Low-degree trisomy 21 mosaicism promotes early-onset Alzheimer disease

Running head: Trisomy 21 mosaicism promotes EOAD

Georg S. Nuebling, MD [a,b‡],Catharina Prix, MD[a,e‡], Matthias Brendel, MD[c], Leonie Beyer, MD[c], Elisabeth Wlasich, [a], Sandra V. Loosli, PhD[a], Henryk Barthel, Prof. [d], Osama Sabri, Prof.[d], Peter Bartenstein, Prof.[c], Jonathan Vöglein, MD [a,e], Adrian Danek, Prof. [a], Axel Rominger, Prof.[f], Dieter Edbauer, Prof.[e], Christian Haass, Prof.[e,g,h], Johannes M. Levin, Prof.[a,e,g*]

‡authors contributed equally

[a]Department of Neurology, Klinikum der Universität München, Ludwig-Maximilians-University Munich, Marchioninistr. 15, 81377 Munich, Germany
[b]Department of Palliative Medicine, Klinikum der Universität München, Ludwig-Maximilians-University Munich, Marchioninistr. 15, 81377 Munich, Germany
[c]Department of Nuclear medicine, Klinikum der Universität München, Ludwig-Maximilians-University Munich, Marchioninistr. 15, 81377 Munich, Germany
[d]Department of Nuclear medicine, University Hospital Leipzig, Liebigstr. 18, 04103 Leipzig, Germany
[e]German Center for Neurodegenerative Diseases (DZNE), Feodor-Lynen-Str. 17, 81377 Munich, Germany
[f]Department of Nuclear medicine, Inselspital Bern, Freiburgstr. 18, 3010 Bern, Switzerland
[g]Munich Cluster for Systems Neurology, Munich, Germany
[h]Metabolic Biochemistry, Biomedical Center (BMC), Faculty of Medicine, Ludwig-Maximilians-University Munich, 81377 Munich, Germany

*Corresponding Author: PD Dr. Johannes Levin
Department of Neurology
Ludwig-Maximilians-University, Munich
Marchioninistr. 15
81377 Munich
Germany
tel.: +49 (0)89 4400 76695
fax.: +49 (0)89 4400 76671
email: johannes.levin@med.uni-muenchen.de

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Highlights

- Causes of Early-Onset Alzheimer Disease apart from APP/PSEN mutations are unknown
- The reported case features EOAD due to previously undiagnosed trisomy 21 mosaicism
- Minimal increases in APP gene dose (21% trisomy 21 mosaicism) suffice to cause EOAD
- PI-2620 tau PET is suitable to detect and monitor tau deposition in EOAD

ABSTRACT

Trisomy-21 mosaicism (mT21) with subclinical intellectual development disorder or physical phenotype has very rarely been associated with early-onset cognitive decline. Notably, early-onset Alzheimer's disease (EOAD) patients' family histories frequently suggest genetic causes other than autosomal-dominant APP/PSEN-1/2 mutations. We present an EOAD patient in his late fifties newly diagnosed with low-degree mT21 (13%/21% blood lymphocytes/ectodermal cells). We applied fluorescence in-situ hybridization to confirm a diagnosis of mT21. Multimodal positron-emission-tomography applying ^{18}F-fluodesoxyglucose (metabolism), ^{18}F-florbetaben (amyloid-β deposits) and ^{18}F-PI-2620 (tau-deposits) tracers was used to confirm a diagnosis of EOAD according to the ATN-criteria of AD. Initial PET-studies revealed marked cerebral amyloid-β- and tau-pathology and parietotemporal hypometabolism, confirming EOAD according to the ATN-criteria of AD. A marked cognitive decline was accompanied by an increase in tau pathology in follow-up studies. This is the first case demonstrating that a low-degree APP gene-dose increase suffices to cause EOAD with prominent amyloid-β/tau pathology.

Keywords: Alzheimer Disease; Amyloid; Neurogenetics; Positron Emission Tomography; Tau; PI-2620

1. INTRODUCTION AND CASE REPORT

Most patients with Down syndrome (DS) carry a triplication of the amyloid precursor protein (APP) gene because of its localization on chromosome 21 and are thus at high risk of developing early-onset Alzheimer dementia (EOAD).(Wiseman et al., 2015) The relevance of this gene-dose alteration to AD pathophysiology was corroborated by two findings: The identification of families with isolated APP triplications and EOAD and the description of DS cases with partial trisomy-21 lacking an APP-triplication who did not develop AD in their lifetime.(Doran et al., 2017; Sleegers et al., 2006) Moreover, it was speculated that sporadic AD may be caused by mosaic neuronal aneuploidy or APP gene copy-number variations.(Lee et al., 2018; Potter et al., 2016) Singular cases were reported where an early-onset AD-like dementia in patients with little or no DS phenotype was ultimately ascribed to trisomy-21 mosaicism (mT21).(Puri et al., 1994; Ringman et al., 2008; Rowe et al., 1989) Consequently, it seems probable that unidentified mT21-carriers are at risk to develop EOAD. However, it is unknown to what degree mT21-associated cognitive decline shares pathophysiologic similarities with AD, since histopathology or imaging data is unavailable. Here, we present Aβ and protein tau positron-emission-tomography (PET) data of a patient with progressive forgetfulness, who was diagnosed with EOAD in association with previously unknown mT21.

1.1 Case report

A patient in his late fifties employed as an untrained worker presented to our memory clinic with progressive forgetfulness for two years. He was currently working part-time due to recurrent back pain caused by scoliosis. His employer recently noted a necessity to surveil his working efforts. He was living alone and managed all housekeeping tasks alone.

His past medical history revealed recurrent deep vein thromboses, hypothyroidism and hypertension. His medication comprised levothyroxine, phenprocoumon, metoprolol, losartan and hydrochlorothiazide. Symptoms indicating depression or psychosis were not present. Clinical examination showed no focal neurological deficit. Vital signs were normal, body mass index was 26 kg/m^2. A mild resemblance to a facial DS phenotype (brachycephaly, short neck, flat bridged nose) was noted, but key features like low-set ears, single palmar crease, epicanthal folds or micrognathia were missing. Laboratory investigations (fbc, thyroid/kidney/liver function tests, vitamin-B12, folate, inflammatory parameters) were normal. Spinal tab was not taken due to oral anticoagulation.

1.2 Early development and family history

The patient's parents reported a mildly delayed mental development in early childhood. Pregnancy and delivery were uneventful. After attending a regular primary school he had transferred to a special-needs school, where he graduated with average marks. He was able to read and write fluently. He obtained

employment as an untrained worker on the regular job market. His family history revealed one possible case of late-onset AD. His parents and brother had apprenticed professions.

2. METHODS

The patient was recruited from our outpatient memory clinic. Exact age, profession and gender (male gender is used) are not given to maintain anonymity. Data acquisition was conducted as part of a biomarker study (AD21), which was approved by the local institutional review board (applications 17-126 and 19-022). The patient gave written informed consent prior to inclusion. Clinical tests comprised a neurological examination and the CAMDEX-DS (Cambridge Examination for Mental Disorders of Older People with Down's Syndrome and Others with Intellectual Disabilities) interview including the CAMCOG-DS neuropsychological examination (German version). (Nubling et al., 2019) Genetic analysis was conducted via Sanger sequencing (primer design: ExonPrimer, sequences see supplement table A.1). Sequencing results (both strands) were analyzed using CLC Workbench (Version 7.9.1). ApoE status was determined with TaqMan Genotyping kits (rs7412/ rs429358, Thermo Fisher, Waltham, MA). PET imaging was performed on a Siemens Biograph-64 system. ^{18}F-florbetaben-PET (295 MBq) images were acquired 90-110 minutes post-injection. ^{18}F-PI-2620-PET (185 MBq) was conducted by dynamic emission recording (0-60 minutes post-injection, 30-60 minute time window for visual interpretation). ^{18}F-fluordesoxyglucose-PET (142 MBq) images were generated 30-50 minutes post-injection after fasting (six hours) and canceling of visual and acoustic stimuli (20 minutes prior to injection). PET and MRI images were co-registered and analyzed after transformation to the (Montreal-Neurology-Institute)-MNI coordinates via PMOD 3.5 (PMOD technologies, Basel, Switzerland). Intensity scaling applied a cerebellar grey matter reference for all tracers.

3. RESULTS

3.1 Neuropsychological assessment

Initially, the patient achieved 14/30 points in the Mini Mental State Examination. After genetic analysis (see below), the patient was re-examined using the CAMDEX-DS. His premorbid IQ was estimated at 75 points using the Colored Progressive Matrices test.(Raven JC, 2002) He scored 73/109 points in the cognitive test battery (CAMCOG-DS). Low scores were obtained regarding memory, whereas abstract thinking, attention and orientation appeared intact (see figure 1B.). Visual perception and praxis showed some impairment. Overall, these findings in conjunction with his medical history were consistent with a diagnosis of EOAD. In the following two years, the patient showed mild decline of overall cognitive function. Of note, memory scores mildly improved after donepezil was commenced. In the third year

however, CAMCOG scores markedly declined, most notably affecting memory, orientation and visual perception (see figure 1B.). At this time, the patient continued to live alone with increasing support by the parents and had entered early retirement.

3.2 Neuroimaging

Since initial neuropsychological assessments were suggestive of AD, multimodal imaging was performed (all shown in figure 1C.). An MRI scan yielded mild parietal atrophy as well as a few unspecific T2-hyperintense white matter lesions. In contrast, ^{18}F-fluordesoxyglucose (FDG) PET revealed marked parieto-temporal hypometabolism suggestive of AD. To corroborate the presence of Aβ pathology, ^{18}F-florbetaben Aβ-PET was conducted, revealing widespread symmetric cortical Aβ deposition with an emphasis on the prefrontal and parieto-temporal regions.(Jennings et al., 2015) In contrast, no striatal deposition was detected. Subsequently, ^{18}F-PI-2620 tau-PET (Mueller et al., 2019) demonstrated abundant parietal, posterior cingular, frontal and temporal tau deposition, consistent with Braak-Stage V/VI.(Braak and Braak, 1991) Upon follow-up after 18 months, tau deposition had further extended especially in the frontal regions (figure 1C, right panel).

3.3 Genetic analyses

Mutations in the EOAD-associated genes *APP*, *PSEN1/2* and TREM2 were excluded. ApoE e3/e4 heterogeneity was confirmed. A lymphocyte karyogram revealed trisomy-21 in 1/15 lymphocytes. Fluorescence *in situ* hybridization (FISH) analysis confirmed a previously undiagnosed mT21 in 13% (33/254) of blood lymphocytes and 21% (45/211) of ectodermal cells (buccal swab, see figure 1A.).

4. DISCUSSION

APP triplications and mutations in *APP*, *PSEN1* and *PSEN2* only account for a fraction of EOAD patients, mostly in cases with a family history suggesting autosomal-dominant inheritance (approximately 13% of all EOAD patients).(An et al., 2016; Campion et al., 1999) However, the frequent finding of a family history positive for dementia (~60-70%) renders further genetic influences on EOAD to be likely.(Campion et al., 1999; Lleo et al., 2002) Low-degree T21 mosaicism, which could be defined as mosaicism with a somatic T21 cell fraction insufficient to yield a DS phenotype, has very rarely been described in association with early-onset cognitive decline (Puri et al., 1994; Ringman et al., 2008; Rowe et al., 1989). Furthermore, an association between T21 and AD is implied by the increase in DS-positive family histories in relatives of AD patients (van Duijn et al., 1991) and the high prevalence of AD in females who gave birth to children with DS at a young age, the latter pointing towards the possibility of a low-degree mosaicism in these mothers or a proneness to form trisomic cells through mechanisms that are not yet understood.(Schupf et al., 2001)

In the present case, the low-degree mT21 detected in both blood and ectodermal cells is the most likely cause for the abundant Aβ and tau deposition and corresponding regional hypometabolism detected by ^{18}F-florbetaben-, ^{18}F-PI-2620- and ^{18}F-FDG-PET, especially in the absence of APP/PSEN mutations. Fulfilling both the ATN-scheme AD biomarker alterations and the neuropsychological criteria for AD with no evidence of confounders such as clinical depression, this case comprises the strongest evidence of the pathophysiological role of low-degree mosaicism to date.(Jack et al., 2018) However, it has to be noted that the prevalence of trisomic brain cells is unknown. Furthermore, the role of the ApoE e3/e4 genotype in this patient has to be discussed. Studies exploring the role of ApoE e4 in DS-AD reported heterogeneous results, with a recent meta-analysis concluding that ApoE e4 encompasses a lower risk than in non-DS patients.(Rohn et al., 2014) Similarly, heterogeneous ApoE e4 is frequently found in EOAD, yielding an estimated 3-4fold increase in EOAD risk. (Sando et al., 2008) Interestingly, the significance of a heterogeneous ApoE e4 genotype appears to be increased in patients with a positive family history, implying synergistic effects with other, yet unknown factors. It is thus possible that in our patient, the ApoE genotype facilitated the development of AD promoted by mosaic trisomy.

Importantly, our results indicate that ^{18}F-PI-2620 PET is a viable biomarker in AD associated with DS for diagnostic and monitoring purposes, which is the largest population of genetically determined AD.(Strydom et al., 2018) These findings further corroborate mT21 as a potential cause of EOAD in patients with little to no DS phenotype. While a coincident occurrence of EOAD and mT21 cannot be completely ruled out in this patient, the negative family history and overall low incidence of EOAD render this possibility highly unlikely. Therefore, FISH analysis should be considered in EOAD patients after exclusion of established differential diagnoses, especially in patients with a suggestive phenotype or developmental history. An mT21 diagnosis in EOAD allows for a more precise genetic counsel since mosaicism as such is not hereditary. However, it has to be noted that the data currently available do not allow predict the likelihood of mT21 in EOAD.

Beyond mT21 being a differential diagnosis in EOAD, the findings presented here corroborate two hypotheses concerning the pathophysiology of AD. In the first paradigm, AD may be the result of insufficient clearance or aberrant generation of aneuploid cells in the developing brain, where neuronal aneuploidy is frequently found and abundant clearance of aneuploid cells takes place during development. This hypothesis is supported by the finding of increased T21-carrying neurons in AD brains (Potter et al., 2016). Secondly, APP gene amplifications in AD neurons were recently identified via single-cell qPCR, resulting in a gene-dose increase of approximately 8%.(Bushman et al., 2015) Therefore, aberrant APP copy-number variations and other DNA content variations may account for an increased APP gene-dose in AD. The case described here further corroborates the hypothesis that even

a mildly increased APP gene-dose suffices to induce abundant AD-like cerebral pathology, and could thus be considered a proof of concept for the pathophysiological relevance of the aforementioned theories.

In summary, the evidence concerning the pathophysiological relevance of low-degree APP gene amplification provided here warrants extending genetic studies in EOAD cases with any evidence for developmental delay or mild DS phenotype by FISH analysis to rule out T21 mosaicism. A similar approach may be considered in EOAD in general, including cases with a positive family history for AD.

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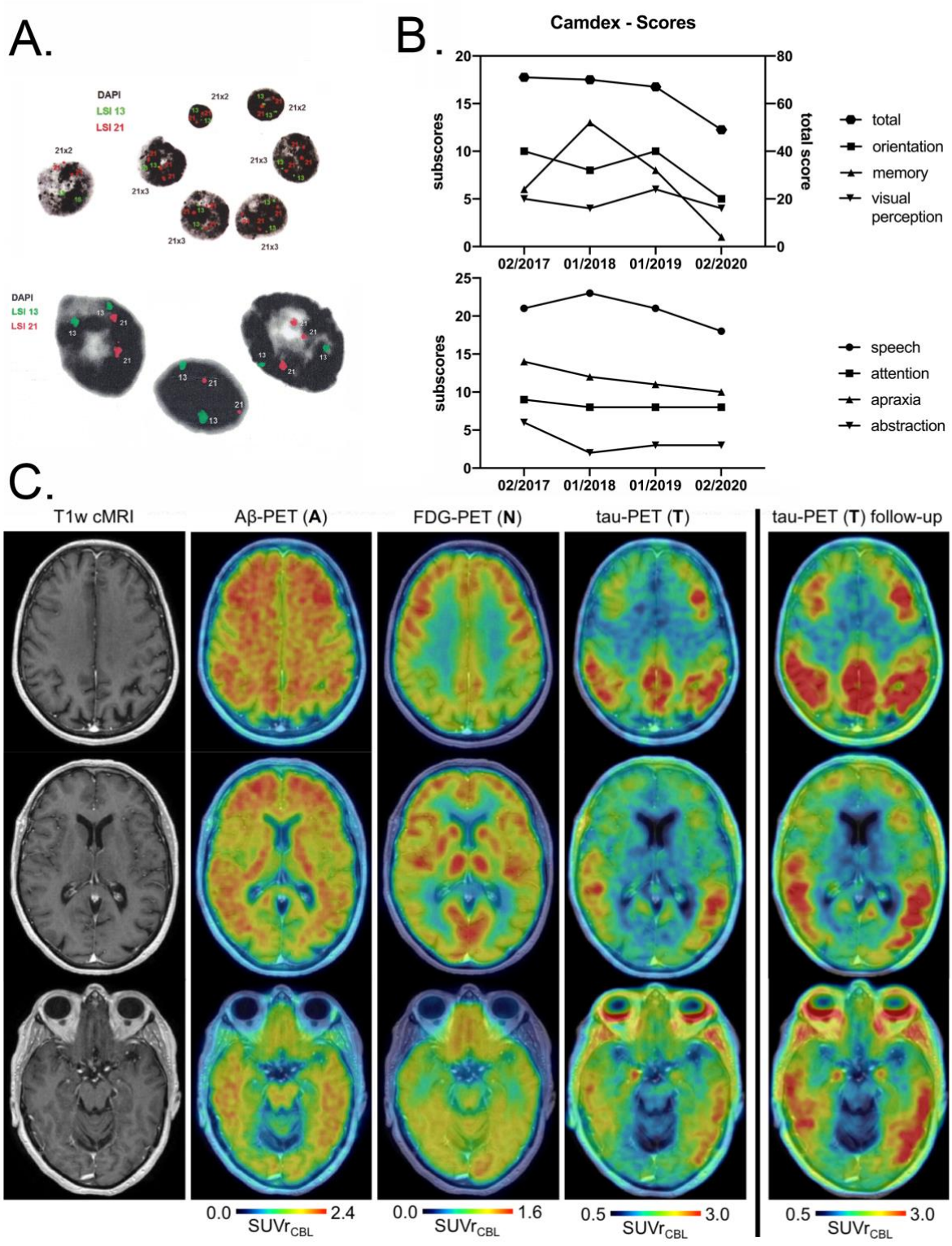

Figure 1 – MRI and PET imaging

A. FISH analysis of peripheral blood lymphocytes (upper cluster) and buccal swab (lower cluster) demonstrating mosaic trisomy 21. B. Progression of CAMCOG total scores and subscores over three years. C. Imaging data corresponding to the ATN diagnostic criteria for Alzheimer disease. MRI imaging (left column) demonstrates only mild parietal atrophy. ^{18}F-florbetaben Aβ-PET (center left column) shows abundant cortical amyloid deposition with emphasis on the frontal and parietotemporal regions.

^{18}F-FDG-PET (center column) shows parieto-temporal hypometabolism. ^{18}F-PI-2620 tau PET (center right column) reveals abundant parietal, posterior cingulate, frontal and temporal tau deposition, which demonstrated a marked increase in a follow-up investigation after 18 months (right column).