Pathophysiological studies in delirium: a focus on genetics

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Publication date
2009

Document Version
Final published version

Citation for published version (APA):

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General discussion and conclusions

Excerpt submitted for publication

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Chapter 10

Introduction

This thesis confirms that delirium is an important neuropsychiatric syndrome in the elderly with frequencies up to 35% in acutely admitted medical patients and even up to 50% in the elderly patients with hip fracture. Yet, the pathophysiological mechanisms underlying the development of delirium are mostly hypothetical. The aim of the research in this thesis was to explore several pathophysiological factors of delirium in elderly patients and especially the role of genetic factors. In this chapter results of these studies will be discussed. Next, some of the pitfalls related to pathophysiological and genetic studies in delirium will be discussed. In addition we offer directions for future research.

Biomarkers for delirium

We have studied possible biomarkers for delirium, both on the basis of former research in delirium, as well as on the basis of a new hypothesis-generating method; i.e. proteomics. By only making use of a priori models of pathophysiology possible alternative mechanisms could be missed. By mass spectrometry, we were able to detect newly developed protein peaks in blood related to delirium. These peaks presumably correspond to different forms of hemoglobin-β. Hemoglobin-β has been found in proteomics research in different types of cancer as well. So far, no plausible explanation for the possible role of hemoglobin-β in the pathophysiology of delirium has come up. The sensitivity of 0.94 in diagnosing delirium of the three peaks offers potential as possible biomarker for the diagnosis of delirium. Hemoglobin-β is unfortunately still difficult to measure in a high throughput setting and therefore its utility as a biomarker is limited so far.

S100B is an interesting and promising marker for delirium. The level of S100B in blood is a measure for the degree of brain cell damage and/or increased permeability of the blood-brain-barrier. Moreover, S100B release by astrocytes can be augmented upon stimulation by, among others, the proinflammatory cytokines tumor necrosis factor (TNF)-α and interleukin (IL)-1β. An increased level of S100B protein during delirium, as was suggested in earlier studies, has been confirmed in both our studies (Chapter 4 and 5). It could be hypothesized that the higher frequency of dementia after delirium reflects irreversible brain damage caused by the detrimental effects related to delirium. If we extrapolate these findings, we could hypothesize that the higher the level of S100B (and thus cerebral damage) in a delirious patient, the higher the risk of dementia after delirium. This cerebral damage could possibly lead to cognitive impairment after delirium via neuroinflammatory mechanisms, as the level of S100B is highest in patients with an infectious disease with activated inflammatory processes. This has also been shown after other neurodegenerative diseases. Alternatively, the higher level of S100B in delirium could be related to active stimulation of astrocytes by cytokines.

The role of inflammatory mechanisms in delirium, i.e. IL-6 and IL-8 in plasma, was studied in more detail in elderly hip fracture patients. Cytokines contribute to a cascade of events typical of inflammation and especially proinflammatory cytokines, such as IL-6 and IL-8, are thought to contribute to the development of sickness behavior.
behaviour during illness shows overlapping symptoms and characteristics with delirium. Weighing the results with former studies investigating the relationship between IL-6 or IL-8 and delirium, the studies are in favour of an association between delirium and both cytokines. Moreover, the higher IL-6 levels found in subtypes of delirium with hyperactive symptoms raise the possibility that different cytokine profiles may lead to different subtypes of delirium. If the IL-6 difference between subtypes is validated, this may provide tools to distinguish between subtypes. Moreover, the resemblance of the figures of the time-course of cytokines and S100B in the surgical population could be another clue for the relation between inflammation and cerebral damage and/or activation of astrocytes.

**Apolipoprotein E gene and delirium**

The apolipoprotein E4 (APOE4) may also play a role in the possible neuroinflammation related to delirium. The meta-analysis (Chapter 9) including our recent unpublished data, showed that the APOE ε4-allele is likely to be associated with delirium in the elderly, especially in a non-cardiac surgical population.

The fact that APOE ε4-allele enhances the risk for delirium is in accordance with the effects the APOE ε4 protein can have on the hypothesized mechanisms that lead to delirium. The major role of APOE is to regulate cholesterol metabolism. In addition, it has been suggested that APOE in the central nervous system (CNS) plays a role in neural plasticity, the repair of damaged neurons, as a neurotrophic factor, and in neural transmission. The three different alleles (ε2, ε3 or ε4) are translated to the different apolipoproteins E (APOE2, APOE3 or APOE4) which differ in functionality and catabolism rates (the APOE4 being faster catabolised). In animal studies it was shown that the APOE ε4 genotype is associated with increased inflammation. Additionally, the APOE ε4 genotype reduces the cholinergic activity in the brain. These theories of reduced cholinergic activity and the pro-inflammatory state are not mutually exclusive since there is interaction between these systems. Animal studies have demonstrated that cytokines can cause a reduction in the acetylcholinergic pathways. All of the above mechanisms can play a role in the enlarged risk on delirium in APOE ε4 carriers.

**Dopamine genes and delirium**

Positive associations with alcohol withdrawal delirium (Chapter 6) were found in two different candidate genes involved in dopamine transmission: the dopamine receptor D3 gene (DRD3) and the dopamine transporter gene (SLC6A3). The findings in the DRD3 gene were not confirmed in our elderly population. In the SLC6A3 gene, the Variable Number of Tandem Repeats (VNTR) was associated with alcohol withdrawal delirium. Although we did not validate the same variation in elderly patients with delirium, we found two other single nucleotide polymorphisms (SNPs), rs393795 and rs1042098 in this gene, to be associated with delirium. Up to now, these polymorphisms have never been described in relation to any disease. On the basis of the first-choice treatment of delirium
Chapter 10

with a dopamine receptor 2 antagonist, we investigated possible associations between the dopamine receptor 2 gene (DRD2) and delirium. One polymorphism in the DRD2 gene (rs6276) was associated with delirium after adjustment for the most important risk factors and the SNPs in the SLC6A3 gene. Interestingly, interaction between the dopamine transporter and the DRD2 gene could possibly play a role since a different variation in the DRD2 gene (rs1800497) is associated with higher density of the dopamine transporter\(^{101}\). The non-coding rs6276 SNP has been described in association with various alcohol related phenotypes\(^{116}\).

In conclusion, we found a protective effect of two different genes involved in dopamine metabolism. The most widely supported theory about the pathophysiology of delirium centers on the neurotransmitter system. This theory states that relative acetylcholine deficiency and dopamine excess could mediate the characteristic symptoms of delirium\(^{186}\). Dopamine is involved in all clinical characteristics of delirium, like changes in attention, locomotion, mood, memory and perception. Moreover, on the one hand, delirium can be evoked by dopamine agonists and anticholinergic medication and, on the other hand, it can be successfully treated with dopamine receptor antagonists and probably cholinesterase inhibitors\(^{5}\). As we found a protective effect of variations in the SLC6A3 and the DRD2 gene, the general hypothesis would be supported by our genetic findings if these variations would reduce the dopaminergic activity in the brain by, for example, changed availability or action of dopamine receptors or reduced attachment to these receptors\(^{132,196}\). Although, the variations found in our study are all non-functional SNPs, they could be in linkage equilibrium with functional SNPs that do influence the dopaminergic activity. As this is yet unknown, it is speculative to state that our findings support the hypothesis of delirium being caused by enhanced dopaminergic activity in the brain.

Genetic risk factors

On the basis of the results in this thesis the SLC6A3, the DRD2 and the APOE gene are associated with delirium in the elderly patients. Although all of these factors need validation, our results showed that only the APOE ε4-allele is a genetic risk factor for delirium, whereas the other two genes showed a protective effect. Genetic research offers two important possibilities: (1) elucidating pathophysiological mechanisms and (2) identification of high risk patients. To continue with the second advantage of genetic research, genetic factors for delirium identified so far do probably not contribute to the identification of high risk patients. Although we did not attempt to make a prediction model for delirium based on a combination of classical risk factors for delirium and genetic factors, we can compare the impact of both types of risk factors by looking at the Odds Ratios (ORs) for the development of delirium. Looking at the risk of APOE ε4-allele (Chapter 9), we observed that delirium was independently associated with carriage of this allele (OR=1.6, 95% Confidence Interval (CI): 1.1-2.2). For the classical risk factors the ORs were remarkably higher. So, next to the major classical risk factors for delirium, older age
and preexistent functional and cognitive impairment, genetic factors described in this thesis would probably not contribute to the identification of delirium. Hopefully, future identification of genetic variations are stronger associated with delirium or provide an approach for adjusting pharmacotherapy at the individual level.

**Difficulties in studying the pathophysiology**
Since delirium is a neuropsychiatric disorder: pathophysiological markers of interest may theoretically best be derived from brain research. Yet, this type of data collection is difficult as methods for brain research in humans are limited and usually incriminating for the patient. Moreover, patients with, or at risk for, delirium often have an impaired capacity to give informed consent for research purposes due to preexisting cognitive impairment, severe illness, and the nature of delirium itself. Additionally, conventional pathophysiological markers can be influenced by the causal combination of predisposing and precipitating factors for delirium. Moreover, the moment in time over the 24 hours observation period may have an effect on the markers, because the symptoms of delirium, as well as concentrations of many markers of interest, fluctuate during the day.

In theory, it is possible that different etiologies may lead to delirium via different pathophysiological pathways. In the same respect, we do not know whether the hyperactive, hypoactive, and mixed subtypes of delirium are all part of the same syndrome; further, we do not know if subtypes alternate during one episode of delirium. Studies of the different subtypes of delirium are scarce, because of lack of a good 24 hour observation period needed to properly classify the patients in different subtypes. These uncertainties may lead to a heterogeneous population of patients with delirium, which reduces the likelihood of significant findings.

**Difficulties related to the phenotype delirium for genetic studies**
A phenotype is any observable characteristic of an organism, such as its morphology, development, biochemical or physiological properties, or behaviour. Phenotypes result from the expression of an organism’s genes, influence of environmental factors, and possible interactions between the two. The phenotype delirium is by definition dependent on environmental factors; a patient needs to cross a certain threshold with a combination of predisposing and precipitating factors to elicit it. Figure 1 suggests a possible relationship between predisposing and precipitating factors in the risk for delirium. While a person with many predisposing factors will only need a minor trigger to develop delirium (i.e. an old demented patient with a mild urinary tract infection), a person without predisposing factors requires a a much more severe trigger (i.e. a young patient with severe sepsis in the Intensive Care Unit). This figure reveals that it is difficult to clearly classify a person as demonstrating a non-delirious phenotype: if there are no precipitating factors, the phenotype of delirium is unknown. If there is a precipitating factor, however, the classification of the phenotype as delirious or not, is strongly influenced by the severity of both the predisposing and precipitating factors. Because the diagnosis of
delirium can also be missed, misclassification can occur in both directions, delirious patients as non-delirious and vice versa.

Figure 1: Relationship between predisposing and precipitating factors.

Darker shading indicates a higher risk for delirium. Genetic factors will play the largest role in the delirium phenotype in the light zone and a smaller role in the darker zone. Oval indicates the location of acutely hospitalized elderly patients.

Figure 1 shows the risk of delirium for different combinations of predisposing and precipitating factors. Predisposing factors vary and grow over time; in addition to age, the incidence of other important factors predisposing to delirium (e.g. cognitive and functional impairment) rises with increasing age. Genetic factors may also be part of these predisposing factors for delirium. Since genetic factors are relatively independent of age, the proportion of phenotypic variation that is attributed to genetic factors declines with increasing age. The diminished role of genetic factors in the etiology of disease at advanced age is not unique for delirium but has also been demonstrated in other old-age diseases like dementia and osteoporosis or cancer at old age. We hypothesize that the contribution of genetics to the development of delirium will be largest in patients who become delirious despite minor predisposing and precipitating factors (lower left corner Figure 1). In analogy, the protective effect of genetic factors or the contribution of genetics to the non-delirium phenotype will be largest in patients without delirium despite the presence of major predisposing and precipitating factors (upper right corner Figure 1). Both of these groups of patients offer ideal study populations for identifying genetic
factors associated with delirium. The first group has the disadvantage of a low incidence of the delirium phenotype, whereas the second could have the disadvantage of a low incidence of the non-delirium phenotype. In an elderly hospitalized population (placed near the upper right corner in Figure 1), we would expect to identify more genetic factors that are protective rather than causative for delirium.

A major pitfall in the comparison of genetic variations between cases and controls is that such a comparison could result in spurious associations if the controls are not completely matched to the cases with respect to factors influenced by an individual’s genetic composition. Delirious patients are significantly older and more frequently have preexisting cognitive and functional impairment than non-delirious patient. Most of these factors are probably not influenced by genetic variations, but many genetic risk factors for cognitive impairment are known. If an association between a genetic factor and delirium is detected, therefore, the association could in fact be related to the association of the genetic factor with cognitive impairment. Adequately diagnosing cognitive impairment during delirium is hard as temporary cognitive impairment is often one of the features of delirium itself. Assessing pre-existent cognitive function during delirium is only possible by interviewing a close relative. Instruments like the ‘Informant Questionnaire on Cognitive Decline-Short Form’ have been developed for this purpose, but they are not as reliable in assessing the patient’s pre-existing cognitive status as tests of cognitive function administered before delirium.

Difficulties related to the study design in genetics
The genotype of an organism is comprised of the inherited instructions it carries within its genes. Variation among individuals may be due to genetic and/or environmental factors. Heritability refers to the proportion of phenotypic variation in a population that is attributed to the genetic profile of the individual. Estimation of heritability is done with twin studies or family studies. The heritability of delirium is unknown, and there are no known families with a high frequency of affected members. However, that delirium might be heritable can be deduced from the estimated heritability of 30% for the occurrence of any psychotic episode in late-onset Alzheimer’s disease. The most important reason accounting for the lack of heritability studies is the fact that delirium develops mainly in elderly people. In a family, therefore, the phenotype will often be unknown for younger members, whereas older siblings already have passed away. This difficulty, in addition to the challenges in the proper classification of the phenotype described earlier, explains why twin and family studies in delirium are hard to perform.

Another problem for successful genetic testing in delirium is the existence of many different hypotheses regarding the pathophysiology of the disease, because these hypotheses result in an abundance of available candidate genes with many possible SNPs. This introduces the risk on finding an association by chance only (i.e. a type 1 error). Correction for multiple tests will partially prevent false associations, but it simultaneously introduces the risk of missing interesting associations. Validation of associations in an
independent cohort of in several of hundreds cases and control patients from different populations offers certainty that a possible finding is not spurious. Usually, a cross-sectional study design is the quickest method for candidate gene studies, since phenotype and genotype are determined at the same time. Nonetheless, classifying the delirium phenotype adequately in this design is impossible. Experienced clinicians can only properly diagnose delirium at the moment the symptoms are present and using a retrospective questionnaire is not reliable as the patients and their relatives are often unaware of whether they have ever experienced delirium. Therefore, a time-consuming prospective study is the only design in which the diagnosis can be made adequately.

**Future studies in pathophysiology**
With respect to the pathophysiology the list of options is endless, so we limit these remarks to research related to this thesis. Apart from validation of our findings, some open ends, may be suitable for future research:

- As the hemoglobin-β is a new hypothesis and our study was performed in a small population of 32 patients, the next logical step would be validation of these results in a larger sample by a large-scale method like an ELISA, which has yet to be developed.
- To investigate the role of S100B as a marker of permanent brain damage after delirium, the impact of increased S100B on changes in cognitive functions several months after the delirious episode should be determined.
- As the levels of IL-6 and S100B showed a comparable course in time, the possible relation between inflammation and brain damage in delirium needs to be studied.
- Causal relationships of all markers with delirium in observational studies remain to be investigated. It would be a major step forward if an adequate animal model for delirium is available. This model is currently under development.

**Future studies in genetics**
The genetics of delirium is still in its infancy, but some progress is being made, and validation of genetic results of this thesis in independent study cohorts is essential. International cooperation for this purpose has already started with some of the known cohorts described in Chapter 9, as well as with some new available cohorts. Because of the small size of the known delirium cohorts, cooperation is warranted to perform future candidate gene studies on a larger scale to permit any further progress. A meta-analysis of different small study cohorts could provide an alternative tool to analyze the data from these separate studies jointly. Table 1 shows the most obvious candidate genes for the different hypotheses of the pathophysiology of delirium. New findings of pathophysiological studies could also be verified by looking at the association between delirium and genetic variations in the genes of interest. For example the gene for
hemoglobin-β described in this thesis is a new candidate gene. Since almost all genes contain several SNPs, the list for future genetic association studies is almost inexhaustible.

While the candidate gene approach examines a single gene, genome-wide association (GWA) studies simultaneously assay hundreds of thousands of SNPs using high-throughput genotyping technologies. Because the entire genome is analyzed, this technique allows the genetics of a disease to be investigated in a non-hypothesis-driven manner. As multiple comparisons are being made, correction for multiple testing is required; this correction implies that even larger study populations are needed to provide sufficient power to detect significant associations.

Table 1: Other possible candidate genes deduced from the hypotheses of the pathophysiological mechanisms of delirium.

<table>
<thead>
<tr>
<th>Function</th>
<th>Candidate gene</th>
<th>Symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytokine</td>
<td>Interleukin 6</td>
<td>IL6</td>
</tr>
<tr>
<td>Cytokine</td>
<td>Interleukin 6 receptor</td>
<td>IL6R</td>
</tr>
<tr>
<td>Cytokine</td>
<td>Interleukin 8</td>
<td>IL8</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>Acetylcholinesterase</td>
<td>ACHE</td>
</tr>
<tr>
<td>Cannabinoid</td>
<td>Cannabinoid receptor 1a</td>
<td>CNR1</td>
</tr>
<tr>
<td>Dopamine/norepinephrine</td>
<td>Catechol-O-methyltransferase</td>
<td>COMT</td>
</tr>
<tr>
<td>Dopamine</td>
<td>Dopamine receptor D1</td>
<td>DRD1</td>
</tr>
<tr>
<td>Dopamine/norepinephrine</td>
<td>Catechol-O-methyltransferase</td>
<td>COMT</td>
</tr>
<tr>
<td>Dopamine/norepinephrine</td>
<td>Tyrosine hydroxylasea</td>
<td>TH</td>
</tr>
<tr>
<td>Glucocorticoid receptor</td>
<td>Nuclear receptor family 3, group C, member 1</td>
<td>NR3C1</td>
</tr>
<tr>
<td>Glucocorticoid receptor</td>
<td>Melanocortin 2 receptor</td>
<td>MC2R</td>
</tr>
<tr>
<td>Glutamate</td>
<td>Glutamate receptor ionotropic, kainate 3a</td>
<td>GRIK3</td>
</tr>
<tr>
<td>Melatonin</td>
<td>Melatonin receptor 1A</td>
<td>MTNR1A</td>
</tr>
<tr>
<td>Neuropeptide</td>
<td>Brain-derived neurotrophic factora</td>
<td>BDNF</td>
</tr>
</tbody>
</table>

a Based on studies showing an association with alcohol withdrawal delirium

Epigenetics refers to heritable changes in phenotype or gene expression caused by mechanisms other than changes in the underlying DNA sequence. Biological, chemical, and physical factors as well as cultural and educational backgrounds influence gene functioning. These gene-environment interactions may lead to epigenetic changes. For example, the methylation of DNA can influence genetic expression and thereby influence the risk of delirium. It will be important to delirium to determine both the genetic polymorphisms and epigenetic alterations in order to provide a comprehensive picture of its genetic etiology.
Conclusion
The studies described in this thesis provide clues for some new and old ideas about the pathophysiological mechanisms leading to delirium. Although these studies are hampered by many issues, we strongly urge the extension of this type of research. If we continue on this road, it may be expected that within a few years a large increment in the understanding of the pathophysiology of delirium will be attained. Based on these findings, development of improved markers for the (early) diagnosis of delirium and surveillance of treatment for this population of vulnerable patients may be expected.