This chapter is under revision as:
De Knecht NC, Schuengel C, Evenhuis HM, Lobbezoo F, Scherder EJA. Apolipoprotein E ε4, cognitive function, and pain experience in Down syndrome.
The presence of the apolipoprotein E (ApoE) ε4 allele appears to be related to cognitive impairment, but little is known about whether this also applies to people with intellectual disabilities, such as Down syndrome (DS). In addition, it has not been examined whether the relationship between cognitive function and pain is moderated by ApoE ε4. The present study addressed the associations among ApoE genotype, cognitive function, and self-reported pain experience in 157 adults with DS. DNA analysis of saliva, neuropsychological tests for memory and executive functioning (EF), and self-reporting pain scales (Facial Affective Scale and Numeric Rating Scale) were used. When controlling for age, ApoE ε4 carriers had a lower EF performance than non-carriers ($p = .044$, $\eta^2 = .04$). The difference in pain experience between ApoE ε4 carriers and non-carriers was not statistically significant and no moderation effect was found. The results extend previous findings and indicate the need for further research.

**Keywords:** cognitive function, Apolipoprotein E, Down syndrome, pain experience.
In the general population, the presence of the Apolipoprotein E (ApoE) ε4 allele is related to a lower performance on a working memory task [59] and a lower performance as well as a faster decline on a memory task [51]. ApoE ε4 carriers also have a faster reduction of hippocampal volume [45], lower rates of glucose metabolism in posterior cingulate, parietal, temporal, and prefrontal areas [57], and more brain activity during memory tasks in hippocampal, parietal, and frontal areas [4,5,23] than non-carriers.

The findings described in the previous paragraph suggest that the presence of the ApoE ε4 decreases cognitive function and influences brain areas involved in pain experience (e.g., prefrontal cortex and hippocampus: [65]). Pain experience is processed in the brain by pathways of white matter that connect grey matter brain areas: the medial and lateral pain systems [65]. Neuroanatomical and neuroendocrinological deviations have been found in these pain systems of people with Down syndrome (DS): small volumes of frontal lobes, hippocampus, amygdala, insula, and anterior cingulate cortex [31,80] and high concentrations of endogenous opioids leu-enkephalin and dynorphin A [58]. Little is known about the pain experience of people with Down syndrome (DS). Communication about pain as well as recognition and interpretation of pain behaviour is complicated in DS by certain factors, including verbal difficulties, a possible delayed pain response, and a tendency to express medical issues such as pain non-verbally instead of complaining about it [12,28,41,68]. Insight into the pain experience of people with DS would provide caregivers and medical professionals guidance for pain diagnostics and management. This is clinically relevant, because caregivers have reported that the detection and treatment of pain in adults with intellectual disabilities is complex and ambiguous [25], and because chronic pain could have a negative influence on emotional well-being, quality of life, and adaptive functioning [6,77].

One approach to obtain insight into the pain experience of adults with DS is to examine the functional relationship with cognitive function. Pain processing and cognitive function often share the same brain areas [46], such as the dorsolateral prefrontal cortex which is involved in both planning and pain inhibition [8,39], and the hippocampus which is involved in both memory and pain affect [7,38]. Indications have been found in elderly with and without
dementia that lower cognitive performance is associated with lower self-reported pain experience [49,50,64]. Establishing a relationship between cognitive function and pain experience in adults with DS could ultimately aid pain assessment, because a (further) decline in cognitive function might indicate an alteration in pain experience.

In establishing a relationship between cognitive function and pain experience in adults with DS, ApoE genotype may be an important genetic component to take into account. The presence of the ApoE \( \varepsilon 4 \) allele is associated in people with Down syndrome (DS) with a worse language comprehension [1] and a faster decline in intelligence [3], although one study showed that cognitive decline was related to chromosome 21 genotype instead of ApoE genotype [20]. The presence of the ApoE \( \varepsilon 4 \) allele in people with DS has also been associated with a faster decline of scores on the Social Functioning Scale (SRZ) [10], which is a measure of adaptive functioning [2].

The findings in the previous paragraph show that the evidence for the association between ApoE and cognitive function is still limited for people with DS. Further, it is largely unknown whether ApoE relates (perhaps in interaction with cognitive function) to pain experience. As far as we know, only one study was performed on this topic, which showed that the presence of an ApoE \( \varepsilon 4 \) allele was unrelated to pain intensity in fibromyalgia [56]. Therefore, the aim of the present study was to examine the associations among ApoE genotype, cognitive function, and self-reported pain experience in adults with DS and to explore moderation of associations by ApoE genotype.

**MATERIAL AND METHODS**

**Study design and ethical approval**
The design was an observational cross-sectional study. The Medical Ethical Committee of the involved university approved the study and informed consent procedure (file NL33540.029.11).

**Participants**
Participants with DS were recruited from 14 care centres for people with intellectual disabilities with locations throughout the Netherlands. Before the start of the
study, the care centres’ caregivers and behavioural specialists assessed inclusion and exclusion criteria per client. Inclusion criteria were: being 18 years of age or older, speaking and understanding Dutch, the capability to verbally answer simple questions, and a clinical impression of testability. This latter inclusion criterion implied that adults with DS could participate, regardless of their level of intellectual disability, as long as they could comprehend the instructions for at least some of the tests. Exclusion criteria were: the presence of neurological disorders such as cerebrovascular accidents, tumors, or dementia; the presence of severe visual impairments or hearing loss; and the use of antipsychotics, anticonvulsants or antidepressants, due to possible cognitive side effects [27,71].

Dementia was an exclusion criterion to remove confounding influences on cognitive function for a more reliable analysis of the relationship between cognitive function and pain experience. To screen participants aged 40 years and older for a possible indication of dementia, scores of the Social Functioning Scale for Intellectual Disability (i.e., SRZ or SRZ-P) [33,34] and the Dementia Questionnaire for Intellectual Disability (DMR) [19] were collected for two moments in time with at least six months between them to assess deterioration over time. The SRZ and SRZ-P assess social and cognitive abilities and activities of daily living, in which the abilities and activities included in the SRZ-P correspond to a higher level of functioning than those included in the SRZ. The caregiver (i.e., caregivers in care centres for people with intellectual disabilities or family members) choose whether the SRZ or SRZ-P was more appropriate for the participant’s level of functioning. When this choice was difficult to make, then both SRZ and SRZ-P were completed and the following criteria from the manuals were applied: the SRZ-P should be used if the SRZ total score is > 100 and the maximal score of at least one subscale is achieved, while the SRZ should be used if the SRZ-P total score is < 8 [33,34]. The DMR consist of the subscales short-term memory, long-term memory, orientation, speech, practical skills, mood, activity and interest, and behavioural disturbance. The questionnaires were completed after the test session and scores of at least six months earlier were derived from files of the care centres. When old scores were missing in the file, then the questionnaires were completed at least six months after the test session (n = 37). were used. A possible indication of dementia was considered to be present if the decrease in
the total scores of both the SRZ/SRZ-P and the DMR over the two moments in time was statistically significant according to criteria in the manuals. On the basis of this procedure, five individuals were excluded.

Participants had to provide informed consent to be included in the study. If there was doubt regarding their capacity to provide informed consent, consent was also required from parents or guardians. All tests were performed in a quiet room of the care centre or home where participants lived.

**Sample size**

According to the statistical program Gpower [21] with $\alpha = .05$, $\beta = .80$, and a medium effect size, the following sample sizes were required: $N = 85$ for a simple model with cognitive function and three covariates, and $N = 98$ for a moderation model with cognitive function, the presence of the ApoE $\varepsilon 4$ allele, the interaction between these two variables, and three covariates. We were able to include 31 – 52 participants in the analyses.

**Assessment of background variables**

*Estimated level of intellectual disability*

The SRZ and/or SRZ-P that were completed by caregivers were also used to estimate the level of intellectual disability. The Committee on Test Affairs Netherlands (COTAN) of the Dutch Institute of Psychologists has rated the reliability and validity of the SRZ and SRZ-P as ‘good’ [16,17]. The SRZ and SRZ-P are widely used in the Netherlands [72]. The caregiver choose whether the SRZ or SRZ-P was more appropriate for the participant’s level of functioning or completed both. In 9 participants, the use of the SRZ or the SRZ-P appeared to be incorrect according to criteria in the manuals. However, the distinction between SRZ and SRZ-P was in the present study mainly important for the possible dementia indication (i.e., comparison between two SRZ measurements or between two SRZ-P measurements). To use only one variable of estimated intellectual disability level, the intellectual disability levels for all participants were based on the SRZ. By using the population norms of the manual, the SRZ total score was converted into a standardized score, which was then converted into a level of intellectual disability by using the “Manual of Psychodiagnosics and Limited Ability” [35].
Participants of whom only the SRZ-P was available were identified as having a mild level of intellectual disability according to the SRZ.

**Estimated intelligence level**

The subtests Block Design and Vocabulary of the Wechsler Preschool and Primary Scale of Intelligence – Revised version (WPPSI-R) [79] were administered to estimate the intelligence level. The level of difficulty increased within both subtests. With Block Design, participants had to copy patterns by constructing them with blocks within a limited time. With Vocabulary, participants had to describe verbally the meaning of words and the most points were given for correct abstract descriptions. Afterwards, the age equivalents in years and months corresponding to the raw scores of the two subtests were retrieved from the “Manual of Psychodiagnostics and Limited Ability” [35], and the mean age equivalent was calculated. A Dutch version of the Vocabulary WPPSI-R subtest was used. However, our Dutch translation of 3 of the 12 words differed from forward-backward translation based on guidelines [47] and data collection was too far advanced to make adaptations. Because our translations seemed easier than the official translations, the mean age equivalent may have been slightly too high.

**Medical information**

Caregivers provided the researcher with file-based information about medication and physical conditions related to pain or pain-related discomfort. Also the presence of thyroid disorders, sleep problems, and symptoms of depression was reported, because of a possible negative influence on cognitive function [11,14,40,73], and the presence of autistic symptoms due to a possible difficulty with using a facial scale to self-report pain. Reported medical information (physical conditions, complaints, and medication administered for painful/discomforting conditions) was used to determine the possible presence of pain or discomfort. One physiotherapist, one general physician, and two specialized physicians for people with intellectual disabilities rated whether the reported physical conditions were expected to cause possible pain or discomfort. The two specialized physicians for people with intellectual disabilities first reached consensus, resulting in one list of ratings from the physiotherapist, one list from the general physician, and one list from the two specialized physicians for people with intellectual disabilities. The raters were blind for the ratings of the other professionals. A Fleiss’ kappa
of .66 was found, indicating a substantial agreement between the three lists [36]. A physical condition was ultimately rated as possibly causing pain or discomfort when at least two of the three professionals indicated that this could be the case.

**Assessment of ApoE genotype**

The Orogene DNA Assisted Collection OG-575 (DNA Genotek Inc., Kanata, Canada) was used. The funnel was screwed on the tube and participants were asked to spit in the funnel until the amount of saliva reached the ‘fill to’ line on the tube. When spitting was impossible because participants did not know how to spit or they thought it not decent to spit, then sponges were used to collect saliva from the buccal fold and from beneath the tongue. The sponges were subsequently used to put the saliva in the tube via the specially designed form of the funnel. When the tube was filled, then the lid from the funnel was closed to add preservation fluid to the saliva, the funnel was screwed from the tube, and the tube was closed with the cap. Due to the special preservation fluid, the samples could be stored at room temperature for years. Genomic DNA was extracted on a chemagic Magnetic Separation Module (PerkinElmer chemagen Technologie, Baesweiler, Germany). DNA samples were quantified using a NanoDrop spectrophotometer (Thermo Scientific, Wilmington, USA). ApoE genotyping was done by Sanger sequencing of codons 112 and 158 of the ApoE gene.

**Neuropsychological assessment**

The Neuropsychological Test series for Elderly with Mild Intellectual Disability (NETOL) [76], a Dutch neuropsychological test battery developed for adults with intellectual disabilities, was used for all participants in the DS group. In 1999, outcomes of the Committee on Test Affairs Netherlands about psychometric aspects of the NETOL were mostly satisfactory to good [15]. The norms were outdated, but the present study required merely the use of raw scores. The internal consistency of two subtests and the interrater reliability of two other subtests were not satisfactory [75], but these subtests were not used in the present study.

**Verbal memory**

Eight Words Test (NETOL) was used to measure encoding, direct recall, delayed recall, and recognition of verbal information [74]. A list with eight words was read aloud and participants were asked to recall the list. This was repeated four times
Visual memory

Visual Memory Test (NETOL) was used to measure encoding, direct recognition, and delayed recognition of visual information, in which abstract line drawings were used to minimalize the involvement of memory for verbal information [74]. Five stimulus drawings were shown; participants had to remember and recognize each one of a set of four drawings. For each stimulus, a maximum of four attempts was provided until the answer was correct: four points were given when the first attempt was successful (total score 0 – 20). Subsequently, the sets of four drawings were shown directly and participants were asked to recognize the five stimulus drawings (score 0 – 5). This last condition was repeated after the Fluency task described below (score 0 – 5).

Executive functioning

Executive functioning (EF) refers to meta-cognitive processes that enable efficient planning, execution, verification, and regulation of goal-directed behaviour [48]. Meander (NETOL) was considered to measure cognitive flexibility [74]. Participants had to copy four alternating patterns of figures such as triangles and squares (total score 0 – 16). Fluency (NETOL) was considered to measure verbal fluency: the ability to name words quickly and efficiently, while inhibiting irrelevant impulses and using strategies [74]. It appeals to semantic memory but also to executive functioning [13,60]. Participants had to name as many animals as possible within one minute and as many first names as possible within one minute. Circle Span backward (NETOL) was considered as a measure for visual-spatial working memory [74]. The researcher tapped drawn circles in series of increasing length, which participants had to repeat backwards (total score 0 – 8). Mazes (WPPSI-R) was considered to measure the ability to plan and follow a visual
pattern [61,67]. Participants had to complete mazes within a limited time per maze while the difficulty increased with each maze (total score 0 – 26).

**Assessment of reported pain**

**Reported presence of pain**

Pain was assessed in five situations during the test session: one rest situation and four situations with active movements. If participants felt pain in more locations per test situation, then they were asked to indicate which location was the most painful. For the rest situation, participants were asked whether they felt any pain at that moment. If this was not the case, then they were asked whether they had felt pain during the day of the test session or in the preceding week. When participants reported pain during any of these questions, then they were asked to point to the painful location on their own body.

Subsequently, participants were asked to imitate four series of active movements as demonstrated by the researcher: 1) movement of the legs and hips (rising from the chair, walking to the end of the room and back, and sitting again), 2) movement of the neck, shoulders, elbows, wrists, and fingers (moving the chin to the ceiling, to the chest, and to the shoulders, stretching the arms upwards and sideways, stretching the arms forwards and touching the shoulders with the hands, and stretching the arms forwards and “playing the piano”), 3) movement of the back (touching the toes with stretched legs and rotating the torso), and 4) movement of the jaw (opening the mouth as far as possible). By encouraging participants to push the maximum limits of their movement capabilities, pain or discomfort of the involved musculoskeletal structures (i.e., muscles and/or joints) was provoked during function. Directly after each series, participants were asked whether they felt any pain during the movements and if so, where this was.

**Self-reported pain affect and intensity**

For pain affect, the Facial Affective Scale (FAS) [43,44] was used. This is an ordinal series of nine drawn faces with expressions ranging from no distress to utter distress, with values that range from 0.04 (maximum positive affect) to 0.97 (maximum negative affect) printed on the back side [44]. Pain affect refers to perceived unpleasantness [55], and is related to pain tolerance and suffering from pain [65]. For pain intensity, the numeric side of the Coloured Analogue
Scale [43,44] was used and this scale is therefore referred to in the rest of the manuscript as “Numeric Rating Scale (NRS)”. It consists of a vertical “ruler” ranging continuously from 0 to 10 with a plastic slide. A higher score indicated more pain.

Participants were only asked to rate their pain if they had sufficient understanding of the pain scale according to their performance on a task. This task was slightly different for the first 29 participants as a result of refining the task to further increase the reliability. Comprehension of the FAS was assessed in the first 29 participants by asking which face represents someone without pain and which face represents someone with the most pain. If participants selected the first or second face and the second to last or last face, respectively, they were considered to understand the least-most extremes (see Figure 1). For the rest of the participants, comprehension of the FAS was assessed by ordering three faces from mild to severe pain (see Figure 2). If participants responded correctly in the task that they performed, they were considered to have at least a global understanding of the FAS and were asked to rate their own pain. The first 29 participants selected one of the nine faces corresponding to their pain. The rest of the participants selected the face that corresponded to their pain by choosing first between three faces (Card A in Figure 3) and then again between three faces (Card B, C or D in Figure 3).

Comprehension of the NRS was assessed in the first 29 participants by asking at what level the slide should be positioned when someone has no pain and at what level when someone has the most pain. Answers that were considered to be correct were 0 or 1 and 9 or 10, respectively (see Figure 4). For the rest of the participants, two questions were added that focused on the magnitude of numbers: “Which is larger: 2 or 8?” and “Which is larger: 6 or 4?”. Participants who answered all questions of their task correctly were asked to rate their own pain by placing the plastic piece of the NRS on the number corresponding to their pain.

For participants who passed the comprehension test according to the intended response but who did not have pain, the FAS value of .04 (corresponding to the face with the lowest pain affect) and the value of 0 (corresponding to the lowest pain intensity) were used, respectively.
FIG. 1
Faces and their corresponding values of the Facial Affective Scale [43]. This is the backside: the front side (faces without letters and numbers) were presented to participants. Photocopy of test material (first author). Patricia A. McGrath, Pain in Children – Appendix: Pain Assessment, Guilford, New York, United States of America, Copyright © 1990.

FIG. 2
Facial Affective Scale: comprehension test with ordering format. Three faces were presented in the order of severe pain, mild pain, and moderate pain. Participants were requested to arrange the faces from “least pain” to “most pain”. The intended order was from mild to severe pain (corresponding to the McGrath’s values of .17, .75, and .85).
**FIG. 3**

Facial Affective Scale, divided into three different parts. Card A was first shown and participants were asked which face corresponded to the reported pain. When the left face of Card A was chosen, the question was repeated while showing Card B. When the middle face of Card A was chosen, the question was repeated while showing Card C. When the right face of Card A was chosen, the question was repeated while showing Card D.

**FIG. 4**

**Statistical analysis**

Statistical analyses were performed using SPSS 21. The level of significance was set at $\alpha = .05$ (two-sided). A domain of memory (Cronbach’s $\alpha = .84$) was formed by standardizing the raw scores of the Eight Word Test and the Visual Memory Test. A domain of EF (Cronbach’s $\alpha = .77$) was formed by standardizing the raw scores of the Meander, Fluency, Circle Span backward, and Mazes tests. Only these composites variables were used as measures of cognitive function. Pain was assessed during the test session in one rest situation and four movement situations. Not all participants were able or willing to perform the four movement situations ($n = 7$), resulting in missing data for some movement situations. Three participants refused to perform one or several situations without specifying the reason or by reporting to be tired, two participants did not perform the jaw situation due to the occurrence of a locked jaw in the past, one participant was not able to perform the transfer and back situations due to the use of a wheelchair, and one participant refused to perform the back situation due to severe back pain. Therefore, the sum of the FAS scores (Cronbach’s $\alpha = .65$) was divided by the number of test situations in which the FAS was used. Similarly, the NRS values of the situations of the test session were summed (Cronbach’s $\alpha = .56$) and divided by the number of test situations in which the NRS was used. These variables were highly correlated ($r_s = .89$, $p < .001$).

Only participants who reported the presence of pain during the test session were included in analyses of pain experience, because the many near-zero values of the participants who did not report the presence of pain could confound the results. Multiple linear regression analyses, of which all assumptions were met [22], were used to analyze whether the relationship between cognitive function and self-reported pain experience is significantly moderated by the presence of ApoE $\varepsilon 4$ allele. This moderation model is shown in Figure 5. The models were selected on theoretically grounds and all variables of a model were entered as a block. All regression analyses with cognitive function were controlled for age (centered to the mean), gender, and the presence of painful or discomforting conditions. Although it was possible to form a domain of Pain Experience (Cronbach’s $\alpha = .97$) and it was desirable to include both cognitive domains in one model ($r_s = .58$), it was decided to perform the multiple linear regression analyses separately.
for Memory and EF and for pain affect and pain intensity due to the otherwise strong reduction of the sample size and statistical power. The four models were first executed without interactions and then with an interaction between cognitive function and presence of ApoE ε4 allele. Bonferroni correction ($p = .05 / 2$) was applied due to the use of two dependent variables Multilevel analysis was not necessary.

RESULTS

Characteristics of the sample and subgroups

Table 1 shows the demographic and medical characteristics of the 157 adults with DS. ApoE allele frequencies were: ε2ε2, $n = 0$ (0%); ε2ε3, $n = 12$ (7.6%); ε2ε4, $n = 7$ (4.5%); ε3ε3, $n = 98$ (62.4%); ε3ε4, $n = 37$ (23.6%); ε4ε4, $n = 3$ (1.9%). Two subgroups were made: 47 (29.9%) carriers and 110 (70.1%) non-carriers of the ε4 allele. Only the subgroup difference in age was statistically significant (see Table 1), whereby carriers were almost five years younger ($M_{\text{carriers}} = 35.6$ years, $M_{\text{non-carriers}} = 40.1$ years).
### TABLE 1
Demographic and medical characteristics of carriers and non-carriers of the Apolipoprotein ε4 allele in adults with Down syndrome

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All participants: ( n = 157 )</th>
<th>Carriers: ( n = 47 )</th>
<th>Non-carriers: ( n = 110 )</th>
<th>Subgroup difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years* ( M ) (SD, range)</td>
<td>( M=38.7 ) (SD=10.2), range 19-64</td>
<td>( M=35.6 ) (SD=8.9), range 19-53</td>
<td>( M=40.1 ) (SD=10.4), range 20-64</td>
<td>( t(155) = 2.55, p = .012^*, r = .20 )</td>
</tr>
<tr>
<td>Gender: male</td>
<td>74 (47%)</td>
<td>21 (45%)</td>
<td>53 (48%)</td>
<td>( \chi^2(1) = 0.16, p = .69, Phi = .03 )</td>
</tr>
<tr>
<td>Living situation: in care centre, or with family</td>
<td>144 (92%), 13 (8%)</td>
<td>43 (92%), 4 (9%)</td>
<td>101 (92%), 9 (8%)</td>
<td>Fisher's exact test, ( p = 1.00, Phi = .01 )</td>
</tr>
<tr>
<td>Intellectual disability: mild, moderate, severe</td>
<td>52 (33%), 91 (58%), 14 (9%)</td>
<td>19 (40%), 26 (55%), 2 (4%)</td>
<td>33 (30%), 65 (59%), 12 (11%)</td>
<td>( \chi^2(2) = 2.80, p = .25, Cramer's V = .13 )</td>
</tr>
<tr>
<td>Estimated intelligence level (age equivalent)</td>
<td>148 (94%), ( M=5.0 ) (SD=1.6)</td>
<td>45 (96%), ( M=4.9 ) (SD=1.4)</td>
<td>103 (94%), ( M=5.1 ) (SD=1.6)</td>
<td>( t(146) = 0.50, p = .62, r = .04 )</td>
</tr>
<tr>
<td>Thyroid disorder</td>
<td>55 (35%); 55 (100%) treatment</td>
<td>14 (30%)</td>
<td>41 (37%)</td>
<td>( \chi^2(1) = 0.81, p = .37, Phi = .07 )</td>
</tr>
<tr>
<td>Present analgesics use</td>
<td>8 (5%)</td>
<td>2 (4%)</td>
<td>6 (6%)</td>
<td>Fisher's exact test, ( p = 1.00, Phi = .03 )</td>
</tr>
<tr>
<td>Physical conditions possible pain/discomfort</td>
<td>80 (51%)</td>
<td>24 (51%)</td>
<td>56 (51%)</td>
<td>( \chi^2(1) = &lt;0.01, p = .99, Phi = &lt;.01 )</td>
</tr>
<tr>
<td>Sleep problems and/or symptoms of depression</td>
<td>11 (7%)</td>
<td>4 (9%)</td>
<td>7 (6%)</td>
<td>Fisher's exact test, ( p = .73, Phi = .04 )</td>
</tr>
<tr>
<td>Reporting pain presence during test session</td>
<td>86 (55%)</td>
<td>23 (49%)</td>
<td>63 (57%)</td>
<td>( \chi^2(1) = 0.92, p = .34, Phi = .08 )</td>
</tr>
</tbody>
</table>

* = statistically significant. Physical conditions possible pain/discomfort: number of participants with a possible presence of pain or discomfort according to reported medical information (physical conditions, complaints, and medication use).
**Associations among ApoE ε4, cognitive function, and pain experience**

The association between age and cognitive function was statistically significant (memory: \( r_s = -.37, p < .001 \); EF: \( r = -.30, p = .001 \)) and was therefore included in the subsequent analysis. When controlling for age (centered to the mean), the difference between carriers and non-carriers was not statistically significant for memory (\( F (1, 119) = 1.29, p = .26, B = 0.14, \eta^2 = .01 \)), but it was for EF (\( F (1, 110) = 4.16, p = .044, B = 0.30, \eta^2 = .04, M_{\text{carriers}} = 0.04, M_{\text{non-carriers}} = 0.21 \)), in which non-carriers had a higher EF performance. The association between carrying the ε4 allele and reporting the presence of pain during the test session was not statistically significant (see Table 1). When selecting participants who reported pain during the test session (\( n = 86 \)), the difference between carriers and non-carriers was not statistically significant for any of the variables in Table 1. Of the participants who reported pain and comprehended self-reporting pain scales, the difference between carriers and non-carriers was not statistically significant for pain affect (\( t (59) = -1.05, p = .30, r = .14, n = 61 \)) nor pain intensity (\( U = 101.50, p = .39, r = -.15, n = 35 \)). Table 2 describes the reported presence of pain, the average self-reported pain experience, and the average cognitive function in carriers and non-carriers.

**Moderator model**

Moderation analyses were performed in those participants who reported pain during the test session (\( n = 86 \)) and comprehended the self-reporting scales (\( n = 61 \) FAS and \( n = 35 \) NRS). The number of participants who reported pain, were included in the memory or EF domain, and who had sleeping problems and/or symptoms of depression (memory: \( n = 5 \); EF: \( n = 4 \)) was too small to permit an analysis of the relationship between such problems/ symptoms and cognitive function. The small numbers of these participants limited the likelihood that their presence would influence the results. The same applied for symptoms of autism (memory: \( n = 0 \); EF: \( n = 1 \)). Of participants who reported pain, the difference in cognitive function between participant with thyroid disorders and those without thyroid disorders was not statistically significant (memory: \( U = 398.00, p = .54, r = -.08 \); EF: \( t (58) = -0.92, p = .36, r = .12 \)). Therefore, thyroid disorder, sleep problems...
## Table 2
Characteristics of self-reported pain and of cognitive function in carriers and non-carriers of the Apolipoprotein ε4 allele in adults with Down syndrome

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Carriers (n = 47)</th>
<th>Non-Carriers (n = 110)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reported presence of pain during test session</td>
<td>49% (rest: 34%, movement 49%), n = 23</td>
<td>57% (rest: 43%, movement 57%), n = 63</td>
</tr>
<tr>
<td>Average pain affect (self-report)</td>
<td>Mdn = 0.04 (IQR = 0.29), n = 35</td>
<td>Mdn = 0.14 (IQR = 0.25), n = 80</td>
</tr>
<tr>
<td>Reported presence of pain: average pain affect</td>
<td>M = 0.36 (SD = 0.20), n = 15</td>
<td>M = 0.30 (SD = 0.18), n = 46</td>
</tr>
<tr>
<td>Average pain intensity (self-report)</td>
<td>Mdn = 1.80 (IQR = 2.70), n = 17</td>
<td>Mdn = 1.10 (IQR = 2.50), n = 43</td>
</tr>
<tr>
<td>Reported presence of pain: average pain intensity</td>
<td>M = 2.74 (SD = 1.22), n = 10</td>
<td>M = 2.79 (SD = 2.15), n = 25</td>
</tr>
<tr>
<td>Memory performance</td>
<td>Mdn = 0.20 (IQR = 0.67), n = 38</td>
<td>Mdn = 0.32 (IQR = 1.05), n = 84</td>
</tr>
<tr>
<td>Executive function performance</td>
<td>M = 0.04 (SD = 0.65), n = 37</td>
<td>M = 0.21 (SD = 0.79), n = 76</td>
</tr>
</tbody>
</table>
and/or symptoms of depression, and symptoms of autism were not included in the analyses.

Table 3 shows the results of the multiple linear regression analyses. Only results concerning cognitive function, the presence of ApoE ε4 allele, and their interaction will be described. The association between cognitive function and pain experience was not statistically significant. Also the interaction between cognitive function and the presence of ApoE ε4 allele did not reach statistically significance in explaining variance in pain experience. The increase of explained variance by the moderation models compared to the simple models was not statistically significant.

**DISCUSSION**

It was found in the present study that ApoE ε4 carriers performed lower on EF tests than non-carriers, while taking age into account. As far as the authors know, the association between ApoE genotype and EF has not yet been examined in people with DS. Although the explained variance was small (4%), the finding is in line with a recent review of 77 studies (22 containing EF measures), in which it was concluded that cognitively intact middle-aged and old adults carrying the ApoE ε4 allele perform lower on EF tests than non-carriers [82]. A possible explanation for the EF impairment is that the frontal cortex of ApoE ε4 carriers has lower rates of glucose metabolism, which could reflect an accelerated ageing process [57], and less choline acetyltransferase [70], which could be related to impaired attentional processes [52]. Another possible mechanism is a mediation by cortisol: a higher cortisol is in ApoE ε4 carriers associated with a lower EF [37]. It remains unclear whether these mechanisms also apply to people with DS.

A main finding of the present study is that the presence of ApoE ε4 was no statistically significant moderator for the association between cognitive function and self-reported pain experience in adults with DS. It is important to notice that the difference in pain experience between ApoE ε4 carriers and non-carriers was not statistically significant. This is in line with the finding of previous research that the presence of an ApoE ε4 allele is unrelated to pain intensity in people with fibromyalgia [56]. It seems that the relationship between ApoE ε4 and EF does not mean that ApoE ε4 carriers also have a different pain experience.
TABLE 3
Simple model (cognitive function) and moderation model (interaction with the presence of Apolipoprotein (ApoE) ε4 allele) to explain pain experience in participants who reported the presence of pain

<table>
<thead>
<tr>
<th>Model</th>
<th>Variables</th>
<th>Memory and pain affect</th>
<th></th>
<th>Executive functioning and pain affect</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>B (SE)</td>
<td>F (df)</td>
<td>n</td>
<td>p</td>
</tr>
<tr>
<td>1</td>
<td>Cognition</td>
<td>-0.08 (0.04)</td>
<td>3.35 (1, 47)</td>
<td>52</td>
<td>.074</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>&lt;0.01 (&lt;0.01)</td>
<td>0.12 (1, 47)</td>
<td>52</td>
<td>.74</td>
</tr>
<tr>
<td></td>
<td>Gender</td>
<td>-0.11 (0.05)</td>
<td>5.26 (1, 47)</td>
<td>52</td>
<td>.026</td>
</tr>
<tr>
<td></td>
<td>Possible pain/discomfort</td>
<td>-0.01 (0.05)</td>
<td>0.01 (1, 47)</td>
<td>52</td>
<td>.93</td>
</tr>
<tr>
<td>2</td>
<td>Cognition</td>
<td>-0.19 (0.14)</td>
<td>3.01 (1, 45)</td>
<td>52</td>
<td>.090</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>&lt;0.01 (&lt;0.01)</td>
<td>0.35 (1, 45)</td>
<td>52</td>
<td>.56</td>
</tr>
<tr>
<td></td>
<td>Gender</td>
<td>-0.12 (0.05)</td>
<td>6.02 (1, 45)</td>
<td>52</td>
<td>.018</td>
</tr>
<tr>
<td></td>
<td>Possible pain/discomfort</td>
<td>&lt;0.01 (0.05)</td>
<td>0.01 (1, 45)</td>
<td>52</td>
<td>.93</td>
</tr>
<tr>
<td></td>
<td>Presence of ApoE ε4 allele</td>
<td>&lt;0.10 (0.06)</td>
<td>2.79 (1, 45)</td>
<td>52</td>
<td>.10</td>
</tr>
<tr>
<td></td>
<td>Cognition x presence ApoE ε4 allele</td>
<td>0.12 (0.15)</td>
<td>0.64 (1, 45)</td>
<td>52</td>
<td>.43</td>
</tr>
</tbody>
</table>

* = statistically significant $p < .025$ ($p = .05 / 2$ due to the use of two dependent variables). ^ = trend $p < .05$. $\eta^2$ = partial eta squared (effect size). Model 1 = simple model, model 2 = moderation model. Age was centered to the mean of the entire group. * = change from model 1 to model 2: memory: 0.05 ($p = .25$); executive functioning: 0.05 ($p = .30$).
<table>
<thead>
<tr>
<th>Model</th>
<th>Variable</th>
<th>Memory and pain intensity</th>
<th></th>
<th></th>
<th>Executive functioning and pain intensity</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>B (SE)</td>
<td>F (df)</td>
<td>n</td>
<td>p</td>
<td>pη2</td>
</tr>
<tr>
<td>1</td>
<td>Cognition</td>
<td>-0.67 (0.62)</td>
<td>1.19 (1, 27)</td>
<td>32</td>
<td>.29</td>
<td>.04</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>0.07 (0.04)</td>
<td>3.18 (1, 27)</td>
<td>32</td>
<td>.086</td>
<td>.11</td>
</tr>
<tr>
<td></td>
<td>Gender</td>
<td>-0.93 (0.66)</td>
<td>1.95 (1, 27)</td>
<td>32</td>
<td>.17</td>
<td>.07</td>
</tr>
<tr>
<td></td>
<td>Possible pain/discomfort</td>
<td>-0.31 (0.68)</td>
<td>0.21 (1, 27)</td>
<td>32</td>
<td>.65</td>
<td>.01</td>
</tr>
<tr>
<td>2</td>
<td>Cognition</td>
<td>1.40 (2.05)</td>
<td>0.06 (1, 25)</td>
<td>32</td>
<td>.80</td>
<td>&lt;.01</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>0.07 (0.04)</td>
<td>3.35 (1, 25)</td>
<td>32</td>
<td>.079</td>
<td>.12</td>
</tr>
<tr>
<td></td>
<td>Gender</td>
<td>-0.72 (0.72)</td>
<td>1.01 (1, 25)</td>
<td>32</td>
<td>.33</td>
<td>.04</td>
</tr>
<tr>
<td></td>
<td>Possible pain/discomfort</td>
<td>-0.36 (0.72)</td>
<td>0.25 (1, 25)</td>
<td>32</td>
<td>.62</td>
<td>.01</td>
</tr>
<tr>
<td></td>
<td>Presence of ApoE ε4 allele</td>
<td>0.05 (0.93)</td>
<td>&lt;0.01 (1, 25)</td>
<td>32</td>
<td>.96</td>
<td>&lt;.01</td>
</tr>
<tr>
<td></td>
<td>Cognition x presence ApoE ε4 allele</td>
<td>-2.26 (2.16)</td>
<td>1.09 (1, 25)</td>
<td>32</td>
<td>.31</td>
<td>.04</td>
</tr>
</tbody>
</table>

* = statistically significant p < .025 (p = .05 / 2 due to the use of two dependent variables). ^ = trend p <.05. pη2 = partial eta squared (effect size). cognitive function. Model 1 = simple model, model 2 = moderation model. Age was centered to the mean of the entire group. * = change from model 1 to model 2: memory: 0.04 (p = .51); executive functioning: 0.13 (p = .13).
In the present study, 29.9% of the adults with DS were ApoE ε4 carrier. This percentage is comparable to the 27.3% ε4 carriers found previously in a large sample of Dutch adults with DS [9]. The frequency of the ApoE ε4 allele in the DS population is similar to that of the general population [3,53,62]. Our finding that ApoE ε4 carriers were younger than non-carriers is in line with a previously reported reduction in ApoE ε4 frequency over age in adults from both the general population [26,63] and DS population [10,26,83]. One of these studies showed that adults with DS carrying an ApoE ε4 allele were three years younger than non-carriers [83].

A possible explanation for the lower age in ApoE ε4 carriers is their increased risk for mortality [69], which has also been demonstrated in adults with DS [54,83]. The increased risk for mortality is in the general population related to an increased risk for diseases in ApoE ε4 carriers: heart disease [18,24,81], atherosclerosis [30], diabetes [24], stroke [24,42], and Alzheimer disease neuropathology [32,78]. As dementia is the major cause of death in adults with DS aged 45 years and older [10], especially an increased risk for Alzheimer neuropathology might explain the lower age in ApoE ε4 carriers with DS.

**Strengths and limitations**

The strength of the present study is that it was the first to examine the genetic variability of the relationship between cognitive function and pain experience in adults with DS. The present study is limited by insufficient power for the moderation analyses. The required sample size was not met due to a small number of participants who both reported pain and were included in the cognitive domains (memory $n = 64$, EF $n = 60$). Another limitation is that the assessment of pain experience in the movement situations was incomplete for seven participants. The missing data may have influenced the average pain ratings of the DS group, because it is possible that some of these participants were refusing to perform the movement due to severe pain.

**Conclusion and recommendations**

In conclusion, the results of the present study show that the presence of ApoE ε4 allele in adults with DS has statistically significant associations with a lower average age and a lower EF performance, although this latter finding had
a small explained variance. The difference in pain experience between ApoE ε4 carriers and non-carriers was not statistically significant. No statistically significant moderation was found for ApoE ε4 allele on the relationship between cognitive function and pain experience. It is recommended that this topic is further examined in a large sample with sufficient statistical power. An interesting additional research question is whether the EF impairment in ApoE ε4 carriers with DS show a fast further decline in those who are physically inactive. Physically inactive carriers of the ApoE ε4 allele have a larger risk for cognitive decline [66], which is important considering the extremely low physical activity levels in older adults with intellectual disabilities [29].

REFERENCES


[29] Hilgenkamp TIM, Reis D, Van Wijck R, Evenhuis HM. Physical activity levels in older adults with intellec-


APOE ε4 RELATED TO COGNITION AND PAIN


