNP 1074F was associated with and protective for follicular lymphoma ($P = 0.0226$). Guo et al. (2014) conducted a similar analysis on the same cohort of women from Connecticut, looking at the interaction between *WRN* polymorphisms, hair dye use and NHL risk (Guo et al., 2014). Hair dyes have been suggested to be a risk factor for hematopoietic cancers, especially for NHL. Many studies have reported significantly elevated risks of NHL associated with duration of hair dye use and the use of dark-colored dyes. One important mechanism underlying this association is that a number of hair dye ingredients included in formulations before 1980, such as phenylenediamines and paraphenylenediamine, are suspected carcinogens that can cause cytogenetic alterations and DNA damage. Women who used hair dyes before 1980 were found to have a significantly higher risk of NHL, particularly for the follicular lymphoma subtype, though not for diffuse large B-cell lymphoma. The WRN 1367C/C genotype and hair dye use prior to 1980 was associated with follicular lymphoma risk, as was overall NHL risk with the WRN SNP 1367R (P for interaction = 0.032) (Guo et al., 2014). In contrast, there was no significant association with risk of NHL for women who began using hair dye ≥ 1980 ($P > 0.05$). The authors suggest one possible explanation for these differences in risk by time period, which was the reformulation of hair dye products beginning in the early 1980s to replace or eliminate dyes reported to produce tumors in animal studies. Alternatively, there may simply have been insufficient time in the post-1980 cohort for full expression of any associated risk. This possibility of a long latent interval and the continued presence of putative carcinogens in contemporary hair dye products prompted the authors to suggest further analysis of exposure-NHL risk associations (Guo et al., 2014).

Body mass index (BMI) has also been linked to the risk of NHL. This may reflect the growing number of associations between BMI and metabolic, endocrine, immune and inflammatory states that may contribute to DNA damage and promote tumorigenesis. Results from epidemiologic studies, however, have been inconsistent: some studies reported a positive association between BMI and NHL risk (Pan et al., 2004; Rapp et al., 2005), whereas others found no association (MacInnis et al., 2005; Samanic et al., 2006). Chen et al., Chen et al. (2013b) conducted a population-based case-control study in Connecticut women (785 cases and 868 controls) to test the hypothesis that *WRN* genetic variants may modify the relationship between BMI and NHL risk (Chen et al., 2013b). Compared to those with BMI < 25, women with BMI ≥ 25 had significantly increased risk of NHL when carrying the WRN SNP 1074 L/L genotype. Furthermore, the WRN SNP 1367R was associated with decreased risk of diffuse large B-cell lymphoma ($P = 0.046$) as previously described (Shen et al., 2006b). However, a significant interaction between BMI and T-cell lymphoma was only observed for WRN SNP 1074F carriers (P for interaction = 0.004). The authors suggested that the WRN 1074F polymorphism might modify the repair of oxidative DNA damage, thereby modifying the association between BMI and risk of NHL (Chen et al., 2013b).

6.7.4. Lung cancer

A large-scale GWAS performed in a UK Caucasian population comprised of 1529 cases and 2707 controls found an association between WRN SNP 1074F and familial though not sporadic lung cancer. Subjects with the 1074F/F genotype had a lower risk of lung cancer (Rudd et al., 2006). In contrast, one study in Nanjing China found that the WRN SNP 114I was associated with survival of Chinese patients with non-small cell lung carcinoma in univariate though not in Cox regression analyses (Dong et al., 2012). Ethnic background may be one explanation for these differences, together with different population size (the Chinese study included fewer than 600 cases).

6.7.5. Gastrointestinal neoplasms

Genetic polymorphisms in DNA repair genes have been associated both with modified repair capacity and cancer risk. Li et al. (2012) determined whether *WRN* SNP variants were associated with esophageal cancer risk in a Chinese hospital-based case-control study that included 117 esophageal cancer cases and 132 controls. They found that individuals who carried at least one WRN 1367R SNP allele (R/C or R/R genotypes) had a 2.21-fold increased risk of developing esophageal cancer compared to those with a 1367C/C genotype ($P = 0.035$). The study also found, perhaps less surprisingly, that tobacco smoking and

alcohol consumption were significantly related to esophageal cancer risk (Li et al., 2012). The authors further examined these possible gene–environment interactions by stratifying the analyses for smoking and drinking status as 'never' or 'ever' smoked or drank alcoholic beverages. This stratified analysis revealed increased risk of esophageal cancer among smokers ($P < 0.001$) and drinkers ($P = 0.004$) carrying at least one WRN 1367R allele. These associations could not be replicated in GWAS studies of Caucasian populations from Europe, North America, and Australia for any non-synonymous WRN coding SNP (Gharahkhani et al., 2016).

6.7.6. Breast cancer

Several studies have associated the WRN SNP 1367R and the 1367R/R genotype with an elevated risk of breast cancer. For example, Zins et al. (2015) found that the WRN 1367R/R genotype was associated with an approximately two-fold elevated breast cancer risk in an Austrian hospital-based case-control study that included 272 breast cancer patients and 254 controls. Moreover, patients with the R/R genotype exhibited a significantly increased risk of developing breast cancer under the age of 55, with 1367R/R patients developing breast cancer five years earlier on average than 1367C/C genotype patients (R/R mean age 55.2 ± 13.3 years versus C/C patient mean age of 60.2 ± 14.7 years) (Zins et al., 2015). A previous German study had revealed a significant association of the WRN SNP 1367R with familial breast cancer risk in families that did not carry either a *BRCA1* or *BRCA2* germ line mutation. The 1367R allele was more frequent among breast cancer cases than controls (Wirtenberger et al., 2006). A third study, of 798 breast cancer cases and 843 controls from the Mayo Clinic Breast Cancer Study, found an elevated risk of breast cancer among WRN SNP 1367R carriers, with further increased risk among women in the upper U.S. Midwest with a 1367R/R genotype (Olson et al., 2011). Finally, a meta-analysis of 2747 cases and 3555 controls confirmed an increased risk of breast cancer in subjects carrying the WRN 1367R allele (Zhang et al., 2011).

Similar associations were sought for the WRN SNP 1074 variants and breast cancer risk in two different Asian populations. One case-control study of 935 primary breast cancer patients and 1545 healthy controls from Taiwan found the WRN SNP 1074F was significantly associated with breast cancer risk ($P = 0.002$), where risk for SNP genotypes 1074L/F and F/F was stronger and more significant in women with a longer interval between menarche and first full-term pregnancy (Ding et al., 2007). A second case-control study of 1004 breast cancer cases and 1008 controls from China found the WRN 1074F/F genotype was significantly associated with a 1.36-fold increased risk of breast cancer (Wang et al., 2009). In addition, a significant gene-environment interaction was evident between WRN SNP 1074F and age at menarche (P for interaction = 0.02): subjects carrying the 1074F/F genotype and with earlier age at menarche had 3.58-fold increased risk of breast cancer (Wang et al., 2009). The authors suggested that high-risk WRN SNPs and an estrogen-related risk factor may act jointly to increase breast cancer risk. In women exposed to a greater cumulative amount of estrogen (with a longer menarche-to first term pregnancy interval), the breast epithelium might have experienced more mitogenic stimulation–and subsequently would have a higher potential to develop breast cancer–if the cells had a WRN SNP-dependent reduction in genomic stability or repair (Ding et al., 2007).

A subsequent multi-ethnic meta-analysis (which included both Asian and European cohorts), found that the WRN 1367R allele was associated with an increased risk of breast cancer ($P < 0.027$). When the analysis was further stratified by ethnicity, WRN SNP 1367R was significantly associated with cancer susceptibility only in Europeans ($P < 0.042$) (Wang et al., 2009). The authors suggested that the smaller sample size of the Asian study population might have contributed to the difference in associations, as could several other potentially confounding variables such as socio-economic and lifestyle factors, differences in ethnic background, and differences in population-specific frequencies of SNP alleles. Wang et al. (2009) provided some support for one of these possibilities by showing a lower frequency of the WRN SNP 1367R allele in Asian than in the European populations (Wang et al., 2009).

6.7.7. Prostate adenocarcinoma

A small university hospital-based study in China identified a lower risk of prostate cancer in men carrying the WRN SNP 1074F. The study cohort was composed of 147 cases and 111 controls, among whom individuals with 1074L/F and L/L genotypes displayed a decreased prevalence of prostate cancer compared to subjects with the 1074F/F genotype ($P = 0.039$). A stratified analysis further revealed a more significant association for carriers of the 1074F/L and L/L genotypes, though only when diagnosed at age ≤ 72 years (decreased prevalence, $P = 0.002$) or diagnosed with localized diseases ($P = 0.003$). No statistically significant SNP associations were identified in patients diagnosed at age > 72 years, or with advanced disease (Wang et al., 2011).

7. WRN polymorphism effects on WRN protein function

The initial discovery of prevalent WRN SNP variants prompted several lines of experimental work to determine the consequences of specific SNP variants on WRN protein and cellular functions. Initial efforts in this direction focused on biochemical activities of WRN and how they are modified by coding region SNPs. For example, the most common non-synonymous SNPs included in many of the association studies reviewed above, including WRN 1074F/L and 1367C/R, were synthesized and purified, though found to minimally affect WRN exonuclease and DNA helicase activities on a range of substrates (Bohr et al., 2004; Kamath-Loeb et al., 2004). These variants, moreover, did not appear to alter WRN sub-cellular localization (Bohr et al., 2004). The WRN 114I SNP is located within the exonuclease domain (Fig. 1), where it has the potential to alter WRN exonuclease function (Mori and Zhou, 2016). However, appropriate experiment to test this hypothesis remains to be conducted. In similar fashion, functional consequences of the WRN SNP 387I located between the WRN exonuclease domain and acidic repeat region (Fig. 1) remain unknown.

Palermo et al. (2016) showed that a purified WRN protein with the WRN SNP 1133A located between the RQC and HRDC domains did not exhibit differences in its exonuclease and DNA helicases activities *in vitro* compared to the WRN protein bearing the more frequent S1133 allele (Palermo et al., 2016). They also found that the relocalization of the WRN 1133A protein to DNA double-strand breaks (DSBs) induced by the topoisomerase I inhibitor camptothecin during the S-phase of the cell cycle was apparently normal. However, they found that loss of CDK1 (Cyclin Dependent Kinase 1) dependent phosphorylation of the WRN residue S1133 can suppress MRE11 focus formation at DSBs in cells (Palermo et al., 2016). Importantly, endogenous and exogenous chemicals constantly damage cellular DNA resulting in numerous DNA lesions throughout the genome. If not repaired properly, these lesions can generate point mutations or chromosomal rearrangements (Rossi et al., 2010) which can eventually lead to either cellular transformation and cancer, to cellular senescence, or even apoptosis. DNA damage induced senescence or apoptosis will affect tissue regeneration and maintenance, one of the many hallmarks of aging (Lopez-Otin et al., 2016).

DSBs are the most harmful lesions that can cause collapse of the replication fork. Cells can exercise either of two main repair pathways at replication fork collapses; homologous recombination (HR) or the nonhomologous end joining (NHEJ). The WRN protein is involved in both pathways as it can resolve a variety of DNA substrates including forks, flaps, displacement loops (D-loops), bubbles, Holliday junctions, and G-quadruplexes (G4), all of which represent intermediates in DNA replication and repair processes (Rossi et al., 2010). WRN protein subcellular localizations and activities can be modulated by post-translational phosphorylation (Pichierri et al., 2011). In turns out that the

phosphorylation of WRN residue S1133 by CDK1 controls the choice of DSB repair pathways at replication fork collapses (Palermo et al., 2016). HR would be the preferred choice for replication fork restart. A crucial step for HR is the resection taking place at DNA ends to produce single stranded DNA required for subsequent strand invasion during the HR process. This initial step implicates the MRE11 DNA repair complex (Palermo et al., 2016; Rossi et al., 2010). The WRN 1133A SNP variant prevents CDK1-dependent phosphorylation of this site, and, as a result, interferes with the correct interaction and formation of MRE11 foci at collapsed replication fork (Palermo et al., 2016). Under such conditions, long range DNA resection followed by subsequent strand invasion for HR is impeded. Cells expressing the 1133A WRN protein exhibit an elevated use of the more error-prone NHEJ pathway at collapsed forks and an increase in chromosome instability (Palermo et al., 2016). These results indicate that the WRN 1133A SNP variant interferes with a normal post-translational phosphorylation site involved in the interaction and regulation of specific DNA repair proteins, thus potentially altering genomic stability and cellular homeostasis in different organs.

Other non-synonymous WRN SNPs might affect how WRN interacts with additional proteins to modulate or disrupt specific biological processes (Li et al., 2012). Gagné et al. (2016) investigated this possibility, and found that the WRN 1074F/1367R protein exhibited higher affinity for the DNA repair proteins DNA-PKc, KU86, KU70, and PARP1 compared to WRN protein containing the 1074L/1367C SNP substitution pair. These differences in affinity were confirmed by western blot analysis of proteins immunoprecipitated with specific WRN variants (Gagné et al., 2016). Moreover, the immunoprecipitated WRN 1074L/1367C protein complex was found to have lower exonuclease activity when compared with WRN 1074F/1367R variant protein. These differences may have *in vivo* functional consequences, as WS fibroblasts expressing WRN 1074F/1367R protein were more resistant to the benzene metabolite hydroquinone than cells expressing WRN 1074L/1367C protein (Gagné et al., 2016). Benzene metabolites can cause both oxidized DNA damage and DSBs. Interestingly, cells expressing WRN protein containing the 1074F/1367R SNP substitutions were more resistant to hydrogen peroxide challenge than cells expressing WRN 1074L/1367C protein, though displayed no difference in sensitivity to the radiomimetic DNA double-strand cleaving agent neocarzinostatin (Gagné et al., 2016). These results led Gagné et al. (2016) to suggest that different combinations of the WRN SNPs 1074L/F and 1367C/R might modulate the cellular response to oxidative DNA damage (Gagné et al., 2016). Major oxidative DNA damage includes 8-oxoguanine and 8-oxoadenine lesions. Such lesions block WRN exonuclease activity, but in the presence of KU proteins, WRN can digest strands containing these lesions (Rossi et al., 2010). In addition, oxidative DNA damage is removed by the base excision repair pathway. PARP1 is a major protein involved in long patch base excision repair by ribosylating and modulating the activities of many DNA repair enzymes. Ribosylation itself is diminished in WRN deficient cells, suggesting that PARP1 itself is modulated by the WRN protein (Rossi et al., 2010). As such, WRN bearing different SNPs will have different affinity for various DNA repair enzymes and thus will impact on the efficiency of cells to cope with a range of DNA lesions.

Further evidence for *in vivo* consequences of specific WRN SNP variants comes from the analysis of peripheral lymphocyte DNA damage, where Jiang et al. (2013) found that carriers of the WRN SNP 1367R allele had less DNA damage as assessed by comet assay than did individuals with a 1367C/C genotype (Jiang et al., 2013). Lymphocytes from subjects heterozygous for the WRN SNP 1367R were also shown to be more resistant to 4-nitroquinoline-1-oxide challenge than lymphocytes from subjects with a 1367C/C genotype (Ogburn et al., 1997). In contrast, a study on 372 human lymphoblastoid cell lines from unrelated healthy Caucasian individuals showed no association between WRN SNP 1367R and resistance to the drugs camptothecin, etoposide, carboplatin, cisplatin, and daunorubicin (Innocenti et al., 2009). In addition to generating DNA damage, most of these chemicals can affect the redox status of cells. For example, both the benzene metabolite hydroquinone and the carcinogen 4-nitroquinoline-1-oxide can significantly increase cellular oxidative as well as direct DNA base damage (Arima et al., 2006; Luo et al., 2008). Of note, oxidative stress or alterations in the redox cellular status appear to be intrinsically associated with every age-related disease process (Lopez-Otin et al., 2016).

Based on the information gained on the WRN enzymatic activities in the context of protein complexes, future association studies will also need to consider the functional consequences of non-synonymous SNPs in proteins known to interact with WRN. For example, Li et al. (2012) suggested a statistically significant variant genotypic interaction between the DNA repair protein MSH2 and WRN and a markedly increased risk of esophageal cancer (Li et al., 2012). MSH2 has been shown to interact physically with the WRN protein (Lachapelle et al., 2011; Saydam et al., 2007). Testing potential interactions of this type will add another level of complexity to analyses of WRN phenotype and disease associations.

Two other aspects of SNP variant effects on WRN function deserve brief mention. First, WS is a recessive loss-of-function phenotype, and as a result there are many carriers of single known pathogenic variants in virtually all populations studied (Fu et al., 2017). It has been reported that heterozygous siblings of WS patients (who express half the amount of WRN protein compared to normal individuals) are more prone to diabetes and heart failure than the normal population (Weirich et al., 1996). Furthermore, genetic instability and hematological disease risk is higher than normal not only in homozygous WS patients but also in heterozygous siblings (Moser et al., 2000). A second consideration is that a subset of functional consequences of WRN genetic variation may be tissue-specific. For example, Ibrahim et al. (2016) showed recently that keratinocytes do not senesce rapidly in culture in the absence of the WRN protein, unlike fibroblasts (Ibrahim et al., 2016).

8. Mechanistic links from WRN polymorphic variants to disease risk

An important question comes to mind when we look at Table 2: What mechanism(s) could link single SNP polymorphisms that alter the *WRN* open reading frame to seemingly different disease risks or associations? WRN protein is known to affect the efficiency and/or fidelity of key DNA metabolic processes such as DNA replication, DNA repair, telomere stability and transcription (Fig. 2). WRN is also known to affect the expression or activity of proteins involved in cell cycle regulation and proliferation (Pichierri et al., 2011), apoptosis (Wang et al., 2001), autophagy (Maity et al., 2014; Saha et al., 2014), lipid metabolism (Aumailley et al., 2015; Turaga et al., 2009), oxidative stress response (Aumailley et al., 2016; Massip et al., 2010; Massip et al., 2006; Talaei et al., 2013), and inflammation (Aumailley et al., 2015; Turaga et al., 2009). Several of these biological processes may be further linked in the context of disease pathogenesis by collectively promoting or inhibiting oxidative stress, protease: anti-protease imbalance and inflammation (Casas et al., 2015).

A recent extensive comparison of gene and miRNA expression in human cells either mutant in or depleted of the WRN protein (Tang et al., 2016) found that the mutational loss or depletion of WRN from human fibroblasts altered the expression of nearly 2500 genes, with an overlap of only 281 genes between WS patient and WRN-depleted cells. Altered WRN-dependent gene expression was strongly linked both to the presence of G4 DNA motifs in the 5′ regulatory regions of genes, with G4 motif-rich genes most often down-regulated in the absence of WRN. Prolonged absence of WRN function, as is experienced in WS patients, was further associated with the altered expression of genes that constitute a wide range of senescence-associated programs: DNA damage/telomere stress, oxidative stress, oncogene-induced and senescence-associated secretory phenotype. Moreover, unexpected functional pathways were strongly perturbed in WS patient fibroblasts, though not in WRN-depleted cells. These pathways included tRNA

Table 2
WRN polymorphisms (or SNPs) associated with age-related phenotypes or diseases.

Polymorphism	Phenotype (comments)	Allele	Population	References
WRN V114I	HDL-cholesterol levels (association not confirmed in a replication cohort)	I allele	European	Kathiresan et al. (2009)
WRN V114I	Increased total cholesterol and LDL (association not confirmed in a replication cohort)	I allele	European	Teslovich et al. (2010)
WRN V114I	Magnitude of HDL-cholesterol change with statins (findings needs to be tested in an independent cohort)	I allele	European	Barber et al. (2010)
WRN V114I	Increased blood levels of Lp-PLA_2 activity (findings needs to be tested in an independent cohort)	I allele	European	Suchindran et al. (2010)
WRN V114I	Increased bone fracture related morbidity (association replicated in independent Japanese cohorts only)	I allele	Japan	Zhou et al. (2016)
WRN V114I	Increased incidence of femoral fracture (validated in an independent Japanese cohort)	I allele	Japan	Mori and Zhou (2016)
WRN V114I	Increased incidence of posterior subcapsular cataract (but association lost after multiple testing)	I allele	Chinese	Jiang et al. (2013)
WRN V114I	Worst effect on refractive error of the eye (validated in 4 out of 5 independent cohorts)	I allele	Multi-ethnic	Stambolian et al. (2013)
WRN V114I	Head circumference in infant (association not validated in replication cohorts)	I allele	European	Taal et al. (2012)
WRN V114I	Decreased risk of Non-Hodgkin Lymphomas (association not validated in independent cohorts)	I allele	European	Hill et al. (2006)
WRN V114I	Decreased survival of patients with non-small cell lung carcinoma (association lost after multiple testing)	I allele	Chinese	Dong et al. (2012)
WRN M387I	Height (association not tested in independent cohorts)	I allele	European	Lango Allen et al. (2010)
WRN M387I	Increased epicardial coronary stenosis (association not validated in independent cohorts)	I allele	European	Luke et al. (2007)
WRN M387I	Serum level of cytostatin C in patients with chronic kidney disease (not validated in independent cohorts)	I allele	European	Kottgen et al. (2010)
WRN L1074F	Increased risk of ischemic stroke (association not validated in a replication cohort)	F allele	European	Christoffersen et al. (2017)
WRN L1074F	Decreased risk of developing sporadic Creutzfeld-Jakob disease (association only found in the UK)	F allele	Multi-ethnic	Mead et al. (2012)
WRN L1074F	Decreased risk of follicular lymphoma due to organic solvent exposure in women (findings need to be tested in an independent cohort)	F allele	European	Jiao et al. (2012)
WRN L1074F	Increased risk of T-cell lymphomas among women with Body Mass Index > 25 (findings need to be tested in an independent cohort)	L allele	European	Chen et al. (2013b)
WRN L1074F	Decreased risk of lung cancer (association not replicated in an independent cohort)	F allele	European	Rudd et al. (2006)
WRN L1074F	Decreased prevalence of prostate cancer in patients aged > 72 years, but not in younger patients (findings need to be tested in independent cohorts)	L allele	Chinese	Wang et al. (2011)
WRN L1074F	Increased risk of breast cancer in women with longer interval between menarche and first pregnancy (association replicated in independent Asian cohorts)	F allele	Taiwanese	Ding et al. (2007)
WRN L1074F	Increased risk of breast cancer in women with earlier age at menarche (association replicated in independent Asian cohorts)	F allele	Chinese	Wang et al. (2009)
WRN S1133A	Decreased longevity (association needs to be tested in independent cohorts)	A allele	European	Kulminski and Culminskaya (2013)
WRN S1133A	Increased total cholesterol (association needs to be tested in independent cohorts)	A allele	European	Kulminski and Culminskaya (2013)
WRN S1133A	Serum level of cytostatin C in patients with chronic kidney disease (not validated in independent cohorts)	A allele	European	Kottgen et al. (2010)
WRN C1367R	Decreased longevity (but association lost after multiple testing; also association not validated in an independent cohort)	R allele	European	Deelen et al. (2011)
WRN C1367R	Body Mass Index (BMI) (association not validated in a replication cohort)	R allele	European	Speliotes et al. (2010)
WRN C1367R	Increased diabetes-free survival rate over the age (findings need to be tested in independent cohorts)	R allele	Japan	Hirai et al. (2005)
WRN C1367R	Increased myocardial infarction (association validated in an independent Japanese cohort only)	C allele	Japan	Ye et al. (1997)
WRN C1367R	Increased myocardial infarction (association validated in an independent Japanese cohort only)	C allele	Japan	Morita et al. (1999)
WRN C1367R	Increased mitral annular calcification (association not validated in a replication cohort)	R allele	European	Thanassoulis et al. (2013)
WRN C1367R	Increased ischemic stroke (association found in two combined cohorts)	C allele	European	Christoffersen et al. (2017)
WRN C1367R	Increased risk for cyanotic congenital heart disease (association not validated in a replication cohort)	R allele	European	Cordell et al. (2013)
WRN C1367R	Beneficial effect of long-term hemodialysis (findings need to be tested in independent cohorts)	R allele	Japan	Yamada et al. (2000)
WRN C1367R	Decreased prevalence of chronic kidney disease in hypertensive individuals (findings need to be tested in independent cohorts)	R allele	Japan	Yoshida et al. (2009)
WRN C1367R	Lower bone density (association not validated in an independent cohort)	R allele	Japan	Ogata et al. (2001)
WRN C1367R	Reduced risk of age-related cortical cataract (association not validated in independent cohorts)	R allele	Chinese	Jiang et al. (2013)
WRN C1367R	Schizophrenia (association not validated in a replication cohort)	R allele	European	Need et al. (2009)
WRN C1367R	Bipolar disorder (association replicated in independent cohorts)	R allele	Multi-ethnic	Chen et al. (2013a)
WRN C1367R	Increased risk of developing sporadic Creutzfeld-Jakob disease (association only found in the UK)	R allele	Multi-ethnic	Mead et al. (2012)
WRN C1367R	Increased education attainment (association not validated in a replication cohort)	R allele	European	Rietveld et al. (2013)
WRN C1367R	Decreased incidence of bone and soft tissue sarcomas (association validated in an independent Japanese cohort only)	R allele	Japan	Nakayama et al. (2008)
WRN C1367R	Decreased incidence of skull base chordoma in Chinese men only (not in Chinese women)	R allele	Chinese	Wang et al. (2014)
WRN C1367R	Decreased risk of Non-Hodgkin Lymphomas in women (not tested in men)	R allele	European	Shen et al. (2006b)
WRN C1367R	Decreased risk lymphomas (association validated in an independent cohort)	R allele	European	Vineis et al. (2009)
WRN C1367R	Increased risk of Non-Hodgkin Lymphomas in women using hair dye before 1980 (findings need to be tested in an independent cohort)	C allele	European	Guo et al. (2014)
WRN C1367R	Increased risk of esophageal cancer among smokers and alcohol drinkers (association not validated in independent cohorts)	R allele	Chinese	Li et al. (2012)
WRN C1367R	Increased risk of developing breast cancer under the age of 55 and in pre-menopausal women (findings need to be tested in an independent cohort)	R allele	European	Zins et al. (2015)
WRN C1367R	Increased risk of developing breast cancer in some families that do not have BRCA1/2 mutations (findings need to be tested in an independent cohort)	R allele	European	Wirtenberger et al. (2006)
WRN C1367R	Increased risk of developing breast cancer (association validated in independent cohorts)	R allele	European	Olson et al. (2011)
WRN C1367R	Increased risk of developing breast cancer (association found in Europeans but not in Asians)	R allele	Multi-ethnic	Zhang et al. (2011)
WRN C1367R	Increased risk of developing breast cancer (association found in Europeans but not in Asians)	R allele	Multi-ethnic	Wang et al. (2009)

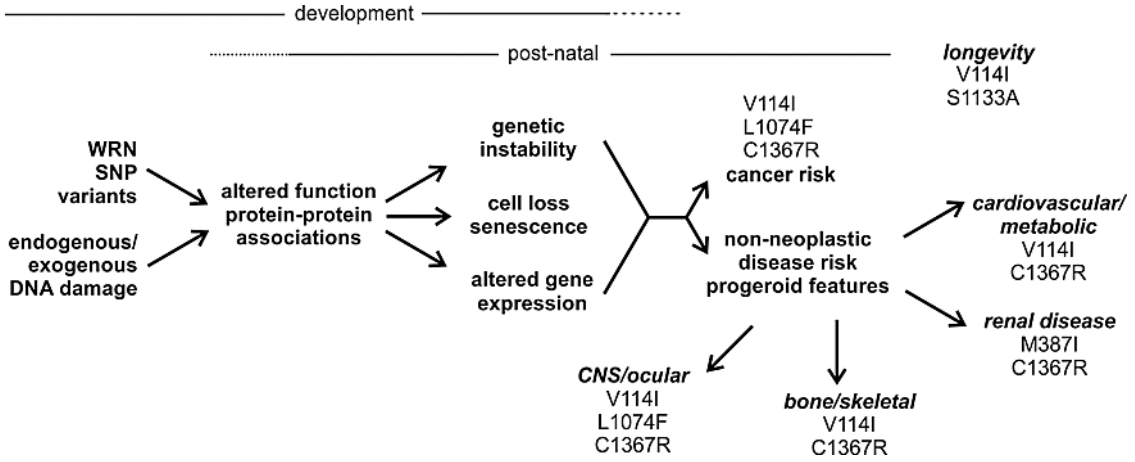

Fig. 2. Model summarizing WRN function in DNA metabolism over the whole of the human lifespan, and how specific WRN SNP variants might affect DNA metabolic function to confer elevated risk or promote the pathogenesis of neoplastic and non-neoplastic disease. Specific WRN SNP variants have also been linked to altered longevity. The mechanism(s) responsible for specific SNP disease or longevity associations remain poorly understood.

charging, as one example of a potentially novel disease-promoting mechanism in WS (Tang et al., 2016).

Cellular processes in addition to DNA metabolism may also be affected by WRN SNP variants. For example, Castro et al. (2008) found that different combinations of the WRN SNPs 1074F and 1367R in fibroblasts derived from 150 non-smoking Mexican males were associated with altered expression of the Plasminogen Inhibitor Activator-1 (PAI-1) mRNA (Castro et al., 2008). Fibroblasts from individuals of SNP genotypes 1367R/R and/or 1074L/L expressed the lowest PAI-1 levels, whereas 1367C/C and/or 1074F/F individuals expressed the highest PAI-1 levels (Castro et al., 2008). The authors suggested further that higher levels of PAI-1 expression an inhibitor of extracellular matrix degradation, might potentiate atherosclerosis risk by promoting abnormal arterial wall intimal growth (Otsuka et al., 2006).

9. Opportunities to improve WRN polymorphism-disease association studies

Table 2 summarizes the WRN SNP associations discussed above. Many of these reported associations were modest or with *P*-values close to 0.05, and few have been replicated in studies of independent populations (Yashin et al., 2015). Many of these association studies foundered on the rocks of small sample size; lack of well-defined case and control groups; a failure to account for co-variables; and a failure to consistently apply multiple testing correction (Pearson and Manolio, 2008). These problems, which are found in many other association analyses, may be further compounded by sub-classification of phenotypes under study or sample stratifications that further decrease sample size and diminish the ability to detect a significant association. Of note, the bar for significance even in conventional GWAS is reasonably high: an association is generally not considered significant until it reaches a genome-wide level of statistical significance or a *P*-value of $\leq 5 \times 10^{-8}$ (Leslie et al., 2014).

A related issue is that many of these studies have focused on only a few–in some instances only one–*WRN* SNP variant. Although several of the common *WRN* SNP variants discussed above are ancient, frequent (enough) and shared across continental populations, many association studies surveyed only the most common variants. This raises the possibility that both less common variant associations or associations linked to additional SNPs have been missed. Notably, a recent update of the mutation spectrum in WS patients by Yokote et al. (2017) includes five non-synonymous coding SNPs with pathogenic consequences (Yokote et al., 2017). The pathogenic mechanisms of some of these variants have been examined using recombinant WRN proteins. For example, two variants changing either the amino acid residue 125 or 135 (K125N and K135E) were shown to cause an increased instability of these WRN proteins (Huang et al., 2006). Homozygous individuals with such variants exhibit WS phenotypes. Another example is the change of residue 574 (G574R) in the WRN protein of a WS patient significantly decreasing its DNA helicase activity (Tadokoro et al., 2013). We expect to discover more non-synonymous coding SNPs in previously unexplored human populations (Yokote et al., 2017). Accordingly, in a recent analysis of *WRN* genetic variants, we identified over 700 base pair level genetic variants in *WRN* by aggregating data across over 60,000 individuals included in the 1000 Genomes, Exome Sequencing Project and Exome Aggregation Consortium datasets (Fu et al., 2017). Among many of the newly discovered WRN variants, one SNP has recently been described for the population of Mexico (Kamath-Loeb et al., 2017). This SNP changes residue 834 of the WRN protein (R834C) and significantly decrease the DNA helicase activity of the protein (Kamath-Loeb et al., 2017). The 834C allele is frequent (up to 5%) only in the Spanish descents of the Mexican population. Interestingly, homozygous 834C subjects do not exhibit the cardinal signs of WS but there is a significant gender bias in favor of males homozygous for this allele (Kamath-Loeb et al., 2017). Although the homozygous subjects do not exhibit WS, association studies of the 834C allele with any age-associated pathology in a much larger Mexican population may reveal new findings. In retrospect, this move to larger sample sizes, together with the use of a more comprehensive list of *WRN* sequence variants, will be the starting point for future association analyses.

Another opportunity to improve our ability to detect *WRN* variant-phenotype and disease associations will draw on data from rapidly developing methods to experimentally determine the functional consequences of large numbers of genetic variants in parallel. These technology-driven functional phenotyping efforts extend the individual variant assays described above, and often have the added virtue of allowing the inclusion of positive and negative controls on a common, defined genetic background. 'Deep mutational scanning' (DMS) methods can easily encompass hundreds or thousands of individual variants in parallel experimental analysis. This approach is an extension of the well-known single amino acid scans that have been used for some time to identify functionally important protein residues (e.g., alanine scans) (Gray et al., 2017). DMS methods thus have the potential to identify the small number of variants to focus on and that have substantial functional consequences as defined by biochemical or cellular assay. The application of these methods to *WRN* will be facilitated by more comprehensive definition of *WRN* sequence variants (Fu et al., 2017), and by rapidly improving methods to generate or capture even large variant libraries for cellular assay (Findlay et al., 2014; Matreyek et al., 2017). Genomic approaches to identify variant-phenotype

associations are also drawing more actively on emerging data on the ethnic and population structure of genetic variation, especially haplotype associations. One early example of the development of these data was reported by Trikka et al. (2002), who studied the haplotype structures of the *WRN* gene in different U.S. ethnic samples including Africans, Caucasians, Hispanics, and Asians. They found that most haplotypes are population-specific, with different patterns of linkage disequilibrium, recombination and haplotype diversity within the *WRN* gene itself in different ethnic samples (Trikka et al., 2002). A good example of the value of haplotype data in association analysis was provided by Shen et al., Shen et al. (2006a), who analyzed the impact of different WRN SNPs on hematotoxicity in Chinese workers exposed to benzene (Shen et al., 2006a). Benzene is a recognized hematotoxicant that leads to chromosome aberrations, apoptosis, and hematopoietic progenitor cell suppression (Lan et al., 2004). The authors analyzed *WRN* haplotype combinations in 250 Chinese workers exposed to benzene and 140 controls. Two WRN SNPs, 114I and 1074F, were associated with a statistically significant decrease in total white blood cell counts (22% and 14%, respectively) among benzene-exposed workers. Moreover, this white cell decrease was greatest in benzene-exposed workers bearing a WRN 114I/1074L/1367C haplotype, 30% lower, versus workers carrying a WRN 114V/1074F/1367R haplotype. These WRN SNP associations remained significant after correcting for multiple testing (Shen et al., 2006a), although this finding requires replication. The authors suggested further that haplotype-associated SNPs might have additive or other effects on function beyond effects attributable to single SNPs (Shen et al., 2006a). Of note, the WRN 114I SNP is located within the exonuclease domain (Fig. 1), where it has the potential to alter WRN exonuclease function (Mori and Zhou, 2016) as well as WRN protein–protein interactions (Gagné et al., 2016).

Finally, other potential confounders in WRN association studies may arise when population stratification is present due to non-random mating between groups of individuals within a specific geographical area or country, and when there are differences in allele frequencies due to drift within a population (Yashin et al., 2015). The recognition and rapid development of population genetic data should allow us to account for, and where possible incorporate, these types of additional data in future association studies.

10. Concluding remarks

As summarized in Table 2, a great deal has already been learned about potential WRN-phenotype or disease associations. These association studies have identified several tantalizing and potentially important associations to pursue, and have provided a much better understanding of the requirements to design and conduct interpretable, well-powered and mechanistically-informative association studies. These studies will be further enabled by the growing number of population-based genomics or precision medicine initiatives, especially those that are attempting to develop longitudinal data on phenotype.

The clinical features of WS, especially the strong risk in WS for clinically important, age-associated disease phenotypes observed in many normal aged individuals, provides a compelling rationale to search for additional *WRN* genotype-disease associations. WS will continue to provide a rich source of new information on the mechanistic basis and pathogenesis of aging, and the development of age-associated disease. In this review, we have emphasized the complexity of genetic variant-disease association studies, and the challenge of interpreting their results. Despite the modest harvest to date, we hope readers will leave with a sense of rapid advances that will improve our ability to link genetic variants in *WRN,* and in many other genes, to disease risk or other phenotypes. Part of the excitement in this reinvigorated area of human disease research stems from advances in a diversity of areas, including new population-based data, methodological developments such as deep mutational scanning, and the rapid advance in our understanding of a growing number of disease mechanisms. Genetic disease associations are indeed complex, though a brighter day–and the identification of more robust associations–lies almost within our grasp.

Acknowledgments

This work was funded by awards from the Canadian Institute of Health Research to ML, and from the U.S. National Cancer Institute to RJMJr as part of Program award P01 CA077852. The content of this article is solely the responsibility of the authors and does not represent the official views of the granting agencies.

References

Arima, Y., Nishigori, C., Takeuchi, T., Oka, S., Morimoto, K., Utani, A., Miyachi, Y., 2006. 4-Nitroquinoline 1-oxide forms 8-hydroxydeoxyguanosine in human fibroblasts through reactive oxygen species. Toxicol. Sci. 91, 382–392.

Au, W.Y., Gascoyne, R.D., Klasa, R.D., Connors, J.M., Gallagher, R.P., Le, N.D., Loong, F., Law, C.K., Liang, R., 2005. Incidence and spectrum of non-Hodgkin lymphoma in Chinese migrants to British Columbia. Br. J. Haematol. 128, 792–796.

Aumailley, L., Garand, C., Dubois, M.J., Johnson, F.B., Marette, A., Lebel, M., 2015. Metabolic and phenotypic differences between mice producing a werner syndrome helicase mutant protein and wrn null mice. PLoS One 10, e0140292.

Aumailley, L., Warren, A., Garand, C., Dubois, M.J., Paquet, E.R., Le Couteur, D.G., Marette, A., Cogger, V.C., Lebel, M., 2016. Vitamin C modulates the metabolic and cytokine profiles, alleviates hepatic endoplasmic reticulum stress, and increases the life span of Gulo-/- mice. Aging (Albany, New York) 8, 458–483.

Auton, A., Brooks, L.D., Durbin, R.M., Garrison, E.P., Kang, H.M., Korbel, J.O., Marchini, J.L., McCarthy, S., McVean, G.A., Abecasis, G.R., 2015. A global reference for human genetic variation. Nature 526, 68–74.

Barber, M.J., Mangravite, L.M., Hyde, C.L., Chasman, D.I., Smith, J.D., McCarty, C.A., Li, X., Wilke, R.A., Rieder, M.J., Williams, P.T., Ridker, P.M., Chatterjee, A., Rotter, J.I., Nickerson, D.A., Stephens, M., Krauss, R.M., 2010. Genome-wide association of lipid-lowering response to statins in combined study populations. PLoS One 5, e9763.

Benjamin, D.J., Cesarini, D., van der Loos, M.J., Dawes, C.T., Koellinger, P.D., Magnusson, P.K., Chabris, C.F., Conley, D., Laibson, D., Johannesson, M., Visscher, P.M., 2012. The genetic architecture of economic and political preferences. Proc. Natl. Acad. Sci. U. S. A. 109, 8026–8031.

Bérubé, J., Garand, C., Lettre, G., Lebel, M., 2013. The non-synonymous polymorphism at position 114 of the WRN protein affects cholesterol efflux in vitro and correlates with cholesterol levels in vivo. Exp. Gerontol. 48, 533–538.

Bethke, L., Murray, A., Webb, E., Schoemaker, M., Muir, K., McKinney, P., Hepworth, S., Dimitropoulou, P., Lophatananon, A., Feychting, M., Lonn, S., Ahlbom, A., Malmer, B., Henriksson, R., Auvinen, A., Kiuru, A., Salminen, T., Johansen, C., Christensen, H.C., Kosteljanetz, M., Swerdlow, A., Houlston, R., 2008. Comprehensive analysis of DNA repair gene variants and risk of meningioma. J. Natl. Cancer Inst. 100, 270–276.

Bohr, V.A., Metter, E.J., Harrigan, J.A., von Kobbe, C., Liu, J.L., Gray, M.D., Majumdar, A., Wilson 3rd, D.M., Seidman, M.M., 2004. Werner syndrome protein 1367 variants and disposition towards coronary artery disease in Caucasian patients. Mech. Ageing Dev. 125, 491–496.

Botto, L.D., Correa, A., Erickson, J.D., 2001. Racial and temporal variations in the prevalence of heart defects. Pediatrics 107, E32.

Briggs, F.B., Goldstein, B.A., McCauley, J.L., Zuvich, R.L., De Jager, P.L., Rioux, J.D., Ivinson, A.J., Compston, A., Hafler, D.A., Hauser, S.L., Oksenberg, J.R., Sawcer, S.J., Pericak-Vance, M.A., Haines, J.L., Barcellos, L.F., 2010. Variation within DNA repair pathway genes and risk of multiple sclerosis. Am. J. Epidemiol. 172, 217–224.

Casas, A.I., Dao, V.T., Daiber, A., Maghzal, G.J., Di Lisa, F., Kaludercic, N., Leach, S., Cuadrado, A., Jaquet, V., Seredenina, T., Krause, K.H., Lopez, M.G., Stocker, R., Ghezzi, P., Schmidt, H.H., 2015. Reactive oxygen-related diseases: therapeutic targets and emerging clinical indications. Antioxid. Redox Signaling 23, 1171–1185.

Castro, E., Ogburn, C.E., Hunt, K.E., Tilvis, R., Louhija, J., Penttinen, R., Erkkola, R., Panduro, A., Riestra, R., Piussan, C., Deeb, S.S., Wang, L., Edland, S.D., Martin, G.M., Oshima, J., 1999. Polymorphisms at the Werner locus: I. Newly identified polymorphisms ethnic variability of 1367Cys/Arg, and its stability in a population of Finnish centenarians. Am. J. Med. Genet. 82, 399–403.

Castro, E., Oviedo-Rodriguez, V., Angel-Chavez, L.I., 2008. WRN polymorphisms affect expression levels of plasminogen activator inhibitor type 1 in cultured fibroblasts. BMC Cardiovasc. Disord. 8, 5.

Chen, D.T., Jiang, X., Akula, N., Shugart, Y.Y., Wendland, J.R., Steele, C.J., Kassem, L., Park, J.H., Chatterjee, N., Jamain, S., Cheng, A., Leboyer, M., Muglia, P., Schulze, T.G., Cichon, S., Nothen, M.M., Rietschel, M., McMahon, F.J., Farmer, A., McGuffin, P., Craig, I., Lewis, C., Hosang, G., Cohen-Woods, S., Vincent, J.B., Kennedy, J.L., Strauss, J., 2013a. Genome-wide association study meta-analysis of European and Asian-ancestry samples identifies three novel loci associated with bipolar disorder. Mol. Psychiatry 18, 195–205.

Chen, Y., Zheng, T., Lan, Q., Kim, C., Qin, Q., Foss, F., Chen, X., Holford, T., Leaderer, B., Boyle, P., Wang, C., Dai, M., Liu, Z., Ma, S., Chanock, S.J., Rothman, N., Zhang, Y., 2013b. Polymorphisms in DNA repair pathway genes, body mass index, and risk of non-Hodgkin lymphoma. Am. J. Hematol. 88, 606–611.

Christoffersen, M., Frikke-Schmidt, R., Nordestgaard, B.G., Tybjaerg-Hansen, A., 2017. Genetic variation in WRN and ischemic stroke: general population studies and meta-

analyses. Exp. Gerontol. 89, 69–77.
Cordell, H.J., Topf, A., Mamasoula, C., Postma, A.V., Bentham, J., Zelenika, D., Heath, S., Blue, G., Cosgrove, C., Granados Riveron, J., Darlay, R., Soemedi, R., Wilson, I.J., Ayers, K.L., Rahman, T.J., Hall, D., Mulder, B.J., Zwinderman, A.H., van Engelen, K., Brook, J.D., Setchfield, K., Bu'Lock, F.A., Thornborough, C., O'Sullivan, J., Stuart, A.G., Parsons, J., Bhattacharya, S., Winlaw, D., Mital, S., Gewillig, M., Breckpot, J., Devriendt, K., Moorman, A.F., Rauch, A., Lathrop, G.M., Keavney, B.D., Goodship, J.A., 2013. Genome-wide association study identifies loci on 12q24 and 13q32 associated with tetralogy of Fallot. Hum. Mol. Genet. 22, 1473–1481.
Croteau, D.L., Popuri, V., Opresko, P.L., Bohr, V.A., 2014. Human RecQ helicases in DNA repair, recombination, and replication. Annu. Rev. Biochem. 83, 519–552.
David, A., Vincent, M., Arrigoni, P.P., Barbarot, S., Pistorius, M.A., Isidor, B., Frampas, E., 2016. Radiographic presentation of musculoskeletal involvement in Werner syndrome (adult progeria). Diagn. Interventional Imaging 98, 373–378.
Deelen, J., Beekman, M., Uh, H.W., Helmer, Q., Kuningas, M., Christiansen, L., Kremer, D., van der Breggen, R., Suchiman, H.E., Lakenberg, N., van den Akker, E.B., Passtoors, W.M., Tiemeier, H., van Heemst, D., de Craen, A.J., Rivadeneira, F., de Geus, E.J., Perola, M., van der Ouderaa, F.J., Gunn, D.A., Boomsma, D.I., Uitterlinden, A.G., Christensen, K., van Duijn, C.M., Heijmans, B.T., Houwing-Duistermaat, J.J., Westendorp, R.G., Slagboom, P.E., 2011. Genome-wide association study identifies a single major locus contributing to survival into old age; the APOE locus revisited. Aging cell 10, 686–698.
Di Stefano, A.F., 2001. VISION 2020: the right to sight: a global initiative for the elimination of avoidable blindness. Optometry 72, 619–622.
Ding, S.L., Yu, J.C., Chen, S.T., Hsu, G.C., Shen, C.Y., 2007. Genetic variation in the premature aging gene WRN: a case-control study on breast cancer susceptibility. Cancer Epidemiol. Biomarkers Prev. 16, 263–269 (A publication of the American Association for Cancer Research. Cosponsored by the American Society of Preventive Oncology).
Dong, J., Hu, Z., Shu, Y., Pan, S., Chen, W., Wang, Y., Hu, L., Jiang, Y., Dai, J., Ma, H., Jin, G., Shen, H., 2012. Potentially functional polymorphisms in DNA repair genes and non-small-cell lung cancer survival: a pathway-based analysis. Mol. Carcinog. 51, 546–552.
Epstein, C.J., Martin, G.M., Schultz, A.L., Motulsky, A.G., 1966. Werner's syndrome a review of its symptomatology, natural history, pathologic features, genetics and relationship to the natural aging process. Medicine (Baltimore). 45, 177–221.
Findlay, G.M., Boyle, E.A., Hause, R.J., Klein, J.C., Shendure, J., 2014. Saturation editing of genomic regions by multiplex homology-directed repair. Nature 513, 120–123.
Forbes, S.A., Tang, G., Bindal, N., Bamford, S., Dawson, E., Cole, C., Kok, C.Y., Jia, M., Ewing, R., Menzies, A., Teague, J.W., Stratton, M.R., Futreal, P.A., 2010. COSMIC (the Catalogue of Somatic Mutations in Cancer): a resource to investigate acquired mutations in human cancer. Nucleic Acids Res. 38, D652–657.
Fu, W., O'Connor, T.D., Jun, G., Kang, H.M., Abecasis, G., Leal, S.M., Gabriel, S., Rieder, M.J., Altshuler, D., Shendure, J., Nickerson, D.A., Bamshad, M.J., Akey, J.M., 2013. Analysis of 6,515 exomes reveals the recent origin of most human protein-coding variants. Nature 493, 216–220.
Fu, W., Ligabue, A., Rogers, K.J., Akey, J.M., Monnat Jr., R.J., 2017. Human RECQ helicase pathogenic variants, population variation and missing diseases. Hum. Mutat. 38, 193–203.
Gagné, J.P., Lachapelle, S., Garand, C., Tsofack, S.P., Coulombe, Y., Caron, M.C., Poirier, G.G., Masson, J.Y., Lebel, M., 2016. Different non-synonymous polymorphisms modulate the interaction of the WRN protein to its protein partners and its enzymatic activities. Oncotarget 7, 85680–85696.
Gharahkhani, P., Fitzgerald, R.C., Vaughan, T.L., Palles, C., Gockel, I., Tomlinson, I., Buas, M.F., May, A., Gerges, C., Anders, M., Becker, J., Kreuser, N., Noder, T., Venerito, M., Veits, L., Schmidt, T., Manner, H., Schmidt, C., Hess, T., Bohmer, A.C., Izbicki, J.R., Holscher, A.H., Lang, H., Lorenz, D., Schumacher, B., Hackelsberger, A., Mayershofer, R., Pech, O., Vashist, Y., Ott, K., Vieth, M., Weismuller, J., Nothen, M.M., Attwood, S., Barr, H., Chegwidden, L., de Caestecker, J., Harrison, R., Love, S.B., MacDonald, D., Moayyedi, P., Prenen, H., Watson, R.G., Iyer, P.G., Anderson, L.A., Bernstein, L., Chow, W.H., Hardie, L.J., Lagergren, J., Liu, G., Risch, H.A., Wu, A.H., Ye, W., Bird, N.C., Shaheen, N.J., Gammon, M.D., Corley, D.A., Caldas, C., Moebus, S., Knapp, M., Peters, W.H., Neuhaus, H., Rosch, T., Ell, C., MacGregor, S., Pharoah, P., Whiteman, D.C., Jankowski, J., Schumacher, J., 2016. Genome-wide association studies in oesophageal adenocarcinoma and Barrett's oesophagus: a large-scale meta-analysis. Lancet Oncol. 17, 1363–1373.
Goto, M., Miller, R.W., Ishikawa, Y., Sugano, H., 1996. Excess of rare cancers in Werner syndrome (adult progeria). Cancer Epidemiol. Biomarkers Prev. 5, 239–246 (A publication of the American Association for Cancer Research. Cosponsored by the American Society of Preventive Oncology).
Goto, M., Ishikawa, Y., Sugimoto, M., Furuichi, Y., 2013. Werner syndrome: a changing pattern of clinical manifestations in Japan (1917 ~ 2008). Biosci. Trends 7, 13–22.
Goto, M., 1997. Hierarchical deterioration of body systems in Werner's syndrome: implications for normal ageing. Mech. Ageing Dev. 98, 239–254.
Gray, M.D., Shen, J.C., Kamath-Loeb, A.S., Blank, A., Sopher, B.L., Martin, G.M., Oshima, J., Loeb, L.A., 1997. The Werner syndrome protein is a DNA helicase. Nat. Genet. 17, 100–103.
Gray, V.E., Hause, R.J., Fowler, D.M., 2017. Analysis of large-scale mutagenesis data to assess the impact of single amino acid substitutions. Genetics 207, 53–61.
Guo, H., Bassig, B.A., Lan, Q., Zhu, Y., Zhang, Y., Holford, T.R., Leaderer, B., Boyle, P., Qin, Q., Zhu, C., Li, N., Rothman, N., Zheng, T., 2014. Polymorphisms in DNA repair genes, hair dye use, and the risk of non-Hodgkin lymphoma. Cancer Causes Control: CCC 25, 1261–1270.
He, M., Zeng, J., Liu, Y., Xu, J., Pokharel, G.P., Ellwein, L.B., 2004. Refractive error and visual impairment in urban children in southern china. Invest. Ophthalmol. Visual Sci. 45, 793–799.
Hemminki, K., Li, X., 2003. Familial risks in nervous system tumors. Cancer Epidemiol. Biomarkers Prev. 12, 1137–1142 (A publication of the American Association for Cancer Research .Cosponsored by the American Society of Preventive Oncology).
Hill, D.A., Wang, S.S., Cerhan, J.R., Davis, S., Cozen, W., Severson, R.K., Hartge, P., Wacholder, S., Yeager, M., Chanock, S.J., Rothman, N., 2006. Risk of non-Hodgkin lymphoma (NHL) in relation to germline variation in DNA repair and related genes. Blood 108, 3161–3167.
Hirai, M., Suzuki, S., Hinokio, Y., Yamada, T., Yoshizumi, S., Suzuki, C., Satoh, J., Oka, Y., 2005. WRN gene 1367 Arg allele protects against development of type 2 diabetes mellitus. Diabetes Res. Clin. Pract. 69, 287–292.
Holden, B.A., Fricke, T.R., Ho, S.M., Wong, R., Schlenther, G., Cronje, S., Burnett, A., Papas, E., Naidoo, K.S., Frick, K.D., 2008. Global vision impairment due to uncorrected presbyopia. Arch. Ophthalmol. 126, 1731–1739.
Huang, S., Lee, L., Hanson, N.B., Lenaerts, C., Hoehn, H., Poot, M., Rubin, C.D., Chen, D.F., Yang, C.C., Juch, H., Dorn, T., Spiegel, R., Oral, E.A., Abid, M., Battisti, C., Lucci-Cordisco, E., Neri, G., Steed, E.H., Kidd, A., Isley, W., Showalter, D., Vittone, J.L., Konstantinow, A., Ring, J., Meyer, P., Wenger, S.L., von Herbay, A., Wollina, U., Schuelke, M., Huizenga, C.R., Leistritz, D.F., Martin, G.M., Mian, I.S., Oshima, J., 2006. The spectrum of WRN mutations in Werner syndrome patients. Hum. Mutat. 27, 558–567.
Ibrahim, B., Sheerin, A.N., Jennert-Burston, K., Bird, J.L., Massala, M.V., Illsley, M., James, S.E., Faragher, R.G., 2016. Absence of premature senescence in Werner's syndrome keratinocytes. Exp. Gerontol. 83, 139–147.
Innocenti, F., Mirkov, S., Nagasubramanian, R., Ramirez, J., Liu, W., Bleibel, W.K., Shukla, S.J., Hennessy, K., Rosner, G.L., Cook Jr., E., Eileen Dolan, M., Ratain, M.J., 2009. The Werner's syndrome 4330T > C (Cys1367Arg) gene variant does not affect the in vitro cytotoxicity of topoisomerase inhibitors and platinum compounds. Cancer Chemother. Pharmacol. 63, 881–887.
Jiang, S., Hu, N., Zhou, J., Zhang, J., Gao, R., Hu, J., Guan, H., 2013. Polymorphisms of the WRN gene and DNA damage of peripheral lymphocytes in age-related cataract in a Han Chinese population. Age (Dordr) 35, 2435–2444.
Jiao, J., Zheng, T., Lan, Q., Chen, Y., Deng, Q., Bi, X., Kim, C., Holford, T., Leaderer, B., Boyle, P., Ba, Y., Xia, Z., Chanock, S.J., Rothman, N., Zhang, Y., 2012. Occupational solvent exposure, genetic variation of DNA repair genes, and the risk of non-Hodgkin's lymphoma. Eur. J. Cancer Prev. 21, 580–584.
Kamath-Loeb, A.S., Welcsh, P., Waite, M., Adman, E.T., Loeb, L.A., 2004. The enzymatic activities of the Werner syndrome protein are disabled by the amino acid polymorphism R834C. J. Biol. Chem. 279, 55499–55505.
Kamath-Loeb, A.S., Zavala-van Rankin, D.G., Flores-Morales, J., Emond, M.J., Sidorova, J.M., Carnevale, A., Cardenas-Cortes, M.D., Norwood, T.H., Monnat, R.J., Loeb, L.A., Mercado-Celis, G.E., 2017. Homozygosity for the WRN Helicase-Inactivating Variant, R834C, does not confer a Werner syndrome clinical phenotype. Sci. Rep. 7, 44081.
Kathiresan, S., Willer, C.J., Peloso, G.M., Demissie, S., Musunuru, K., Schadt, E.E., Kaplan, L., Bennett, D., Li, Y., Tanaka, T., Voight, B.F., Bonnycastle, L.L., Jackson, A.U., Crawford, G., Surti, A., Guiducci, C., Burtt, N.P., Parish, S., Clarke, R., Zelenika, D., Kubalanza, K.A., Morken, M.A., Scott, L.J., Stringham, H.M., Galan, P., Swift, A.J., Kuusisto, J., Bergman, R.N., Sundvall, J., Laakso, M., Ferrucci, L., Scheet, P., Sanna, S., Uda, M., Yang, Q., Lunetta, K.L., Dupuis, J., de Bakker, P.I., O'Donnell, C.J., Chambers, J.C., Kooner, J.S., Hercberg, S., Meneton, P., Lakatta, E.G., Scuteri, A., Schlessinger, D., Tuomilehto, J., Collins, F.S., Groop, L., Altshuler, D., Collins, R., Lathrop, G.M., Melander, O., Salomaa, V., Peltonen, L., Orho-Melander, M., Ordovas, J.M., Boehnke, M., Abecasis, G.R., Mohlke, K.L., Cupples, L.A., 2009. Common variants at 30 loci contribute to polygenic dyslipidemia. Nat. Genet. 41, 56–65.
Kitano, K., 2014. Structural mechanisms of human RecQ helicases WRN and BLM. Front. Genet. 5, 366.
Klein, B.E., Klein, R., Linton, K.L., 1992. Prevalence of age-related lens opacities in a population. The Beaver Dam Eye Study. Ophthalmology 99, 546–552.
Kottgen, A., Pattaro, C., Boger, C.A., Fuchsberger, C., Olden, M., Glazer, N.L., Parsa, A., Gao, X., Yang, Q., Smith, A.V., O'Connell, J.R., Li, M., Schmidt, H., Tanaka, T., Isaacs, A., Ketkar, S., Hwang, S.J., Johnson, A.D., Dehghan, A., Teumer, A., Pare, G., Atkinson, E.J., Zeller, T., Lohman, K., Cornelis, M.C., Probst-Hensch, N.M., Kronenberg, F., Tonjes, A., Hayward, C., Aspelund, T., Eiriksdottir, G., Launer, L.J., Harris, T.B., Rampersaud, E., Mitchell, B.D., Arking, D.E., Boerwinkle, E., Struchalin, M., Cavalieri, M., Singleton, A., Giallauria, F., Metter, J., de Boer, I.H., Haritunians, T., Lumley, T., Siscovick, D., Psaty, B.M., Zillikens, M.C., Oostra, B.A., Feitosa, M., Province, M., de Andrade, M., Turner, S.T., Schillert, A., Ziegler, A., Wild, P.S., Schnabel, R.B., Wilde, S., Munzel, T.F., Leak, T.S., Illig, T., Klopp, N., Meisinger, C., Wichmann, H.E., Koenig, W., Zgaga, L., Zemunik, T., Kolcic, I., Minelli, C., Hu, F.B., Johansson, A., Igl, W., Zaboli, G., Wild, S.H., Wright, A.F., Campbell, H., Ellinghaus, D., Schreiber, S., Aulchenko, Y.S., Felix, J.F., Rivadeneira, F., Uitterlinden, A.G., Hofman, A., Imboden, M., Nitsch, D., Brandstatter, A., Kollerits, B., Kedenko, L., Magi, R., Stumvoll, M., Kovacs, P., Boban, M., Campbell, S., Endlich, K., Volzke, H., Kroemer, H.K., Nauck, M., Volker, U., Polasek, O., Vitart, V., et al., 2010. New loci associated with kidney function and chronic kidney disease. Nat. Genet. 42, 376–384.
Krapohl, E., Rimfeld, K., Shakeshaft, N.G., Trzaskowski, M., McMillan, A., Pingault, J.B., Asbury, K., Harlaar, N., Kovas, Y., Dale, P.S., Plomin, R., 2014. The high heritability of educational achievement reflects many genetically influenced traits, not just intelligence. Proc. Natl. Acad. Sci. U. S. A. 111, 15273–15278.
Kulminski, A.M., Culminskaya, I., 2013. Genomics of human health and aging. Age (Dordr.) 35, 455–469.
Kuningas, M., Slagboom, P.E., Westendorp, R.G., van Heemst, D., 2006. Impact of genetic variations in the WRN gene on age related pathologies and mortality. Mech. Ageing Dev. 127, 307–313.
Lachapelle, S., Gagné, J.P., Garand, C., Desbiens, M., Coulombe, Y., Bohr, V.A., Hendzel, M.J., Masson, J.Y., Poirier, G.G., Lebel, M., 2011. Proteome-wide identification of WRN-interacting proteins in untreated and nuclease-treated samples. J. Proteome

Res. 10, 1216–1227.
Lan, Q., Zhang, L., Li, G., Vermeulen, R., Weinberg, R.S., Dosemeci, M., Rappaport, S.M., Shen, M., Alter, B.P., Wu, Y., Kopp, W., Waidyanatha, S., Rabkin, C., Guo, W., Chanock, S., Hayes, R.B., Linet, M., Kim, S., Yin, S., Rothman, N., Smith, M.T., 2004. Hematotoxicity in workers exposed to low levels of benzene. Science 306, 1774–1776.
Lango Allen, H., Estrada, K., Lettre, G., Berndt, S.I., Weedon, M.N., Rivadeneira, F., Willer, C.J., Jackson, A.U., Vedantam, S., Raychaudhuri, S., Ferreira, T., Wood, A.R., Weyant, R.J., Segre, A.V., Speliotes, E.K., Wheeler, E., Soranzo, N., Park, J.H., Yang, J., Gudbjartsson, D., Heard-Costa, N.L., Randall, J.C., Qi, L., Vernon Smith, A., Magi, R., Pastinen, T., Liang, L., Heid, I.M., Luan, J., Thorleifsson, G., Winkler, T.W., Goddard, M.E., Sin Lo, K., Palmer, C., Workalemahu, T., Aulchenko, Y.S., Johansson, A., Zillikens, M.C., Feitosa, M.F., Esko, T., Johnson, T., Ketkar, S., Kraft, P., Mangino, M., Prokopenko, I., Absher, D., Albrecht, E., Ernst, F., Glazer, N.L., Hayward, C., Hottenga, J.J., Jacobs, K.B., Knowles, J.W., Kutalik, Z., Monda, K.L., Polasek, O., Preuss, M., Rayner, N.W., Robertson, N.R., Steinthorsdottir, V., Tyrer, J.P., Voight, B.F., Wiklund, F., Xu, J., Zhao, J.H., Nyholt, D.R., Pellikka, N., Perola, M., Perry, J.R., Surakka, I., Tammesoo, M.L., Altmaier, E.L., Amin, N., Aspelund, T., Bhangale, T., Boucher, G., Chasman, D.I., Chen, C., Coin, L., Cooper, M.N., Dixon, A.L., Gibson, Q., Grundberg, E., Hao, K., Juhani Junttila, M., Kaplan, L.M., Kettunen, J., Konig, I.R., Kwan, T., Lawrence, R.W., Levinson, D.F., Lorentzon, M., McKnight, B., Morris, A.P., Muller, M., Suh Ngwa, J., Purcell, S., Rafelt, S., Salem, R.M., Salvi, E., et al., 2010. Hundreds of variants clustered in genomic loci and biological pathways affect human height. Nature 467, 832–838.
Laroche, M., Ricq, G., Cantagrel, A., Amigues, J.M., Mazieres, B., 1997. Bone and joint involvement in adults with Werner's syndrome. Rev. Rhum. Engl. Ed. 64, 843–846.
Lauper, J.M., Krause, A., Vaughan, T.L., Monnat Jr., R.J., 2013. Spectrum and risk of neoplasia in Werner syndrome: a systematic review. PLoS One 8, e59709.
Lek, M., Karczewski, K.J., Minikel, E.V., Samocha, K.E., Banks, E., Fennell, T., O'Donnell-Luria, A.H., Ware, J.S., Hill, A.J., Cummings, B.B., Tukiainen, T., Birnbaum, D.P., Kosmicki, J.A., Duncan, L.E., Estrada, K., Zhao, F., Zou, J., Pierce-Hoffman, E., Berghout, J., Cooper, D.N., Deflaux, N., DePristo, M., Do, R., Flannick, J., Fromer, M., Gauthier, L., Goldstein, J., Gupta, N., Howrigan, D., Kiezun, A., Kurki, M.I., Moonshine, A.L., Natarajan, P., Orozco, L., Peloso, G.M., Poplin, R., Rivas, M.A., Ruano-Rubio, V., Rose, S.A., Ruderfer, D.M., Shakir, K., Stenson, P.D., Stevens, C., Thomas, B.P., Tiao, G., Tusie-Luna, M.T., Weisburd, B., Won, H.H., Yu, D., Altshuler, D.M., Ardissino, D., Boehnke, M., Danesh, J., Donnelly, S., Elosua, R., Florez, J.C., Gabriel, S.B., Getz, G., Glatt, S.J., Hultman, C.M., Kathiresan, S., Laakso, M., McCarroll, S., McCarthy, M.I., McGovern, D., McPherson, R., Neale, B.M., Palotie, A., Purcell, S.M., Saleheen, D., Scharf, J.M., Sklar, P., Sullivan, P.F., Tuomilehto, J., Tsuang, M.T., Watkins, H.C., Wilson, J.G., Daly, M.J., MacArthur, D.G., 2016. Analysis of protein-coding genetic variation in 60,706 humans. Nature 536, 285–291.
Leslie, R., O'Donnell, C.J., Johnson, A.D., 2014. GRASP: analysis of genotype-phenotype results from 1390 genome-wide association studies and corresponding open access database. Bioinformatics 30, i185–194.
Li, T., Suo, Q., He, D., Du, W., Yang, M., Fan, X., Liu, J., 2012. Esophageal cancer risk is associated with polymorphisms of DNA repair genes MSH2 and WRN in Chinese population. J. Thoracic Oncol. 7, 448–452 Official publication of the International Association for the Study of Lung Cancer.
Lin, L.L., Shih, Y.F., Hsiao, C.K., Chen, C.J., 2004. Prevalence of myopia in Taiwanese schoolchildren: 1983–2000. Ann. Acad. Med. Singapore 33, 27–33.
Lopez-Otin, C., Galluzzi, L., Freije, J.M., Madeo, F., Kroemer, G., 2016. Metabolic control of longevity. Cell 166, 802–821.
Luke, M.M., Kane, J.P., Liu, D.M., Rowland, C.M., Shiffman, D., Cassano, J., Catanese, J.J., Pullinger, C.R., Leong, D.U., Arellano, A.R., Tong, C.H., Movsesyan, I., Naya-Vigne, J., Noordhof, C., Feric, N.T., Malloy, M.J., Topol, E.J., Koschinsky, M.L., Devlin, J.J., Ellis, S.G., 2007. A polymorphism in the protease-like domain of apolipoprotein(a) is associated with severe coronary artery disease. Arterioscler. Thromb. Vasc. Biol. 27, 2030–2036.
Luo, L., Jiang, L., Geng, C., Cao, J., Zhong, L., 2008. Hydroquinone-induced genotoxicity and oxidative DNA damage in HepG2 cells. Chem. Biol. Interact. 173, 1–8.
MacInnis, R.J., English, D.R., Hopper, J.L., Giles, G.G., 2005. Body size and composition and the risk of lymphohematopoietic malignancies. J. Natl. Cancer Inst. 97, 1154–1157.
Maity, J., Bohr, V.A., Laskar, A., Karmakar, P., 2014. Transient overexpression of Werner protein rescues starvation induced autophagy in Werner syndrome cells. Biochim. Biophys. Acta 1842, 2387–2394.
Massip, L., Garand, C., Turaga, R.V., Deschênes, F., Thorin, E., Lebel, M., 2006. Increased insulin, triglycerides, reactive oxygen species, and cardiac fibrosis in mice with a mutation in the helicase domain of the Werner syndrome gene homologue. Exp. Gerontol. 41, 157–168.
Massip, L., Garand, C., Paquet, E.R., Cogger, V.C., O'Reilly, J.N., Tworek, L., Hatherell, A., Taylor, C.G., Thorin, E., Zahradka, P., Le Couteur, D.G., Lebel, M., 2010. Vitamin C restores healthy aging in a mouse model for Werner syndrome. FASEB J. 24, 158–172 Official publication of the Federation of American Societies for Experimental Biology.
Matreyek, K.A., Stephany, J.J., Fowler, D.M., 2017. A platform for functional assessment of large variant libraries in mammalian cells. Nucleic Acids Res. 45, e102.
Mead, S., Uphill, J., Beck, J., Poulter, M., Campbell, T., Lowe, J., Adamson, G., Hummerich, H., Klopp, N., Ruckert, I.M., Wichmann, H.E., Azazi, D., Plagnol, V., Pako, W.H., Whitfield, J., Alpers, M.P., Whittaker, J., Balding, D.J., Zerr, I., Kretzschmar, H., Collinge, J., 2012. Genome-wide association study in multiple human prion diseases suggests genetic risk factors additional to PRNP. Hum. Mol. Genet. 21, 1897–1906.
Mori, S., Zhou, H., 2016. Implementation of personalized medicine for fracture risk assessment in osteoporosis. Geriatrics Gerontol. Int. 16 (Suppl 1), 57–65.
Morita, H., Kurihara, H., Sugiyama, T., Hamada, C., Yazaki, Y., 1999. A polymorphic variant C1367R of the Werner helicase gene and atherosclerotic diseases in the Japanese population. Thromb. Haemost. 82, 160–161.
Moser, M.J., Bigbee, W.L., Grant, S.G., Emond, M.J., Langlois, R.G., Jensen, R.H., Oshima, J., Monnat Jr., R.J., 2000. Genetic instability and hematologic disease risk in Werner syndrome patients and heterozygotes. Cancer Res. 60, 2492–2496.
Murata, K., Nakashima, H., 1982. Werner's syndrome: twenty-four cases with a review of the Japanese medical literature. J. Am. Geriatr. Soc. 30, 303–308.
Nakayama, R., Sato, Y., Masutani, M., Ogino, H., Nakatani, F., Chuman, H., Beppu, Y., Morioka, H., Yabe, H., Hirose, H., Sugimura, H., Sakamoto, H., Ohta, T., Toyama, Y., Yoshida, T., Kawai, A., 2008. Association of a missense single nucleotide polymorphism, Cys1367Arg of the WRN gene, with the risk of bone and soft tissue sarcomas in Japan. Cancer Sci. 99, 333–339.
Need, A.C., Ge, D., Weale, M.E., Maia, J., Feng, S., Heinzen, E.L., Shianna, K.V., Yoon, W., Kasperaviciute, D., Gennarelli, M., Strittmatter, W.J., Bonvicini, C., Rossi, G., Jayathilake, K., Cola, P.A., McEvoy, J.P., Keefe, R.S., Fisher, E.M., St Jean, P.L., Giegling, I., Hartmann, A.M., Moller, H.J., Ruppert, A., Fraser, G., Crombie, C., Middleton, L.T., St Clair, D., Roses, A.D., Muglia, P., Francks, C., Rujescu, D., Meltzer, H.Y., Goldstein, D.B., 2009. A genome-wide investigation of SNPs and CNVs in schizophrenia. PLoS Genet. 5, e1000373.
Ogata, N., Shiraki, M., Hosoi, T., Koshizuka, Y., Nakamura, K., Kawaguchi, H., 2001. A polymorphic variant at the Werner helicase (WRN) gene is associated with bone density, but not spondylosis, in postmenopausal women. J. Bone Miner. Metab. 19, 296–301.
Ogburn, C.E., Oshima, J., Poot, M., Chen, R., Hunt, K.E., Gollahon, K.A., Rabinovitch, P.S., Martin, G.M., 1997. An apoptosis-inducing genotoxin differentiates heterozygotic carriers for Werner helicase mutations from wild-type and homozygous mutants. Hum. Genet. 101, 121–125.
Olson, J.E., Wang, X., Pankratz, V.S., Fredericksen, Z.S., Vachon, C.M., Vierkant, R.A., Cerhan, J.R., Couch, F.J., 2011. Centrosome-related genes, genetic variation, and risk of breast cancer. Breast Cancer Res. Treat. 125, 221–228.
Oshima, J., Sidorova, J.M., Monnat Jr., R.J., 2017. Werner syndrome: clinical features, pathogenesis and potential therapeutic interventions. Ageing Res. Rev. 33, 105–114.
Otsuka, G., Agah, R., Frutkin, A.D., Wight, T.N., Dichek, D.A., 2006. Transforming growth factor beta 1 induces neointima formation through plasminogen activator inhibitor-1-dependent pathways. Arterioscler. Thromb. Vasc. Biol. 26, 737–743.
Oyen, N., Poulsen, G., Boyd, H.A., Wohlfahrt, J., Jensen, P.K., Melbye, M., 2009. Recurrence of congenital heart defects in families. Circulation 120, 295–301.
Palermo, V., Rinalducci, S., Sanchez, M., Grillini, F., Sommers, J.A., Brosh Jr., R.M., Zolla, L., Franchitto, A., Pichierri, P., 2016. CDK1 phosphorylates WRN at collapsed replication forks. Nat. Commun. 7, 12880.
Pan, S.Y., Johnson, K.C., Ugnat, A.M., Wen, S.W., Mao, Y., 2004. Association of obesity and cancer risk in Canada. Am. J. Epidemiol. 159, 259–268.
Passarino, G., Shen, P., Van Kirk, J.B., Lin, A.A., De Benedictis, G., Cavalli Sforza, L.L., Oefner, P.J., Underhill, P.A., 2001. The Werner syndrome gene and global sequence variation. Genomics 71, 118–122.
Payao, S.L., de Labio, R.W., Gatti, L.L., Rigolin, V.O., Bertolucci, P.H., Smith Mde, A., 2004. Werner helicase polymorphism is not associated with Alzheimer's disease. J. Alzheimer's Dis.: JAD 6, 591–594 (discussion 673-581).
Pearson, T.A., Manolio, T.A., 2008. How to interpret a genome-wide association study. JAMA 299, 1335–1344.
Perls, T., Shea-Drinkwater, M., Bowen-Flynn, J., Ridge, S.B., Kang, S., Joyce, E., Daly, M., Brewster, S.J., Kunkel, L., Puca, A.A., 2000. Exceptional familial clustering for extreme longevity in humans. J. Am. Geriatr. Soc. 48, 1483–1485.
Pichierri, P., Ammazzalorso, F., Bignami, M., Franchitto, A., 2011. The Werner syndrome protein: linking the replication checkpoint response to genome stability. Aging (Albany, New York) 3, 311–318.
Pizzarello, L., Abiose, A., Ffytche, T., Duerksen, R., Thulasiraj, R., Taylor, H., Faal, H., Rao, G., Kocur, I., Resnikoff, S., 2004. VISION 2020: The Right to Sight: a global initiative to eliminate avoidable blindness. Arch. Ophthalmol. 122, 615–620.
Polosak, J., Kurylowicz, A., Roszkowska-Gancarz, M., Owczarz, M., Puzianowska-Kuznicka, M., 2011. Aging is accompanied by a progressive decrease of expression of the WRN gene in human blood mononuclear cells. J. Gerontol. Ser. A, Biol. Sci. Med. Sci. 66, 19–25.
Rapp, K., Schroeder, J., Klenk, J., Stoehr, S., Ulmer, H., Concin, H., Diem, G., Oberaigner, W., Weiland, S.K., 2005. Obesity and incidence of cancer: a large cohort study of over 145,000 adults in Austria. Br. J. Cancer 93, 1062–1067.
Rietveld, C.A., Medland, S.E., Derringer, J., Yang, J., Esko, T., Martin, N.W., Westra, H.J., Shakhbazov, K., Abdellaoui, A., Agrawal, A., Albrecht, E., Alizadeh, B.Z., Amin, N., Barnard, J., Baumeister, S.E., Benke, K.S., Bielak, L.F., Boatman, J.A., Boyle, P.A., Davies, G., de Leeuw, C., Eklund, N., Evans, D.S., Ferhmann, R., Fischer, K., Gieger, C., Gjessing, H.K., Hagg, S., Harris, J.R., Hayward, C., Holzapfel, C., Ibrahim-Verbaas, C.A., Ingelsson, E., Jacobsson, B., Joshi, P.K., Jugessur, A., Kaakinen, M., Kanoni, S., Karjalainen, J., Kolcic, I., Kristiansson, K., Kutalik, Z., Lahti, J., Lee, S.H., Lin, P., Lind, P.A., Liu, Y., Lohman, K., Loitfelder, M., McMahon, G., Vidal, P.M., Meirelles, O., Milani, L., Myhre, R., Nuotio, M.L., Oldmeadow, C.J., Petrovic, K.E., Peyrot, W.J., Polasek, O., Quaye, L., Reinmaa, E., Rice, J.P., Rizzi, T.S., Schmidt, H., Schmidt, R., Smith, A.V., Smith, J.A., Tanaka, T., Terracciano, A., van der Loos, M.J., Vitart, V., Volzke, H., Wellmann, J., Yu, L., Zhao, W., Allik, J., Attia, J.R., Bandinelli, S., Bastardot, F., Beauchamp, J., Bennett, D.A., Berger, K., Bierut, L.J., Boomsma, D.I., Bultmann, U., Campbell, H., Chabris, C.F., Cherkas, L., Chung, M.K., Cucca, F., de Andrade, M., De Jager, P.L., De Neve, J.E., Deary, I.J., Dedoussis, G.V., Deloukas, P., Dimitriou, M., Eiriksdottir, G., Elderson, M.F., Eriksson, J.G., et al., 2013. GWAS of 126,559 individuals identifies genetic variants associated with educational attainment. Science 340, 1467–1471.
Rossi, M.L., Ghosh, A.K., Bohr, V.A., 2010. Roles of Werner syndrome protein in protection of genome integrity. DNA Repair 9, 331–344.

Rubin, C.D., Zerwekh, J.E., Reed-Gitomer, B.Y., Pak, C.Y., 1992. Characterization of osteoporosis in a patient with Werner's syndrome. J. Am. Geriatr. Soc. 40, 1161–1163.
Rudd, M.F., Webb, E.L., Matakidou, A., Sellick, G.S., Williams, R.D., Bridle, H., Eisen, T., Houlston, R.S., 2006. Variants in the GH-IGF axis confer susceptibility to lung cancer. Genome Res. 16, 693–701.
Saha, B., Cypro, A., Martin, G.M., Oshima, J., 2014. Rapamycin decreases DNA damage accumulation and enhances cell growth of WRN-deficient human fibroblasts. Aging Cell 13, 573–575.
Samanic, C., Chow, W.H., Gridley, G., Jarvholm, B., Fraumeni Jr., J.F., 2006. Relation of body mass index to cancer risk in 362,552 Swedish men. Cancer Causes Control: CCC 17, 901–909.
Saydam, N., Kanagaraj, R., Dietschy, T., Garcia, P.L., Pena-Diaz, J., Shevelev, I., Stagljar, I., Janscak, P., 2007. Physical and functional interactions between Werner syndrome helicase and mismatch-repair initiation factors. Nucleic Acids Res. 35, 5706–5716.
Schoenmaker, M., de Craen, A.J., de Meijer, P.H., Beekman, M., Blauw, G.J., Slagboom, P.E., Westendorp, R.G., 2006. Evidence of genetic enrichment for exceptional survival using a family approach: the Leiden Longevity Study. Eur. J. Hum. Genet.: EJHG 14, 79–84.
Sebastiani, P., Solovieff, N., Dewan, A.T., Walsh, K.M., Puca, A., Hartley, S.W., Melista, E., Andersen, S., Dworkis, D.A., Wilk, J.B., Myers, R.H., Steinberg, M.H., Montano, M., Baldwin, C.T., Hoh, J., Perls, T.T., 2012. Genetic signatures of exceptional longevity in humans. PLoS One 7, e29848.
Shamanna, R.A., Croteau, D.L., Lee, J.H., Bohr, V.A., 2017. Recent advances in understanding werner syndrome. F1000Research 6, 1779.
Shen, M., Lan, Q., Zhang, L., Chanock, S., Li, G., Vermeulen, R., Rappaport, S.M., Guo, W., Hayes, R.B., Linet, M., Yin, S., Yeager, M., Welch, R., Forrest, M.S., Rothman, N., Smith, M.T., 2006a. Polymorphisms in genes involved in DNA double-strand break repair pathway and susceptibility to benzene-induced hematotoxicity. Carcinogenesis 27, 2083–2089.
Shen, M., Zheng, T., Lan, Q., Zhang, Y., Zahm, S.H., Wang, S.S., Holford, T.R., Leaderer, B., Yeager, M., Welch, R., Kang, D., Boyle, P., Zhang, B., Zou, K., Zhu, Y., Chanock, S., Rothman, N., 2006b. Polymorphisms in DNA repair genes and risk of non-Hodgkin lymphoma among women in Connecticut. Hum. Genet. 119, 659–668.
Shiraki, M., Aoki, C., Goto, M., 1998. Bone and calcium metabolism in Werner's syndrome. Endocr. J. 45, 505–512.
Smith, M.A., Silva, M.D., Araujo, L.Q., Ramos, L.R., Labio, R.W., Burbano, R.R., Peres, C.A., Andreoli, S.B., Payao, S.L., Cendoroglo, M.S., 2005. Frequency of Werner helicase 1367 polymorphism and age-related morbidity in an elderly Brazilian population. Braz. J. Med. Biol. Res. 38, 1053–1059 Revista brasileira de pesquisas medicas e biologicas.
Speliotes, E.K., Willer, C.J., Berndt, S.I., Monda, K.L., Thorleifsson, G., Jackson, A.U., Allen, H.L., Lindgren, C.M., Luan, J., Magi, R., Randall, J.C., Vedantam, S., Winkler, T.W., Qi, L., Workalemahu, T., Heid, I.M., Steinthorsdottir, V., Stringham, H.M., Weedon, M.N., Wheeler, E., Wood, A.R., Ferreira, T., Weyant, R.J., Segre, A.V., Estrada, K., Liang, L., Nemesh, J., Park, J.H., Gustafsson, S., Kilpelainen, T.O., Yang, J., Bouatia-Naji, N., Esko, T., Feitosa, M.F., Kutalik, Z., Mangino, M., Raychaudhuri, S., Scherag, A., Smith, A.V., Welch, R., Zhao, J.H., Aben, K.K., Absher, D.M., Amin, N., Dixon, A.L., Fisher, E., Glazer, N.L., Goddard, M.E., Heard-Costa, N.L., Hoesel, V., Hottenga, J.J., Johansson, A., Johnson, T., Ketkar, S., Lamina, C., Li, S., Moffatt, M.F., Myers, R.H., Narisu, N., Perry, J.R., Peters, M.J., Preuss, M., Ripatti, S., Rivadeneira, F., Sandholt, C., Scott, L.J., Timpson, N.J., Tyrer, J.P., van Wingerden, S., Watanabe, R.M., White, C.C., Wiklund, F., Barlassina, C., Chasman, D.I., Cooper, M.N., Jansson, J.O., Lawrence, R.W., Pellikka, N., Prokopenko, I., Shi, J., Thiering, E., Alavere, H., Alibrandi, M.T., Almgren, P., Arnold, A.M., Aspelund, T., Atwood, L.D., Balkau, B., Balmforth, A.J., Bennett, A.J., Ben-Shlomo, Y., Bergman, R.N., Bergmann, S., Biebermann, H., Blakemore, A.I., Boes, T., Bonnycastle, L.L., Bornstein, S.R., Brown, M.J., Buchanan, T.A., et al., 2010. Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. Nat. Genet. 42, 937–948.
Stambolian, D., Wojciechowski, R., Oexle, K., Pirastu, M., Li, X., Raffel, L.J., Cotch, M.F., Chew, E.Y., Klein, B., Klein, R., Wong, T.Y., Simpson, C.L., Klaver, C.C., van Duijn, C.M., Verhoeven, V.J., Baird, P.N., Vitart, V., Paterson, A.D., Mitchell, P., Saw, S.M., Fossarello, M., Kazmierkiewicz, K., Murgia, F., Portas, L., Schache, M., Richardson, A., Xie, J., Wang, J.J., Rochtchina, E., Viswanathan, A.C., Hayward, C., Wright, A.F., Polasek, O., Campbell, H., Rudan, I., Oostra, B.A., Uitterlinden, A.G., Hofman, A., Rivadeneira, F., Amin, N., Karssen, L.C., Vingerling, J.R., Hosseini, S.M., Doring, A., Bettecken, T., Vatavuk, Z., Gieger, C., Wichmann, H.E., Wilson, J.F., Fleck, B., Foster, P.J., Topouzis, F., McGuffin, P., Sim, X., Inouye, M., Holliday, E.G., Attia, J., Scott, R.J., Rotter, J.I., Meitinger, T., Bailey-Wilson, J.E., 2013. Meta-analysis of genome-wide association studies in five cohorts reveals common variants in RBFOX1, a regulator of tissue-specific splicing, associated with refractive error. Hum. Mol. Genet. 22, 2754–2764.
Suchindran, S., Rivedal, D., Guyton, J.R., Milledge, T., Gao, X., Benjamin, A., Rowell, J., Ginsburg, G.S., McCarthy, J.J., 2010. Genome-wide association study of Lp-PLA(2) activity and mass in the Framingham Heart Study. PLoS Genet. 6, e1000928.
Taal, H.R., St Pourcain, B., Thiering, E., Das, S., Mook-Kanamori, D.O., Warrington, N.M., Kaakinen, M., Kreiner-Moller, E., Bradfield, J.P., Freathy, R.M., Geller, F., Guxens, M., Cousminer, D.L., Kerkhof, M., Timpson, N.J., Ikram, M.A., Beilin, L.J., Bonnelykke, K., Buxton, J.L., Charoen, P., Chawes, B.L., Eriksson, J., Evans, D.M., Hofman, A., Kemp, J.P., Kim, C.E., Klopp, N., Lahti, J., Lye, S.J., McMahon, G., Mentch, F.D., Muller-Nurasyid, M., O'Reilly, P.F., Prokopenko, I., Rivadeneira, F., Steegers, E.A., Sunyer, J., Tiesler, C., Yaghootkar, H., Breteler, M.M., Decarli, C., Breteler, M.M., Debette, S., Fornage, M., Gudnason, V., Launer, L.J., van der Lugt, A., Mosley Jr., T.H., Seshadri, S., Smith, A.V., Vernooij, M.W., Blakemore, A.I., Chiavacci, R.M., Feenstra, B., Fernandez-Banet, J., Grant, S.F., Hartikainen, A.L., van der Heijden, A.J., Iniguez, C., Lathrop, M., McArdle, W.L., Molgaard, A., Newnham, J.P., Palmer, L.J., Palotie, A., Pouta, A., Ring, S.M., Sovio, U., Standl, M., Uitterlinden, A.G., Wichmann, H.E., Vissing, N.H., DeCarli, C., van Duijn, C.M., McCarthy, M.I., Koppelman, G.H., Estivill, X., Hattersley, A.T., Melbye, M., Bisgaard, H., Pennell, C.E., Widen, E., Hakonarson, H., Smith, G.D., Heinrich, J., Jarvelin, M.R., Jaddoe, V.W., 2012. Common variants at 12q15 and 12q24 are associated with infant head circumference. Nat. Genet. 44, 532–538.
Tadokoro, T., Rybanska-Spaeder, I., Kulikowicz, T., Dawut, L., Oshima, J., Croteau, D.L., Bohr, V.A., 2013. Functional deficit associated with a missense Werner syndrome mutation. DNA Repair 12, 414–421.
Takemoto, M., Mori, S., Kuzuya, M., Yoshimoto, S., Shimamoto, A., Igarashi, M., Tanaka, Y., Miki, T., Yokote, K., 2013. Diagnostic criteria for Werner syndrome based on Japanese nationwide epidemiological survey. Geriatrics Gerontol. Int. 13, 475–481.
Talaei, F., van Praag, V.M., Henning, R.H., 2013. Hydrogen sulfide restores a normal morphological phenotype in Werner syndrome fibroblasts, attenuates oxidative damage and modulates mTOR pathway. Pharmacol. Res. 74, 34–44.
Tang, W., Robles, A.I., Beyer, R.P., Gray, L.T., Nguyen, G.H., Oshima, J., Maizels, N., Harris, C.C., Monnat Jr., R.J., 2016. The Werner syndrome RECQ helicase targets G4 DNA in human cells to modulate transcription. Hum. Mol. Genet. 25, 2060–2069.
Teslovich, T.M., Musunuru, K., Smith, A.V., Edmondson, A.C., Stylianou, I.M., Koseki, M., Pirruccello, J.P., Ripatti, S., Chasman, D.I., Willer, C.J., Johansen, C.T., Fouchier, S.W., Isaacs, A., Peloso, G.M., Barbalic, M., Ricketts, S.L., Bis, J.C., Aulchenko, Y.S., Thorleifsson, G., Feitosa, M.F., Chambers, J., Orho-Melander, M., Melander, O., Johnson, T., Li, X., Guo, X., Li, M., Shin Cho, Y., Jin Go, M., Jin Kim, Y., Lee, J.Y., Park, T., Kim, K., Sim, X., Twee-Hee Ong, R., Croteau-Chonka, D.C., Lange, L.A., Smith, J.D., Song, K., Hua Zhao, J., Yuan, X., Luan, J., Lamina, C., Ziegler, A., Zhang, W., Zee, R.Y., Wright, A.F., Witteman, J.C., Wilson, J.F., Willemsen, G., Wichmann, H.E., Whitfield, J.B., Waterworth, D.M., Wareham, N.J., Waeber, G., Vollenweider, P., Voight, B.F., Vitart, V., Uitterlinden, A.G., Uda, M., Tuomilehto, J., Thompson, J.R., Tanaka, T., Surakka, I., Stringham, H.M., Spector, T.D., Soranzo, N., Smit, J.H., Sinisalo, J., Silander, K., Sijbrands, E.J., Scuteri, A., Scott, J., Schlessinger, D., Sanna, S., Salomaa, V., Saharinen, J., Sabatti, C., Ruokonen, A., Rudan, I., Rose, L.M., Roberts, R., Rieder, M., Psaty, B.M., Pramstaller, P.P., Pichler, I., Perola, M., Penninx, B.W., Pedersen, N.L., Pattaro, C., Parker, A.N., Pare, G., Oostra, B.A., O'Donnell, C.J., Nieminen, M.S., Nickerson, D.A., Montgomery, G.W., Meitinger, T., McPherson, R., McCarthy, M.I., et al., 2010. Biological, clinical and population relevance of 95 loci for blood lipids. Nature 466, 707–713.
Thanassoulis, G., Campbell, C.Y., Owens, D.S., Smith, J.G., Smith, A.V., Peloso, G.M., Kerr, K.F., Pechlivanis, S., Budoff, M.J., Harris, T.B., Malhotra, R., O'Brien, K.D., Kamstrup, P.R., Nordestgaard, B.G., Tybjaerg-Hansen, A., Allison, M.A., Aspelund, T., Criqui, M.H., Heckbert, S.R., Hwang, S.J., Liu, Y., Sjogren, M., van der Pals, J., Kalsch, H., Muhleisen, T.W., Nothen, M.M., Cupples, L.A., Caslake, M., Di Angelantonio, E., Danesh, J., Rotter, J.I., Sigurdsson, S., Wong, Q., Erbel, R., Kathiresan, S., Melander, O., Gudnason, V., O'Donnell, C.J., Post, W.S., 2013. Genetic associations with valvular calcification and aortic stenosis. N. Engl. J. Med. 368, 503–512.
Trikka, D., Fang, Z., Renwick, A., Jones, S.H., Chakraborty, R., Kimmel, M., Nelson, D.L., 2002. Complex SNP-based haplotypes in three human helicases: implications for cancer association studies. Genome Res. 12, 627–639.
Turaga, R.V., Paquet, E.R., Sild, M., Vignard, J., Garand, C., Johnson, F.B., Masson, J.Y., Lebel, M., 2009. The Werner syndrome protein affects the expression of genes involved in adipogenesis and inflammation in addition to cell cycle and DNA damage responses. Cell Cycle 8, 2080–2092.
Vineis, P., Manuguerra, M., Kavvoura, F.K., Guarrera, S., Allione, A., Rosa, F., Di Gregorio, A., Polidoro, S., Saletta, F., Ioannidis, J.P., Matullo, G., 2009. A field synopsis on low-penetrance variants in DNA repair genes and cancer susceptibility. J. Natl. Cancer Inst. 101, 24–36.
Walton, N.P., Brammar, T.J., Coleman, N.P., 2000. The musculoskeletal manifestations of Werner's syndrome. J. Bone Joint Surg. 82, 885–888 British volume.
Wang, X.W., Tseng, A., Ellis, N.A., Spillare, E.A., Linke, S.P., Robles, A.I., Seker, H., Yang, Q., Hu, P., Beresten, S., Bemmels, N.A., Garfield, S., Harris, C.C., 2001. Functional interaction of p53 and BLM DNA helicase in apoptosis. J. Biol. Chem. 276, 32948–32955.
Wang, Z., Xu, Y., Tang, J., Ma, H., Qin, J., Lu, C., Wang, X., Hu, Z., Wang, X., Shen, H., 2009. A polymorphism in Werner syndrome gene is associated with breast cancer susceptibility in Chinese women. Breast Cancer Res. Treat. 118, 169–175.
Wang, L., Kaku, H., Huang, P., Xu, K., Yang, K., Zhang, J., Li, M., Xie, L., Wang, X., Sakai, A., Watanabe, M., Nasu, Y., Shimizu, K., Kumon, H., Na, Y., 2011. Single nucleotide polymorphism WRN Leu1074Phe is associated with prostate cancer susceptibility in Chinese subjects. Acta Med. Okayama 65, 315–323.
Wang, K., Wang, L., Feng, J., Hao, S., Tian, K., Wu, Z., Zhang, L., Jia, G., Wan, H., Zhang, J., 2014. WRN Cys1367Arg polymorphism is not associated with skull base chordoma. Biomed. Rep. 2, 521–524.
Weirich, H.G., Weirich-Schwaiger, H., Kofler, H., Sidoroff, A., Fritsch, P., Schachtschabel, D.O., Schweiger, M., Hirsch-Kauffmann, M., 1996. Werner syndrome: studies in an affected family reveal a cellular phenotype of unaffected siblings. Mech. Ageing Dev. 88, 1–15.
Wirtenberger, M., Frank, B., Hemminki, K., Klaes, R., Schmutzler, R.K., Wappenschmidt, B., Meindl, A., Kiechle, M., Arnold, N., Weber, B.H., Niederacher, D., Bartram, C.R., Burwinkel, B., 2006. Interaction of Werner and Bloom syndrome genes with p53 in familial breast cancer. Carcinogenesis 27, 1655–1660.
Yamada, H., Yamada, Y., Fukatsu, A., Miura, N., Aoki, T., Futenma, A., Kakumu, S., 2000. Polymorphism of Werner helicase-associated gene in long-term hemodialysis patients. Nephron 86, 543.
Yashin, A.I., Wu, D., Arbeeva, L.S., Arbeev, K.G., Kulminski, A.M., Akushevich, I., Kovtun, M., Culminskaya, I., Stallard, E., Li, M., Ukraintseva, S.V., 2015. Genetics of aging, health, and survival: dynamic regulation of human longevity related traits. Front. Genet. 6, 122.

Ye, L., Miki, T., Nakura, J., Oshima, J., Kamino, K., Rakugi, H., Ikegami, H., Higaki, J., Edland, S.D., Martin, G.M., Ogihara, T., 1997. Association of a polymorphic variant of the Werner helicase gene with myocardial infarction in a Japanese population. Am. J. Med. Genet. 68, 494–498.

Yokote, K., Chanprasert, S., Lee, L., Eirich, K., Takemoto, M., Watanabe, A., Koizumi, N., Lessel, D., Mori, T., Hisama, F.M., Ladd, P.D., Angle, B., Baris, H., Cefle, K., Palanduz, S., Ozturk, S., Chateau, A., Deguchi, K., Easwar, T.K., Federico, A., Fox, A., Grebe, T.A., Hay, B., Nampoothiri, S., Seiter, K., Streeten, E., Pina-Aguilar, R.E., Poke, G., Poot, M., Posmyk, R., Martin, G.M., Kubisch, C., Schindler, D., Oshima, J., 2017. WRN mutation update: mutation spectrum, patient registries, and translational prospects. Hum. Mutat. 38, 7–15.

Yoshida, T., Kato, K., Yokoi, K., Watanabe, S., Metoki, N., Satoh, K., Aoyagi, Y., Nishigaki, Y., Nozawa, Y., Yamada, Y., 2009. Association of candidate gene polymorphisms with chronic kidney disease in Japanese individuals with hypertension. Hypertens. Res.: Off. J. Jap. Soc. Hypertens. 32, 411–418.

Yu, C.E., Oshima, J., Fu, Y.H., Wijsman, E.M., Hisama, F., Alisch, R., Matthews, S., Nakura, J., Miki, T., Ouais, S., Martin, G.M., Mulligan, J., Schellenberg, G.D., 1996. Positional cloning of the Werner's syndrome gene. Science 272, 258–262.

Zhang, B., Beeghly-Fadiel, A., Long, J., Zheng, W., 2011. Genetic variants associated with breast-cancer risk: comprehensive research synopsis, meta-analysis, and epidemiological evidence. Lancet Oncol. 12, 477–488.

Zhou, H., Mori, S., Tanaka, M., Sawabe, M., Arai, T., Muramatsu, M., Mieno, M.N., Shinkai, S., Yamada, Y., Miyachi, M., Murakami, H., Sanada, K., Ito, H., 2015. A missense single nucleotide polymorphism, V114I of the Werner syndrome gene, is associated with risk of osteoporosis and femoral fracture in the Japanese population. J. Bone Miner. Metab. 33, 694–700.

Zhou, H., Mori, S., Ishizaki, T., Tanaka, M., Tanisawa, K., Mieno, M.N., Sawabe, M., Arai, T., Muramatsu, M., Yamada, Y., Ito, H., 2016. Genetic risk score based on the lifetime prevalence of femoral fracture in 924 consecutive autopsies of Japanese males. J. Bone Miner. Metab. 34, 685–691.

Zins, K., Frech, B., Taubenschuss, E., Schneeberger, C., Abraham, D., Schreiber, M., 2015. Association of the rs1346044 polymorphism of the werner syndrome gene RECQL2 with increased risk and premature onset of Breast cancer. Int. J. Mol. Sci. 16, 29643–29653.