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Genes determining the severity of cerebral palsy: the role of single nucleotide polymorphisms on the amount and structure of apolipoprotein E

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Abstract

Aim—ApolipoproteinE (apoE) influences repair and other processes in the brain and the apoE4 variant is a risk factor for Alzheimer's disease and for prolonged recovery following traumatic brain injury. We previously reported that specific single nucleotide polymorphisms in the *APOE* or *TOMM40* genes affecting the structure and production of apoE were associated with epilepsy, more impaired hand function and gastrostomy tube feeding in children with cerebral palsy (CP). This study explored how various combinations of the same polymorphisms may affect these clinical manifestations.

Methods—Successful DNA analyses of *APOE* and *TOMM40* were carried out on 227 children. The CP Register of Norway provided details of gross and fine motor function, epilepsy and gastrostomy tube feeding. Possible associations between these clinical manifestations and various combinations of the *APOE*ε2, ε3 or ε4 alleles and of the rs59007384 polymorphism in the *TOMM40* gene were explored.

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CONFLICT OF INTEREST

None declared

Results—Epilepsy, impaired fine motor function and gastrostomy tube feeding were less common in children carrying the combination of rs59007384 GG and *APOE*ε2 or ε3 than in children with other combinations.

Conclusion—Our findings suggest that specific combinations of genes influence the structure and production of apoE differently and affect the clinical manifestations of CP.

Keywords

apoE; APOE; regulation of apoE synthesis; rs59007384 polymorphism; cerebral palsy

INTRODUCTION

Apolipoprotein E (apoE) is the major transporter of lipids and cholesterol in the brain, substances which are necessary for myelin synthesis, membrane and synapse formation and maintenance (1). In addition, the protein has been shown to have a number of regulatory functions in the central nervous system (CNS), including stimulation of dendritic branching, axonal growth and guidance, regeneration and further development of neuronal circuits after a developmental disturbance and modulating effects on inflammation processes (2).

Three isoforms of apoE, E2, E3 and E4, coded by three corresponding alleles of the *APOE* gene, ε2, ε3 and ε4, have been described (2). The gene and the resulting proteins have been extensively investigated, primarily because of the strong association between the *APOE*ε4 allele and the increased risk of Alzheimer's disease. *APOE*ε4 has also been associated with a prolonged recovery phase and worse outcome after traumatic brain injury in both adults and children (3).

With regard to cerebral palsy (CP), studies of a possible relationship between *APOE*ε4 and the aetiology of CP have reported conflicting results (4-6). However, in a previous study we found that children with CP who carried the *APOE*ε4 allele were more likely to have epilepsy, reduced fine motor function and feeding difficulties that made a gastrostomy tube necessary (7). These associations may be explained by less efficient functions of the apoE4 isoform compared to the other isoforms.

The processes involved in developmental brain disturbance may be influenced by the amount of apoE in cerebrospinal fluid (CSF). The amount of the protein produced seems to be regulated by variations in specific transcription enhancer elements (8). The single nucleotide polymorphism (SNP) rs59007384, previously known as n17664883, which is located in the *TOMM40* gene, near and centromeric to the *APOE* gene, has been reported to be influential (8). In a previous study, we found that having certain genotypes - GT or TT - of this SNP was associated with adverse clinical outcomes similar to those observed to be associated with the ε4 allele of the *APOE* gene (7). These complications were more commonly seen among children carrying at least one rs59007384 T allele than among children with the more prevalent GG genotype. The *TOMM40* gene encodes the TOM40 protein, which is a pore subunit of the mitochondrial outer membrane protein translocator, and variation within intron 4 may contribute to increased CSF apoE protein levels by mechanisms that are only partly understood (8).

The combined findings of our previous studies suggest that these clinical manifestations of CP are not only dependent on the structure of apoE, as determined by variants of the *APOE* gene, but also on the amount of the protein in the CNS, partly determined by variants of the TOMM40 rs59007384 polymorphism (8).

In this study, therefore, we wanted to explore whether various combinations of the *APOE* genotypes and the TOMM40 rs59007384 polymorphism increased or decreased the risk for epilepsy, gastrostomy tube feeding and impaired bimanual function in children with cerebral palsy.

We hypothesised that these clinical manifestations would be least common among children with the rs59007384 GG genotype without an ϵ 4 allele of the *APOE* gene, whereas the same complications would be most commonly observed among children who were carriers of at least one T allele of rs59007384 and at least one ϵ 4 allele of the *APOE* gene. We also hypothesised that carriers of other combinations would have an intermediate risk.

METHODS

Study design and population

This study was an extension of two earlier studies describing the association of polymorphisms of the *APOE* gene, and in nearby genes affecting apoE expression, with the severity of cerebral palsy (7, 9). The design and eligible population of these cross-sectional studies have previously been described in detail (7). Briefly, 703 children who were born between 1996 and 2003 and enrolled in the Cerebral Palsy Register of Norway (CPRN) were invited to participate. Positive responses and informed consent to participate were obtained from 281 families (40%). Details of gender, CP subtype and gross and fine motor function levels for participants and nonparticipants are listed in Table 1, as well as data concerning epilepsy and the use of a gastrostomy feeding tube.

DNA-analysis

Catch-All™ buccal swabs (Epicentre® Biotechnologies, Madison, WI, USA) were used to collect buccal epithelial cells for DNA extraction and analysis. The swabs were sent to the children's families with a detailed instruction sheet, information about the study and a consent form. In addition to the written instructions, an instructional video was made and linked to the CPRN home page.

Cells with DNA for analysis were collected on buccal swabs by the parents from February to April 2011 and returned swabs were refrigerated at 4-6°C until analysis in April and May 2011. DNA extraction and regulator SNP genotyping were performed at the DNA Sciences Core, University of Virginia School of Medicine, Charlottesville, Virginia, USA, following procedures previously described (10). Briefly, SNP genotyping by the TaqMan Allelic Discrimination method was employed. All genotyping was performed in triplicate on an Applied Biosystems 7900HT Sequence Detection System (Life Technologies Corp, Carlsbad, CA, USA.) Genotype calling was by the SDS software, which provided the allele type and confidence for each call, all according to the manufacturer's instructions. The genotyping of *APOE* and TOMM40 rs59007384 were carried out as previously described.

Briefly, observed allele combinations of the *APOE* gene were $\epsilon 2/\epsilon 3$, $\epsilon 3/\epsilon 3$, $\epsilon 2/\epsilon 4$, $\epsilon 3/\epsilon 4$ or $\epsilon 4/\epsilon 4$, whereas the observed combinations of rs59007384 were GG, TT or GT. In the present study we only included children who were successfully genotyped both for *APOE* and TOMM40 rs59007384.

Outcome variables

The main outcome variables, gross motor function, fine motor function, epilepsy and severe feeding problems were obtained from the CPRN. Fine motor function was assessed with the Bimanual Fine Motor Function scale (BFMF) (11). This details fine motor function on a five-level scale, with level I indicating the least severe activity limitation and level V the most. For children born before 1999, BFMF levels were retrospectively estimated by the registry into five categories, based on qualitative information on hand function reported by contact paediatricians (12). Fine motor function was then dichotomised into mild impairment (BFMF levels I and II) and severe impairment (levels III, IV and V).

The CPRN records information on epilepsy and the use of antiepileptic drugs. Active epilepsy was defined as the current use of antiepileptic drugs in children with epilepsy. The register also records information on feeding difficulties and severe feeding problems were defined as the presence of a gastrostomy feeding tube (12, 13).

Exposure variables

The exposure variable was the combination of the presence or absence of an $\epsilon 4$ allele of the *APOE* gene with the presence or absence of a TOMM40 rs59007384 T allele. This specifically means that both the *APOE* and the rs59007384 genotypes were dichotomised as follows. In the case of *APOE*, the combinations $\epsilon 2/\epsilon 4$, $\epsilon 3/\epsilon 4$ or $\epsilon 4/\epsilon 4$ were labelled as APOE4(+), while the combinations $\epsilon 2/\epsilon 3$ or $\epsilon 3/\epsilon 3$ were categorised as APOE4(−). In the case of TOMM40 rs59007384, the genotypes were dichotomised into those with the GG genotype, versus those with either the GT or the TT genotype.

Other variables

Information on CP subtype, gross motor function and sex was also obtained from the CPRN. In the registry, CP is defined according to the criteria set by the Surveillance of Cerebral Palsy in Europe, while gross motor function is assessed according to the Gross Motor Function Classification System, GMFCS (14, 15). The distinction between GMFCS level I and level II was not made for children born during 1996-1998.

Characteristics of responders vs. non-responders

The proportion of children with more severe impairments was higher among non-responders, than among responders (Table 1).

Statistics

STATA version 11 (College Station, TX) was used for all data analysis and statistical significance was defined as $p < 0.05$.

To test the relationship between TOMM40 rs59007384 genotypes and *APOE* $\epsilon 4$ and measures of cerebral palsy severity - epilepsy, gastrostomy tube feeding and impaired motor function - logistic regression was used to calculate the odds ratios (OR) and 95% confidence intervals (CI). For each model, participants with the rs59007384 GG genotype and APOE4(–) were considered the reference. One logistic model compared each TOMM40 rs59007384/APOE4 combination - rs59007384 GG/APOE4(+), rs59007384 GT/TT/APOE4(–) and rs59007384 GT/TT/APOE4(+) - back to the reference group and included *post-hoc* comparisons between all combination groups. A second logistic model joined all TOMM40 rs59007384/APOE4 combinations and compared them back to the reference group. In addition to these *a priori* defined main outcome measures the association with gross motor function assessed by the GMFCS was also studied.

ETHICS

The present study was approved by the Regional Committee for Medical and Health Research Ethics in Central Norway (approval number 2010/398). Written informed consent was obtained from the parents of participants younger than 16-years-of-age and from the participants themselves if they were older.

RESULTS

In all, 227 children were successfully genotyped for both *APOE* and the *TOMM40* rs59007384 polymorphism. No significant associations were seen with these genotypes and sex, CP subtype or gross motor function (data not shown). The genotype combination of rs59007384 GG/APOE4(–) was found in 125 children, around 50% of the total number of children genotyped, 64 children had rs59007384 GT/TT with APOE4(+), whereas only 10 children had the combination of rs59007384 GG with APOE4(+). The combination of rs59007384 GT/TT and APOE4(–) was carried by 28 children. Table 2 shows that, compared with the reference group of children with the TOMM40 rs59007384 GG/APOE4(–) combination, all other combinations had higher odds for adverse outcomes. Furthermore, Table 2 shows that children who were carriers of the hypothesised worst combination of rs59007384 GT/TT with APOE4(+), did not have higher odds for adverse outcomes than the children who were carriers of the combinations hypothesised to be of intermediary risk, that is rs59007384 GT/TT and APOE4(–) or rs59007384 GG/APOE4(+). Given the relatively large confidence intervals seen and the widely unbalanced numbers of children in each TOMM40 rs59007384/APOE4 group, we re-analysed the data by comparing the children in the reference group - rs59007384 GG/APOE4(–) - with children with any other combination. These analyses (Table 3) showed that children in the reference group were significantly more likely to have a favourable outcome than children in the combined group.

DISCUSSION

Our findings were partly consistent with our primary hypothesis, in that we found that children with the combination of the TOMM40 rs59007384 GG genotype and $\epsilon 2$ or $\epsilon 3$ alleles of *APOE* were less likely to have epilepsy, to be in need of gastrostomy tube feeding

and to have severely impaired hand function compared with children with other allele combinations. However, we were unable to confirm the second part of our hypothesis, that the same clinical manifestations would be most commonly observed among children who were carriers of at least one T allele of rs59007384, as well as at least one ϵ 4 allele of the APOE gene, and that carriers of other combinations had an intermediate risk. Instead, we found that any other combination of these polymorphisms had essentially the same odds for more severe clinical manifestations of CP

The strength of this study is its population-based design, an ethnically homogeneous population and the collection of clinical data before the genetic analyses. Significance indicated by the low *p*-values suggests chance to be an unlikely explanation of our main findings (Table 3).

The proportion of children with the most severe fine and gross motor impairments, as well as the proportion in need of gastrostomy tube feeding, was lower among responders than among non-responders. However, the association between genotypes and the clinical outcomes would need to be completely different among non-responders and responders in order to affect our findings. Moreover, the most consistent finding in this study, and in our two previous studies, was related to epilepsy and the occurrence of this complication did not differ between responders and non-responders. We therefore also consider it unlikely that selection bias can explain our results.

The use of the GMFCS and BFMF to assess gross and fine motor function is well documented and unlikely to differ between reporting clinicians. There may however, be some misclassification of BFMF levels for children born before 1999, since BFMF levels were assessed retrospectively in these children (12). Moreover, gastrostomy tube feeding may be influenced by local practices and availability and show regional variations (13). Thus, there may have been some misclassification of this variable as an indicator of severe feeding problems. However, these potential misclassifications are likely to diminish the differences between children with different genotypes as regional differences in availability may have led to lower numbers of gastrostomies. In contrast, the use of anti-epileptic drugs, which were recorded on a specific tick-off box in the registration form, was likely to be a more robust variable. The effect sizes for the associations between the unfavourable genotypes and the adverse clinical outcomes were relatively large, as suggested by ORs ranging between 1.93 and 3.21 (Table 3), which may be consistent with a cause-effect relationship. We were unable to demonstrate a dose-response effect, whereby children with the combination of a T allele of TOMM40 rs59007384 and an ϵ 4 allele of the APOE gene would have the poorest clinical outcome, children with the rs59007384 GG genotype and no APOE ϵ 4 allele would have the most favourable outcome and children with the other combinations would have an intermediate outcome. Thus, our results may suggest that either the presence of a T allele of TOMM40 rs59007384 or presence of an ϵ 4 allele of APOE are as unfavourable as the combination of both alleles.

The low proportions of children with the combinations rs59007384 GG/APOE4(+) and rs59007384 GT/TT with APOE4(–) were most likely explained by strong linkage disequilibrium between TOMM40 and the APOE alleles (8, 16). The APOE ϵ 4 allele will in

most instances be passed on as a unit together with the *TOMM40* rs59007384 T allele because of the close proximity of the two genes and the strong linkage between SNPs in this region.

In the present study we have applied a novel approach by addressing the possible association between different combinations of genetic variants of the *TOMM40* gene and *APOE* genotypes with clinical outcome. Thus, the study expands the results of our previous studies where the association between clinical outcome and these genotypes were investigated separately (7, 9).

We are not aware of other studies of a similar design, but our results may be consistent with Bekris et al, who suggested that an *APOE* regulatory element could exist in the region proximal to *APOE* that may influence apoE levels in CSF. They proposed that the region in intron 4 of *TOMM40* may contain regulatory elements for *APOE* expression that could contribute to high CSF apoE levels when the major homozygote genotype rs59007384 GG (non-variant) was present whereas the minor homozygote TT or the heterozygote GT (variant) would result in lower levels (8). A more recent study by Bekris et al confirmed these results (17). In line with these findings, we find it plausible that the rs59007384 GG genotype of *TOMM40*, located in the proposed region, may contribute to higher levels of apoE in the CSF than other genotypes following a developmentally adverse event. This higher level may be favourable in the absence of an APOE ϵ 4 allele, while the presence of a larger amount of apoE4 would be unfavourable for outcome and severity of cerebral palsy. Correspondingly, lower levels of apoE as a consequence of the rs59007384 GT or TT genotype are unfavourable regardless of the structure of apoE, that is whether there is an ϵ 4 allele present or not.

Similar events or a combinations of risk factors may result in varying clinical manifestations of CP and this observation may be explained by differences in individual susceptibility to injury, potentially caused by genetic factors (18). A more detailed understanding of the genetic mechanisms influencing the severity of cerebral palsy may thus provide future targets for pharmacological or other specific interventions. The conversion of the three-dimensional structure of the apoE4 molecule into an apoE3 configuration is already being explored as part of the attempts to find treatments for Alzheimer's disease. This may well be of interest in the context of CP treatment, provided that the suggested protective effect of apoE4 in children can be ruled out (19-21).

CONCLUSION

Among children with CP, the combination of being homozygous for the *TOMM40* rs59007384 G allele and absence of the APOE ϵ 4 allele was favourable in terms of hand function, epilepsy and feeding abilities, while all other allele combinations were equally unfavourable. Our findings suggest that certain combinations of genetic variation in the *TOMM40* and *APOE* genes are associated with differences in the clinical manifestations of cerebral palsy. The results are consistent with the concept of genetic influence on the final functional outcome after developmentally adverse events in the immature brain. The findings of the present study need to be confirmed in a different cohort of children with CP.

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Abbreviations

apoE	apolipoprotein E
BFMF	Bimanual Fine Motor Function
CNS	central nervous system
CP	cerebral palsy
CPRN	Cerebral Palsy Register of Norway
CSF	cerebrospinal fluid
GMFCS	Gross Motor Function Classification System
GTF	gastrostomy tube feeding
SNP	single nucleotide polymorphism

References

1. Mahley RW, Weisgraber KH, Huang Y. Apolipoprotein E4: a causative factor and therapeutic target in neuropathology, including Alzheimer's disease. *Proc Natl Acad Sci U S A*. 2006; 103:5644–51. [PubMed: 16567625]
2. Hauser PS, Narayanaswami V, Ryan RO. Apolipoprotein E: From lipid transport to neurobiology. *Prog Lipid Res*. 2011; 50:62–74. [PubMed: 20854843]
3. Brichtova E, Kozak L. Apolipoprotein E genotype and traumatic brain injury in children--association with neurological outcome. *Childs Nerv Syst*. 2008; 24:349–56. [PubMed: 17932679]
4. O'Callaghan ME, MacLennan AH, Haan EA, Dekker G. The genomic basis of cerebral palsy: a HuGE systematic literature review. *Hum Genet*. 2009; 126:149–72. [PubMed: 19238444]
5. Kuroda MM, Weck ME, Sarwark JF, Hamidullah A, Wainwright MS. Association of apolipoprotein E genotype and cerebral palsy in children. *Pediatrics*. 2007; 119:306–13. [PubMed: 17272620]
6. McMichael GL, Gibson CS, Goldwater PN, Haan EA, Priest K, Dekker GA, et al. Association between Apolipoprotein E genotype and cerebral palsy is not confirmed in a Caucasian population. *Hum Genet*. 2008; 124:411–6. [PubMed: 18810496]
7. Lien E, Andersen GL, Bao Y, Gordish-Dressman H, Skranes JS, Vik T, et al. Apolipoprotein E polymorphisms and severity of cerebral palsy: a cross-sectional study in 255 children in Norway. *Developmental Medicine & Child Neurology*. 2013; 55:372–7. [PubMed: 23384326]
8. Bekris LM, Millard SP, Galloway NM, Vuletic S, Albers JJ, Li G, et al. Multiple SNPs within and surrounding the apolipoprotein E gene influence cerebrospinal fluid apolipoprotein E protein levels. *J Alzheimers Dis*. 2008; 13:255–66. [PubMed: 18430993]

9. Lien E, Andersen GL, Bao Y, Gordish-Dressman H, Skranes J, Blackman JA, et al. Gene sequences regulating the production of apoE and cerebral palsy of variable severity. *Eur J Paediatr Neurol*. 2014; 18:591–6. [PubMed: 24786335]
10. Blackman JA, Gurka MJ, Bao Y, Dragulev BP, Chen WM, Romness MJ. Apolipoprotein E and functional motor severity in cerebral palsy. *J Pediatr Rehabil Med*. 2009; 2:67–74. [PubMed: 21791796]
11. Beckung E, Hagberg G. Neuroimpairments, activity limitations, and participation restrictions in children with cerebral palsy. *Dev Med Child Neurol*. 2002; 44:309–16. [PubMed: 12033716]
12. Andersen GL, Irgens LM, Haagaas I, Skranes JS, Meberg AE, Vik T. Cerebral palsy in Norway: prevalence, subtypes and severity. *Eur J Paediatr Neurol*. 2008; 12:4–13. [PubMed: 17574886]
13. Dahlseng MO, Finbraten AK, Juliusson PB, Skranes J, Andersen G, Vik T. Feeding problems, growth and nutritional status in children with cerebral palsy. *Acta Paediatr*. 2012; 101:92–8. [PubMed: 21767308]
14. Surveillance of cerebral palsy in Europe: a collaboration of cerebral palsy surveys and registers. Surveillance of Cerebral Palsy in Europe (SCPE). *Dev Med Child Neurol*. 2000; 42:816–24. [PubMed: 11132255]
15. Palisano R, Rosenbaum P, Walter S, Russell D, Wood E, Galuppi B. Development and reliability of a system to classify gross motor function in children with cerebral palsy. *Dev Med Child Neurol*. 1997; 39:214–23. [PubMed: 9183258]
16. Yu CE, Seltman H, Peskind ER, Galloway N, Zhou PX, Rosenthal E, et al. Comprehensive analysis of APOE and selected proximate markers for late-onset Alzheimer's disease: patterns of linkage disequilibrium and disease/marker association. *Genomics*. 2007; 89:655–65. [PubMed: 17434289]
17. Bekris LM, Lutz F, Yu CE. Functional analysis of APOE locus genetic variation implicates regional enhancers in the regulation of both TOMM40 and APOE. *J Hum Genet*. 2012; 57:18–25. [PubMed: 22089642]
18. Stoknes M, Andersen GL, Elkamil AI, Irgens LM, Skranes J, Salvesen KÅ, et al. The effects of multiple pre- and perinatal risk factors on the occurrence of cerebral palsy. A Norwegian register based study. *European Journal of Paediatric Neurology*. 2012; 16:56–63. [PubMed: 22104566]
19. Mahley RW, Huang Y. Small-molecule structure correctors target abnormal protein structure and function: structure corrector rescue of apolipoprotein E4-associated neuropathology. *J Med Chem*. 2012; 55:8997–9008. [PubMed: 23013167]
20. Blackman JA, Worley G, Strittmatter WJ. Apolipoprotein E and brain injury: implications for children. *Developmental Medicine & Child Neurology*. 2005; 47:64–70. [PubMed: 15686292]
21. Blackman JA. Apolipoprotein E genotype and cerebral palsy. *Dev Med Child Neurol*. 2010; 52:600. [PubMed: 20059521]

Key notes

- Genetic influences on repair processes may affect the outcome of early cerebral injuries.
- Our study found that specific combinations of gene variants that influence the structure and production of apolipoprotein E (apoE), which plays a role in repair processes, have different effects on the clinical manifestations of cerebral palsy.
- apoE may influence fine motor function, the presence of epilepsy and gastrostomy tube feeding in children with cerebral palsy.

Table 1

Characteristics of children with cerebral palsy (CP) born 1996-2003 who returned swabs for DNA analyses (responders) compared with children who did not return swabs (non-responders).

	Responders	Non-responders
	<i>n</i> (%) 281(100%)	<i>n</i> (%) 422 (100%)
Gender		
Boys	156 (55.5 %)	244 (57.8 %)
Girls	125 (44.5 %)	178 (42.2 %)
CP subtype according to SCPE^a-classification		
Unilateral	115 (40.9 %)	154 (36.5 %)
Bilateral	129 (45.9 %)	190 (45.0 %)
Dyskinetic	16 (5.7 %)	35 (8.3 %)
Ataxic	11 (3.9 %)	25 (5.9 %)
Non-Classified	10 (3.6 %)	18 (4.3 %)
GMFCS^b level		
I-II	177 (63.0 %)	247 (58.5 %)
III	36 (12.8 %)	44 (10.4 %)
IV	39 (13.9 %)	58 (13.7 %)
V	22 (7.8 %)	61 (14.5 %)
Missing	7 (2.5 %)	12 (2.8 %)
BFMF^c level		
I	84 (29.9 %)	103 (24.4 %)
II	81 (28.8 %)	122 (28.9 %)
III	31 (11.0 %)	32 (7.6 %)
IV	28 (10.0 %)	44 (10.4 %)
V	25 (8.9 %)	69 (16.4 %)
Missing	32 (11.4 %)	52 (12.3 %)
Use of antiepileptic drugs		
Yes	67 (23.8 %)	117 (27.7 %)
No	121 (43.1 %)	186 (44.1 %)
Missing	93 (33.1%)	119 (28.2 %)
Gastrostomy tube feeding		
Yes	26 (9.3 %)	68 (16.1 %)
No	242 (86.1 %)	328 (77.7 %)
Missing	13 (4.6 %)	26 (6.1 %)

^a Surveillance of Cerebral Palsy in Europe

^b Gross Motor Function Classification System

^c Bimanual Fine Motor Function

Table 2

Different combinations of the *TOMM40* rs59007384 and *APOE* genotypes in 227^b children with CP and their fine motor function (BFMF level I-II, versus III-V), use of antiepileptic drugs (AED), and need for gastrostomy tube feeding (GTF). The table also shows the odds ratios (OR) with 95% confidence intervals (CI) for more adverse outcome in children with the various genotype combinations, using children with the *TOMM40* rs59007384GG/*APOE*4(-) genotype as reference.

		Adverse outcome			
Clinical measure	Genotype	No	Yes		
	<i>TOMM40</i> ^a <i>APOE</i>	n	n	OR (CI)	P-value
BFMF^b (n=220)	GG E4(-)	85	34	1.0	
	GG E4(+)	5	4	2.00 (0.51-7.90)	0.320
	GT/TT E4(-)	14	14	2.50 (1.08-5.80)	0.033
	GT/TT E4(+)	38	26	1.71 (0.90-3.24)	0.090
AED^b (n=221)	GG E4(-)	97	24	1.0	
	GG E4(+)	6	4	2.69 (0.70-10.31)	0.150
	GT/TT E4(-)	17	10	2.38 (0.97-5.85)	0.060
	GT/TT E4(+)	41	22	2.16 (1.09-4.30)	0.027
GTF^b (n=225)	GG E4(-)	117	8	1.0	
	GG E4(+)	7	3	6.27 (1.36-28.96)	0.019
	GT/TT E4(-)	23	5	3.18 (0.95-10.59)	0.060
	GT/TT E4(+)	52	10	2.81 (1.05-7.53)	0.040

^a *TOMM40* SNP rs 59007384 (G>T)

^b Due to differences in available information on BFMF, AED and GTF, the numbers differ slightly.

Table 3

The number of children with the genotype TOMM40 rs59007384GG/APOE4(-) with any of the three other combinations of rs59007384 and *APOE* genotypes (all other combinations), their fine motor function (BFMF level I-II, versus III-V), their use of antiepileptic drugs (AED), and their need to be fed through a gastrostomy tube (GTF). The table also shows odds ratios (OR) with 95% confidence intervals (CI) for more adverse outcome in children with the various genotype combinations, using children with the genotype TOMM40 rs59007384GG/APOE4(-) as reference.

Clinical measure/genotype		APOE4(-) with TOMM40 GG ^a	All other combinations	
		n	n	p-value
BFMF^b (n=220)	Level I-II	85	57	
	Level III-V	34	44	
	OR (CI)	1.00	1.93 (1.10-3.37)	0.021
AED^b (n=221)	No	97	64	
	Yes	24	36	
	OR (CI)	1.00	2.27 (1.24-4.16)	0.008
GTF^b (n= 225)	No	117	82	
	Yes	8	18	
	OR (CI)	1.00	3.21 (1.33-7.73)	0.009

^aTOMM40 SNP rs59007384 (G>T)

^bDue to differences in available information on BFMF, AED and GTF, the numbers differ slightly