Prevalence of *C9ORF72* expansion in a large series of patients with idiopathic normal pressure hydrocephalus

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Abstract:

Background/Aims: The *C9ORF72* expansion is known to cause frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis (ALS). We aim to identify the prevalence of the *C9ORF72* expansion in idiopathic normal pressure hydrocephalus (iNPH) **Methods:** We analysed the *C9ORF72* expansion in a large cohort of patients with possible iNPH (n=487) and cognitively intact elderly controls (n=432; age > 65 years). **Results:** While the *C9ORF72* expansion was detected in 1.6 % (n=8/487) of cases with possible iNPH, no control subject was found to carry the mutation. The mean age at onset of symptoms of *C9ORF72* expansion carriers was 59 years (range: 52-67 years), 11 years less than non-carriers (p=0.0002). The most frequent initial/main symptom pertained to gait difficulties. Despite identified mutation, only three of the patients fulfilled the criteria for FTLD-ALS spectrum. Clinically significant shunt response was detected in 6 out of 7 shunted *C9ORF72* expansion carriers. **Conclusion:** This is the first study cohort identifying the underlying *C9ORF72* expansion in patients with iNPH providing evidence for the potential comorbidity between iNPH and FTLD-ALS spectrum. Analysis of the *C9ORF72* expansion should be considered for patients with probable iNPH presenting with frontal atrophy and personality changes or other severe psychiatric symptoms.

Running title: Prevalence of *C9ORF72* expansion in iNPH

82 Introduction

83

84 Idiopathic normal pressure hydrocephalus (iNPH) and frontotemporal lobar degeneration (FTLD)
85 are cognitive disorders presenting with overlapping clinical features. INPH is characterized by a
86 triad of core symptoms, namely gait difficulties, cognitive impairment, and urinary incontinence.
87 The symptoms are progressive in nature, and patients may express the triad only partially or fully
88 (1,2). In iNPH, ventricular enlargement comprises a key radiological finding alongside the
89 observable characteristic clinical symptoms (3,4) and the observed cognitive impairment is usually
90 characterized by psychomotor slowness, impairment in frontal executive functions, as well as other
91 symptoms reflecting frontal-subcortical dysfunction (1,4,5). By contrast, FTLN is a clinically
92 heterogeneous group of syndromes that includes behavioural variant frontotemporal dementia
93 (bvFTD), semantic variant primary progressive aphasia (svPPA), and non-fluent variant primary
94 progressive aphasia (nfvPPA). BvFTD is the most common clinical subtype of FTLN, which
95 presents with progressive changes in personality and behaviour, together with deficits in executive
96 functions (6). Hexanucleotide repeat expansion in the *C9ORF72* is the most common genetic cause
97 of bvFTD (7,8). Although amyotrophic lateral sclerosis (ALS) represents the most frequent motor
98 symptom in patients with the *C9ORF72* expansion, extrapyramidal symptoms have also been
99 documented (9,10).

100 On the basis of the similarity of clinical symptoms in bvFTD and iNPH, it was hypothesized that
101 bvFTD may demonstrate comorbidity with iNPH. Importantly, we have previously documented a
102 bvFTD case suffering from comorbid iNPH (11). To this end, the aim of the study was to evaluate
103 the role of the *C9ORF72* expansion in a large cohort of iNPH patients. This is the first study
104 addressing systematically the prevalence of the *C9ORF72* expansion in iNPH.

105

106 Methods

107 All experimental procedures complied with the standards of the Declaration of Helsinki and further,
108 the Kuopio University Hospital Research Ethics Board approved the study. All patients provided
109 informed consent. The clinical cohort comprised of 487 patients with possible iNPH (*Fig. 1; Table*
110 *1*). The control group consisted of 432 subjects (mean age at evaluation 72 ± 3.4 ; range 66-87; 159
111 male), who underwent a thorough evaluation by an experienced neurologist specialized in memory
112 disorders, to rule out neurodegenerative diseases.

113

114 INPH diagnosis was carried out by neurologist in accordance with the Relkin et al. (2005)(1)
115 criteria. The diagnosis of bvFTD was based on the Rascovsky et al. (2011) (6) criteria. Patients who
116 simultaneously presented with at least one of the three core symptoms associated with iNPH,
117 together with enlarged ventricles disproportionate to the size of the sulci of the cerebral convexities
118 (Evan's index >0.30) (14) based on CT or MRI scans, were referred to a neurosurgeon.
119 Subsequently, a 24-hour intraventricular pressure (IVP) monitoring, spinal tap, or lumbar infusion
120 test was performed by a neurosurgeon.

121

122 A ventriculoperitoneal shunt was placed in 409 patients, out of whom 44% (n=181) were shunted
123 on the basis of the results of the 24-hour IVP monitoring. The remaining patients were shunted
124 according to the following protocol: 32% (n=132) of patients were shunted based on a positive tap-
125 test (at least 20% increase in walking speed in a 20-meter test, including one turn); 16% (n=66)
126 based on a pathological finding (conductance ≤ 10) in a lumbar infusion test; and 7% (n=30) based
127 on the combination of clinical evaluation and radiological findings.

128

129 Shunt response was defined as an improvement in at least two of the classical triad of symptoms, or
130 improvement in one classical symptom accompanied with a significant improvement in headaches,

131 vertigo, and/or balance. Additionally, objective shunt response evaluation, where pre and post-shunt
132 gait speed was used as a determining variable, was available for some of the patients. Gait speed
133 improvement over 20 % was considered significant (*Table 2*).

134

135 The patients' cognitive functioning was evaluated by the Consortium to Establish a Registry for
136 Alzheimer's Disease Neuropsychological Battery (CERAD-NB) or MMSE. Behavioral symptoms
137 were evaluated by Frontal Behavioural Inventory (FBI). Additionally, extensive follow-up data and
138 medical records were carefully re-evaluated.

139

140 The patients' first-degree family history was carefully mapped and history of iNPH, ALS, dementia,
141 or psychiatric diseases were evaluated (reported in table 2).

142

143 Brain biopsy was extracted from 425 patients from the right frontal cortex during the insertion of
144 the ventricular catheter for shunt or IVP monitoring. One biopsy was excluded from the cohort due
145 to technical problem (#6). A neuropathologist evaluated immunohistochemistry for beta-amyloid
146 (6F/3D), hyperphosphorylated tau (AT8), p62, and TDP-43.

147

148 Previous MRI (1.5T or 3T) (n=6) or CT-scans (n=2) of all *C9ORF72* expansion carriers were re-
149 evaluated by an experienced neuroradiologists, in order to both characterize and quantify the
150 apparent morphological changes of the brain described in Tables 2 and 3.

151

152 The presence of the *C9ORF72* repeat expansion was detected utilizing the Repeat-Primed PCR
153 (RP-PCR) (8). The results of the RP-PCR were confirmed using the Amplicon length analysis (12),
154 and verified in a collaborating laboratory, where the analysis was repeated in a blinded manner.
155 All of the identified living cases with the *C9ORF72* repeat expansion were clinically re-evaluated
156 by an experienced neurologist (AMR) specialized in FTLN (patient #3 deceased during the follow-
157 up period).

158

159 Results

160 The *C9ORF72* expansion was detected in 1.6% (8/487) of cases with possible iNPH. Seven of them
161 fulfilled the clinical diagnostic criteria for probable iNPH, who subsequently underwent the
162 shunting procedure. (Table 2). Additionally, one identified iNPH case with the *C9ORF72* expansion
163 failed to fully fulfil the diagnostic criteria for probable iNPH, thus having finally an unlikely iNPH
164 status. The patient in question suffered from severe and widespread cognitive impairment and was
165 diagnosed with both clinically and neuropathologically (#3) confirmed Alzheimer's disease (AD);
166 thus, ventriculoperitoneal shunt was not placed in this case. One shunted patient was retrospectively
167 diagnosed to be suffering from neuropathologically confirmed FTLN (#7). None of the control
168 subjects was found to carry the *C9ORF72* expansion.

169 The mean onset age of symptoms in patients with the *C9ORF72* expansion was significantly lower
170 compared to non-carriers (59 vs 70 years, 95 % CI 5.1-16.7, $p=0.0002$). The mean Evan's Index
171 was 0.43. Disproportion between the sylvian and suprasylvian subarachnoid spaces was present in
172 all but one (#8) of the patients, with the results ranging from mild (#2, #3, #4, #6) to severe (#1, #5,
173 #7) (Table 3, Fig 2).

174 Six patients were found to present with the full classical triad of symptoms associated with iNPH
175 together with the enlarged ventricles as indicated by brain imaging. In five out of the eight patients,
176 gait difficulty was the leading symptom. The mean pre-operative MMSE score of the sample was
177 20.8/30 [14-25, median 22]. Results from the CERAD-NB overall showed that five patients were
178 found to exhibit mild to moderate cognitive impairment (#1, #4, #6, #7 and #8), with one remaining
179 patient demonstrating unimpaired cognition (#2), and the other remaining patient showing profound
180 deficits due to severe dementia (#5) (*Table 4*). Moreover, two patients suffered from mild
181 depression. The mean follow-up time was 7.4 years.

182 Two patients demonstrated mild behavioral changes, and one patient displaying moderate
183 deterioration of behaviour. Out of the eight patients, only two cases fulfilled the clinical diagnostic
184 criteria for bvFTD (6), with one of the remaining patients being diagnosed with ALS based on
185 ENMG (#1). All patients had a family history of symptoms linked to iNPH, ALS, dementia, or
186 psychiatric diseases (*Table 2*).

187 Positive clinically verified or objectively measured shunt response was detected in almost all cases
188 when evaluated during multiple follow-ups. (*Table 2*). The most frequent benefit attributable to the
189 shunt pertained to improvement in gait followed by improvement in urinary incontinence. Some of
190 the patients showed a modest improvement in both episodic memory and executive functions (#1,
191 #6, #7) even though no clinically relevant shunt response was evident in their cognitive abilities.
192 The patient's cognitive abilities showed a tendency to deteriorate over the mean cognitive follow-up
193 period of 10.6 (range 4-70 months) years but some of the patients remained cognitively stable
194 (*Table 4*).

195 Beta-amyloid reactivity was common but only one patient presented with both beta-amyloid and tau
196 protein concomitantly (#3), with the patient subsequently being diagnosed with AD. All of the
197 patients testing positive for beta-amyloid were *ApoE4* carriers. P62 and TDP-43
198 immunohistochemistry were frequently detected but not in all cases (*Table 2*, *Figure 3*).

200 Discussion

201 This is the first study addressing the presence of the *C9ORF72* repeat expansion in an iNPH cohort.
202 Surprisingly, the *C9ORF72* expansion was found in 1.6 % of patients primarily diagnosed with
203 possible iNPH. Almost all of the *C9ORF72* expansion carriers presented with gait difficulties.
204 Recently it has been suggested that the full or intermediate (20-30 repeats) *C9ORF72* expansion
205 could be associated with Parkinson's disease (PD) (13) but the results were not replicated in
206 autopsy confirmed study setting or meta-analysis of published research (14,15). However,
207 connection between atypical Parkinsonian disorders (progressive supranuclear palsy, multiple
208 system atrophy and cortico-basal degeneration) and intermediate number of repeats have been
209 found in many papers (16–20).

210 The differential diagnostics in iNPH is based on clinical and radiological findings. Response to
211 shunt plays a significant role in confirming the diagnosis, which causes most likely both missed and
212 false diagnoses (4,21). It should be noted that ventriculomegaly can be an early sign of
213 neurodegeneration even when parasagittal sulci are obliterated. In the current study, all shunted
214 patients fulfilled the criteria for probable iNPH but only three of the *C9ORF72* expansion carriers
215 were determined to additionally suffer from comorbid bvFTD or ALS. It could be that the clinical
216 spectrum of *C9ORF72* expansion is variable and the current clinical criteria for bvFTD might not
217 be sensitive enough to identify atypical bvFTD patients (6,22). However, interestingly 6/7 of the
218 shunted *C9ORF72* expansion carriers in the study cohort were observed to demonstrate a clinically
219 verified shunt response, suggesting that the presence of the *C9ORF72* expansion did not hamper the
220 shunt response. However, the shunt surgery failed to significantly impact upon the patients
221 cognitive functioning and the shunt response showed a tendency toward diminishing during the

222 follow-up period, with the average duration of 7.4 years. These findings are in line with previous
223 reports on the shunt response (23,24)(15,16,17,18).

224 Neuropathological evaluation of the frontal cortical biopsies was available for seven of the cases
225 carrying the *C9ORF72* expansion. Unfortunately, neuropathological analyzes were not
226 systematically performed for p62 and TDP-43 pathology thereby diminishing both the validity and
227 the generalizability of the current results. The *C9ORF72* expansion has been associated with TDP-
228 43 pathology with or without p62 immunoreactive inclusions (27). In the current cohort, p62
229 immunoreactive inclusions were detected more frequently as compared to TDP-43 pathology,
230 which may either be due to the small size of the frontal cortical biopsy (1mm x 3-5 mm in size),
231 and/or some phenomena associated with the *C9ORF72* expansion. It has previously been shown
232 that the presence of ubiquitin together with p62 positive and TDP-43 negative neuronal inclusions,
233 especially in the cerebellum, hippocampus and thalamus, are highly specific to the *C9ORF72*
234 expansion (27,28).

235 In the current study, AD-related neuropathology characterized by beta-amyloid plaques without tau-
236 pathology was observed in two cases. Each of the beta-amyloid positive cases was an *ApoE4* allele
237 carrier. It has been previously reported that AD-associated neuropathology is occasionally linked to
238 the *C9ORF72*-associated FTLN (29). In addition, similarly to the AD patients, abnormally low CSF
239 A β_{1-42} levels have been identified in over 20% of the FTLN patients with the *C9ORF72* expansion
240 (30). As AD and vascular dementia are the most frequent cognitive disorders showing comorbidity
241 with iNPH, the pathologic features perceived in iNPH most commonly represent both vascular and
242 AD-related changes (31). Thus, the current results are consistent with the previous evidence of
243 typical TDP-43- and p62-positive inclusions in patients with the *C9ORF72* expansion. Furthermore,
244 the present findings provide supporting evidence to the assumption that mixed neuropathology may
245 be present both in cases with the *C9ORF72* expansion, as well as in iNPH patients.

Differential diagnosis between iNPH and bvFTD can be challenging due to the overlapping clinical and imaging features. For example, psychomotor slowness and deficits in executive functions represent typical features of both diseases (1,4–6). Difficulties with gait and balance form the core symptomatology of iNPH (1), and bvFTD patients also may suffer from extrapyramidal symptoms, such as gait impairment, bradykinesia, urinary incontinence, and tremor. Moreover, psychiatric symptoms are commonplace in bvFTD patients resulting in erroneous diagnoses of psychiatric disease at the beginning of the disease course (6). It is possible that also iNPH is associated with a constellation of psychiatric manifestations, including aggression, disturbances of impulse control, and even psychosis (1,3). Furthermore, Schizophrenia is an over-represented comorbidity in iNPH (32). The diagnostic process is further complicated by the fact that neuroradiological differential diagnostics are not clear in all cases. Ventricular enlargement is a key radiological finding in iNPH (1), and may also be present in the *C9ORF72* expansion-associated bvFTD (9,33).

Despite the growing evidence, the etiology of iNPH remains elusive. However, accruing literature suggests that iNPH likely is associated with a genetic component (34–39). The current findings showing that the *C9ORF72* expansion is associated with the iNPH phenotype in a subset of patients, appears to represent a further genetic variation with potential linkages to iNPH. Normal ventricular wall – ependyma – is covered by motile cilia and primary ciliary dyskinesias have been linked with congenital hydrocephalus (40). The *C9ORF72* expansion could, through yet unknown mechanisms, relate to ependymal dysfunction or the impaired clearance of harmful metabolites and therefore advance the progression of iNPH.

The main strengths of the current study include the large cohort of iNPH patients, which provides a unique opportunity to capture even the rather sporadic potential comorbid features that may be shared between iNPH and other neurodegenerative disorders. Secondly, the assessment of the shunt response combined with the evaluation of clinical characteristics of iNPH allows for reliable diagnosis of probable iNPH. Moreover, brain biopsies from the frontal cortex offer a rare albeit a

271 powerful window into the neuropathological changes in the living brain tissue. The limitations
272 include a potential for type one error due to the low frequency of the *C9ORF72* expansion and the
273 absence of the *C9ORF72* expansion in the cognitively healthy elderly population. However, the
274 diagnosis of bvFTD and iNPH was based on the evaluation of a neurologist and a neurosurgeon and
275 there was a consensus on the diagnoses regarding all patients.

276 **Conclusion**

277 This study provides the first known systematic evidence of the *C9ORF72* expansion among iNPH
278 patients. To this end, the current findings strongly support clinical similarity or even potential
279 comorbidity between iNPH and FTLD-ALS spectrum in a subset of patients carrying the *C9ORF72*
280 expansion. The clinical symptomatology associated with iNPH and bvFTD appear overlapping,
281 which complicates the differential diagnostics of these two diseases. However, in the current study,
282 the positive shunt response supports the idea of potential comorbidity, rather than unsuccessful
283 differential diagnostics between iNPH and FTLD-ALS spectrum. On the basis of the current
284 evidence, clinicians are encouraged to routinely inquire their patients about the existence of possible
285 behavioral and/or psychiatric problems, together with a family history of ALS/FTLD, to allow for
286 the consistent evaluation of the potential role of FTLD-ALS spectrum in iNPH patients. It is
287 additionally recommended that iNPH patients with cortical atrophy and personality changes, severe
288 neuropsychiatric and/or psychiatric symptoms, would be submitted to an analysis for the potential
289 presence of the *C9ORF72* expansion. On the other hand, FTLD patients with enlarged ventricles
290 and severely impaired gait should be evaluated by a neurosurgeon for a potential shunt benefit.
291 Despite the fact that the presence of the *C9ORF72* expansion in iNPH patients appears quite rare,
292 the frequency of the *C9ORF72* expansion in the present iNPH cohort was unexpectedly high. Thus,
293 further investigations into the potential role of the *C9ORF72* expansion in other iNPH cohorts are
294 warranted.

295 **Supplementary material**

296 No supplementary material.

297

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Fig 1: Flowchart

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Flow chart of 487 consecutive patients suspected with iNPH

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Fig 2: Neuroradiology

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CT or MR images of the 6 C9ORF72 expansion carriers. All patients (a, b, c, d, e, f) showed

423

enlarged ventricles with Evan’s index of 0.36-0.50. Evident frontotemporal (g, same patient as in

424

b), and frontoparietal cortical atrophy (h, same patient as in d).

425

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Figure 3: Neuropathology

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p62 immunopositivity (A, B), thin TDP-43 immunoreactive neuritis (C) and moderate amount of Aβ-

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immureactive plaques (D). Arrowheads marking example neuropathological changes.

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Table 1: Demographics of the study cohort and controls

431

Table 2: Clinical characteristics and pathological findings of the iNPH patients with the C9ORF72

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expansion

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Table 3: Neuroradiological features of the C9ORF72 expansion carriers

434 Table 4: Cognitive profile of the *C9ORF72* expansion carriers

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