



## Conference on ‘Nutrition and healthy ageing’ Symposium 1: Biology of ageing

### Genomics of ageing in twins

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Ageing is a complex multifactorial process, reflecting the progression of all degenerative pathways within an organism. Due to the increase of life expectancy, in recent years, there is a pressing need to identify early-life events and risk factors that determine health outcomes in later life. So far, genetic variation only explains ~20–25% of the variability of human survival to age 80+. This clearly implies that other factors (environmental, epigenetic and lifestyle) contribute to lifespan and the rate of healthy ageing within an individual. Twin studies in the past two decades proved to be a very powerful tool to discriminate the genetic from the environmental component. The aim of this review is to describe the basic concepts of the twin study design and to report some of the latest studies in which high-throughput technologies (e.g. genome/epigenome-wide assay, next generation sequencing, MS metabolic profiling) combined with the classical twin design have been applied to the analysis of novel ‘omics’ to further understand the molecular mechanisms of human ageing.

#### Twins: Ageing: Omics: Heritability

Human ageing is a multifactorial process which represents the final outcome of the interaction among genetic, environmental, stochastic and lifestyle factors.

In the past decade, probably fuelled by the news in the press, there has been an increased interest of the public opinion in the ageing process. Indeed, in the past century, in the developed world, there was a remarkable increase of life expectancy<sup>(1)</sup>. This phenomenon was the result of advances in medical science, improved lifestyle (hygiene and nutrition) and considerable decline of mortality rates among young<sup>(2)</sup>.

In 2006, the European Community reported that by 2050 the number of residents aged 65+ will increase by 70% and the 80+ age group will increase by 170% in the same period<sup>(3)</sup>. If healthy life expectancy evolves broadly in line with the change in age-specific life expectancy, then the projected increase in spending on health-care due to ageing would be halved. A healthy, active ageing population can only be supported through effective health policy across the lifecycle. Such a policy requires an understanding of the ageing process including

the identification of robust markers of cellular senescence and investigation of their role in ageing.

In the past decade, the introduction of high-throughput technologies, in particular when combined with the classical study design, provided a powerful tool to decipher the molecular mechanisms of genes/pathways involved in the ageing processes<sup>(4,5)</sup>.

The aim of this review is to provide a brief introduction to twin study design as well as to report some of the latest findings in ageing research using this powerful method.

#### Classic twin design

Twins have fascinated human communities since the beginning of recorded history and feature in the legends and myths of many cultures<sup>(6)</sup>.

Twin studies are an invaluable tool to investigate trait variability in the normal population. Classic twin studies compare concordance of the trait/disease between

**Table 1** Genetic component of the most relevant age-related disease/risk-factors

	Heritability (%)	Reference
Age-related hearing impairment	40	Christensen <i>et al.</i> <sup>(18)</sup>
Age-related macular degeneration	45	Hammond <i>et al.</i> <sup>(19)</sup>
Alzheimer's	79	Gatz <i>et al.</i> <sup>(14)</sup>
Apo A1	46 (M)–55 (F)	Snieider <i>et al.</i> <sup>(20)</sup>
Apo B	65 (M)–59 (F)	Snieider <i>et al.</i> <sup>(20)</sup>
BMI	83 (M)–74 (F)	Lajunen <i>et al.</i> <sup>(21)</sup>
Bone mineral density – femoral neck	84	Arden <i>et al.</i> <sup>(22)</sup>
Bone mineral density – lumbar	78	Arden <i>et al.</i> <sup>(22)</sup>
Bread intake	18	Pimpin <i>et al.</i> <sup>(16)</sup>
CAD (mortality)	57 (M)–38 (F)	Marenberg <i>et al.</i> <sup>(13)</sup>
Cancer – breast	27	Lichtenstein <i>et al.</i> <sup>(15)</sup>
Cancer – colorectum	35	Lichtenstein <i>et al.</i> <sup>(15)</sup>
Cancer – lung	26	Lichtenstein <i>et al.</i> <sup>(15)</sup>
Cancer – ovary	22	Lichtenstein <i>et al.</i> <sup>(15)</sup>
Cancer – prostate	42	Lichtenstein <i>et al.</i> <sup>(15)</sup>
Cognitive function	80	Pedersen <i>et al.</i> <sup>(23)</sup>
C-reactive protein	52	MacGregor <i>et al.</i> <sup>(24)</sup>
Diabetes type II	75	Kaprio <i>et al.</i> <sup>(25)</sup>
Diastolic blood pressure	48	Evans <i>et al.</i> <sup>(26)</sup>
Dairy intake	17	Pimpin <i>et al.</i> <sup>(16)</sup>
Egg intake	6	Pimpin <i>et al.</i> <sup>(16)</sup>
Forced expiratory volume in 1 s	36	Hukkinen <i>et al.</i> <sup>(27)</sup>
Frailty	43	Dato <i>et al.</i> <sup>(28)</sup>
Fruit intake	10	Pimpin <i>et al.</i> <sup>(16)</sup>
Grip strength	62	Frederiksen <i>et al.</i> <sup>(29)</sup>
HDL	69	Snieider <i>et al.</i> <sup>(20)</sup>
Lipoprotein(a)	89	Snieider <i>et al.</i> <sup>(20)</sup>
Longevity	26 (M)–23 (F)	Herskind <i>et al.</i> <sup>(11)</sup>
LDL	77 (M)–69 (F)	Snieider <i>et al.</i> <sup>(20)</sup>
Meat and fish intake	9	Pimpin <i>et al.</i> <sup>(16)</sup>
Osteoarthritis	54	Spector <i>et al.</i> <sup>(30)</sup>
Plasma homocysteine	57	Siva <i>et al.</i> <sup>(31)</sup>
Systolic blood pressure	53	Evans <i>et al.</i> <sup>(26)</sup>
Total cholesterol	77 (M)–66 (F)	Snieider <i>et al.</i> <sup>(20)</sup>
TAG	69 (M)–51 (F)	Snieider <i>et al.</i> <sup>(20)</sup>
Vegetable intake	15	Pimpin <i>et al.</i> <sup>(16)</sup>

CAD, coronary artery disease; M, male; F, female.

monozygotic (MZ) twin pairs, which are virtually genetic clones (as they result from a single fertilised egg) *v.* dizygotic (DZ) or non-identical twin pairs, which share on average 50 % of the genetic information providing the estimate of the genetic and environmental components of a given trait.

The environmental variables contribute to the expression of complex traits/diseases. Therefore, because MZ and DZ twin pairs are exposed to similar pre- and post-natal environmental factors (equal environment assumption)<sup>(7,8)</sup>, if the MZ are more similar than the DZ when compared for a particular trait/disease, then the observed phenotypic variation is likely to be the result of the genetic component. In this context, it is possible to estimate both the heritability ( $h^2$ ; twice the difference between the correlation between the MZ ( $r_{MZ}$ ) and the correlation between DZ twins ( $r_{DZ}$ )<sup>(9)</sup> and proportion of the variance that is due to a shared environment for MZ ( $r_{MZ}-h^2$ ) and DZ ( $r_{DZ}-h^2/2$ ) of the analysed trait/disease<sup>(9)</sup>.

Heritability studies of twin cohorts provided the first evidence of the genetic component for most

diseases/traits. It was common belief, for example, that some diseases (e.g. autism, multiple sclerosis and attention deficit hyperactivity disorder) were the result of family/environmental exposure. Twin studies highlighted their genetic component, thus pointing the research towards new directions<sup>(10)</sup>.

### Twin studies of longevity and age-related diseases

In the past century, the unique genetic make-up of twins was successfully used to explore the genetic and environmental contribution underlying a number of traits/diseases related to ageing. In particular, twin studies defined the genetic component of longevity<sup>(11,12)</sup>, CVD<sup>(13)</sup>, Alzheimer's disease<sup>(14)</sup> and cancer<sup>(15)</sup> as well as the influence on dietary intake<sup>(16,17)</sup> (Table 1).

The analysis of a Danish twin cohort estimated that the heritability for human lifespan was between 20 and 26 % depending on the sex and the model used<sup>(11)</sup>. In other words, one quarter of the human longevity variation is due to genetic factors. A further study based

on a larger twin dataset of North European descent not only confirmed the previous results but also highlighted that the genetic influences are minimal prior to age 60 years and increase thereafter<sup>(12)</sup>.

In 1994, Marenberg *et al.*<sup>(13)</sup> studied the concordance on 10 502 Swedish twin pairs to investigate the genetic basis of coronary artery disease (CAD) and myocardial infarct mortality. Their findings clearly highlighted the presence of a genetic component in CAD/myocardial infarct mortality. The authors showed that the relative hazard (RH) of death by early onset (before age 55 years) CAD was double in male MZ twins (RH=8.1) compared with male DZ twins (RH=3.8), with a RH nearly six times higher in females (RH<sub>MZ</sub>=15; RH<sub>DZ</sub>=2.6). These results were further validated in a follow-up study of the same cohort, with the heritability of CAD/myocardial infarct mortality, ranging from 0.38 in females to 0.57 in males<sup>(32)</sup>. In the past 40 years, twin studies have also highlighted the genetic component of other age-related cardiovascular risk factors including lipid levels<sup>(20)</sup>, blood pressure<sup>(26)</sup>, C-reactive protein<sup>(24)</sup>, plasma homocysteine<sup>(31)</sup> and diabetes<sup>(25)</sup> (Table 1).

An increasingly large body of evidence highlights the link between early years dietary intake and risk of obesity and ultimately increase of CVD risk later in life<sup>(33)</sup>. Indeed, to discriminate between genetic components and environmental elements of the food preference is an essential first step to identify interventional targets to improve dietary intake. A recent study<sup>(16)</sup> based on 21-month-old twins, revealed that the shared environment was the predominant determinant of the dietary intake variation (ranging from 66 to 97%). These results were consistent with previous studies in older twin<sup>(17)</sup>, emphasising the importance of the family environment in the healthy dietary pattern to prevent health problems later in the adult life.

Upon the completion of the Human Genome Project in 2003, the fast development of high-throughput SNP genotyping technology has revolutionised the way age-related trait/disease are analysed, opening the so-called 'genomic gold rush'<sup>(34)</sup>. Genome-wide association studies enabled the identification of a large number of loci/genes involved in age-related traits/diseases. To date, up to 1669 papers have reported genome-wide association analysis/meta-analysis results; of these more than half (925) had as main target one age-related trait/disease<sup>(35)</sup>.

Despite their pivotal role in understanding the genetic component of age-related traits, twin studies could not be fully exploited during the genome-wide association era.

In fact, because MZ share their entire genome they cannot be fully employed in gene mapping and DZ twins are not different from ordinary siblings. However, it should be emphasised that the use of DZ twin studies for gene mapping are theoretically more powerful than sibling studies because they can take advantage of the unique characteristics of twins: shared pre/post-natal factors, matched age and non-paternity (an important cause of error in sib-pair analysis) reduced to almost nil.

### When classic twin design met new 'omics'

As in other multifactorial traits, the genetic component of human ageing represents only one aspect of it. Over time, lifestyle and environmental factors also play an important role. Since we are not able to control the genetic element, it is paramount to understand how environmental and lifestyle factors influence the ageing process in order to manage a healthy ageing policy.

Epigenetic mechanisms, under the influence of environmental/lifestyle factors, modify gene expression and ultimately have an impact over the possibility to develop a particular disease.

The latest research highlighted how exposure to particularly adverse conditions (e.g. starvation) in early stages of development has an impact on epigenetic markers later in life<sup>(36–38)</sup>, and is associated with increased risk of developing age-related diseases (e.g. CAD/myocardial infarct, obesity, hypertension and diabetes)<sup>(39–44)</sup>.

Over the past decade the development of new high-throughput tools to study epigenetic markers (e.g. microarrays and next generation sequencing) opened the door to DNA methylation analysis on a genome-wide scale<sup>(45)</sup>.

One of the first indications that epigenetic modifications change with age was provided by a MZ twin study in 2005<sup>(46)</sup>. The authors found that during the early years of their life MZ twins are epigenetically indistinguishable. However, the analysis of older MZ twins exhibited remarkable differences of both local and global methylation patterns. These differences affect their gene-expression portrait<sup>(46)</sup>.

In a recent study using a the newly developed microarray methylation assay, the authors analysed the genome-wide methylation profile of both MZ and DZ twin couples<sup>(47)</sup>. They found that 23% (96 over 431) of the CpG sites yielded a significant heritability ranging from 94 to 57%<sup>(47)</sup>. They also identified fifty-eight probes that were significantly related to age<sup>(47)</sup>.

Using a similar approach, Bell *et al.*<sup>(48)</sup> not only reported novel age-related differentially methylated regions, but also provided the first evidence that age-related differentially methylated region changes occur throughout the individual's lifespan and that a proportion of these may start from an early age stage.

Metabolomics is another 'omics' which recently has generated much interest in the age-related disease/trait analysis. Metabolomics is the unbiased analysis (quantitative and qualitative) of the complete set of small, low molecular weight metabolites present in cells, body fluids and tissues (the metabolome)<sup>(49)</sup>.

The origin of this field goes back at least as far as ancient Greece<sup>(50)</sup>, but it has become the latest tool to investigate molecular changes related to the ageing process, helped by the latest advances in technology, which now allow us to measure thousands of metabolites per individual at once.

The special advantage of this new 'omics' is to combine the effect of both environmental and lifestyle factors with genomics. Metabolite concentrations result from external factors (e.g. as dietary patterns), while their



associations with genes, proteins and SNP come from the metabolome interactions within the bigger biological network. In this scenario, changes in the metabolome are amplified compared with changes in the genome and transcriptome, providing more power to detect new pathways for age-related traits<sup>(51)</sup>.

Indeed, changes in individual's human metabolic phenotype (metabotype) over longer time periods can be indicative of disorder-related modifications, possibly preceding overt disease symptoms<sup>(52)</sup>.

In a recent study based on a large twin population (*n* 6055), Menni *et al.*<sup>(4)</sup> identified a panel of twenty-two independent metabolites associated with age. These twenty-two metabolites, which combined explain 59% of the variance, can be used as surrogate measure of chronological age<sup>(4)</sup>. In particular one metabolite, C-glycosyl tryptophan, not only correlates strongly with age and with some age-related traits (e.g. the forced expiratory volume and the bone mineral density) but is also associated with birth weight. This may indicate that this metabolite may be involved in one of the early development processes that determine the health status in midlife and old age<sup>(4)</sup>.

Finally, two emerging 'omics' (glycomics and metagenomics) have already provided the first evidence of substantial correlation with age<sup>(53–57)</sup>. Applying the twin model to study these new fields may provide, in the near future, new insights into the ageing process.

### Conclusions

In less than a decade the development of new biomedical and bioinformatic technologies (e.g. microarray and next generation sequencing) revolutionised ageing and medical research. The new available tools facilitated the genome-wide measure of biological entities (e.g. genes, proteins, epigenetic signatures and metabolites) through large datasets.

In the post-genomic era, classical twin design, taking advantage of its unique characteristics, will supply an invaluable tool for the combined analysis of all the new 'omics' (epigenomics, metabolomics and in the near future glycomics and metagenomics) in order to decipher nature and nurture signatures characteristic of the ageing process.

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### Conflicts of Interest

None.

### Authorship

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