Brief Communication

Neuro–degenerative Diseases

Neurodegenerative Dis 2006;3:334–337
DOI: 10.1159/000097302

Received: June 19, 2006
Accepted after revision: August 21, 2006

Genetic Association Study on Colony-Stimulating Factor 1 in Alzheimer's Disease

M. Axel Wollmer Roger M. Nitsch Christoph Hock Andreas Papassotiropoulos

Division of Psychiatry Research, University of Zurich, Zurich, Switzerland

Key Words
Microglia · β-Amyloid · Polymorphism · Macrophage

Abstract
Background: Colony-stimulating factor 1 (CSF1) regulates the proliferation and differentiation of myelomonocytic cells. Microglial cells of CSF1-deficient mice are reduced in number and are functionally impaired. CSF1-deficient mice exhibit subtle neurodevelopmental defects, enhanced neuronal vulnerability. Moreover, it has been reported that these mice may have amyloid-plaque-like depositions in the brain at an early age. The human *CSF1* gene maps to chromosome 1p21–p13, a region previously linked to Alzheimer's disease (AD). Thus, *CSF1* is a functional and positional candidate gene for AD. ***Objective:*** We assessed if genetic variability of *CSF1* may influence the risk for AD. ***Methods:*** We conducted a population-based case-control association study with 3 single nucleotide polymorphisms (SNPs) across the *CSF1* locus in a sample of n = 185 (rs3093054, rs756325) and n = 327 (rs1058885) individuals. ***Results:*** None of the 3 investigated SNPs was associated with the risk for AD in our sample. ***Conclusion:*** These data do not support the hypothesis that genetic variability of *CSF1* influences the risk for AD.

Colony-stimulating factor 1 (CSF1) regulates the proliferation and differentiation of myelomonocytic cells and is involved in the initiation and maintenance of the macrophage immune response. Microglia are the resident macrophages of the CNS. They are supposed to contribute to the removal of β-amyloid (Aβ) from the brain [1]. Especially brain macrophages of myelomonocytic origin seem to restrict cerebral amyloid deposition [2]. Aβ increases neuronal expression of CSF1 [3], and neuronal damage leads to up-regulation of both CSF1 and its receptor in microglial cells [4]. CSF1 enhances the capacity of microglial cells to phagocytose Aβ [5]. Thus, up-regulation of CSF1 in Alzheimer's disease (AD) and in APP-transgenic mice [3, 6] may represent the, admittedly insufficient, activation of an Aβ clearance pathway. CSF1-deficient osteopetrotic *(op/op)* mice have reduced numbers and functional impairment of myelomonocytic cells including microglia [7–9]. Observations suggesting that these mice may deposit congophilic, Aβ antibody-reactive material in the brain resembling amyloid plaques of AD patients and APP-transgenic mice have been reported [10, 11]. These depositions may be associated with reduced density of pyramidal cells in the CA1 and CA3 regions of the hippocampus. Moreover, *op/op* mice exhibit subtle neurodevelopmental defects [12] and increased neuronal vulnerability [13, 14], suggesting that CSF1 may also have trophic effects on neurons [15].

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1660–2854/06/0036–0334$23.50/0

Accessible online at:
www.karger.com/ndd

M. Axel Wollmer, MD
Division of Psychiatry Research, University of Zurich
August Forel Strasse 1
CH–8008 Zurich (Switzerland)
Tel. +41 44 384 2658, Fax +41 44 384 2686, E-Mail awollmer@bli.unizh.ch

Table 1. Characteristics of the investigated samples

	Group size	Age years	Females %	*APOE ε4* positive, %
Sample 1 (n = 185)	129/56	66.6 ± 9.3/70.7 ± 9.1	49.6/53.6	31.0/57.1
Sample 2 (n = 327)	183/144	67.0 ± 9.1/70.2 ± 8.0	49.2/51.4	32.2/57.9

Sample 1 was used for all 3 investigated SNPs. Sample 2 includes sample 1 and was used only for rs1058885. Results are those of healthy control subjects/AD patients.

We assumed that genetic variability of *CSF1* may modify amyloid deposition and neurodegeneration in the human brain and thereby the risk for Alzheimer's disease. To test this hypothesis we conducted a genetic case-control association study in a series of n = 327 individuals (183 healthy control subjects and 144 cases of sporadic AD, sample 2, table 1) from Switzerland. Diagnoses were made according to the National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer's Disease and Related Disorders Associations (NINCDS-ADRDA) criteria for probable AD. Dementia and memory deficits in control subjects were excluded by neuropsychological testing, consisting of the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) neuropsychological test battery and the Mini-Mental State Examination (MMSE). The sample is characterized in table 1. The local ethics committee approved the study, and informed consent was obtained from all participants prior to the investigation.

The human *CSF1* gene maps to chromosome 1p21–p13, ~4.3 cM off the peak marker D1S1678 of a region previously linked to AD [16]. It comprises 18,898 bp (base pairs 110165499–1101843397 of the chromosomal sequence) with 10 exons and encodes a 554-amino-acid protein. To span the whole locus we used 1 marker upstream (rs3093054) and 1 marker downstream (rs756325) of the gene. We genotyped rs1058885 (T/C, Leu/Pro) to tag the highly variable exon 6, which harbours 6 of 8 described non-synonymous *CSF1* single nucleotide polymorphisms (SNPs) within a sequence of ~470 bp. Information on SNPs was derived from the NCBI SNP database (http://www.ncbi.nlm.nih.gov/SNP). Genotypes were determined from genomic DNA by Pyrosequencing™. The following oligonucleotides were used for PCR and sequencing: rs1058885, forward AGGCTCTCCCAGGATCTCAT, reverse biotin-TTCACTTGCTGGTCCTCCTT, sequencing CCCAGGATCTCATCAC; rs3093054, forward biotin-GGAGGGTGGAGAGAAGAACA, reverse AGTGGGACTTGCAGCGTCT, sequencing GGATCTGCTTGATGTGG; rs756325, forward biotin-TTCCTCCCCTCAAAAGGATT, reverse GGGTCACAAAGGACTCAAGC, sequencing CCTGGTGGATTTAGGG. The investigated SNPs and their haplotypes are characterized in table 2.

Table 2. Pairwise linkage disequilibrium of the 3 investigated *CSF1* SNPs

	rs3093054	rs1058885	rs756325
rs3093054 [pos. –2,494 bp C/G, MAF 0.35 (G) HWE p = 0.1]		D′ = 0.078 r^2 = 0.006 χ^2 = 1.935 p = 0.164	D′ = 0.221 r^2 = 0.015 χ^2 = 5.493 p = 0.019
rs1058885 [pos. 13,009 bp (AA 408) T/C (L/P), MAF 0.4 (C) HWE p = 0.18]			D′ = 0.839 r^2 = 0.204 χ^2 = 72.777 p = 0.000
rs756325 [pos. +5,709 bp A/G, MAF 0.14 (G) HWE p = 0.77]			

rs105885 and rs756325 formed haplotypes for which the gametic phase could be predicted with a probability of >95% for all individuals with genotypes for both contributing SNPs. Three haplotypes were common (T/A, 84.3%; C/A, 39.9%; C/G, 25.3%), and 1 haplotype was rare (T/G, 2.2%). None of the 4 haplotypes was associated with AD (T/A, p = 1.0; C/A, p = 0.62; C/G, p = 1.0; T/G, p = 0.08; Fischer's exact test). rs3093054 was not in linkage disequilibrium with rs1058885 and was weakly linked to rs756325. The left column characterizes the investigated SNPs. Pos. = position relative to the gene; – = a position 5′ upstream; + = a position 3′ downstream of the gene; AA = affected amino acid; MAF = minor allele frequency. The p value is the significance of the deviation from Hardy-Weinberg equilibrium (HWE).

Table 3. Genotypic distribution of the three *CSF1* SNPs between AD cases and healthy control subjects

rs3093054	AD (n = 54)	HCS (n = 123)	Total (n = 177)
C/C	22 (40.7%)	49 (39.8%)	71 (40.1%)
C/G	26 (48.1%)	63 (51.2%)	89 (50.3%)
G/G	6 (11.1%)	11 (8.9%)	17 (9.6%)
	OR = 1.03, 95% CI = 0.64–1.65, Pearson χ^2 = 0.01, 1 d.f., p = 0.91 (p = 0.95)		
rs1058885	AD (n = 144)	HCS (n = 183)	Total (n = 327)
T/T	53 (36.8%)	70 (38.3%)	123 (37.6%)
T/C	65 (45.1%)	81 (44.3%)	146 (44.6%)
C/C	26 (18.1%)	32 (17.5%)	58 (17.7%)
	OR = 1.04, 95% CI = 0.76–1.43, Pearson χ^2 = 0.07, 1 d.f., p = 0.79 (p = 0.80)		
rs756325	AD (n = 54)	HCS (n = 124)	Total (n = 178)
A/A	37 (68.5%)	92 (74.2%)	129 (72.5%)
A/G	15 (27.8%)	31 (25.0%)	46 (25.8%)
G/G	2 (3.7%)	1 (0.8%)	3 (1.7%)
	OR = 1.39, 95% CI = 0.75–2.58, Pearson χ^2 = 1.11, 1 d.f., p = 0.29 (p = 0.31)		

There was no significant association of the investigated SNPs with the diagnosis of AD. Statistics refer to the comparison of the 2 alleles of the respective SNPs between the AD and the HCS group. p values in brackets were obtained by forward unconditional logistic regression, correcting for age, sex, presence or absence of at least 1 *APOE* ε4 allele and the respective other *CSF1* SNPs. HCS = Healthy control subjects.

None of the 3 markers or their haplotypes were associated with AD risk in a sub-sample of n = 185 individuals (sample 1, table 1), using χ^2 tests and forward unconditional logistic regression controlling for apolipoprotein E (*APOE*), age and sex (tables 2, 3). Because linkage to AD on chromosome 1 was predominantly observed in *APOE* ε4 allele carriers [16], we also stratified for *APOE* ε4. In the ε4-positive subset (n = 71) we found a borderline significance of rs1058885 (linear-by-linear χ^2 test, p = 0.03). To corroborate this observation we increased the sample size to n = 327 (sample 2, table 1). In the enlarged sample the effect was disrupted (linear-by-linear χ^2 test, p = 0.06), and rs1058885 also remained negative in the combined sample of *APOE*-ε4-allele-positive (n = 140) and -negative participants (table 3).

Based on functional and positional criteria, *CSF1* was selected as a candidate risk gene for AD. In summary our data do not support the hypothesis that genetic variability of *CSF1* may contribute to the risk for sporadic AD in the investigated population. We emphasize that the present study does not exclude *CSF1* as an AD susceptibility gene because of 2 main reasons. (I) Low statistical power: the relative risk associated with the minor alleles of the 3 SNPs that would have been detectable with 80% power at a significance level of p = 0.05 (n = 185) was between 0.13 and 0.37 or 2.47 and 3.10, respectively. Conversely, the power to reach a significance level of p = 0.05 for the observed relative risks was between 4 and 11%. (II) Low resolution: with 3 SNPs across the *CSF1* locus the resolution was about 9 kb. The linkage disequilibrium and allele frequencies of the investigated markers did not capture the whole genetic variability of *CSF1*.

CSF1 may play an important role in the regulation of the microglial response to amyloid pathology in AD. Over-expression of the CSF1 receptor facilitates the phagocytosis of antibody-opsonized Aβ by microglial cells [17], which is a potentially important mechanism in immune therapy of AD. Future studies could assess if *CSF1* SNPs are associated with responsiveness to active or passive Aβ vaccination, if CSF1 may serve as a biomarker to monitor the microglial response to this treatment, or if CSF1 has a therapeutic potential alone or as an adjuvant in the immune therapy of AD.

Acknowledgement

We thank Ms. Esmeralda Gruber and Ms. Christin Wilde for their patient care and sampling and Ms. Kim-Dung Huynh for her excellent technical assistance. This work was supported by the National Center for Competence in Research (NCCR), Neuronal Plasticity and Repair, and by the EU APOPIS programme (contract LSHM-CT-2003-503330).

References

1 Rogers J, Strohmeyer R, Kovelowski CJ, Li R: Microglia and inflammatory mechanisms in the clearance of amyloid β peptide. Glia 2002;40:260–269.

2 Simard AR, Soulet D, Gowing G, Julien JP, Rivest S: Bone marrow-derived microglia play a critical role in restricting senile plaque formation in Alzheimer's disease. Neuron 2006;49:489–502.

3 Du Yan S, Zhu H, Fu J, Yan SF, Roher A, Tourtellotte WW, Rajavashisth T, Chen X, Godman GC, Stern D, Schmidt AM: Amyloid-β peptide-receptor for advanced glycation endproduct interaction elicits neuronal expression of macrophage-colony stimulating factor: a proinflammatory pathway in Alzheimer disease. Proc Natl Acad Sci USA 1997;94:5296–5301.

4 Hao AJ, Dheen ST, Ling EA: Expression of macrophage colony-stimulating factor and its receptor in microglia activation is linked to teratogen-induced neuronal damage. Neuroscience 2002;112:889–900.

5 Mitrasinovic OM, Vincent VA, Simsek D, Murphy GM Jr: Macrophage colony stimulating factor promotes phagocytosis by murine microglia. Neurosci Lett 2003;344:185–188.

6 Murphy GM Jr, Zhao F, Yang L, Cordell B: Expression of macrophage colony-stimulating factor receptor is increased in the AβPP(V717F) transgenic mouse model of Alzheimer's disease. Am J Pathol 2000;157:895–904.

7 Wegiel J, Wisniewski HM, Dziewiatkowski J, Tarnawski M, Kozielski R, Trenkner E, Wiktor-Jedrzejczak W: Reduced number and altered morphology of microglial cells in colony stimulating factor-1-deficient osteopetrotic op/op mice. Brain Res 1998;804:135–139.

8 Raivich G, Moreno-Flores MT, Moller JC, Kreutzberg GW: Inhibition of posttraumatic microglial proliferation in a genetic model of macrophage colony-stimulating factor deficiency in the mouse. Eur J Neurosci 1994;6:1615–1618.

9 Kalla R, Liu Z, Xu S, Koppius A, Imai Y, Kloss CU, Kohsaka S, Gschwendtner A, Moller JC, Werner A, Raivich G: Microglia and the early phase of immune surveillance in the axotomized facial motor nucleus: impaired microglial activation and lymphocyte recruitment but no effect on neuronal survival or axonal regeneration in macrophage-colony stimulating factor-deficient mice. J Comp Neurol 2001;436:182–201.

10 Kaku M, Tsutsui K, Motokawa M, Kawata T, Fujita T, Kohno S, Tohma Y, Ohtani J, Tenjoh K, Tanne K: Amyloid β protein deposition and neuron loss in osteopetrotic (op/op) mice. Brain Res Brain Res Protoc 2003;12:104–108.

11 Kawata T, Tsutsui K, Kohno S, Kaku M, Fujita T, Tenjou K, Ohtani J, Motokawa M, Shigekawa M, Tohma Y, Tanne K: Amyloid β protein deposition in osteopetrotic (op/op) mice is reduced by injections of macrophage colony stimulating factor. J Int Med Res 2005;33:654–660.

12 Michaelson MD, Bieri PL, Mehler MF, Xu H, Arezzo JC, Pollard JW, Kessler JA: CSF-1 deficiency in mice results in abnormal brain development. Development 1996;122:2661–2672.

13 Fedoroff S, Berezovskaya O, Maysinger D: Role of colony stimulating factor-1 in brain damage caused by ischemia. Neurosci Biobehav Rev 1997;21:187–191.

14 Bruccoleri A, Harry GJ: Chemical-induced hippocampal neurodegeneration and elevations in TNFα, TNFβ, IL-1α, IP-10, and MCP-1 mRNA in osteopetrotic (op/op) mice. J Neurosci Res 2000;62:146–155.

15 Mitrasinovic OM, Grattan A, Robinson CC, Lapustea NB, Poon C, Ryan H, Phong C, Murphy GM Jr: Microglia overexpressing the macrophage colony-stimulating factor receptor are neuroprotective in a microglial-hippocampal organotypic coculture system. J Neurosci 2005;25:4442–4451.

16 Myers A, Wavrant De-Vrieze F, Holmans P, Hamshere M, Crook R, Compton D, Marshall H, Meyer D, Shears S, Booth J, Ramic D, Knowles H, Morris JC, Williams N, Norton N, Abraham R, Kehoe P, Williams H, Rudrasingham V, Rice F, Giles P, Tunstall N, Jones L, Lovestone S, Williams J, Owen MJ, Hardy J, Goate A: Full genome screen for Alzheimer disease: stage II analysis. Am J Med Genet 2002;114:235–244.

17 Mitrasinovic OM, Murphy GM Jr: Microglial overexpression of the M-CSF receptor augments phagocytosis of opsonized Aβ. Neurobiol Aging 2003;24:807–815.