“Omic” Studies and Cadasil

Subjects: Neurosciences
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CADASIL (Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy; OMIM#125310) is a systemic arteriopathy of non-atherosclerotic and non-amyloid cause. It is a rare disease affecting fewer than 2/1000 individuals, caused by mutations in the NOTCH3 gene. It has autosomal dominant inheritance, although it can also occur due to de novo mutations.

Keywords: CADASIL ; genomic ; transcriptomic ; proteomic

1.Overview

CADASIL (Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy) is a small vessel disease caused by mutations in *NOTCH3* that lead to an odd number of cysteines in the epidermal growth factor (EGF)-like repeat domain, causing protein misfolding and aggregation. The main symptoms are migraines, psychiatric disorders, recurrent strokes, and dementia. Omic technologies allow the massive study of different molecules for understanding diseases in a non-biased manner or even for discovering targets and their possible treatments. We analyzed the progress in understanding CADASIL that has been made possible by omics sciences. For this purpose, we included studies that focused on CADASIL and used omics techniques, searching bibliographic resources, such as PubMed. We excluded studies with other phenotypes, such as migraine or leukodystrophies. A total of 18 articles were reviewed. Due to the high prevalence of *NOTCH3* mutations considered pathogenic to date in genomic repositories, one can ask whether all of them produce CADASIL, different degrees of the disease, or whether they are just a risk factor for small vessel disease. Besides, proteomics and transcriptomics studies found that the molecules that are significantly altered in CADASIL are mainly related to cell adhesion, the cytoskeleton or extracellular matrix components, misfolding control, autophagia, angiogenesis, or the transforming growth factor β (TGFβ) signaling pathway. The omics studies performed on CADASIL have been useful for understanding the biological mechanisms and could be key factors for finding potential drug targets.

2. Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy

CADASIL (Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy; OMIM#125310) is a systemic arteriopathy of non-atherosclerotic and non-amyloid cause. It is a rare disease affecting fewer than 2/1000 individuals, caused by mutations in the *NOTCH3* gene. It has autosomal dominant inheritance, although it can also occur due to *de novo* mutations [1].

The etiopathogenesis of the disease is not well understood. It is thought to be triggered by mutations in *NOTCH3* that cause an odd number of cysteines in the domain hosting the epidermal growth factor-like repetitions (EGFr) of the receptor encoded by this gene, leading to disruption of disulfide bonds and protein aggregation [2][3].

CADASIL is characterized by the following symptoms: migraine with aura; psychiatric disorders; recurrent small subcortical infarcts; and dementia at an early age [4]. It is the most common cause of stroke and dementia of genetic origin. On MRI scans, white matter hyperintensities (WMH) in the temporal lobe and external capsule are characteristic of the disease [5][6].

The presence of protein aggregates known as granular osmiophilic material (GOMs) in skin biopsies of patients, assessed by electron microscopy, has 100% specificity for its diagnosis [5]. However, due to the focal nature of GOMs, false negatives may occur [7]. Therefore, the definitive diagnosis is established through genetic testing with the identification of pathogenic mutations, which affect the number of cysteines in EGFr.

Once the diagnosis has been made, it is difficult to determine the patients' clinical course. Patients can progress differently, even if they have the same mutation, belong to the same family, or even if they are monozygotic twins [8][9].

There is no curative or disease-modifying treatment, only symptomatic treatments are available. Hence the importance of deepening our understanding of the disease in order to find therapeutic targets whose modulation can improve the quality of life of these patients.

Omic technologies are used to detect genes (genomics), mRNA (transcriptomics), proteins (proteomics), and metabolites (metabolomics) in a specific biological sample in a non-targeted and non-biased manner. The integration of these techniques is called systems biology [10]. Unlike with traditional studies, with these techniques, it is possible to generate hypotheses, which are mostly driven or reductionist hypotheses [10].

Omic technologies can be used in screening, diagnosis, and prognosis, as well as for aiding our understanding of the etiology of diseases or identifying biomarkers [10][11]. Moreover, they are used in target [12] and drug discovery and in the assessment of their toxicity and efficacy [10].

With this review, we aim to take a closer look at the progress made in recent years in CADASIL thanks to omics technologies, as well as to explore what therapeutic possibilities these technologies could offer through a comprehensive resource for omics research on drugs, such as the DrugBank (https://go.drugbank.com, accessed on 27 May 2021).

The use of omics technologies in the field of CADASIL allows for a more efficient diagnosis of the disease. From an epidemiological point of view, it has been found that in genomic repositories, there is a high prevalence of individuals with variants affecting the number of EGFr cysteines. This then raises the question of whether they all really are pathogenic mutations, which was previously considered to be the case. From an etiological point of view, the massive study of data has highlighted the importance of metabolic functions/pathways related to the extracellular matrix, cell adhesion, autophagy, misfolding control, angiogenesis, or TGFβ signaling. These technologies have allowed us to understand the histopathological findings in the disease or identify which molecules or pathways may be of interest for drug targeting, opening a wide range of possibilities for the development of future clinical trials.

3. Conclusions

Due to the introduction of omics techniques in CADASIL studies, we have been able to gain insight into several aspects of the disease.

Nowadays, the diagnosis of CADASIL is more efficient, leading to a lower cost and time for analyzing more exons, which means that a larger number of patients can be screened in a more comprehensive way.

From an epidemiological point of view, we have gone from establishing a minimum CADASIL prevalence of 1.32–4.1/100,000 adults [13][14][15] to a prevalence of 1.4–3.4/1000 carriers of pathological variants in *NOTCH3* [16][17][18][19][20] and 9/1000 in Taiwan [21].

Currently, comparing patients with a mutation in the EGFr domains 1–6 with patients with a mutation in EGFr domains 7–34 in the above-mentioned biobanks, subjects with pathogenic variants were correlated with spatial distribution (the anterior temporal lobe and external capsule) and extent of WMH, a higher frequency and number of lacunes and microbleeds, family history of stroke, and vascular dementia [18][19][20].

This finding highlights the importance of the use of these technologies. The role of mutations considered pathogenic has been questioned. If they all produce CADASIL, then CADASIL is no longer a rare disease. Therefore, depending on the altered structural or functional domain, the patient could have a more or less torpid course. Another hypothesis is that perhaps not all pathogenic variants cause CADASIL. Since SNPs in the *NOTCH3* gene were not found to be associated with lacunar stroke or WMH volume [22], maybe only the variants that affect the number of cysteines in EGFr are risk factors for developing small vessel disease, instead of CADASIL.

From the etiology perspective, omics techniques have made it possible to find molecules whose levels/expression are altered in patients with CADASIL, and which may be amenable to pharmacological repositioning. The most highlighted molecules by the authors in the articles were Notch3, HTRA1, TIMP3, VTN, endostatin, and serum amyloid P-component. The different studies showed that these proteins are co-localized with Notch3, supporting the etiopathogenic interest in them.

Mutations in HTRA1 are also the cause of small vessel cerebral arteriopathy in heterozygosis and homozygosis. In the latter case, they cause CARASIL (Cerebral Autosomal Recessive Arteriopathy with Subcortical Infarcts and Leukoencephalopathy), leading to TGFβ signaling impairment. TGFβ is closely related to small vessel disease in the brain [23]. The fact that several of the substrates of this enzyme are elevated in patients with CADASIL compared with controls, as in the case of VTN, suggests that its proteolytic activity may be diminished [24], as is the case of CARASIL [25].

The remaining molecules found in the proteomic studies, with the same direction of effect in at least two different studies, are likely to be of special interest in the disease. In fact, they have been related to angiogenesis, protein processing and vesicular trafficking, nervous system, or extracellular matrix, among others. To confirm their interest, targeted studies on these proteins would be of important value. Those proteins with contradictory effects in the studies reviewed may be false-positive associations and, therefore, should be taken with caution.

From transcriptomic studies, *E2F4* has been found to be overexpressed in CADASIL patients, not knowing whether this elevated expression is due to a compensatory mechanism for lack of protein production or elevated levels of the protein.

E2F4 belongs to the E2F family of transcription factors. E2F members have been involved in neuronal migration, and activation of E2Fs in VSMCs promotes migration [26]. E2F4 is involved in the process of IH [27]. Mice lacking E2F4 exhibit increased IH following arterial damage [27].

Besides, a diminished expression of *E2F4* attenuates the endothelial cell migration, and its subsequent overexpression could rescue normal endothelial migration [26].

Likewise, E2F4 is part of the signaling pathway of TGFβ [28]. As we have just mentioned, TGFβ is related to cerebral small vessel disease. On the one hand, mutations in *HTRA1* produce a TGFβ signaling impairment and lead to the appearance of CARASIL. On the other hand, in CADASIL, HTRA1, and LTBP-1 (protein that regulates bioavailability of TGFβ [23]) are present in GOMs, the histopathological hallmark of the disease.

It is, therefore, important that all this information help us understand the reason for the alterations found in CADASIL, such as changes in cell adhesions and components of the extracellular matrix or angiogenesis, as well as highlighting other pathways that thus far only a few studies have considered, and which can be decisive, such as alterations in autophagy, mitochondria, or the TGFβ signaling pathway. This could encourage researchers to find drugs that could directly modify pathways that are clearly involved in CADASIL, instead of looking for drugs that target a specific molecule.

Therefore, thanks to this vanguard technology, progress has been made in understanding the extent of the disease and its etiopathogenesis, but there is still a long way to go before we can fully comprehend what happens in CADASIL. Omic studies will allow further investigation of the etiopathogenesis of the disease in a cost-effective manner, since the costs of this type of technology are becoming increasingly lower, allowing the analysis of a larger number of patients and molecules at once, which with conventional techniques would entail a high expenditure of resources and time. In addition, it will allow the exploration of new working hypotheses in an unbiased manner and develop disease-modifying drugs in the future.

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