Published in final edited form as:

Neurosci Lett. 2017 January 01; 636: 254–257. doi:10.1016/j.neulet.2016.11.023.

Cognitive ability in Down Syndrome and its relationship to urinary neopterin, a marker of activated cellular immunity

Panagiotis Zis^{1,2}, André Strydom^{1,3}, David Buckley⁴, Daniel Adekitan⁴, and Patrick C McHugh⁴

¹University College London, Division of Psychiatry, London, United Kingdom

²Sheffield Teaching Hospitals NHS Trust, Department of Neurology, Sheffield, United Kingdom

³The LonDowns Consortium

⁴Centre for Biomarker Research, School of Applied Sciences, University of Huddersfield, Huddersfield, United Kingdom

Abstract

Background—Neopterin is an unconjugated pteridine that is secreted in large quantities by activated macrophages and can be used as a clinical marker of activated cellular immunity and oxidative stress. We aimed to investigate whether urinary neopterin levels are associated with cognitive function in people with Down syndrome (DS).

Methods—Out of 32 adults with DS who originally participated in a longitudinal study, 25 were followed up at 4 years. Informants rated their adaptive behavior (ABAS) and the adults with DS attempted assessments of language skills and memory at both baseline and follow-up time points (Modified Memory Object Task, MOMT), and receptive vocabulary (British Picture Vocabulary Scale, BPVS).

Results—Neopterin/creatinine levels were negatively correlated with change in the MOMT total score (Spearman's $Rho = -0.517$, $p = 0.020$) and change in the MOMT delayed recall score (Spearman's $Rho = -0.577$, $p = 0.008$) over time, i.e. higher neopterin/creatinine level was associated with worse performance on a test of cognitive ability over time.

Conclusion—Urine neopterin may have potential as a biomarker for memory decline in Down syndrome, and could potentially also help to track progression of mild cognitive impairment (MCI) to Alzheimer's disease in other high risk populations.

Keywords

Down syndrome; aging; Alzheimer's disease; memory; neopterin; oxidative stress

1 Introduction

Oxidative stress is related to the imbalance between oxidant production and the organism's defenses against its potential damage. In oxidative stress, the organism lacks the ability to detoxify the system of reactive oxygen species (ROS) and repair the damage they cause. Levels of ROS above the clearance capacity of the body can cause mitochondrial

dysfunction, cellular damage, and cell death [1]. Oxidative stress has been implicated in the pathogenesis of many diseases, including Alzheimer's disease (AD) [2,3].

In people with Down syndrome (DS), the most common genetic cause of intellectual disability (ID), the prevalence of AD increases significantly with age [4]. By the age of 50, almost all people with DS have neuropathological [5–7] and neuroimaging changes characteristic of AD [5, 6]. Although this may be in large part due to the triplication of the APP gene in DS, increasing evidence suggests that oxidative stress and inflammation may also play a role in AD pathogenesis associated with the syndrome [8].

Neopterin is an unconjugated pteridine that is secreted in large quantities by activated macrophages and can be used as a clinical marker of activated cellular immunity [9]. Plasma neopterin levels have been found to be higher in patients with AD [10, 11] and people with DS [12], with [13] or without [14, 15] dementia. Moreover, urinary neopterin levels correlate with cerebrospinal fluid neopterin levels in specific patient populations, such as multiple sclerosis patients with positive oligoclonal bands [16].

We aimed to investigate whether urinary neopterin levels are associated with cognitive function in people with DS and whether these levels correlate with isoprostane 8,12-iso-iPF₂α (iPF₂α), a urinary marker of oxidative stress.

2 Methods and Materials

2.1 Study population, iPF₂α levels, neopterin levels and psychometric measurements

We used a within-syndrome design to examine the association between urinary markers of oxidative stress and changes in cognitive functioning over time in adults with DS. We have previously established a cohort of 32 individuals with DS who underwent cognitive testing and donated urine samples for iPF₂α [17]. The patients were followed-up 4 years later [18] when they also donated a urine sample for iPF₂α [19] and neopterin analysis. Urine spot samples were collected in the morning and were stored within 6 hours of collection at -80°C.

Methods for measuring iPF₂α (corrected for creatinine concentrations) have been described elsewhere for both the baseline [17] and the follow-up time points [19].

Urine neopterin levels were measured at follow-up. Urine stored at -80°C was initially allowed to thaw at room temperature in the dark, mixed thoroughly by repeated inversion, and diluted 1/100 with mobile phase (15mM Potassium Phosphate; pH 6.4). Five hundred microlitres of diluted urine was then processed through a 0.45μm RC membrane (4mm) syringe filter. Each sample was analysed in triplicate with an injection volume of 20μl and the coefficients of variation for neopterin were 4.4% (intra-assay) and 8.6% (inter-assay). Each sample was analysed on an Agilent Technologies 1100 system at a flow rate of 1 mL/min using a reversed-phase SphereClone ODS(2) (4.6 x 250 mm, 5 μm) column coupled to a SecurityGuard guard cartridge (Phenomenex). Fluorescence detection of neopterin was performed using a 1260 Infinity Fluorescence Detector with an excitation and emission wavelength of 353 nm and 438 nm, respectively. Creatinine was detected using an Agilent

1100 series UV detector with a wavelength of 235 nm. The concentration of neopterin in the urine was then normalised per mole of creatinine.

Details of the psychometric assessments and population characteristics have also been described elsewhere [17 – 19]. In brief, informants (who were in most cases family members) completed a measure of adaptive behavior (Adaptive Behaviour Assessment Scale; ABAS [20]) and a dementia screen (the cognitive scale of the Dementia Scale for people with Learning Disabilities; DLD [21]). The DLD identifies individuals who may benefit from a clinical assessment for dementia, but does not provide a formal diagnosis. If a difference of 7 points or more on the sum of cognitive scores (i.e. short- and long-term memory and orientation) and/or 5 points or more on the sum of social scores (i.e. speech, activity, mood, behaviour and practical skills) is found between the baseline assessment and subsequent assessments, assessment for dementia should be considered [22].

Participants who were able to, completed a measure of receptive vocabulary (British picture vocabulary scale; BPVS II [23]), and a modification of an object memory task (MOMT) based on the Fuld object memory test [24, 25]. We modified the task by reducing the number of objects to 6, and the number of trials to two.

2.2 Ethics statement and consent procedures

The study was approved by the National NHS research ethics committee and we followed the Mental Capacity Act, UK (2005) if participants did not have the capacity to consent for themselves, by gaining agreement from carers. Written informed consent or carer's agreement for participation was obtained for all participants.

2.3 Data analyses

Data was analysed with Statistical Package for the Social Sciences (SPSS) [26]. Mann Whitney U tests were used to compare scores between groups. Correlations between neopterin levels and baseline factors were examined using Spearman's correlations. Correlations between the changes in psychometric and functional assessments scores (BPVS, ABAS, MOMT) were examined using Spearman's correlations. The significance level was set at 0.05.

3 Results

3.1 Study population

The original cohort consisted of 32 individuals with DS. Two of the original participants refused to participate again, and four were lost to follow-up. All of the remaining 26 individuals were followed up. Twenty-five urine samples were available for neopterin analysis. The 25 participants (52% females) had a mean age of 36.6 ± 7.2 years. None was diagnosed with Alzheimer's disease at baseline. At follow up, 4 of the 26 individuals screened above threshold on DLD, but did not have a clinical diagnosis of AD and upon assessment did not present with sufficient features for a DSM-IV or ICD10 diagnosis.

3.2 Neopterin, creatinine and iPF2alpha measurements

At follow-up, urinary neopterin levels ranged from 0.13 to 6.49 $\mu\text{mol/ml}$ (mean 1.79 ± 1.28), urinary creatinine levels ranged from 1.23 to 37.50 mmol/ml (mean 12.75 ± 9.38) and neopterin levels adjusted for creatinine ranged from 76.82 to 434.89 $\mu\text{mol/mol creatinine}$ (mean 162.91 ± 84.32). No significant correlation was observed between age and urinary neopterin (Spearman's ρ 0.107, $p=0.610$) or urinary creatinine (Spearman's ρ 0.083, $p=0.694$).

Data on urinary iPF2alpha were available for 24/26 patients at baseline and for 25/26 patients at follow-up. At baseline, the range of iPF2alpha was 0.16 to 3.18 ng/mg creatinine (mean 1.40 ± 0.81) and at follow-up 0.28 to 5.27 ng/mg creatinine (mean 1.35 ± 1.22). For the 23/26 patients that had measurement on both time points the change between iPF2alpha levels at follow up and at baseline ranged from -2.60 to +4.30 ng/mg creatinine (mean 0.007 ± 1.60).

3.3 Relationship between neopterin and urinary iPF2alpha

Neopterin/creatinine levels were not correlated significantly with the iPF2alpha/creatinine levels (Spearman's ρ 0.312, $p=0.129$). However, neopterin/creatinine levels were positively correlated with the change in urinary iPF2alpha/creatinine levels over the 4-year period (Spearman's ρ 0.445, $p=0.034$).

3.4 Relationship between urinary neopterin/creatinine and cognitive ability changes

Table 1 lists the correlations between levels of neopterin/creatinine and performance over 4 years on tests of functional ability, memory or language ability. There were no statistically significant correlations between levels of neopterin/creatinine and change in ABAS total score, change in ABAS functional academic score, or change in BPVS score. However, neopterin/creatinine levels were negatively correlated with change in the MOMT total score (Spearman's $\rho = -0.517$, $p=0.020$) and change in the MOMT delayed recall score (Spearman's $\rho = -0.577$, $p=0.008$).

4 Discussion

We have previously shown that change in urinary iPF2alpha/creatinine over time is associated with memory decline in people with DS, suggesting that sequential measurements of urinary iPF2alpha may have potential as biomarker for cognitive decline and progression to AD in this population [19]. In the present study, we have explored the relationship between cognitive change over time in adults with DS and urinary neopterin, a marker of immune activation, and its relationship to oxidative stress. Compared to other markers of activated cellular immunity and oxidative stress, such as isoprostanes, neopterin analysis is relatively low cost which is an advantage in both the clinical and the research setting.

Our results suggest that neopterin/creatinine levels correlate with cognitive ability over time suggesting that urinary neopterin may be an early marker of cognitive decline in DS. In our study, higher neopterin/creatinine level was associated with worse performance on a test of cognitive ability over time (positive difference in the MOMT score means improvement and

negative difference in the MOMT score means deterioration). This finding is consistent with other studies where it has been shown that plasma neopterin levels are higher in demented individuals with DS [13] and that a high plasma neopterin level is an independent determinant of the risk of dementia in persons with Down syndrome [27]. Plasma neopterin has been shown to be increased in healthy adults with DS compared to age and sex matched healthy controls [15]. Our results extend these findings by demonstrating that neopterin levels may be associated with memory decline before the onset of dementia in this population.

It has been suggested that oxidative stress is an important consequence of the specific biology of DS, and is likely to play a role in some of the co-morbidities associated with DS, including the tendency to develop AD [28]. Oxidative damage may lead to enhanced amyloid beta peptide (A β) production [29–33]. Although the precise mechanism is still unknown it has been shown that oxidation of proteins is an early event in DS and might contribute to neurodegenerative phenomena [34].

Although neopterin may play a role as a measure of oxidative stress, since the amount of neopterin produced by activated monocytes/macrophages correlates with their capacity to release ROS [35], neopterin is primarily a measure of immune response [9, 35–37]. Although oxidative damage may be one of the causes of inflammation or immune response, it is not necessarily the only cause and, therefore, the relationship between neopterin and markers of oxidative stress may be partially related to other, unmeasured factors. This may account for our observation that there was not direct relationship between neopterin and iPF2alpha levels; however, there was a relationship between neopterin levels at follow-up and a change in iPF2alpha levels between baseline and follow-up.

The strength of our study is that it is – to our knowledge – the first to show that urinary neopterin may be used as a biomarker of immune activation due to the pathogenic processes involved in AD in DS, including the effects of oxidative stress, and which correlates with cognitive decline over time in this population.

Our results should be interpreted with some caution given the limitations of our design. Firstly, our sample size was relatively small, and our results need to be confirmed with a replication larger study. As we have not measured the neopterin level at baseline we can only indirectly correlate the neopterin level with cognitive decline over time. A prospective cohort study is needed to confirm our findings.

In conclusion, for the first time, it is suggested that urinary neopterin is correlated with cognitive decline DS individuals before the diagnosis of AD. This finding strengthens the evidence that peripheral neopterin levels may have potential as a biomarker in DS, and in AD. The evidence for the role of immune response and oxidative stress in DS is growing and also suggests potential treatment strategies to reduce the high risk for AD in this population.

Acknowledgments

We wish to thank the Down Syndrome Association (UK) for their assistance with recruitment, and all the participants and their carers for their time and efforts. Funding for the baseline work was provided by the north central London research consortium (NoCloR). Support for follow-up analysis has been through a Wellcome Trust

Strategic Award (grant number: 098330/Z/12/Z) awarded to The London Down Syndrome (LonDownS) Consortium).

References

1. Patten DA, Germain M, Kelly MA, Slack RS. Reactive oxygen species: stuck in the middle of neurodegeneration. *J Alzheimers Dis.* 2010; 20(2):S357–S367. [PubMed: 20421690]
2. Bennett S, Grant MM, Aldred S. Oxidative stress in vascular dementia and Alzheimer's disease: A common pathology. *J Alzheimers Dis.* 2009; 17:245–257. [PubMed: 19221412]
3. Ansari MA, Scheff SW. Oxidative stress in the progression of Alzheimer disease in the frontal cortex. *J Neuropathol Exp Neurol.* 2010; 69:155–167. [PubMed: 20084018]
4. Wiseman FK, Al-Janabi T, Hardy J, Karmiloff-Smith A, Nizetic D, Tybulewicz VL, Fisher EM, Strydom A. A genetic cause of Alzheimer disease: mechanistic insights from Down syndrome. *Nat Rev Neurosci.* 2015 Sep; 16(9):564–74. [PubMed: 26243569]
5. Roizen NJ, Patterson D. Down's syndrome. *Lancet.* 2003; 361:1281–1289. [PubMed: 12699967]
6. Teipel SJ, Hampel H. Neuroanatomy of Down syndrome in vivo: A model of preclinical Alzheimer's disease. *Behav Genet.* 2006; 36:405–415. [PubMed: 16485178]
7. Hyde LA, Crnic LS. Age-related deficits in context discrimination learning in Ts65Dn mice that model Down syndrome and Alzheimer's disease. *Behav Neurosci.* 2001 Dec; 115(6):1239–46. [PubMed: 11770055]
8. Yang H, Zhuo JM, Chu J, Chinnici C, Praticò D. Amelioration of the Alzheimer's disease phenotype by absence of 12/15-lipoxygenase. *Biol Psychiatry.* 2010; 68(10):922–9. [PubMed: 20570249]
9. Armstrong RA, Cattell RJ, Jones SA, Winsper S, Blair JA. Elevated urinary neopterin suggests immune activation in Alzheimer's disease and Down's syndrome. *Neuroscience research communications.* 1994; 14(2):63–9.
10. Parker DC, Mielke MM, Yu Q, Rosenberg PB, Jain A, Lyketsos CG, Fedarko NS, Oh ES. Plasma neopterin level as a marker of peripheral immune activation in amnesic mild cognitive impairment and Alzheimer's disease. *Int J Geriatr Psychiatry.* 2013 Feb; 28(2):149–54. [PubMed: 22539447]
11. Savas S, Kabaroglu C, Alpman A, Sarac F, Yalcin MA, Parildar Z, Ozkinay F, Kumral E, Akcicek F. No relationship between lipoprotein-associated phospholipase A2, proinflammatory cytokines, and neopterin in Alzheimer's disease. *Exp Gerontol.* 2016 May; 77:1–6. [PubMed: 26828804]
12. Mehta PD, Patrick BA, Dalton AJ, Patel B, Mehta SP, Pirttila T, Coyle PK. Increased serum neopterin levels in adults with Down syndrome. *J Neuroimmunol.* 2005 Jul; 164(1–2):129–33. [PubMed: 15908015]
13. Coppus AM, Fekkes D, Verhoeven WM, Tuinier S, van Duijn CM. Plasma levels of nitric oxide related amino acids in demented subjects with Down syndrome are related to neopterin concentrations. *Amino Acids.* 2010 Mar; 38(3):923–8. [PubMed: 19455393]
14. Mehta PD, Capone G, Jewell A, Freedland RL. Increased amyloid beta protein levels in children and adolescents with Down syndrome. *J Neurol Sci.* 2007 Mar; 254(1–2):22–7. [PubMed: 17275850]
15. Coppus AW, Fekkes D, Verhoeven WM, Tuinier S, Egger JI, van Duijn CM. Plasma amino acids and neopterin in healthy persons with Down's syndrome. *J Neural Transm (Vienna).* 2007; 114(8):1041–5. [PubMed: 17401539]
16. Leuzzi V, Carducci C, Chiarotti F, D'Agnano D, Giannini MT, Antonozzi I, Carducci C. Urinary neopterin and phenylalanine loading test as tools for the biochemical diagnosis of segawa disease. *JIMD Rep.* 2013; 7:67–7. [PubMed: 23430498]
17. Strydom A, Dickinson MJ, Shende S, Praticò D, Walker Z. Oxidative stress and cognitive ability in adults with Down syndrome. *Prog Neuropsychopharmacol Biol Psychiatry.* 2009; 33(1):76–80. [PubMed: 18983885]
18. Zis P, Dickinson M, Shende S, Walker Z, Strydom A. Oxidative stress and memory decline in adults with Down syndrome: longitudinal study. *J Alzheimers Dis.* 2012; 31(2):277–83. [PubMed: 22561328]

19. Zis P, McHugh P, McQuillin A, Praticò D, Dickinson M, Shende S, Walker Z, Strydom A. Memory decline in Down syndrome and its relationship to iPF2alpha, a urinary marker of oxidative stress. *PLoS One*. 2014 Jun.9(6):e97709. [PubMed: 24901945]
20. Harrison, PT, Oakland, T. Adaptive Behaviour Assessment System (ABAS). Harcourt Assessment; USA: 2000.
21. Evenhuis HM. Further evaluation of the Dementia Questionnaire for Persons with Mental Retardation (DMR). *J Intel Dis Res*. 1996; 40:369–373.
22. Walker B, MacBryer S, Jones A, Law J. Interinformant agreement of the dementia questionnaire for people with learning disabilities. *British Journal of Learning Disabilities*. 2015 Sep 1; 43(3): 227–33.
23. Dunn, LM, Dunn, LM. 2nd edition. NFER-Nelson; London: 1997. British Picture Vocabulary Scale.
24. Fuld PA, Masur DM, Blau AD, Crystal H, Aronson MK. Objectmemory evaluation for prospective detection of dementia in normal functioning elderly: predictive and normative data. *Journal of Clinical and Experimental Neuropsychology*. 1990; 12(4):520–528. [PubMed: 2211974]
25. Burt DB, Aylward EH. Test battery for the diagnosis of dementia in individuals with intellectual disability. *Journal of Intellectual Disability Research*. 2000; 44(2):175–180. [PubMed: 10898382]
26. SPSS inc. SPSS for Windows Release 11.0.0. SPSS Inc; 2001.
27. Coppus AM, Fekkes D, Verhoeven WM, Evenhuis HM, van Duijn CM. Neopterin and the risk of dementia in persons with Down syndrome. *Neurosci Lett*. 2009 Jul 17; 458(2):60–4. [PubMed: 19442877]
28. Lott IT. Antioxidants in Down syndrome. *Biochim Biophys Acta*. 2012; 1822(5):657–63. [PubMed: 22206998]
29. Nunomura A, Perry G, Pappolla MA, Friedland RP, Hirai K, Chiba S, et al. Neuronal oxidative stress precedes amyloid- β deposition in Down syndrome. *J Neuropathol Exp Neurol*. 2000; 59:1011–1017. [PubMed: 11089579]
30. Sultana R, Perluigi M, Butterfield DA. Lipid peroxidation triggers neurodegeneration: A redox proteomics view into the Alzheimer disease brain. *Free Radical Biology and Medicine*. 2012; doi: 10.1016/j.freeradbiomed.2012.09.027
31. Reddy PH. Amyloid precursor protein-mediated free radicals and oxidative damage: implications for the development and progression of Alzheimer's disease. *J Neurochem*. 2006; 96:1–13.
32. Praticò D, Uryu K, Leight S, Trojanowski JQ, Lee VM. Increased lipid peroxidation precedes amyloid plaque formation in an animal model of Alzheimer amyloidosis. *J Neurosci*. 2001; 21:4183–4187. [PubMed: 11404403]
33. Cenini G, Dowling AL, Beckett TL, Barone E, Mancuso C, Murphy MP, et al. Association between frontal cortex oxidative damage and beta-amyloid as a function of age in Down syndrome. *Biochim Biophys Acta*. 2012; 1822(2):130–8. [PubMed: 22009041]
34. Di Domenico F, Coccia R, Cocciolo A, Murphy MP, Cenini G, Head E, Butterfield DA, Giorgi A, Schinina ME, Mancuso C, Cini C, Perluigi M. Impairment of proteostasis network in Down syndrome prior to the development of Alzheimer's Disease neuropathology: Redox proteomics analysis of human brain. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*. *Biochim Biophys Acta*. 2013; 1832(8):1249–1259. [PubMed: 23603808]
35. Murr C, Fuith LC, Widner B, Wirleitner B, Baier-Bitterlich G, Fuchs D. Increased neopterin concentrations in patients with cancer: indicator of oxidative stress? *Anticancer Res*. 1999 May-Jun;19(3A):1721–8. [PubMed: 10470106]
36. Svoboda P, Ko SH, Cho B, Yoo SH, Choi SW, Ye SK, Kasai H, Chung MH. Neopterin, a marker of immune response, and 8-hydroxy-2'-deoxyguanosine, a marker of oxidative stress, correlate at high age as determined by automated simultaneous high-performance liquid chromatography analysis of human urine. Neopterin, a marker of immune response, and 8-hydroxy-2'-deoxyguanosine, a marker of oxidative stress, correlate at high age as determined by automated simultaneous high-performance liquid chromatography analysis of human urine.
37. Firth CA, Laing AD, Baird SK, Pearson J, Gieseg SP. Inflammatory sites as a source of plasma neopterin: measurement of high levels of neopterin and markers of oxidative stress in pus drained from human abscesses. *Clin Biochem*. 2008 Sep; 41(13):1078–83. [PubMed: 18619433]

Table 1
Correlations between performance in psychometric measures over 4 years and levels of neopterin/creatinine.

		Neopterin/creatinine (t2)
Change in ABAS total score	Spearman's Rho	0.163
	P	0.437
	N	25
Change in ABAS functional academic score	Spearman's Rho	-0.116
	P	0.581
	N	25
Change in BPVS raw score	Spearman's Rho	-0.415
	P	0.062
	N	21
Change in MOMT total score	Spearman's Rho	-0.517
	P	0.02 *
	N	20
Change in MOMT delayed recall score	Spearman's Rho	-0.577
	P	0.008 **
	N	20

*
 $p < 0.05$.

**
 $p < 0.01$.