Ageing Research Reviews 12 (2013) 182–187

Contents lists available at SciVerse ScienceDirect

Ageing Research Reviews

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

Review

Twins for epigenetic studies of human aging and development

Qihua Tan[a,b,*], Lene Christiansen[a,b], Mads Thomassen[b], Torben A. Kruse[b], Kaare Christensen[a,c]

[a] The Danish Twin Registry and The Danish Aging Research Center, Institute of Public Health, University of Southern Denmark, J. B. Winsløws Vej 9B, DK-5000, Odense C, Denmark
[b] Department of Clinical Genetics, Odense University Hospital, Sdr. Boulevard 29, DK-5000, Odense C, Denmark
[c] Department of Clinical Biochemistry and Pharmacology, Odense University Hospital, Sdr. Boulevard 29, DK-5000, Odense C, Denmark

ARTICLE INFO

Article history:
Received 18 March 2012
Received in revised form 16 June 2012
Accepted 21 June 2012
Available online 29 June 2012

Keywords:
Twins
Aging
Epigenetics
Environments
Genomics

ABSTRACT

Most of the complex traits including aging phenotypes are caused by the interaction between genome and environmental conditions and the interface of epigenetics may be a central mechanism. Although modern technologies allow us high-throughput profiling of epigenetic patterns already at genome level, our understanding of genetic and environmental influences on the epigenetic processes remains limited. Twins are of special interest for genetic studies due to their genetic similarity and rearing-environment sharing. The classical twin design has made a great contribution in dissecting the genetic and environmental contributions to human diseases and complex traits. In the era of functional genomics, the valuable sample of twins is helping to bridge the gap between gene activity and the environments through epigenetic mechanisms unlimited by DNA sequence variations. We propose to extend the classical twin design to study the aging-related molecular epigenetic phenotypes and link them with environmental exposures especially early life events. Different study designs and application issues will be highlighted and novel approaches introduced with aim at making uses of twins in assessing the environmental impact on epigenetic changes during development and in the aging process.

1. Introduction

The human life expectancy experienced a remarkable increase during the last century with an unprecedented gain in the developed world (Christensen et al., 2009) and similar improvement has been observed or expected in the developing countries. At the same time, researches have shown even larger improvement in health expectancy than in lifespan (Jeune and Brønnum-Hansen, 2008; Robine, 2006). These phenomena suggest the important role of improving environment in determining individual health status and survival. Although social-economic development and consequently advances in biomedical technology together with improved healthcare and disease treatment can be considered in explaining these changes, studying the biological mechanism of how environmental attributes affect individual health is of central importance to public health. In the literature, recent studies have shown that the impact of environmental factors can be acquired via the epigenome or genome in epigenetics (Fraga et al., 2005; Wong et al., 2005; Poulsen et al., 2007; Szyf et al., 2008; Ling and Groop, 2009; Tan et al., 2010; Petronis, 2010), one of the current topics in cancer and complex disease studies drawing active research. Although the molecular evidences are interesting and current techniques such as those offered by Affymetrix and Illumina allow genome-wide epigenetic (DNA methylation) profiling, identifying and understanding the epigenetic patterns under a given genetic predisposition and environmental exposures impose new challenges to traditional epidemiology both in experimental design and in methodological issues.

Twins are of special interest for genetic studies due to their genetic similarity and rearing-environmental sharing. The last century witnessed successful uses of twins in exploring the genetic and environmental contributions to human diseases and other complex traits like, e.g. life span and aging. The twin design makes use of the unique genetic make-ups in twins and infers the genetic importance in human diseases or traits. By comparing phenotype correlation in identical or monozygotic (MZ) and fraternal or dizygotic (DZ) twin pairs, various genetic and environmental components can be assessed using the classical twin design. For example, the heritability for human lifespan was estimated about 25% using Danish twins (Herskind et al., 1996; Hjelmborg et al., 2006) meaning that about one quarter of the human life span variation can be accounted for by genetic factors. In our recent review (Tan et al., 2010), the use of twins in functional genomic studies of human complex diseases has been summarized and new approaches that expand the traditional twin design to molecular genetic studies proposed. Here, we emphasize the novel use

* Corresponding author at: Epidemiology, Institute of Public Health, University of Southern Denmark, Winsløws Vej 9B, DK-5000 Odense C, Denmark.
Tel.: +45 6550 3536.
E-mail address: qtan@health.sdu.dk (Q. Tan).

1568-1637/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.arr.2012.06.004

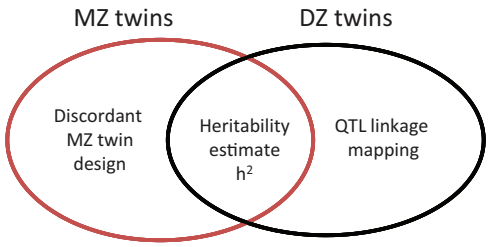

Fig. 1. The uses of twins in epigenetic studies. To the left, MZ twin pairs discordant for one trait or phenotype can be sampled and analyzed for identifying loci under differential epigenetic modification. To the right, non-parametric quantitative trait loci linkage mapping can be applied to molecular epigenetic phenotypes in DZ twins to map genes showing *trans*- or *cis*- regulatory mechanisms. In the middle, with both MZ and DZ twins, the classical twin model can be extended to molecular epigenetic phenotypes for assessing the genetic and environmental contributions in epigenetic control over gene activities.

of identical twins discordant in disease phenotypes in epigenetic studies which can be a powerful approach in linking disease with gene regulation patterns and environmental exposures. Likewise, the human aging phenotypes are complex phenotypes that can be affected by a large number of both genetic and environmental factors together with their interplay. With proper and efficient design, twins can offer remarkable opportunities for functional genomic studies of human aging and development. In this paper, we highlight the different twin study designs and application issues and summarize the newest development in making uses of twins in assessing the environmental impact on epigenetic changes during development and in the aging process (Fig. 1).

2. New technology and new opportunities

Right at conception, the genomic DNA sequence is identically fixed. However, the functional profile of a gene is determined not only by its sequence but also by the way in which it is regulated by epigenetic mechanisms including DNA methylation, histone modification, different species of non-coding RNAs, etc. It is anticipated that, perhaps the most significant change in the 21st century genetics will be the shift from structural genomics, where genes are regarded as a static concept, to functional genomics, where the dynamic patterns of gene activity are analyzed jointly from gene interaction to gene regulation and to functional genomics analysis (Peltonen and McKusick, 2001). Among the various mechanisms, DNA methylation is the major form of epigenetic modification that is most robust and readily measurable using high throughput techniques. For example, the Affymetrix high-density tiling arrays using chromatin immunoprecipitation (ChIP-on-chip), Illumina Bead Arrays, and Illumina Solexa sequencing based DNA methylation analysis by sequencing bisulphite-treated DNA (Zhang and Jeltsch, 2010). All these techniques enable massive epigenetic profiling at genome scale.

The epigenetic modification involves binding of small molecules to specific sites in DNA or chromatin (Foley et al., 2009) which acts as 'volume controls' that up or down regulate a gene's expression without changing its DNA sequence. For example, a gene is generally silenced by methylation at the 5′ position of the cytosine pyrimidine ring in DNA at a CpG (cytosine and guanine nucleotides linked by phosphate) dinucleotide, known as CpG islands, clustered in the promoter region of many genes. At certain times during development, specialized cellular machinery scours the genome and erases its epigenetic tags in order to restore a genetic "blank state" during which a small minority of genes make it through this process and pass their epigenetic tags unchanged from parent to offspring (Pál and Hurst, 2004). This phenomenon suggests that the molecular mechanisms of heritability may not be limited to DNA sequences. The inheritance of epigenetic patterns could help to explain, in part, the missing heritability in current GWAS studies on complex diseases or phenotypes (McCarthy and Hirschhorn, 2008).

Of important properties for epigenetic regulation of gene activities is that it is under control of both genetic and environmental factors. For example, individual variations at the methylenetetrahydrofolate reductase gene can vary the levels of DNA methylation (Castro et al., 2004; Friso et al., 2002). Dissecting the genetic and environmental components in the measured epigenetic status and linking it with disease conditions or the aging process imposes a new challenge to biostatisticians and bioinformaticians in health and aging research.

3. Modern use of a classical design

Aging is a natural process that everyone must undergo at his or her own time and pace. Although genetics may initially determine how a person ages, environment could over time play a higher role than the genes during aging.

Environments especially early life events are important modifiers of the aging process (Szyf, 2012, 2009; Murgatroyd and Spengler, 2011). Since the genetic mechanisms behind aging are not controllable, understanding how environmental factors retard or accelerate the aging process is of more practical impact. For that purpose, it is essential to dissect the genetic and environmental components for which twins can contribute. In brief, the relative importance of genetic and environmental components in the variation of a phenotype can be assessed by comparing phenotype correlation within identical twin pairs who share their nuclear DNA with that within DZ twin pairs who share only 50% of DNA sequence on average. In the literature, the classical twin design has been applied to multiple aging phenotypes including for example, physical (Frederiksen et al., 2002) and cognitive (Greenwood et al., 2011; Reynolds et al., 2005) functions in the elderly. A similar twin method was applied to gene expression data from elderly Danish twins (Tan et al., 2005). This analysis gave relatively high heritability estimates for the activity levels of the top most active genes.

Recently, the twin method has been applied to studying global DNA methylation profiles in MZ and DZ twins (Kaminsky et al., 2009) with highly significant epigenetic differences reported in DZ twins as compared with that in MZ twins. The result suggests that both genetic and environmental factors influence epigenetic regulation of gene activities and provides a model for measuring their relative contributions. Note that DNA methylation levels are dynamic and change throughout an individual's life course. However, both animal and human data suggest early life milieu (including intrauterine life) have a great impact on the epigenome which can be linked to the development of obesity (Lillycrop and Burdge, 2011), diabetes (Fradin and Bougnères, 2011), stress (Murgatroyd and Spengler, 2011) and late-onset mental illnesses (Szyf et al., 2007; McGowan and Szyf, 2010). This can be due to the fact that many of the differential gene expressions in determining the structural and functional differentiation of cells in a multicellular organism arise during development. Epigenetic changes triggered by early life environment offer a plausible mechanism by which early experiences could be integrated into the genome to program adult hormonal and behavioral responses, thus facilitating the adaptation of an organism to changing environment through alterations in gene activity. Of special interest in the classic twin model that contains additive genetic (A), common environmental (C) and unique environmental (E) effects, i.e. the ACE model, the C component reflects the common rearing environment in their early lives. When applied to epigenetic measurement data