Acta Neuropathologica (2022) 144:1171–1174
https://doi.org/10.1007/s00401-022-02500-w

CORRESPONDENCE

# Expanding the spectrum of amyloid-β plaque pathology: the Down syndrome associated 'bird-nest plaque'

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Received: 3 June 2022 / Revised: 9 September 2022 / Accepted: 10 September 2022 / Published online: 16 September 2022


**Keywords** Alzheimer's disease · Amyloid-beta · Down syndrome · Plaque

Cotton wool plaques are the characteristic feature of hereditary Alzheimer's disease (AD) with *PSEN1* mutations [7]. Recently, Boon et al. reported a new type of Aβ plaque, referred as the 'coarse-grained plaque (CGP)' associated with early onset AD (EOAD) [1]. Thus, several factors including age, genetic abnormalities, and disease progression may influence the morphology of Aβ plaques.

Down syndrome (DS) is caused by triplication of chromosome 21 and most patients develop severe AD-like pathology early in their life [6]. In the frame of our study in DS patients [4], we identified an unusual morphological form of Aβ plaques. Here, we evaluated frontal, temporal, and parietal cortices from 11 DS patients (38–66 years) [4] (further details in Supplementary Table 1, online resource).

We observed plaques clearly identifiable in H&E staining showing a (1) distinct border, (2) relatively large diameter (mean: ~ 70 µm, range: 36–118 µm), (3) a dense fibrillar structure in all 10 cases with severe Aβ deposition, and in 7 cases, a frequently visible amyloid core (Fig. 1a). The plaques were observed in clusters, predominantly in layers II–IV of sulcal depths and were associated with cerebral amyloid angiopathy (CAA). In thioflavin S staining, plaques were composed of thick and dense amyloid fibrils (Fig. 1b). Immunostaining against Aβ (aa 8–17; 6F/3D) revealed dense fibrous structures with a distinct circumscribed border reminiscent of a bird-nest (Fig. 1c). Thus, we named these plaques as 'bird-nest plaque (BNP)'. Unlike Aβ plaques characteristic of AD, the BNP is strongly immunoreactive for $A\beta_{40}$ (Fig. 1d), but less immunoreactive for $A\beta_{42}$ (Fig. 1e) (Supplementary Table 2, online resource, summarizes additional Aβ antibodies). Phosphorylated (p)-tau-immunopositive dystrophic neurites and reactive astrocytes were observed bordering the plaques (Fig. 1f, g), and microglia were observed around and within the plaques (Fig. 1h). Except for glial fibrillary acidic protein (GFAP), the immunoreactive properties of the BNP and CGP were similar (Fig. 1i–p), and 7 DS cases also showed plaques consistent with the CGP. To ensure distinction of CGP from BNP in H&E sections, the following two criteria were most informative:

1. a distinct border (also detectable in Aβ-immunostaining) formed by reactive (eosinophilic) astrocytic processes, and
2. formation of thick and dense amyloid fibrils within the plaque (see Table 1 [1, 2], Supplementary Figs. 1–3, online resource).

We found several BNPs fulfilling both criteria, defined as 'typical BNP', in 7 out of 10 DS cases. In addition, all 10 cases contained distinct plaques, which lacked the unequivocal astrocytic border and/or amyloid core, but contrasting CGP, showed thick and dense amyloid fibrils. We interpreted these as early forms ("immature") of a BNP. Typical BNPs were predominantly found in frontal and temporal cortices and to a lesser extent the parietal cortex contrasting CGPs

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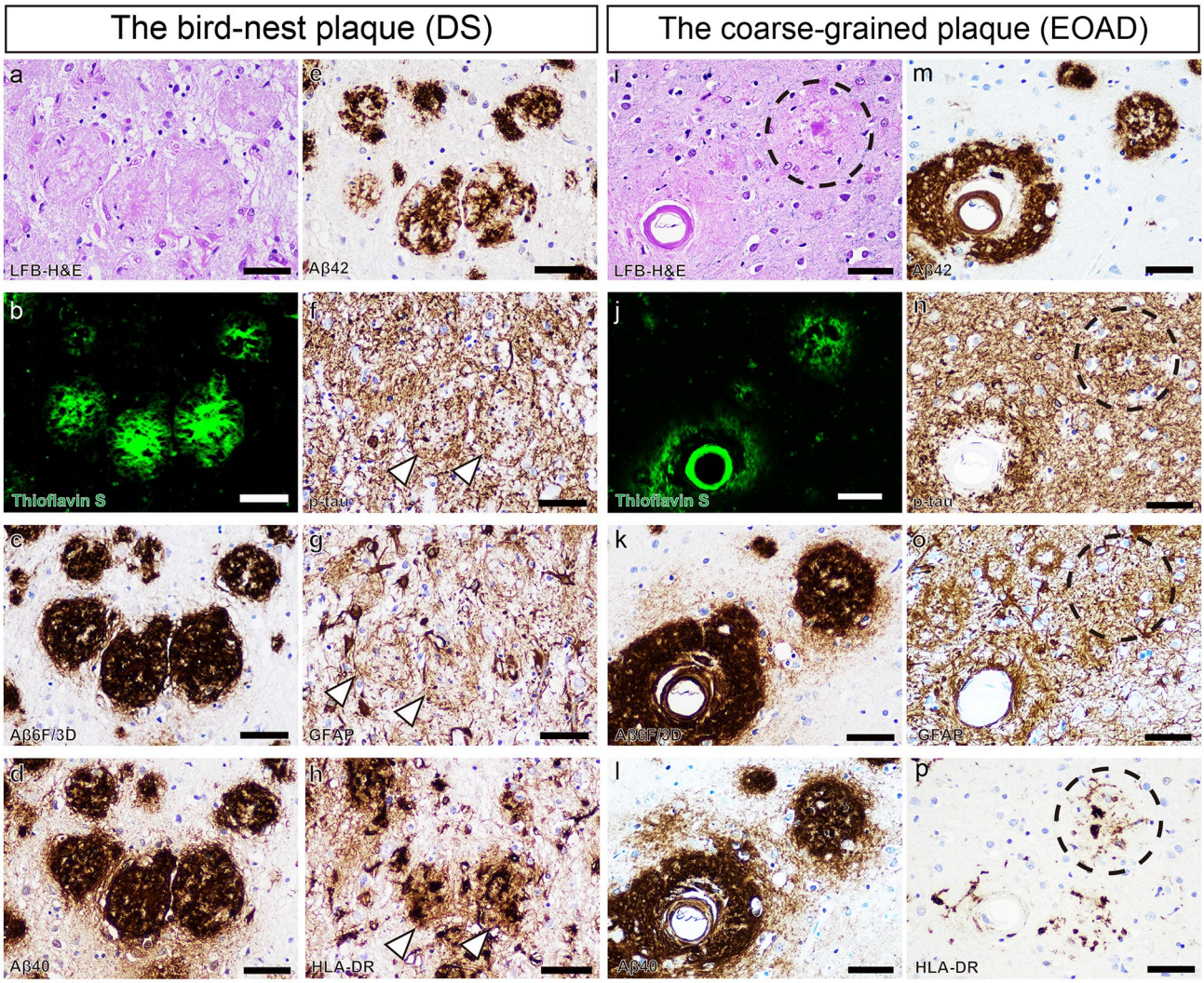
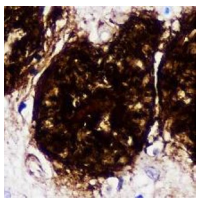
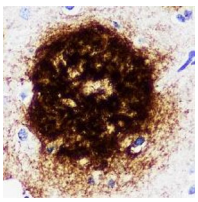
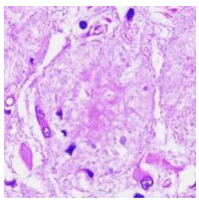
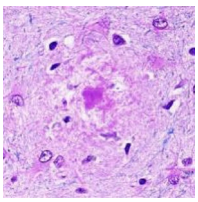
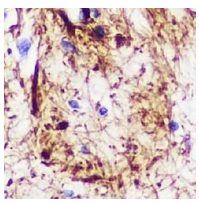
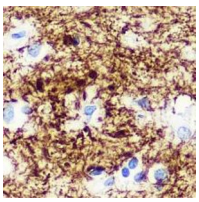



**Fig. 1** Representative microphotographs of the bird-nest plaques (BNP) and the coarse-grained plaques (CGP). **a**–**h** The BNP in a Down syndrome case (Case 4). **i**–**p** The CGP in an EOAD disease case. **a**, **i** Luxol fast blue-hematoxylin and eosin (LFB-H&E) staining. **b**, **j** Thioflavin S staining. **c**–**h**, **k**–**p** Immunohistochemistry for Aβ (6F/3D; c, k), $Aβ_{40}$ (**d**, **l**), $Aβ_{42}$ (**e**, **m**), p-tau (AT8; **f**, **n**), GFAP (**g**, **o**), and HLA-DR (**h**, **p**). In LFB-H&E staining and GFAP immunostaining, the BNP shows a distinct circumscribed border formed by reactive astrocytic processes embracing the plaques, whereas the CGP exhibits tissue distortion without a clear astrocytic border. In thioflavin S staining, amyloid fibrils in the BNP are dense compared with the CGP. Immunostaining for Aβ confirms these observations and show similar antigen profiles for BNP and CGP (c–m). Note amyloid angiopathy beside the CGP, suggesting a relationship between the two [1]. Arrowheads indicate the BNPs. Scale bar = 50 μm (**a**–**p**)

[1]. Thus, using the criteria, we examined temporal cortex specimens from 19 non-DS-related early onset (age of death: 47–75 years) and 10 late-onset AD cases (age of death: 75–96 years), and found only one EOAD case (66 year-old female) exhibited a single typical BNP.

This study identified an Aβ plaque (BNP) reminiscent of the "fibrous plaques" reported previously [5], supporting the notion that DS-related AD pathology is distinct. Although the morphology of the typical BNP can be distinguished from the CGP in EOAD, Aβ immunohistochemical properties are similar [1]. We hypothesize that EOAD and DS are associated with a distinct process of plaque formation in sulcal depths associated with CAA, representing a spectrum of plaques including CGP and BNP. Overexpression of APP, differences in composition of the constituent peptides of the plaques between DS, EOAD, and late-onset AD, and/or distinct neuroinflammation and astrocytic function/s may be responsible for the morphological differences [2, 3, 6]. Based on our study, the presence of BNPs, particularly typical BNPs, in the temporal cortex can be suggestive of DS-related AD pathology and indicate more accelerated Aβ accumulation. Since the shape and border of plaques in Aβ-immunostained sections can be subjective to the observer, identification of surrounding astrocytes by H&E

**Table 1** Comparison of the histological and immunohistochemical findings between the BNP and CGP. *IR* immunoreactivity, *ND* not done

| Staining | Bird-nest plaque | | Coarse-grained plaque [1, 2] (Our results/previous study's results) | |
|---|---|---|---|---|
| **Aβ (6F/3D)** |  | Strong IR with clearly defined border |  | Strong IR with poorly defined border |
| **H&E** |  | Dense fibrillar deposition with clearly-defined border formed by embracing reactive astrocytes |  | Poorly-defined border with tissue distortion |
| **GFAP** |  | +; astrocytic processes bordering the outer plaque, forming the border |  | +; cell bodies are often found within the plaque and the border is unclear |
| Thioflavin S | ++; Thick fibrillar amyloid throughout the plaque | | +; Fibrillar amyloid throughout the plaque/+~++ | |
| $A\beta_{40}$ | ++ | | ++/++ | |
| $A\beta_{42}$ | +; weaker than $A\beta_{40}$ | | +/±; weaker than $A\beta_{40}$ | |
| $A\beta_{Np3E}$* | ++ | | ND/++ | |
| $A\beta_{pSer8}$* | + | | ND/++ | |
| P-tau | +; dystrophic neurites | | ±/±; dystrophic neurites | |
| Microglia | ++ | | +; HLA-DR/+; MHC-II | |

The staining methods indicated in bold are the most useful for differentiating between BNP and CGP

*The results are shown in Supplementary Fig. 2

IR grading is indicated as follows: ±, some positive staining + positive staining. + + prominent positive staining

staining and GFAP immunostaining facilitates accurate identification of BNPs.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s00401-022-02500-w.

**Acknowledgements** Brain samples were obtained through appropriate consenting procedures with Local Ethic Committee approval and approved by the University Health Network Research Ethics Board (Nr. 20–5258). This study was supported by the Edmond J Safra Philanthropic Foundation and the Rossy Family Foundation and Canadian Foundation for Innovation (40480) and the Ontario Research Fund.

## Declarations

**Conflict of interest** GGK is member of the Acta Neuropathologica Editorial Board, but was not involved in the editorial handling of this article. SI, IMV, and SF declare no competing interest.

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