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Same ages, same genes: same brains, same pathologies?:

Dementia Timings, Co-occurring Brain Pathologies, ApoE Genotypes in identical and fraternal age-matched twins at autopsy.

Diego Iacono, MD, PhD^{1,2,3,4}, Inga Volkmann, BSc¹, Inger Nennesmo, MD, PhD⁵, Nancy L. Pedersen, PhD^{6,7}, Laura Fratiglioni, MD, PhD⁶, Boo Johansson, PhD⁸, David Karlsson, MD⁹, Bengt Winblad, MD, PhD¹, and Margaret Gatz, PhD^{6,10}

¹Karolinska Institutet, Department NVS, Center for Alzheimer Research, Neurogeriatrics, Huddinge, Sweden

²Neuropathology Research, Biomedical Research Institute of New Jersey, BRInj, Cedar Knolls, NJ, USA

³Atlantic Neuroscience Institute, Atlantic Health System, Overlook and Morristown Medical Center, NJ, USA

⁴Icahn School of Medicine at Mount Sinai, Department of Neurology, NY, USA

⁵Department of Pathology, Division of Neuropathology, Karolinska Institutet, Huddinge Hospital, Huddinge, Sweden

⁶Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden

⁷Aging Research Center (ARC), Karolinska Institutet/Stockholm University, Stockholm, Sweden

⁸Department of Psychology, University of Göteborg, Göteborg, Sweden

⁹Department of Geriatric Rehabilitation, Värnamo Hospital, Värnamo, Sweden

¹⁰Department of Psychology, University of Southern California, Los Angeles, CA, USA

Introduction

Twin studies assume exceptional relevance when we aim to analyze the brain pathologies associated with diagnosis of AD. It is now clear that multiple brain pathologies frequently co-occur in the same aged individual¹, showing different levels of severity and anatomical distribution, and that cannot be linearly correlated with the specific cognitive manifestations observed in a single AD patient.² Even MZ twins within a pair can show different degrees of clinico-pathologic variability.^{3–5} If we could assess autopsy-brains from clinically well-characterized MZ and DZ twins with or without dementia, and deceased during the same year, the epidemiological, clinical, pathologic, genetic, and molecular findings that could derive from these assessments would be potentially impressive. Although obtaining both brains from temporally-synchronized MZ and DZ twins, that is twins deceased at the same

age and that received a brain autopsy, are events more unique than rare, we had such a chance.

We report cognitive diagnoses, neuropathologic findings, and semi-quantitative immunohistochemistry-based measurements, of a series of co-occurring pathologies found in multiple regions of the brain of two twin pairs, one identical (MZ) and one fraternal (DZ), deceased at the same age. The age at death for the older male MZ pair was 98 years; the age at death for the younger opposite sex DZ pair was 79 years. With respect to ApoE genotype, where the allele ApoE4 is the major genetic risk factor for AD known so far⁶, the MZ twins were heterozygotes for the ApoE2 allele (ApoE2/3), while DZ twins were both heterozygotes for the ApoE4 allele (ApoE3/4). The MZ twins were then heterozygotes for the rarest ApoE allele, the ApoE2, which is also known to have protective properties against AD.⁷

The aim of the study was to assess and report semi-quantitative neuropathologic findings of co-occurring brain pathologies in these two temporally-synchronized-at death, MZ and DZ twin pairs.

Material and Methods

Both pairs of twins were initially part of the Swedish Twin Registry (STR).⁸ STR represents one of the oldest and largest twin registries available in the world. For a more detailed description of STR and its derivative studies, refer to previous publications.⁹

Selection criteria for this investigation were: a) brain autopsy for both twins of the same pair; b) one or both twins of the same pair diagnosed with dementia; c) death of both twins occurred during the same year. Among a total of 140 individual twin autopsies available, only 2 twin pairs met the above criteria.

Clinical and cognitive diagnosis

Clinical diagnoses of dementia followed DSM-III-R¹⁰ clinical diagnosis and NINCDS/ADRDA criteria [11], reflecting the year in which diagnoses were made. AD diagnoses were also reviewed accordingly to NIA-AA clinical criteria.¹² Furthermore, psychiatric symptoms were evaluated using the Neuropsychiatric Inventory for dementia (NPI).¹³

Neuropathologic criteria, brain regions, immunostains, semi-quantitative scorings

All four brains were stored and examined for standard neuropathologic assessments at the Brain Bank at Karolinska Institutet, Karolinska University Hospital at Huddinge, Stockholm, Sweden. Each autopsy was obtained in accordance with Swedish law. A detailed description of the methods, criteria, and antibodies used to assess these twin brains have been previously described.¹⁴ The following brain regions were selected for semi-quantitative assessments of each brain: middle frontal gyrus (MFG), medial temporal gyrus (MTG), anterior cingulate gyrus (ACG), posterior hippocampus (PH; at the level of the corpus geniculate lateral), amygdala (AMY), mesencephalon (MES; including the substantia nigra, SN), and pons (PONS; including the locus coeruleus, LC).

The following pathologic lesions were considered: β -amyloid diffuse-, neuritic-, and cored-plaques ($A\beta$ -DP, $A\beta$ -NP, $A\beta$ -CP); hyperphosphorylated-tau tangles (tau-NFT), tau-threads (tau-th), tau-dystrophic neurites (tau-N), and tau-plaques (tau-P); alpha-synuclein positive Lewy Body (LB), and alpha-synuclein positive dystrophic neurites (LN); ubiquitin positive (ubiq) neuronal cytoplasmatic inclusions (ubiq-NCI), ubiq-neuropil threads (ubiq-th), and ubiq-dystrophic neurites (ubiq-N); protein p62 positive (p62) neuronal cytoplasmatic inclusions (p62-NCI), p62-neuropil threads (p62-th), and p62-dystrophic neurites (p62-N); TDP43 positive (TDP43) neuronal cytoplasmatic inclusions (TDP43-NCI), TDP43-neuropil threads (TDP43-th), and TDP43-dystrophic neurites (TDP43-N); phosphorylated-TDP43 (pTDP43-NCI), pTDP43-neuropil threads (pTDP43-th), and pTDP43-dystrophic neurites (pTDP43-N). As for pathologic criteria, each brain was evaluated using the “*practical approach*” of NIA-AA pathologic criteria for AD.¹⁵

The semi-quantitative system used was defined as: no lesions= negative (N), 2 lesions= sparse (S), <2–6> lesions= moderate (M), and >6 lesions= frequent (F). For NCIs, number of lesions were referred to the number of neurons containing at least one cytoplasmatic inclusion independently on its shape (i.e. spheroidal or lentiform). For statistical purposes the frequency-based semi-quantitative system was transformed in a numerical system with N=0, S=1, M=2, F=3.

Descriptive statistics

After transformation to numerical values (0–3) for each brain region and immunostain, all lesions were aggregated per type of pathology:

- β -amyloid pathology= $A\beta$ -DP+ $A\beta$ -NP+ $A\beta$ -CP;
- tau pathology=tau-NFT+tau-th+tau-N+tau-P;
- Lewy pathology=LB+LN;
- ubiq pathology=ubiq-NCI+ubiq-th+ubiq-N;
- p62 pathology=p62-NCI+p62-th+p62-N;
- TDP43 pathology=TDP43NCI+TDP43-th+TDP43-N;
- pTDP43-NCI+pTDP43-th+pTDP43-N=pTDP43 pathology.

Neuropil threads (th) and dystrophic neurites (N) TDP43 and pTDP43 pathology were also evaluated separately to determine possible different p-/TDP43 pathology subtypes as previously proposed.¹⁶ Cumulative scores (sum of all single numerical scores obtained for each brain region, and for each immunostain) are shown in Figure 1.

Results

Main demographic, zygosity, attained years of education, clinical diagnoses, age of dementia-onset, interval between dementia-onset and death, and neuropsychiatric findings are summarized in Table 1a and 1b. Autopsy-confirmed diagnoses (CERAD criteria)¹¹ were: probable AD (probAD) for TwinA1 and TwinA2, and definite AD (def AD) for TwinB1 and TwinB2. None of the twins presented clinical or autopsy-diagnosis of: PD, DLB, VaD, PDD

or FTD. Levels of AD neuropathologic change (NIA-AA pathologic criteria-based) were “intermediate” for all four twins. All twins presented psychiatric symptoms (Table 1b).

For the ApoE2/3 MZ pair, the intra-pair difference between twins’ ages at dementia-onset was 7 years. Both twins showed marked TDP43 and pTDP43 pathology, defined as TDP43-pathology of *type D*¹⁶, with greater pathology in the twin with earlier onset. The twin with later onset (and shorter survival with dementia) showed greater tau pathology in particular, but also a higher β -amyloid cumulative score compared to his twin brother. Other co-occurring pathologies were minimal. For the ApoE3/4 DZ pair, there was marked β -amyloid and tau pathology in both twins, with both cumulative scores higher in the twin with the slightly later onset, as well as substantial co-occurring brain pathologies, ubiq in particular. In contrast, there was very little TDP43 and pTDP43 pathology in either DZ twin. As for the anatomical distribution, the only cerebral regions constantly positive to both TDP43 and pTDP43 pathologies, as for other pathologies (i.e. p62, ubiq), were PH and AMY.

Figure 1 shows time-scales, cognition-deaths intervals, and cumulative scores-histograms, for all pathologies in both twin pairs. Table 2a, b, c (supplementary material) shows single pathologic score for all types of pathology in each cerebral region considered in this study.

Discussion

To the best of our knowledge, clinico-pathologic correlations with semi-quantitative immunohistochemistry-based neuropathologic analyses on twins deceased at the same age has never been described before.

Our autopsy findings from this unique set of same-age twin brains confirm previous larger studies supporting ApoE4 as an accelerating factor and ApoE2 as protective factor for AD [7]. ApoE2 was associated indeed with dementia, but at a quite advanced age. At the same time, in the MZ twin pair, the interval between dementia onsets suggests that other modifying factors should be taken in account¹⁷ to better explain why their dementia onsets and clinical manifestations differed within the twin pair. Dementia in both MZ twins was associated with relatively low levels of amyloid, tau and all other considered co-occurring brain pathologies, except for p/TPD43 pathology, with frequent NCI among diffuse TDP43/pTDP43 positive nuclei in both members of the pair. As novelty, this last observation suggests a possible correlation between TDP-43 pathology and duration of disease. Intriguingly, a possible new modifying effect (protective) of ApoE2 on TDP-43 pathology in very old subjects could be hypothesized based on these novel clinico-pathologic observations. This possible correlation between TDP-43 pathology and duration of disease in very old subjects could acquire actually even higher relevance considering the discrepancy between duration of disease and other “classic” AD pathologies, like tau pathology, for example.

The association of ApoE2 with reduced amounts of various brain pathologies: β -amyloid, tau, LB, ubiq, and p62 pathology is coherent with previous neuropathologic observations on the relationship between ApoE2 vs ApoE4 allele and AD pathology, especially for amyloid.¹⁸ The TDP43 and pTDP43 findings may indicate differential positivity at different

stages of disease, which could be also related to the ageing *per se* and not necessarily to a clinical form of FTD. Studies on TDP43/pTDP43 lesions, their anatomical distributions, age-related accumulations, and possible correlations to ageing-related neurodegenerative processes are largely missing.¹⁹ Relatively less accumulation of TDP43/pTDP43 pathology was seen in the other twin pair, who were ApoE4 heterozygotes and had a younger age of onset. Based on these rare twin cases, and with the limitation of the sample size, a differential TDP43/pTDP43 nuclear/NCI distribution pattern in AD, and in other neurodegenerative diseases as well, could be hypothesized. This new possible clinico-pathologic correlation (TDP-43 vs. duration of disease) needs to be confirmed by larger clinico-pathologic studies. These studies, however, should take also in account: the possible correlation between TDP-43 pathology accumulation and aging *per se*, the preferential deposition of TPD-43 pathology in specific brain regions, other co-occurring age-related brain pathologies, as well as possible modifying factors such as ApoE2 and other biological or environmental factors, which remain still unknown.

These clinico-pathologic findings on age-matched-at autopsy twins provide a rare and unusual example of the complexity of AD. Although genetic factors, especially ApoE genotype, may play an important accelerating role in the pathogenesis of dementia, and specifically for AD, other non-genetic factors are necessarily involved to explain the clinicopathologic variability observed among AD cases, even between identical twins with a genetic protective factor as ApoE2.

We cannot assume that these single pairs of MZ and DZ twins are representative of MZ and DZ twins in general, but together, the four cases offered a unique natural experiment and a rare investigative window into the variability of brain pathologies associated with AD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Life times-scale, ApoE genotypes, dementia-onsets/death intervals, co-occurring pathologies, and cumulative scores in age-at death matched twins

Fig. 1.

The figure shows the main life-cognitive events, intervals, and histograms of cumulative scores of all pathologies considered in the study. $a=7$, $b=1.5$ years. DO= dementia-onset, AOD= age of death. $\beta A=\beta$ -amyloid, tau=hyperphosphorylated-tau protein, Lewy=Lewy pathology (bodies and neurites), ubiqu=ubiquitin, p62= protein p62, TDP43= transactive response DNA binding protein 43, pTDP43=phosphorylated-TDP43. MZ= monozygotic, DZ=dizygotic.

Part a shows main demographic, educational, ApoE genotype, age of death, dementia-onset, interval of time between dementia-onset and death (interval dementia onset-death), difference of age between twins at the dementia-onset (difference between twins in age at dementia onset, and clinical diagnosis of Alzheimer’s disease (AD) following three different set of criteria: DSM, NINCDS/ADRDA, and NIA-AA clinical criteria. **Part b** shows clinical diagnosis of AD and related scores of the last Mini Mental Status Examination (MMSE) and Neuropsychiatric Inventory (NPI) before death, diagnostic probabilistic criteria for AD (NIA-Reagan, ABC scores, and level of AD neuropathologic change) for each twin. All scores are based on immunohistochemistry. Ages and intervals are expressed in years.

Table 1

Table 1a											
Twin ID	Zyg	Sex	Education	ApoE genotype	Age at death	Age at dementia-onset	Interval dementia onset-death	Difference between twin in age at dementia-onset	Clinical diagnosis (DSM-III-R)	Clinical diagnosis (NINCDS/ADRDA)	Clinical diagnosis (NIA-AA)
TwinA1	MZ	M	5	23	98	85	13	7	Dementia	ProbAD	ProbAD
TwinA2		M	6	23	98	92	6		Dementia	PossAD	PossAD
TwinB1	DZ	M	6	34	79	71.5	7.5	1.5	Dementia	ProbAD	ProbAD
TwinB2		F	6	34	79	70	9		Dementia	PossAD	PossAD

Table 1b											
Twin ID	Last MMSE	NPI assessment	Psychiatric symptoms (NPI items)	Type (NPI items)		CERAD age-related plaques score	CERAD diagnosis	NFT-Braak stage	NIA-Reagan criteria	ABC score	Level of AD neuropathologic change
TwinA1	16 (5)	5	Yes	delusions, hallucinations, apathy, eating disturbance apathy, lability, sleep and eating disturbances		B	Prob AD	IV	Intermediate	A1B2C3	Intermediate
TwinA2	14 (1)	4	Yes			B	Prob AD	IV	Intermediate	A1B2C3	Intermediate
TwinB1	9 (5)	5	Yes	delusions, hallucinations, agitation, anxiety, apathy, sleep disturbance delusions, hallucinations, anxiety, apathy, motor disturbance, eating disorder		C	Def AD	V	High	A1B3C3	Intermediate
TwinB2	9 (4)	5	Yes			C	Def AD	V	High	A1B3C3	Intermediate