

Genetic susceptibility to ageing-associated diseases

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Abstract – The constant and rapid increase of life expectancy in western countries is associated with a major ageing of our populations. In these conditions, we can expect an epidemic progression of most chronic diseases, especially cardiovascular, neurodegenerative and metabolic disorders, the main causes of death in the world. The global burden of these diseases will have a dramatic impact on the health and on the socio-economical context of our societies. From a global point of view, the occurrence and progression of these multifactorial diseases rely upon the nature and intensity of the environmental determinants we are exposed to all life long, but also to our individual genetic susceptibility. Through the determination of this higher susceptibility to an environmental risk factor and the understanding of its mechanisms of action, prevention and management efforts will be better focused. In such multifactorial affections, the development and the transmission of the disease do not follow the simple laws of monogenic Mendelian models. The complexity of this transmission is associated with the influence, at various degrees, of several genes and of a close interaction between this particular genetic susceptibility and environmental risk factors. With the recent development of automated and high throughput molecular biology techniques and their use in epidemiological studies, gene expression regulation and post genomic studies, the determination of sub-groups facing a higher individual genetic susceptibility has begun. This determination will offer new clues for a better-targeted disease management. *To cite this article: P. Amouyel, C. R. Biologies 325 (2002) 741–745.* © 2002 Académie des sciences / Éditions scientifiques et médicales Elsevier SAS

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Résumé – **Susceptibilité génétique aux maladies associées au vieillissement.** L'augmentation constante et rapide de l'espérance de vie dans les pays occidentaux s'accompagne d'un vieillissement majeur de nos populations. Aussi, devons-nous nous attendre à une croissance épidémique de la plupart des maladies chroniques, et particulièrement des affections cardiovasculaires, neurodégénératives et métaboliques, principales causes de décès dans le monde. L'explosion généralisée de ces maladies aura un lourd impact sur la santé et sur l'environnement socio-économique de nos sociétés. D'un point de vue global, la survenue et la progression de ces maladies multifactorielles reposent sur la nature et l'intensité des déterminants auxquels nous sommes exposés tout au long de notre vie, mais également à notre susceptibilité génétique individuelle. À partir de la caractérisation de cette susceptibilité plus élevée à un facteur de risque environnemental et de la compréhension de ses mécanismes d'action, les efforts de prévention et de prise en charge seront mieux ciblés. Dans de telles affections multifactorielles, le développement et la transmission de la maladie ne suivent pas les lois simples des modèles mendéliens monogéniques. La complexité de cette transmission est associée à l'influence, à des degrés divers, de plusieurs gènes et aux interactions étroites entre une susceptibilité génétique particulière et des facteurs de risque environnementaux. Grâce aux développements récents de l'automatisation et du haut débit des techniques de biologie moléculaire, ainsi que de leurs applications aux enquêtes épidémiologiques, à la régulation de l'expression des gènes et aux études post-génomiques, la caractérisation de sous-groupes présentant une susceptibilité génétique importante aux maladies liées au vieillissement a commencé. Cette caractérisation offrira de nouvelles pistes pour une prise en charge mieux ciblée de ces maladies. *Pour citer cet article : P. Amouyel, C. R. Biologies 325 (2002) 741–745.* © 2002 Académie des sciences / Éditions scientifiques et médicales Elsevier SAS

susceptibilité génétique / interactions gène-environnement / maladies liées au vieillissement / génomique / pharmacogénomique

1. Introduction

During the last thirty years, life expectancy has continuously increased in most western countries. The estimated trends of population ageing in today's 15 European countries suggest that in 2050, the size of the European population aged 50 years and over will reach 145 to 185 millions (vs 120 millions in 1995) according to different demographic simulations [1]. This significant increase in life expectancy in western countries is accompanied by an increase in the frequency of degenerative chronic diseases, whose impact on the health of populations constitutes a major stake for the society. This global burden of ageing-associated diseases (cardiovascular, neurodegenerative and metabolic diseases, cancers...) will also have a dramatic impact on the socio-economical conditions of our European society [2]. A way to reverse this trend is to speed up research in the field of ageing and longevity to better approach the questions of chronic diseases treatments. Indeed, improving knowledge and finding solutions to compress the morbidity will help to increase life expectancy without disability and fight against this epidemic threat [3].

The risk of occurrence of ageing-associated diseases depends on the nature and intensity of environmental factors to which more or less susceptible individuals are submitted. The customary approach to the assessment of the impact of an environmental risk factor for a multifactorial disease in a population consists in appraising an average risk linked to this factor, adjusted to the demographic variables and the main confounding factors. However, if in a population taken as a whole the relative risk of becoming affected by this disease represents, for example, an average of 1.5 for the people exposed to this factor, this relative risk may today be 5.2 for 10% of the population and 1.1 for the remaining 90%. The characterisation of this individual susceptibility added to a risk factor and the understanding of its modes of action will give the possibility to focus on prevention and case management endeavours.

These gene–environment interactions can now be tackled thanks to the integration of epidemiology and to the control of molecular biology tools adapted to population approaches [4]. The recent development of automated molecular biology techniques and the use of their results in epidemiological studies has allowed starting characterising these sub-groups constitutionally exposed to this high relative risk. Such research endeavours should lead to a better knowledge of causal factors of ageing diseases, and bring new primary and secondary prevention as well as therapeutic case management prospects.

2. Research strategies

Many degenerative chronic diseases occur more frequently on people related to individuals affected than in the general population [5]. Several approaches have given the possibility to show that such family impact is partly of genetic origin. However, the occurrence and transmission of such pathologies do not follow the simple laws of the Mendelian monogenic patterns [6]. The complexity of this transmission is related to the intervention, at various degrees, of several genes and to the close interaction of genetic susceptibility factors so defined with the environmental risk factors. For example, it is important to understand how certain genetic factors involved in dyslipemias, myocardial infarction or hypertension seem to have different effects, whether or not the individuals are obese [7].

The concept of genetic susceptibility factors could be comprehended thanks to the developments of the study on genetic polymorphisms, enabling to appraise the variability of the genome. The genome is generally constant for any given species. However, a significant variability degree in the nucleotide sequence ensures the diversity of individuals in the species. This variability is expressed through the existence of mutations in precise points on the genome. These mutations can be the direct cause for the variation of a phenotypic trait: it is then a functional mutation; or these mutations may have no direct effect on a trait, but be linked, through evolutionary accidents, to a phenotype or a pathology: thus, these are non-functional markers, very useful to localise susceptibility genes on the genome.

The characterisation of genetic risk factors relies on two strategies. The first one consists in searching for the co-segregation, in families, of most often anonymous polymorphic genetic markers, placed at regular intervals on the genome, and of the disease. As a consequence, it is theoretically possible to locate the sequence of a gene, known or most of the time unknown, making segregation with the disease on a chromosome, and then on a precise area on this chromosome. The second approach relies on genes a priori selected for their possible links with the disease. These genes, called candidate genes, are usually involved in coding for proteins implicated in the pathophysiology of the disease studied. This last strategy can apply to studies of the family type, but also to studies of association in samples of unrelated individuals [8]. Such methods consist in comparing the frequency of the polymorphism of a candidate gene in people affected with the disease to that of the same polymorphism in control.

To perform these comparisons, it is important to be able to set up studies with a significant number of subjects necessary to obtain a sufficient statistical power. This number depends on the frequency of the genetic polymorphism in the population. A number of 200 subjects per group (case or control subjects) represents a minimum for a candidate gene, given the customary frequency of the polymorphisms studied. If we want to appraise the risk linked to one or more susceptibility factors and the risk linked to environmental factors simultaneously, a minimum number of 1000 subjects per group then becomes necessary [9].

The association thus described remains a statistical association and requires that a number of causality presumption criteria be reviewed. The qualitative or quantitative measure of the protein coded by the candidate gene allows defining what is called an intermediate phenotype. The association of the genetic polymorphism to any variation in this intermediate phenotype and to the disease is an argument that reinforces the plausibility of the link found. The multicentric recruitment of a number of groups of affected persons and control subjects originating from various populations in one single study authorises the study of the homogeneity of the relationship in various populations. Finally, it is important to search for an external consistency for the association, which means that several studies conducted completely separately lead to the same results.

3. The example of the apolipoprotein E polymorphism

Among the numerous candidate genes studied as potential susceptibility risk factors of chronic diseases, the apolipoprotein E and its frequent polymorphism offers a special example of the complex relationships that may exist between ageing diseases and genetic determinants.

Apolipoproteins are lipid transporters. Among these, the apolipoprotein E (APOE) plays a central role [10]. This apolipoprotein exists as three frequent isoforms in human populations: APOE2, APOE3 and APOE4. These three isoforms are coded by three different alleles: $\epsilon 2$, $\epsilon 3$ and $\epsilon 4$. APOE is expressed in liver, macrophages, kidney, lung and brain. These isoforms are associated with heterogeneity of lipid and lipoprotein plasma levels. APOE3, the most frequent isoform (70 to 80%), is characterised by a cystein residue at position 112 and an arginin residue at position 158 of the coding sequence, and considered as the reference isoform. APOE4, characterised by arginin residues at position 112 and 158, is associated with increased plasma

concentrations of low-density lipoprotein (LDL) cholesterol levels. Conversely, APOE2, characterised by cystein residues at position 112 and 158, is associated with lower levels of LDL-cholesterol. APOE4 is associated with an increased risk of myocardial infarction and, although discussed, APOE2 is linked with a decreased risk of cardiovascular disease [11]. Moreover, APOE is present in senile plaques of human brains affected with Alzheimer's disease (AD). The $\epsilon 4$ allele was found to be a major risk factor of AD and of various other neurodegenerative processes related to cognitive impairment [12]. Similarly, to what is reported for cardiovascular diseases, $\epsilon 2$ allele is considered as a major protective factor for AD [13]. Thus, a common molecular determinant is associated with deleterious and protective effects in two different diseases affecting the cardiovascular system and the central nervous system.

Lipids are involved in multiple metabolic pathways and may be related to other pathophysiological mechanisms. For instance, age-related macular degeneration (AMD) is the most common cause of blindness in the elderly in Europe and in the United States [14]. In western countries, more than five millions individuals aged 85 years and over will be affected by AMD in 2020. The diagnosis of AMD is suspected on eye examination of patients [15]. This examination, combined with fluorescein angiography detects several lesions. Irregular pigmentation of the retina and choroid, and accumulations of debris can be seen on retinal examination. Such debris are called drusen, from a German word meaning stony nodule. Drusen are yellow deposits in the deep part of the retina [16]. These drusen are seen in 50% of the white population over 50 years and represent accumulation of debris from the metabolic process of vision. These debris accumulate beneath retinal pigment epithelium through Bruch's membrane and basal lamina. Drusen are characterised by protein and lipid deposits. They contain cholesteryl esters, unsaturated fatty acids and sometimes phospholipids. This observation suggests that lipid trafficking may be an interesting metabolic pathway to identify candidate genes in AMD. In a case-control study comparing patients with AMD to controls of similar age and gender, we analysed the influence of APOE alleles on the occurrence of AMD [17]. An unexpected protective effect of the presence of at least one APOE $\epsilon 4$ allele was observed in AMD with an odds ratio of 0.34 (95% CI = [0.17 – 0.68]). An independent study developed in a Dutch population replicated these results [18].

Thus, APOE is involved in several chronic diseases in human. This involvement was approached by the

existence of a genetic polymorphism, frequent in humans. However, according to the type of the organ implicated in the disease, the effect of each allele may significantly vary. APOE4 is a risk factor of dyslipidemia, of coronary heart disease, of Alzheimer's disease and other neurodegenerative diseases, but is a protective factor for AMD. On the other hand, APOE2 is associated with low LDL-cholesterol levels, a lower risk of AD and with better cognitive functioning. These observations are not systematically verified for all subjects. For instance, all patients carrying an APOE ϵ 4 allele do not develop Alzheimer's disease. Recent studies suggest that quantitative effects [19], associated with the qualitative effects of APOE isoforms are important in the setting of disease risk level. These quantitative variations seem to be supported by other APOE mutations located in the promoter region [20, 21]. Moreover, interactions with environmental factors as fat consumption or light exposition may modulate the levels of risk.

Thus, the study of a frequent polymorphism of a common protein opens new area of knowledge in the field of the classification of the diseases, of the molecular mechanisms involved in these pathologies and presumably in prevention and treatments.

4. Perspective and contribution

These general research approaches of the genetic susceptibility of ageing diseases contribute to the progress of knowledge of chronic diseases regarding at least three main areas.

Firstly, these approaches allow generating new hypotheses on the modes of occurrence. Given the rapid evolution of the environment within western societies, it is possible to comprehend the occurrence of certain chronic diseases as being linked to the deleterious effects of genes initially selected for beneficial effects in quite different environmental conditions. This theory initially developed by Neel [22] suggests indeed that certain genes, which he qualifies as 'thrifty genes', favourable to survival in hard conditions (malnutrition, large-scale migrations), would become unfavourable in the current environmental conditions (overfeeding, lack of physical activity). Illustrations for such hypotheses exist for such diseases, as diabetes or high blood pressure. Comparisons, which we are developing in large-scale population surveys, allow us to detect such major interactions between genetic susceptibility factors and the environment implied in the genesis of diseases and to deduce potential mechanisms for their prevention.

Secondly, a different classification of diseases based on molecular physiopathological mechanisms can be

proposed. The characterisation of the response to a causal factor according to the susceptibility of an individual tends to modify the classification of heterogeneous pathologies, the clinic and evolution constituting the main classification elements so far. For example, a genetic susceptibility factor such as the APOE polymorphism plays a part both in the circulatory system and in the nervous system. From properties of transport of lipids and other substances, the APOE is involved in cellular regeneration mechanisms subsequently to an injury [23]. A quantitative or qualitative variation of this transport function may have a harmful or favourable effect on the injured tissue, slowing down or accelerating its repair. In the case of the E4 isoform of the APOE, it may be an over-accumulation of atherogenic lipoproteins in the vascular endothelium after a dietary intake containing an excessive amount of saturated fats, or of amyloid peptide in the cerebral tissue after the axonal regeneration following a cranial trauma. The concept of pathologies linked to tissue repair disorders could thus be defined. This approach to the classification of diseases through molecular epidemiology also allows making hypotheses concerning the physiopathological mechanisms at the origin of degenerative chronic diseases that recent genomic and proteomic techniques allow to tackle more efficiently today.

Finally, a better targeted management of chronic diseases and more adapted prevention measures can be potentially achieved. The approach to pathologies through gene–environment interactions allows us to define new management ways. Indeed, treatments may be considered as environmental factors. Thus gene–treatment interactions can now be studied, leading to new approaches in pharmacology. Supported by an exciting breakthrough and by the recent access to automated and high throughput techniques, global strategies based on genomics and genetics have been set to unravel the complexity of ageing diseases and to speed up research process and drug development. This evolution opens a new research field: the pharmacogenomics [24,25].

These research targets related to ageing diseases must now be integrated into the technological and conceptual revolution linked to the development of genomics and post-genomics. Indeed, the short-term outcome of the Human Genome Project, the development of high-throughput analysis techniques concerning both genes and their transcripts, the optimisation of proteomics methods open important investigation fields for the understanding of the genetic susceptibility to ageing diseases.

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