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Original Article

The molecular genetics of sirtuins: association with human longevity and age-related diseases

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Abstract: The sirtuins are a family of proteins remarkably conserved from yeast to humans. In organisms such as yeast, worms and flies it is quite well established that the activity of sirtuins prolongs lifespan. As a result of promising findings in simple organisms, sirtuins are now investigated in higher organisms in relation to the ageing process. In mammals there are seven different sirtuin proteins each encoded by individual genes (*SIRT*1-7). Although sirtuins share a highly conserved catalytic domain, they differ in their biological function. Some mammalian sirtuins have been implicated in different ageing pathways and their modulation has been deemed to be beneficial in different models of age-associated diseases. Overall, sirtuins could contribute to mechanisms of human longevity and avoid or delay the onset of age-associated disorders. Here we review and discuss the potential impact of genetic variation in the sirtuin genes in relation to human longevity and age-related diseases.

Keywords: Ageing, longevity, sirtuin, SIRT1, SIRT3, SIRT4, genetic variation

Introduction

The sirtuins belong to a family of conserved genes found in organisms ranging from bacteria to mammals [1]. The mammal genome contains seven sirtuin genes (*SIRT1-7*) which share a highly conserved NAD^+-binding catalytic domain [2]. Despite their homology, sirtuins seem to have specific and individual biological functions due to their different substrates, subcellular localization and patterns of expression, as summarized in **TABLE 1** [3-20]. The first member of this gene family to be identified was *SIR2*, from yeast *Saccharomyces cerevisiae* (*Sc*). In 1999, not long after its identification, *SIR2* was shown to extend the lifespan of *Sc* by repressing genome instability [21, 22]. A functional role of *SIR2* in ageing was later demonstrated in other model organisms including *Caenorhabditis elegans* and *Drosophila melanogaster* [23]. Recent findings have further highlighted the involvement of mammalian sirtuins in age-associated disorders such as metabolic diseases, cancer, cardiovascular disease, neurodegeneration and inflammation, thus suggesting a promising role for sirtuins as therapeutic targets against ageing-linked pathologies (reviewed in [24]). In the light of these findings, a growing interest to understand the involvement of sirtuins in ageing and age-associated diseases has arisen. This review summarizes the impact of variation in the sirtuin genes that support a role for human sirtuins in modulating lifespan and disease susceptibility at older ages. In particular, we focus on *SIRT1*, the most researched sirtuin to date, while the relevance of the other sirtuins is less well established and a matter of debate.

In order to undertake a review on this topic as comprehensive as possible, we conducted a literature review using PubMed (http://www.ncbi.nlm.nih.gov/pubmed/) with the keywords "sirtuin", "SIRT1", "SIRT2", "SIRT3", "SIRT4", "SIRT5", "SIRT6", "SIRT7", "polymorphism", "longevity", "ageing" and "age-associated disease".

Table 1. Sirtuin localization, activity, cellular targets, biological effect and possible pathologic area

Sirtuin	Localization	Activity	Targets	Biological effect	Disease area
SIRT1	Nucleus	Deacetylase	PGC-1α, FOXO1, LXR, PPARγ, UCP2, PTP1B, MyoD	Gluconeogenesis (+), Fatty acid oxidation (+), Cholesterol scavenging (+), Adipogenesis (-), Fat storage (-), Mitochondrial activity (+), Thermogenesis (+), Insulin secretion (+), Myogenesis (-) [3]	Metabolic
			NF-Kb, Ku70, FOXO3, FOXO4, p53	Stress response (+) , cell survival (+) [4]	Neurological
			P53, c-Myc	Cell proliferation (+,-) [5, 6]	Cancer
SIRT2	Cytosol	Deacetylase	Tubulin, Histone H4, FOXO3a	Microtubule stability (+) [7], Cell cycle (+) [8], Stress response (+) [9]	Neurological
		ART	FOXO1	Adipogenesis (-) [10]	Metabolic
SIRT3	Mitochondria	Deacetylase	ACS2, GDH	Metabolism [11, 12]	Metabolic
			PGC-1α	Thermogenesis (+) [13]	
SIRT4	Mitochondria	ART	GDH, IDE	Insulin secretion (-) [14, 15]	Metabolic
SIRT5	Mitochondria	Deacetylase	CPS1	Urea cycle (+) [16]	Neurological
SIRT6	Nucleus	Deacetylase	Histone H3k9	DNA repair (+) [17]	Cancer
		ART	Histone H3k9	Glucose homeostasis (+) [18]	Metabolic
SIRT7	Nucleolus	Deacetylase	p53	Stress resistance (+) [19]	Cardiovascular
			Pol I	rDNA transcription (+) [20]	

ART, ADP-ribosyl-transferase; PGC-1α, peroxisome proliferator-activated receptor γ coactivator 1 α, FOXO, forkhead box O; LXR, liver X receptor; PPARγ, peroxisome proliferator-activated receptor γ; UCP2, uncoupling protein 2; PTP1B, protein tyrosine phosphatase 1B; NF-kb, nuclear factor kappa B; ACS2, acetyl-CoA synthetase; GDH, glutamate dehydrogenase; IDE, insulin degrading enzyme; CPS1, carbamoyl phosphate synthetase 1; PolI, DNA polymerase I.
+, - positive or negative regulation

Sirtuin gene variability and human longevity

Mammalian sirtuins were first investigated for their involvement in calorie restriction (CR), a nutritional *regimen* that involves the reduction of daily food intake by approximately 30%, suggested to prolong life expectation and delay age -associated disorders [25]. Several studies support a link between sirtuins and the CR phenotype. It has been shown that SIRT1 null mice have a shorter lifespan than their wild-type (WT) littermates and do not benefit from CR to increase their lifespan [26]. Other *in vitro* evidence has also indicated the involvement of the other mammalian sirtuins (e.g SIRT3, SIRT4 and SIRT5) in CR phenotype [27, 28].

The life-prolonging effects that genes homologous to *SIR2* exert on various model organisms and the emerging evidence of links between

sirtuins and CR has prompted a number of investigations into whether common allelic variations in the SIRT genes are associated with exceptional longevity in humans.

SIRT1 and longevity

Flachsbart and coworkers compared 1026 unrelated German long-lived individuals (mean age: 98.3 years) with 547 German control 'younger' subjects (mean age: 67.2 years) [29]. They analyzed five known single nucleotide polymorphisms (SNPs) (four of which were tagging SNPs) distributed across the entire *SIRT1* gene, designed to capture the majority of the variation in *SIRT1* which has been shown to have high levels of linkage disequilibrium (LD) across the gene [29]. No evidence of a genetic association was detected between any of the SNPs tested individually and longevity nor with any of the five haplotypes identified (with a frequency > 1%). A similar result was observed in a Japanese population [30] and by two other European based studies (the Leiden 85-Plus study [31] and the Rotterdam study [32]), where *SIRT1* variation was not associated to a reduced risk of mortality.

SIRT3 and longevity

SIRT3 may have a role in modulating longevity not due to its homology to *SIR2* but also because it maps to chromosome 11p15.5 that has previously been implicated with life extension by association studies [33]. Rose and coworkers analyzed the genotypic distribution of the *SIRT3* silent variation G477T (corresponding to Ser159Ser; AF083108) in relation to longevity [34]. They found a sex-specific relationship between this polymorphism and survival in the elderly in an Italian cohort from Calabria. The association was strongest in elderly males where the homozygous genotype for the minor allele (TT) increased survival (p= 0.0272) while the heterozygous GT genotype appeared to be associated with decreased survival (p= 0.0391). Because of the silent nature of the polymorphism, no functional effect could be suggested for the G477T variation, however, the authors also found another polymorphism, consisting of a variable number of tandem repeats (VNTR) with a 72-bp repeat core which resided in intron 5 of *SIRT3* which was highly associated with G477T and appeared to have a putative enhancer function. The authors suggested that this might be the 'true' variant responsible for the association [35]. The VNTR polymorphism has six possible alleles depending on the number of core repeats; four alleles were relatively common (alleles 1 to 4 which corresponded to 1-4 repeats), while two alleles of 5 or 6 repeats were more rare. Moreover, the authors also described a functional single nucleotide substitution (T/C) located in the second repeat, 63 base pairs from the starting point of the VNTR. The VNTR region showed an allele-specific enhancer activity depending on the presence of the T/C variant and the number of repeats. Interestingly, by comparing the genotypic and allelic frequencies between two groups of subjects aged 20 to 80 years and 90 to 106-years, the allele lacking the enhancer activity was found to be absent in males older than 90 years, while it was still present in the younger group. This finding suggested that this allele may have a deleterious effect on male longevity.

In a different multicentre study that recruited elderly participants from Central Italy, South Italy and Germany (for a total of 1321 centenarians and 1140 younger subjects) to investigate the 11p15.5 chromosomal region in conjunction with longevity, different SNPs on *SIRT3* showed significant association with longevity in the Italian females and in the German male subgroups [36]. However, this result was not supported by an attempted replication study that tested eight polymorphisms in the *SIRT3* gene (rs2293168, rs511744, rs3782115, rs7934919, rs1045288, rs939915, rs559422, rs3817630) in a French sample of 546 centenarians and 315 younger subjects [36]. An additional meta-analysis of *SIRT3* genetic variation showed a positive association for one polymorphisms only (rs939915) [36].

Other sirtuins and longevity

None of the other sirtuins (*SIRT2, SIRT4 to 7*) have yet been investigated in relation to human longevity. Thus, the main genetic evidence implicating sirtuins to longevity arises from *SIRT3* variability in modulating lifespan in males. This gender-specific contribution to longevity has already been reported in genetic studies addressing this topic [37-39]. Although the evidence is somewhat limited, not least because there are currently only a limited number of ageing cohorts that can be investigated, the current findings still allow the formulation of a hypothe-

sis that these observed associations may be related to the enhancer activity of the VNTR polymorphism but more than likely only contributes to a small extent to what is an highly complex trait like longevity. For this reason, the limited availability of study cohorts and the considerable number of confounders, successful replications may be unlikely and may also explain current inconsistencies in the data.

What is perhaps more surprising is the current lack of evidence of association between longevity and *SIRT1* variation. The failure to find any association with longevity despite the high coverage of *SIRT1* variability and the large number of subjects used in the German study [29], serves to undermine the likelihood of *SIRT1* modulating human longevity. Yet, if *SIRT1* were to exert only a small effect (which may still be possible), the issue surrounding the likely number of confounders requiring consideration would suggest this study would actually be considerably underpowered. Indeed, other evidence supporting possible links between *SIRT1* and modulatory effects on longevity comes from the evaluation of the genetic variability of FOXO3a, a molecular target of SIRT1 and transcription factor that regulates the scavenging of reactive oxygen species (ROS) and resistance to oxidative stress [40]. *FOXO3A* variation has also been strongly associated with longevity in two independent studies performed in Japanese [30] and German populations [41]. These findings may represent indirect support for SIRT1 involvement in longevity although it is perhaps more likely that any perceived association between SIRT1 action and longevity could be as a result of its interaction with FOXO3a which may be the true effector of longevity modulation.

Sirtuin variability and age-related diseases

Sirtuins post-translationally modulate the function of many important cellular proteins, such as p53, forkhead box class O (FOXO) transcription factors, peroxisome proliferator activated receptor PPARγ co-activator 1α (PGC-1α), nuclear factor NF-kB and others, which are closely linked to some age-related disorders (reviewed in [42, 43]). Moreover, sirtuin genetic or pharmacological modulation has shown efficacy in preclinical models of metabolic, inflammatory and cardiovascular diseases, cancer and neurodegeneration (reviewed in [44]).

One study which focused on factors affecting the prevalence of age-related diseases (the Leiden 85-Plus study) evaluated *SIRT1* genetic variation in two separate elderly cohorts, both including subjects older than 85 years, for a total of 1245 participants [31]. The study outcomes were mortality and different parameters concerning the metabolic profile, cardiovascular pathologies and diabetes. All participants were analyzed for 5 SNPs (rs3758391, rs3740051, rs2236319, rs2273773 and rs3818291) covering the *SIRT1* gene region in equally spaced intervals. Two of the selected SNPs were the same as those genotyped in the German study previously described [29]. The analysis of mortality risk and *SIRT1* haplotypes revealed no effect on total mortality rates, but highlighted a trend towards lower cardiovascular mortality in subjects carrying the TAATG haplotype compared to the reference haplotype (i.e. the most frequent one) [31]. The same association was also found for heterozygote of rs3758391 following univariate analysis, however, this association did not correspond to differences in the prevalence of cardiovascular pathologies. No association was also found between *SIRT1* haplotypes and various metabolic profile parameters tested, except for LDL cholesterol and the TAATA haplotype where carriers of this haplotype had lower LDL levels than carriers of the more common reference haplotype [31]. Moreover, an interesting association was found between rs3758391 and cognitive performance, where people homozygous for the T allele of rs3758391 performed better on all tests measuring cognitive function for immediate and delayed memory.

Sirtuins and metabolic diseases

As previously stated, sirtuins are involved in the regulation of metabolism. Abnormal metabolic homeostasis can have severe consequences and it is an important risk factor for the development of severe diseases like obesity and diabetes. Moreover, signs of metabolic imbalance, such as insulin resistance, are increasing during ageing [45].

SIRT1 and metabolic diseases

SIRT1 has been widely investigated in the field of metabolism showing a pleiotropic activity in different key organs. *SIRT1* promotes gluconeogenesis through the deacetylation of PGC-1α

and FOXO1 in liver [46, 47]. *SIRT1* also enhances fatty acid oxidation in liver and skeletal muscle acting again through PGC-1α [48, 49]; attenuates adipogenesis and fat storage in white adipose tissue [50] and improves mitochondrial activity in brown adipose tissue and skeletal muscle [51]. Furthermore, *SIRT1* enhances insulin secretion by repressing UCP2 in the pancreas [52] and increases insulin sensitivity in the skeletal muscle [53].

On the basis of these collective findings, *SIRT1* variation has been explored in the context of metabolism. A study in 2006 found that the minor allele of three *SIRT1* SNPs (rs3740051, rs2236319 and rs2272773) was significantly associated with higher basal energy expenditure in a Finnish cohort of healthy, normal weight non-diabetic offspring (mean age: 34 years) of patients with type II diabetes [51]. Association was also observed, but in the opposite direction, in another study based on 917 non-diabetic individuals from southern Germany (TUEF cohort) [54]. In this case, carriers of the minor allele of two different SNPs (rs12413112 and rs7069102) presented with lower basal energy expenditure (p= 0.04 and p= 0.05 respectively) compared to people who were homozygous for the major allele. In the same study, no significant association of *SIRT1* variants with prediabetic traits was found. However, the authors evaluated also 196 individuals belonging to a population which was at risk of diabetes (aged 45.8 ± 11.2 years – TULIP cohort) involved in a lifestyle intervention program for 9 months [54]. The longitudinal evaluation highlighted that rs12413112 minor allele carriers were resistant against lifestyle-induced improvement of fasting plasma glucose (p= 0.04), had a weaker increase in insulin sensitivity (p= 0.05) and remarkably attenuated decline in liver fat content (p= 0.01). Thus, the authors concluded that *SIRT1* does not affect prediabetic traits but influences the response to a lifestyle intervention. The authors also suggested that the different findings between their data and that derived from the Finnish cohort may be due to the very low linkage disequilibrium between the different SNPs in both studies. This would allow the associated SNPs in the two studies to act in the opposite direction as for the measured outcome (energy expenditure). Of course another explanation is that the associations observed were type I (false positive) or type II (false negative) statistical errors.

In a more recent investigation based on a longitudinal study in a cohort of 4573 participants (the Rotterdam study, mean age: 67.6 ± 7.7 years), *SIRT1* variation (assessed by three tagging SNPs: rs7895833, rs1467568, and rs497849) did not influence the all-cause mortality risk [32]. Instead, in a subgroup with type II diabetes at baseline (n= 413), *SIRT1* variability was related to survival rate. Diabetic subjects homozygous for the most common haplotype had a higher mortality risk (HR 1.5, 95% CI 1.1-2.1) compared to non homozygous. In particular, cardiovascular mortality in diabetic subjects was higher than in homozygous carriers. This risk further increased for diabetic participants when evaluated in association with smoking and dietary niacin consumption [32]. The same *SIRT1* SNPs were also analyzed in an intended replication cohort of 2347 participants (Erasmus Rucphen Family study) in relation to BMI and risk of obesity (defined as BMI≥ 30 Kg/m^2) [55], two well-known risk factors for different age-related pathologies (diabetes, cadiovascular diseases, etc) and early death. Minor alleles of rs7895833 and rs1467568 were associated with lower BMI (p= 0.002 for both SNPs in the two cohort combined) and with a 0.2-0.4 Kg/m^2 decrease in BMI per allele copy. Moreover, carriers of these alleles had a 13-18% decreased risk of obesity (for rs7895833 p= 0.02 and for rs1467568 p= 0.0009 in the two studies combined). In the Rotterdam Study, the two variants were also found to be associated with a lower BMI increase during a 6.4 year follow-up (p= 0.01 and 0.08 respectively) [55].

Further evidence implicating *SIRT1* in metabolic diseases came from another Belgian cohort in which an association between the *SIRT1* gene and obesity was found. In 1068 obese subjects and 313 individuals older than 20 years, carriers of the C-allele of rs7069102 had a lower risk of being obese than non-carriers (p= 0.025). Moreover, in a multifactorial study that analyzed 3575 participants (20-59 years) for a relationship between obesity and 327 SNPs across 239 genes that belong to pathways that regulate fatty acid and glucose metabolism, the rs2273773 SNP of *SIRT1* gene was associated with BMI but in women only, supporting a potential role of *SIRT1* in obesity [56].

Overall, the literature supports the involvement of *SIRT1* genetic variants in influencing metabolic parameters such as energy expenditure,

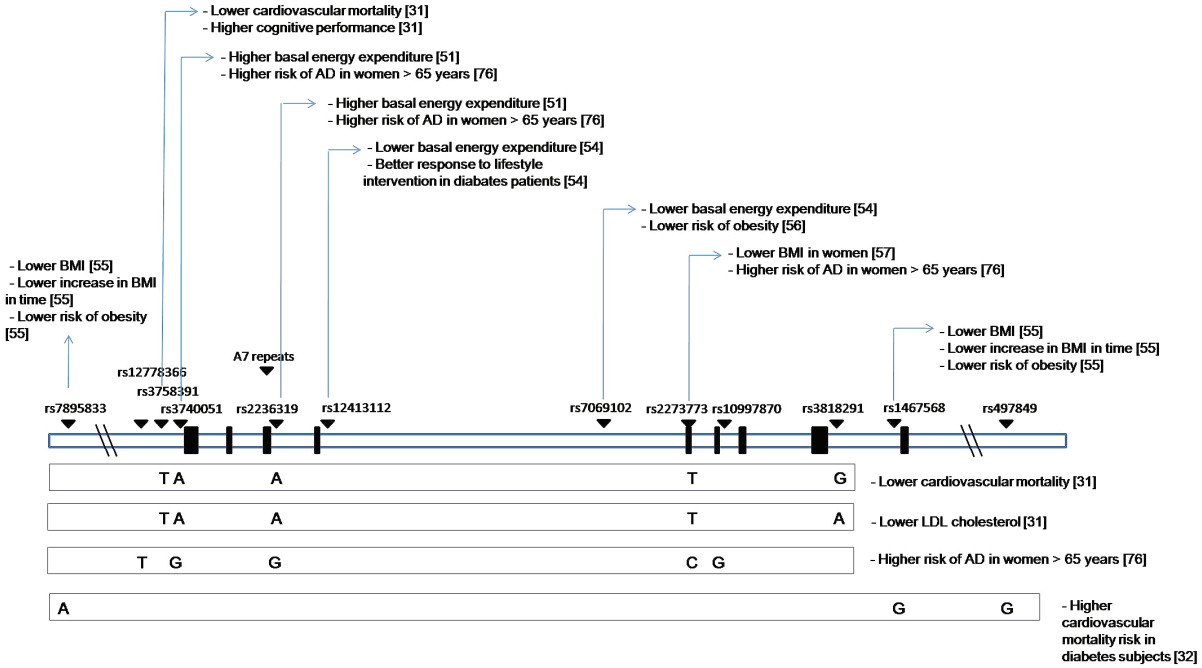

Figure 1. *SIRT1* genetic variability related to ageing and age-associated diseases. Schematic structure of the *SIRT1* gene where introns and exons are represented by white and black boxes respectively. *SIRT1* SNPs reported to be associated with ageing and age-related diseases are represented across *SIRT1* gene by black triangles and the relative rs number. *SIRT1* haplotypes associated with age-related diseases are reported below the gene scheme.

BMI, LDL cholesterol and risk of obesity. Moreover, although SIRT1 is not directly involved in glucose metabolism, variability in the gene that encodes for it may modulate the outcome of a lifestyle intervention in diabetic patients and could also be relevant for the risk of cardiovascular mortality or the responsiveness of people to different forms of cardiovascular treatment. In this respect, it is not clear whether the perceived risk is concomitant with metabolic diseases, since in the Rotterdam study an association was found in diabetic patients only, whereas in the Leiden 85-Plus Study there was no correspondence with metabolic parameters.

Mitochondrial sirtuins and metabolic diseases

Among the seven sirtuins, SIRT3, SIRT4 and SIRT5 were reported to prevalently localise in the mitochondria. Due to their localisation, mitochondrial-associated sirtuins are potentially involved in metabolism and metabolic disfunction. The investigation of 58 tagging SNPs in 13 genes involved in mitochondrial function for an association with type II diabetes mellitus (T2DM) revealed that only rs2522138 in *SIRT4* was significantly associated p= 0.01 [57]. The study was performed with a two-stage design. The first stage examined 519 controls and 480 cases (mean age: 65±8 and 67±8 years, respectively) for all 58 SNPs, while the second stage included 7620 cases and 2544 controls (mean age: 63±12 and 68±12 years, respectively) but only focused on the SNPs showing evidence for association with T2DM from the first stage. Meta-analysis of the data derived from the two cohorts resulted in a significant association between the G allele of rs2522138 in *SIRT4* and T2DM. However, an effort to further replication these findings in an independent sample population (514 controls and 706 cases, 57±10 and 59±10 years, respectively) was not found to be significant although evidence toward an effect could be suggested (p= 0.06). The authors suggested that in the context of testing multiple SNPs, their positive result should be interpreted with caution and could be deemed consistent with statistical noise [57]. Besides, meta-analysis of three genome wide associations studies (GWAS) of T2DM, intended to offer greater sensitivity to detect modest effect size variations (the DIAGRAM GWAS meta-

analysis), no evidence to support association between *SIRT4* and diabetes (p= 0.72 for the G allele of rs2522138 of *SIRT4*) was observed [58]. Similarly, no positive association was found between *SIRT3* and T2DM in a previous study [57].

In summary, these studies collectively suggest that an association between mitochondrial-related sirtuins and diabetes is, if present, likely to be only of small effect. Yet, the inconsistencies of the findings for *SIRT4* and the *in vitro* evidence linking SIRT3 and SIRT4 to metabolism suggest that this route of investigation may warrant further study until there is less ambiguity in the data.

Sirtuins and cancer

SIRT1 involvement in cancer is still contentious. Recent findings show that *SIRT1* is a tumor suppressor gene [59], whereas *SIRT1* has been previously suggested to be a tumor promoter [60, 61]. It may be the case that *SIRT1* may have dual functions in this manner in different tissue contexts as has also been suggested [62].

SIRT1 sequence variation has been investigated in gastric cancer (GC) and in colorectal cancer (CRC) characterized by gene microsatellite instability (MSI). The attention was focused on a region in exon III of *SIRT1* characterized by two sets of 7A repeats (nucleotides 703-709 and 760-766). Analyzing 45 patients with GC and 48 patients with CRC a new deletion mutation was found in two cancer patients in the A7 repeat sequences. This deletion resulted in a frameshift and the introduction of a premature stop codon [63]. However, this study was small and the frequency of the new mutation rare and thus is unlikely to play a significant role in cancer pathogenesis involving MSI. To better assess the role of *SIRT1* in cancer development, mutational analysis of the entire coding region of *SIRT1* in groups of people with and without human cancer might be a strategy worthy of future pursuit.

Sirtuins and neurodegenerative disorders

Sirtuins have been found to work as disease-modifiers in various models of neurodegeneration such as in Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD) and Amyotrophic lateral sclerosis (ALS) (review in [64]). Some genetic studies have also been published.

The candidacy *SIRT1* as a possible risk factor in relation to AD risk was due to its mapping to chromosome 10 (10q21.3), a region previously reported to be important to AD according to linkage studies [65, 66] and where other novel AD-associated genes have been reported, some of which have yet to be replicated and others not replicated [67-73]. *SIRT1* association to late-onset AD was evaluated in a genetic screen of novel candidate genes on chromosome 10. The screening was performed on 1160 AD patients (mean age at onset 75.6 ± 6.84 years) and 1389 control subjects from the British population. No evidence supporting association between *SIRT1* variation and AD risk was found [74]. These findings were supported by an independent study in the Finnish population on 326 AD cases (mean age at onset 72.1 ± 6.8 years) and 463 controls (mean age 69.6 ± 5.1 years). In this study five *SIRT1* SNPs were genotyped (rs12778366; rs3740051; rs2236319; rs2273773 and rs10997870) [75]. A weak positive association with AD was reported in the subgroup of women older than 65 years for three different genotypes in the *SIRT1* gene: rs3740051 (A/G), rs2236319 (A/G), rs2273773 (C/T). Moreover the TGGCG haplotype from the 5 SNPs studied was over-represented in AD women older than 65 years compared to controls (p= 0.026). However, these associations may be a false positive because of the stratification with gender and age and indeed, following Bonferroni's correction of the data there no longer was evidence to support any association [75].

Collectively these data suggest that *SIRT1* variability is not important with respect to AD risk, however given the increasing amount of *in vivo* and cellular data about sirtuins and AD, sirtuin associated pathways may still be implicated and an initial starting point might be the investigation of all seven sirtuin genes as risk factors for AD onset. In keeping with this, *SIRT3* promoter methylation state has been studied in AD patients but no evidence supporting the existing of any peculiar methylation state in comparison to controls was found [76].

While the majority of genetic studies of neurodegenerative disease to-date have focused on AD, the same arguments can be applied to

other forms of neurodegenerative disease, such as Parkinson's disease where evidence from *in vitro* studies suggests the involvement of SIRT2 [77]. Thus study of the sirtuin genes in these other diseases could be worthwhile.

Conclusions

Despite the increasing amount of cellular and *in vivo* evidence linking sirtuins to age-associated diseases, only weak, often unreplicated evidence has been found to support the sirtuin involvement as being related to genetic variability. However, in some ways the failure to find association may be due to the complexity of ageing and other conditions they are involved with that are likely to be influenced by many other non-genetic factors, such as social behavior, diet, physical exercise and environment. The genetic contribution in such instances is likely to only be a small component. Indeed susceptibility genes of large effect in any complex disease are very rare as has been suggested following on from recent large GWAS studies of AD where other genes of similar effect size to the well recognized apolipoprotein-E epsilon 4 allele (*APOE* ε4) have not materialised [78]. Even if genetic variability in sirtuins is not found to be important in various clinical contexts, where much more work can be done given the paucity of information on sirtuins other than *SIRT1* (**Figure 1**), the non-genetic evidence implicating sirtuins in various ageing and disease models suggests that sirtuin related pathways are important and this might direct future research focus towards other molecular targets upstream or downstream of sirtuin action.

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