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Environmental Neurology

Gene-environment interaction and Mendelian randomisation

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ABSTRACT

Genetic factors only account for up to a third of the cases of Parkinson's disease (PD), while the remaining cases are of unknown aetiology. Environmental exposures (such as pesticides or heavy metals) and the interaction with genetic susceptibility factors (summarized in the concept of impaired xenobiotic metabolism) are believed to play a major role in the mechanisms of neurodegeneration. Beside of the classical association studies (e.g. genome-wide association studies), a novel approach to investigate environmental risk factors are Mendelian randomisation studies. This review explores the gene-environment interaction and the gain of Mendelian randomisation studies in assessing causalities of modifiable risk factors for PD.

1. Introduction

Parkinson's disease (PD) is the most common movement disorder and the second most frequent neurodegenerative disease after Alzheimer's disease (AD) [1,2]. First described two centuries ago, the underlying cause of PD still remains unknown for the majority of the cases, however, there are recognized factors which increase the risk for PD, including aging, environmental exposure (pesticides or heavy metals) and genetic factors (genetic risk variants and monogenic Mendelian traits) – currently up to 30% of all PD cases can be referred to a genetic contribution [3].

Fundamentally, the motor symptoms of PD – as a prototype for neurodegenerative disorders– are mainly characterized by a progressive loss of dopaminergic neurons linked with an accumulation of aggregation-prone alpha-synuclein protein. These intracellular alpha-synuclein inclusions are a more generalized process affecting different types of neurons in the central and peripheral nervous system contributing to the spectrum of non-motor symptoms in PD and forming the pathognomonic Lewy bodies in affected brain regions (reviewed in [4]). Therefore, specific clearance pathways are involved in PD, that include on one hand impaired protein degradation via the ubiquitin-proteasome system [5] and on another hand impaired lysosomal clearance of proteins and

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organelles via autophagy [6,7]. The subsequent aggregation of misfolded alpha-synuclein and its prion-like spread within the central and autonomous nervous system is supposed to be a critical step in disease progression [4,8,9]. Whether these aggregates or the preceding (proto-)fibrillary structures of alpha-synuclein interfere with the neuron's vital functions is currently subject of debate [9]. However, the sequestration of other proteins and transcription factors into aggregates, as well as impaired mitochondrial function also increase the cells' vulnerability to excitotoxicity and oxidative stress, leading to energy depletion. In addition, due to deficiency of the ubiquitin-proteasome system, the autophagy lysosome pathway plays an important role for degradation of misfolded proteins and defect mitochondria [10]. These mechanisms lead in fine to the activation of the apoptotic cascade and cell death related to neurodegeneration. Some neuronal populations seem to more vulnerable to these pathogenic mechanisms than others within the nervous system, thus determining the clinical phenotype of the disease according to the preferential sites of neuronal dysfunction and subsequent degeneration [11,12].

In the last two decades the investigation of rare familial forms of PD with monogenetic cases showing classical Mendelian inheritance provided the first major insights into the underlying molecular mechanisms involved in PD, by analysing in vitro and in vivo the resulting dysfunction of the proteins encoded by these genes, e.g. alpha-synuclein. This allowed to dissect pathophysiological mechanisms of impaired protein degradation, oxidative stress and mitochondrial dysfunction, and therefore rare monogenic forms of PD served as an entry point to understand the more common sporadic form of PD [13]. Indeed, some of the mutations identified in monogenic forms of PD were also observed in patients without a positive family history or sometimes in unaffected individuals, so apparently not sufficient to cause the disease. This indicates reduced penetrance for some of the mutations. Carriers of the *G2019S* mutation in the *LRRK2* gene are a good example, where patients were diagnosed with sporadic PD mirroring the reduced penetrance of certain genetic risk factors [14–20]. Unbiased genome-wide association studies (GWAS) have demonstrated a role of more common genetic variants in the pathogenesis of idiopathic PD [20–25]. The "graded risk" concept (Fig. 1) includes Mendelian mutations, low frequency genetic variants and common polymorphisms and introduces the concept of a continuum from more common genetic variants to rare disease-causing mutations with an associated more or less strong impact on the expressivity and contribution to the development of the disease [3,26].

Besides the participation of genetic susceptibility factors in the pathogenesis of neurodegeneration, these findings on reduced penetrance and potential polygenic contribution to disease risk indicate an important role of additional, most probably environmental, risk factors and an important interplay between both genetic and environmental factors in modulating the clinical expression of the disease [27].

2. Gene-environment interactions

2.1. *Aging and DNA methylation*

Aging, at the cellular level, reflects an accumulation of changes affecting the physiological functioning of the cell and the whole organism, leading to an increased vulnerability to death. "Chronologic age" does not necessarily reflect necessarily "biological age", as additional variables such as individual genetic background, lifestyle and disease processes influence cellular aging. Hallmarks of aging are genetic alterations including telomere attrition, increased genomic instability and epigenetic alterations involving changes in DNA methylation patterns, histone modifications and chromatin remodeling [28].

Different biomarkers previously proposed for defining the biological age of a specific cell or tissue, such as telomere length [29] or age-dependent deletions of mitochondrial DNA [30], were insufficient in their precision (e.g. sensitivity, specificity) and practicality (e.g. accessibility, cost-effectiveness) [31]. Epigenetic changes, especially the DNA methylation status provides a more reliable biomarker in this regard [32–34]. Especially some CpG sites (5'-cytosine-phosphate-guanine-3' sites) exhibit more linear DNA methylation changes throughout aging and are thus valuable biomarkers for age prediction [35–37].

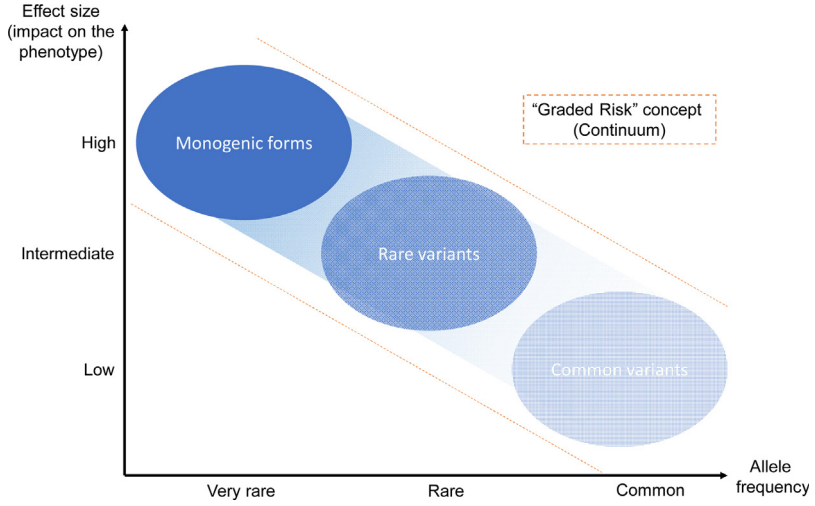

Fig. 1 – The graded-risk concept (adapted from Manolio et al., 2009; [26]).

Interestingly, ''epigenetic drift'' – the divergence of the epigenome due to random changes in methylation (or errors in the maintenance of the epigenome) over a lifespan – is influenced substantially by environmental factors, as shown in twin studies [36,37]. This is believed to cause the individual susceptibilities, prognoses, treatment outcomes and in a larger sense the individual clinical phenotypes of common diseases.

Over time, the accumulation of epigenetic changes – or epimutations – is believed to induce instability of the genome, to facilitate genetic mutations and finally pathological processes defining a disease. These changes in DNA methylation and their effect on gene expression give us new insight in age-related diseases such as PD [38,39].

2.2. *Gene-environment interaction*

Within the last two decades, genetic causes for PD have more and more come to the forefront in PD research [40]. However, most cases cannot be explained yet by a monogenetic form of PD, nor by risk variants. Inversely, even though environmental factors are recognized to contribute to PD, not everyone with PD has been exposed to one of the currently known environmental risk factors and vice versa not everyone exposed to an environmental risk factor is developing the disease. Commonly, pesticides are thought to alter mitochondrial function and increase oxidative stress in dopaminergic neurons, but also to accelerate the formation of alpha-synuclein fibrils and interfere with the function of the ubiquitin-proteasome system [41–43]. Professional or occupational pesticide usage may increase expression of the dopamine transporter (DAT) and lead to an accumulation of toxicants in dopaminergic neurons, affecting dopaminergic neurotransmission in subjects carrying DAT susceptibility alleles [44,45]. Exposure to organophosphates of subjects carrying polymorphisms in the ACHE/PON1 locus might increase the risk for insecticide-induced PD via a neurotransmission imbalance due to impaired acetylcholinesterase (AChE) and paraoxonase (PON1) activity (and in fine impaired organophosphate degradations) [46,47]. The combination and interaction of genetic susceptibility factors and environmental exposure is summarized in the concept of impaired xenobiotic metabolism and sheds first light into the mechanisms of neurodegeneration. These initial insights may translate into new therapies for PD patients that take into account the individual genetic backgrounds reflected in enzymatic activities [48–50].

Even once associations of PD with environmental factors were established, it was yet not clear whether there is (inverse) causality between PD and these factors, e.g. the reverse association between PD and smoking [51]. One way to investigate environmental factors and their causality for PD might be by using so called Mendelian randomisation (MR) methods.

3. Mendelian randomisation

3.1. *Principles of Mendelian randomisation*

Association studies are a common tool to investigate aetiologies of pathologies, however it is now known that association and causality are not equivalent by no means, as an association between a disease and a risk factor can be caused by unknown factors (confounders) or the disease can cause the increased presence of a risk factor (reverse causation), as represented in Fig. 2A. In order to avoid confounding factors (selection bias, cofounders, reverse causation...), randomised control trials (RCT), by randomly assigning study participants in two or more groups, are considered as gold standard for investigating causality in prospective clinical trials. Besides the ethical concerns of exposing subjects deliberately to the investigated risk factor, these studies have the downside that they are expensive and time consuming as a longitudinal observation period is needed. Additionally, RCTs are limited in the number of risk factors investigated and they only reflect the effect of an exposure during a certain period of time in the life of the study participant.

The aim of a MR study is to assess whether a non-genetic/modifiable environmental exposure is associated with the investigated pathology by introducing a randomisation method into an observational study and avoiding the above-mentioned downsides of RCTs [52]. As shown in a directed acyclic graph (Fig. 2A), MR is able to test the null hypothesis that the pathology (outcome) is not caused by an exposure, by assuming that genetic variants used as instrumental variables (IV) are robustly associated with the investigated exposure, are independent of confounders and are associated with the investigated outcome solely (and linearly, unaffected by statistical interactions) via the exposure [53]. The randomisation method in MR studies is based on Mendelian principles of inheritance [54]. MR studies use genetic variants, such as single nucleotide polymorphisms (SNPs) as IVs to measure the effect of an non-genetic exposure on an outcome (Fig. 2A) [55,56]. This randomisation method of using germline genetic variants (which are supposed to be randomly distributed during conception in the general population) as variables, is used for proving causal associations and to accurately estimate the effect of a lifelong exposure to an environmental/lifestyle risk factor on an outcome [57], by being less susceptible to the biases of observational studies [58].

3.2. *Mendelian randomisation in Parkinson's disease*

In the past decades, classical epidemiological studies (including potential recall biases) could determine some environmental risk factors for PD, for example pesticide usage or head trauma [59–62]. But also, protective factors having a negative association with the PD risk, such as smoking, caffeine drinking or elevated urate levels in serum were identified in such studies. These negative associations (or potential protective factors) are widely discussed and MR studies provide a way to analyse these associations for their causalities and to shed light on potential protective factors.

Potentially due to its antioxidative effect, elevated plasma urate levels were shown to have a negative association with PD risk [63]. A Japanese clinical study had already investigated whether inosine (a urate precursor) would increase the plasma urate level of PD patients, without proving causality [64]. Two independent Danish studies recently used MR methods to assess causality of PD incidence and metabolic data such as

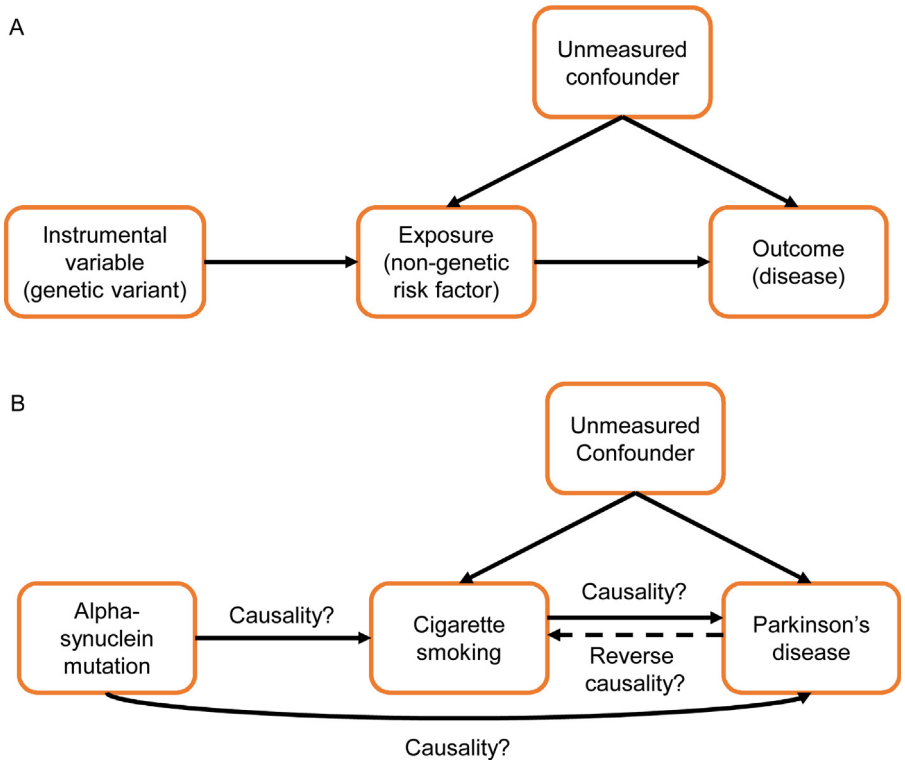

Fig. 2 – The principles of Mendelian randomisation represented in a directed acyclic graph (A). The example of Parkinson's disease is represented in B. Genetic variants of alpha-synuclein (or other) can potentially cause the PD phenotype or even a "primary personality" for a tendency to expose to non-genetic factors (such as cigarette smoking). If cigarette smoking is "protective factor" for PD (causality) or due to this "primary personality" (reverse causality) is debated.

plasma urate levels [65,66]. The epidemiological association between elevated plasma urate and a reduced risk for PD was shown, but MR analysis remained negative and provided no evidence for a causal relationship between this modifiable risk factor and the outcome. The initially demonstrated epidemiological association might therefore be attributed to other unknown factors than the elevated urate levels, making the rationale of clinical inosine studies potentially less relevant [67].

The negative association of PD risk and coffee drinking or nicotine smoking was previously shown in different epidemiological studies [68], raising the controversy over whether, for example, smoking cigarettes reduces the risk for PD, or PD patients are less likely to become addicted to smoking because of their underlying "primary personality" (Fig. 2B). Serum caffeine and its metabolites were even investigated as potential diagnostic biomarkers for early PD, consistent with its supposed neuroprotective effect [69]. However, the underlying causality is not known yet (neuroprotective substances absorbed by the lungs or digestive tract are speculated) and an RCT exposing intentionally subjects to cigarette smoking to investigate the risk for PD would be highly unethical. Many MR studies have been conducted as a new approach to investigate potential protective environmental factors, such as coffee consumption, and their effect on health [70]. Also high serum iron levels and a high body mass index (BMI) were shown in MR studies to have a negative causal effect on PD risk [71,72]. Low LDL cholesterol levels on the other hand, might have a causal effect on lowering the risk for AD, without an effect on PD risk [73].

A recent MR study on daily coffee consumption and PD risk could not define a causal effect between these two, but did find a positive causal association between being a "morning person" and increased PD risk [74]. The circadian rhythm is highly determined by genetic variants and the presumption is that the resulting phenotype "morning person" is more active in the morning and does not need to drink as much coffee to start its day. However, the "night owl" (having a lower risk for PD) would need more coffee in the morning to cope with the "8 a.m. to 5 p.m." work schedule dictated by the society, explaining the reduced risk for PD associated with coffee consumption as an epiphenomenon. This causal association of the sleep-wake rhythm and PD (or neurodegenerative diseases in general) makes sense in light of studies showing amyloid-beta accumulation in sleep deprived brains [75].

3.3. *Limitations of Mendelian randomisation*

Complementing the classical observational studies by avoiding their biases, MR is not without its limitations. In order to be used to assess the effect of a non-genetic risk factor on an outcome, MR relies on the availability of robust genetic data. Many GWAS studies have enlightened in the last decades not only associations with diseases, but also complex measurable parameters such as body mass index (BMI; [76]), serum level of metabolites (such as serum uric acid or cholesterol levels;

[77,78]), cognitive features or many other behaviour patterns like diets and habits [79–81]. For example, Yengo and colleagues analysed GWAS data of about seven hundred subjects using MR and identified hundreds of exposure-associated SNPs (data available because of the increasing number of genetic association studies and data banks) [76]. The discovery of new genetic traits and SNPs and the assessment of causalities are more accurate if a large sample of genetic data is available. Therefore, MR studies cannot be used if no SNPs have been identified to be associated for the exposure, or they don't have sufficient power to detect causality if the SNPs don't explain the integral exposure variation [57].

4. Conclusion

Mendelian randomisation studies are an efficient method, complementary to classical association studies, to assess causalities of modifiable environmental risk factors if sufficient genetic data is available. Therefore, cohorts assessing a huge array of different clinical data and providing a detailed genetic characterization are needed in the future to enhance the power of these MR studies [82]. Avoiding confounder and reverse causality biases of association studies, MR thus enables to investigate gene-environmental interactions and determine potential protective factors to be implemented in future preventive medicine campaigns. They might offer a good tool for guiding future clinical RCTS, by avoiding errancy and waste of research resources.

5. Disclosure of interest

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