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On the identification of low allele frequency mosaic mutations in the brains of Alzheimer disease patients

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Abstract

Background: The cause of sporadic Alzheimer's disease (AD) remains unclear. Given the growing evidence that protein aggregates can spread in a "prion-like" fashion, we reasoned that a small population of brain cells producing such "prion-like" particles due to a postzygotic acquired mutation would be sufficient to trigger the disease. Deep DNA sequencing technology should in principle allow the detection of such mosaics.

Methods: To detect the somatic mutations of genes causing AD present in a small number of cells, we developed a targeted deep sequencing approach to scrutinize the genomic loci of *APP*, *PSEN1*, and *PSEN2* genes in DNA extracted from the entorhinal cortex, one of the brain regions showing the earliest signs of AD pathology. We also included the analysis of the *MAPT* gene because mutations may promote tangle formation. We validated candidate mutations with an independent targeted ultradeep amplicon sequencing technique.

Results: We demonstrate that our approach can detect single-nucleotide mosaic variants with a 1% allele frequency and copy number mosaic variants present in as few as 10% of cells. We screened 72 AD and 58 control brain samples and identified three mosaic variants with low allelic frequency (~1%): two novel *MAPT* variants in sporadic AD patients and a known *PSEN2* variant in a Braak II control subject. Moreover, we detected both novel and known pathogenic nonmosaic heterozygous variants in *PSEN1* and *PSEN2* in this cohort of sporadic AD patients.

Conclusion: Our results show that mosaic mutations with low allelic frequencies in AD-relevant genes can be detected in brain-derived DNA, but larger samples need to be investigated before a more definitive conclusion with regard to the pathogenicity of such mosaics can be made.

Keywords: Somatic mutation; Mosaicism; Alzheimer's disease; Prion-like spread; Genetics

1. Introduction

The concepts of somatic disease-causing mutations and of mosaic genomic heterogeneity are well known in the etiology of cancer [1–3]. Recently, several studies have highlighted the role of such acquired mutations as

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pathogenic drivers for neurodevelopmental diseases [4–7]. The possibility that mosaic mutations contribute to neurodegenerative diseases should also be considered [8–11]. Indeed, neurons accumulate a wide spectrum of somatic mutations, in the forms of single nucleotide variants (SNVs), insertion/deletions (indels), retrotranspositions, copy number variants (CNVs), and whole-chromosomal aneuploidies [4,5,12–14]. Although the mutation rate of human cells varies for different kind of mutations and for different tissues, a rate of 1×10^{-10} de novo point mutations per base per cell cycle is a reasonable estimate [15,16], implying approximately one new mutation per cell division. The brain contains $\sim 10^{11}$ neurons and about a similar number of nonneuronal cells [17], thus it is easily conceivable that pathogenic mutations may arise de novo in a mosaic fashion during its ontogenesis. Depending on the time point of the mutation appearance in the cell lineage tree descending from the zygote, the sequencing of DNA isolated from blood may only exceptionally detect such mutation [18] (Fig. 1). This explains why this potentially important phenomenon has not been systematically investigated for Alzheimer's disease (AD).

Most AD patients are sporadic (SAD), i.e., characterized by a late onset and unclear familial inheritance. The biochemical and clinical features of SAD resemble those of familial AD (FAD), which is characterized by a clear autosomal dominant inheritance of causative mutations in mainly three genes (*APP*, *PSEN1*, and *PSEN2*) [19,20]. Growing evidence that protein aggregates of Aβ or Tau (encoded by *MAPT* gene) can spread in the brain and act as local initiators of further aggregation of normal proteins in a "prion-like" fashion [21–25], provides a mechanistic framework to understand how somatic mutations in the brain could spark neurodegenerative disease. De novo mosaic mutations of AD-relevant genes would create a *nidus* of mutant cells mixed between normal cells that would continuously produce and release proaggregating proteins. Such aggregates could act as seeds for further protein aggregation at sites distal from their origin (Fig. 1).

Detection of low-grade mosaic mutations has been hindered by the low sensitivity of classical Sanger sequencing, which allows the detection of mosaic mutations only with an allelic frequency of at least 20% [26]. Recent attempts to identify mosaic pathogenic mutations in Parkinson's disease used high-resolution melting analysis, which allows the detection of mutations with 5% to 10% allelic frequency [11]. Here, we deep sequenced DNA libraries enriched for AD-relevant genes to achieve high sequencing depth, followed by an amplicon ultradeep sequencing validation: this approach enabled the detection of mosaic SNVs having an allelic fraction as low as 1%.

2. Materials and methods

2.1. Samples

Small blocks (~100 mg) of entorhinal cortex were obtained from Lille NeuroBank (BB-0033-00030) and

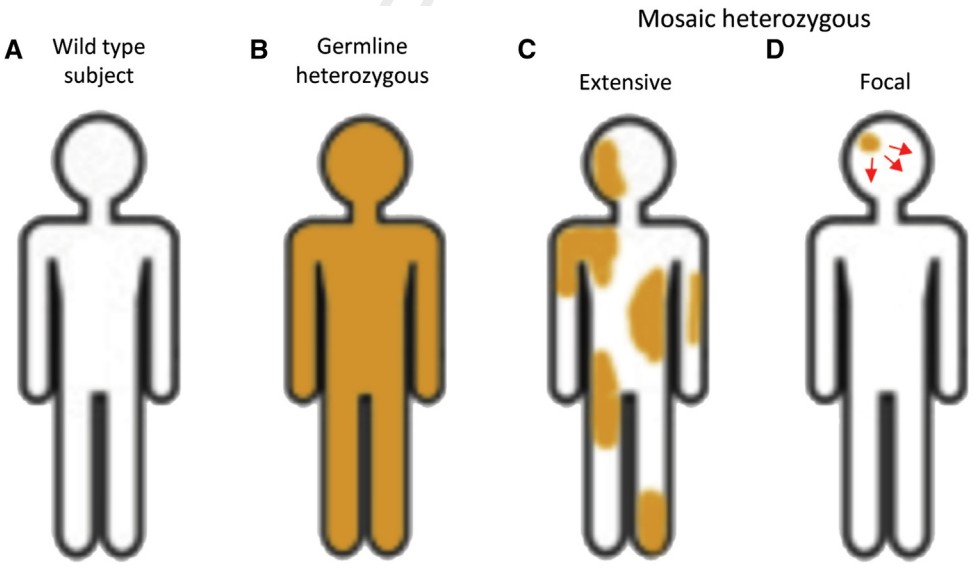

Fig. 1. Somatic mutations and hypothesis of pathology spread in sporadic Alzheimer's disease (AD). An inherited mutation will be carried by all cells of a human body (B), this is the typical case of a familial AD patient. In case of mutations arising in a postzygotic stage, an individual will be a genetic mosaic Q19 for such mutation, with cells either carrying the mutation (orange) or not (white). Depending on the developmental time point of the appearance of the mutation, genetic mosaics can be either extensive (C), with mutant cells appearing in several organs/tissues, or focal (D), when mutant cells are localized in a single organ/tissue. Our working hypothesis is that some sporadic AD patients are focal mosaics for mutations in AD-relevant genes appearing in brain cells. Amyloid beta (Aβ) and/or tau aggregates produced locally as consequence of the mosaic mutation can then spread (red arrows in (D) and seed further aggregation in other brain areas in a "prion-like" fashion, thus leading to full blown AD.