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SHC2 gene copy number in multiple system atrophy (MSA)

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Abstract

Purpose—Multiple system atrophy (MSA) is a sporadic, late onset, rapidly-progressing neurodegenerative disorder, which is characterized by autonomic failure, together with parkinsonian, cerebellar, and pyramidal motor symptoms. The pathologic hallmark is the glial cytoplasmic inclusion with alpha-synuclein aggregates. MSA is thus an alpha synucleinopathy. Recently, Sasaki et al. reported that heterozygosity for copy number loss of Src homology 2 domain containing-transforming protein 2 (SHC2) genes (heterozygous SHC2 gene deletions) occurred in DNAs from many Japanese individuals with MSA. Because background copy number variation (CNV) can be distinct in different human populations, we assessed SHC2 allele copy number in DNAs from a US cohort of individuals with MSA, to determine the contribution of SHC2 gene copy number variation in an American cohort followed at a US referral center for MSA. Our cohort included 105 carefully phenotyped individuals with MSA.

Methods—We studied 105 well characterized patients with MSA and 5 control subjects with reduced SHC2 gene copy number. We used two TaqMan Gene Copy Number Assays, to determine the copy number of two segments of the SHC2 gene that are separated by 27 Kb.

Results—Assay results of DNAs from all of our 105 subjects with MSA showed two copies of both segments of their SHC2 genes.

Conclusion—Our results indicate that SHC2 gene deletions underlie few, if any, cases of well characterized MSA in the US population. This is in contrast to the Japanese experience reported by Sasaki et al., likely reflecting heterogeneity of the disease in different genetic backgrounds.

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Keywords

Multiple System Atrophy; genetics; copy number variation; movement disorders; SHC2

Introduction

Multiple system atrophy (MSA) is an adult-onset, rapidly progressive neurodegenerative disease that presents with autonomic failure in combination with parkinsonism or cerebellar ataxia, and ultimately, pyramidal involvement. Patients are sometimes subclassified as having MSA-P when parkinsonian features predominate or MSA-C if cerebellar ataxia predominates. Whether these two forms of MSA constitute a single disease or two or more distinct entities must await more fundamental elucidation of the underlying pathophysiology. The neuropathological features consist of neuronal loss in the basal ganglia, cerebellum, pons, inferior olivary nuclei, the intermediolateral column, and Onuf's nucleus typically accompanied by gliosis.

The neuropathological hallmark of MSA is the glial cytoplasmic inclusion (GCI), particularly prominent in the oligodendroglia, but with neuronal involvement as well. These structures contain misfolded, hyperphosphorylated, fibrillar α-synuclein as their main component. Thus the GCIs of MSA, like the Lewy bodies of Parkinson's disease (PD) and Dementia with Lewy bodies (DLB), contain deposits of α-synuclein. Unlike PD and DLB, however, MSA is a primary oligodendroglial α-synucleinopathy that leads to neurodegeneration[14;22;23].

In recent years genetic alterations associated with MSA-C have been investigated in several families with autosomal recessive inheritance as suggested by affected siblings in three or more families [8;13;14;23]. Studies to determine the molecular basis of MSA targeting the α-synculein, apolipoprotein E, dopamine beta-hydroxylase, ubiquitin C-terminal hydrolase-1, fragile X mental retardation 1 and leucine-rich kinase 2 genes did not detect variations that could reasonably give rise to the pathophysiology of MSA[8;13;14;23]. However Scholz et al reported a single-nucleotide polymorphism (rs111931074) associated with α-synculein in pathologically confirmed cases of MSA.[18]

MSA development, however, has been proposed to be associated with polymorphisms in genes involved with inflammatory processes and oxidative stress [3;4;8;11;12;20], but the evidence on this remains limited.

In a recent report, Sasaki et al. described heterozygosity for a SHC2 (SHC-transforming protein 2) gene deletion in a Japanese individual with MSA whose unaffected identical twin did not have a deletion [17]. They also found that 10/31 (32%) of unrelated Japanese patients with MSA were heterozygous for SHC2 gene deletions based on CAN beadchip and custom tiling microarray analysis [17]. SHC-transforming protein 2 (SHC2) is a 582 amino acid gene product believed to function as a signaling adapter that couples activated growth factor receptors to signaling pathways in neurons, including signal transduction pathways of neurotrophin-activated Trk receptors in cortical neurons.

These findings suggest a potential genetic mechanism for MSA. Heterozygosity for *de novo* gene deletions could possibly explain the limited familial pattern of MSA commonly encountered by clinicians involved in the diagnosis and care of such patients. For example, in the experience of the Vanderbilt Autonomic Dysfunction Center which has followed more than 400 MSA patients over a period of 30 years, no patient with the MSA diagnosis was known to have a first degree relative who also met the criteria for this disease, though a number had relatives with Parkinson's Disease or related disorders.

Copy number variation (CNV) in the human genome has emerged recently as a potential causative mechanism, particularly in neuropsychiatric disease [15;24]. With studies of increasing numbers of human genomes, large numbers of deletions, duplications and inversions have been found. While most of these are the common and presumably benign polymorphisms, some are rare, large, or even *de novo* CNVs which have been found to cause a number of complex genetic diseases [16]. In 2007, Sebat [19] reported a ten-fold increase in large *de novo* CNVs in autism, and others reported similar contributions of CNVs to schizophrenia [9], epilepsy [7], bipolar disorder [2] and intellectual impairment of unknown etiology.

The report of copy number loss in some patients with MSA from Hokkaido University is important because it describes how a genetic etiology might exist in some patients with MSA even in the absence of a detectible familial pattern in the clinic. This prompted our group to determine the contribution of SHC2 gene deletions in our MSA patient population. It was recognized that genetic determinants in human populations living for extended periods in different geographic locations might well yield differences in the nature of presentation and genetic/environmental influences in neurodegenerative diseases like MSA. Such potential genetic differences might themselves be informative.

The aim of this study was to determine the contribution of SHC2 gene deletions in a US cohort of MSA patients. Another advantage of our study was our comparatively large MSA patient population.

Methods

DNA was isolated from peripheral blood samples of 105 unrelated MSA patients seen at Vanderbilt University's Autonomic Dysfunction Center over the period 1992–2010 [5]. Of 14 patients who ultimately had autopsies, MSA was confirmed by the identification of glial cytoplasmic inclusions in each, suggesting a strong clinicopathological concordance in clinical and pathological diagnosis of MSA in our center. For control purposes, DNA was obtained from 5 unrelated control subjects, who were known to have a reduced copy number of the SHC2 gene. Written consent was obtained from all participants, in accordance with protocols approved by the Institutional Review Board at Vanderbilt University.

DNA was genotyped for SHC2 gene copy number using TaqMan Gene Copy Number Assays (Applied Biosystems, Foster City, California). Two regions of the SHC2 gene were assayed in each case. FAM dye based assays Hs04020716 and Hs04032620 targeted SCH2 on locations Chr19:417177 binding at the 5′ end and Chr19:444480 binding within the middle of the gene respectively (on NCBI Build37, hg19.v10). A Vic dye based copy number reference assay against the RNaseP gene (4403326) was used as the internal reference.

Each DNA sample was run in triplicate with each assay repeated twice. Each 20 μl assay contained 20 ng of genomic DNA, 1 μl of the specific Taqman copy number assay and 1 μl of the reference gene assay and 10 μl aliquots were run on an ABI 7500 real-time instrument using the following thermal-cycling conditions: 10 minutes at 95°C, followed by 40 cycles of 15 seconds at 95°C and 60 seconds at 60°C. Real-time data was collected by the SDS 2.3 software (ABI) and then further analyzed by the Copycaller software (ABI). The method involves relative quantification of the test sequence versus a reference gene known to have two copies per diploid genome. Relative quantity was determined by the ΔΔCt [(FAM Ct − VIC Ct) sample − (FAM Ct − VIC Ct) calibrator] method, where a reference sample or calibrator known to have two copies of the test sequence is used as the basis for comparative results [10].

Results

In total, 105 patients with MSA (63 men; age, 62.9±9.0 years; 42 women; age 57.8 ±9.0 years were enrolled. Table 1 lists the baseline characteristics of the cases. The results of SHC2 gene copy number analysis for each patient are presented in Figure 1. None of the sample results fell into the ranges expected for heterozygous or homozygous SHC2 gene deletions.

Discussion

Clinical experience in European and US centers where substantial numbers of MSA patients are evaluated has not generally elicited family histories or other evidence suggestive of a significant genetic basis for MSA. However, efforts to elucidate environmental influences leading to MSA have also met with limited success. The vast majority of MSA patients have no first degree family members with illnesses recognizably like MSA. While occasional MSA patients are encountered who have a relative with parkinsonism, these seem to be no more common in MSA than in patients without neurological disease. Despite this, there are reports of rare familial cases that involved affected sibs (MIM 146500). It is also possible that MSA causing mutant alleles of low penetrance with delayed age of onset and variable expressivity could contribute to MSA but prevent diagnosis of multiple affected family members because of skipped generations, death before onset of signs and symptoms and mild phenotypic expression.

It is recognized, however, that clinical presentation does not always provide strong evidence in some cases. It is noteworthy that 40 years ago, Parkinson’s Disease was considered to be almost entirely sporadic in nature. However, with increased evidence from GWAS and exome sequencing a dozen or more genes have been identified that convey risk for the disease. It is possible that such observations will emerge with further genetic studies of MSA.

In a recent review of the role of genetics in MSA, Wenning et al. provided substantial, albeit necessarily fragmentary, evidence of a genetic component in the etiology of MSA[21]. Genes involved in oxidative stress, inflammatory processes, and mitochondrial dysfunction, as well as genes implicated in other ataxia- and parkinsonian neurodegenerative diseases have all been suggested. Nevertheless, while strong evidence exists for involvement of one or more of these proposed genes in other neurodegenerative disorders, definitive evidence has been difficult to find in MSA patients. A recent manuscript by the Multiple System Atrophy Research Collaboration identified an impaired variant of COQ2 with strong association to MSA in multiplex families and sporadic MSA patients in Japanese but not North American or European patients [25].

Taken together these observations require that the contribution of CNVs to MSA be carefully studied in multiple genetic backgrounds. Fundamentally, disease-related CNVs presumably can operate by dosage sensitivity, at the simplest level, but it remains unclear how specific CNVs can result in such disparate phenotypes. However several aspects already seem clear in certain well-studied instances mentioned above [24]. In certain cases, CNV breakpoints may not be mapped to the nucleotide level, so overlapping variants could exhibit different phenotypes. Also, a CNV deletion could unmask a recessive variant on the trans allele or there could be cis effects where the CNV influences the expression of nearby loci.

Alternatively, as in known imprinting disorders, the loci within the CNV may exhibit parent-of-origin effects with consequent differing levels of expression of the now hemizygous genes on the second allele. Another possibility is that there are disease-associated CNVs,

which could lower susceptibility thresholds depending on the genomic background and/or environmental influences resulting in phenotypes on a clinical continuum. Eichler has, for example proposed a two-hit model [6]. Here a second CNV, elsewhere in the genome, influences penetrance and expressivity of the first CNV. Evidence for this model is best illustrated by a 600 kb microdeletion at 16p12.1 that has been found in children with intellectual disability and/or autism. Unlike most disease-associated CNVs, the 16p12.1 deletion is rarely *de novo*, being inherited from one parent in 95% of the cases. The carrier parent, while cognitively intact, might display milder phenotypes such as depression, or mild learning disability. In 25% of cases, the child with the more severe phenotype has a second large CNV elsewhere in the genome, a 40-fold increase over the predicted rate. The key element may paradoxically be that instead of a second CNV, a second conventional mutation elsewhere in the genome tips the balance to a severe phenotype through gene-gene interactions.

Clearly, genetic studies on CNVs influencing neurological diseases like MSA may provide analogous possibilities. The report of Sasaki et al. [17] of heterozygosity for an SHC2 deletion in a Japanese individual with MSA whose unaffected identical twin sibling did not have a deletion provides another potential explanation of the lack of apparent Mendelian inheritance, e.g., the occurrence of new mutations as evidenced by a discordant identical twin. Thus, some cases of MSA might arise due to *de novo* SHC2 gene deletions. These patients would have no family history in preceding generations and the reduced and delayed penetrance could greatly reduce the chances of detecting MSA in any of their offspring. Finally, environmental and/or additional genetic factors may be necessary to trigger MSA and these might have somehow eluded investigators.

Our results are based on TaqMan Gene Copy Number Assays. This assay is a robust method to determine copy number variations and provides a rapid, relatively inexpensive alternative to the methods used by Sasaki et al. [1;12;17]. The heterozygous copy number deletions in the SHC2 gene of our control samples displayed similar to the results of the MSA patients found in the Sasaki et al manuscript [17]. Currently, only Sasaki et al. have reported SHC2 gene deletions in MSA [17]. We infer that their subjects were of Japanese extraction, while our subjects were 94% or North American Caucasians and 5% of African American. Only one individual of Korean American extraction was included in our population. Our lack of Asian, much less Japanese, subjects may be an important factor in our failure to find any SHC2 deletion heterozygotes. Negative data in our distinct American population does not detract from the important discovery by Sasaki et al. (2011) in the Japanese MSA population [17].

The mean age of onset of MSA is about 53 years, and no proven case has had onset earlier than age 30 years. Onset after age 75 years is uncommon, but cases over this age are seen. Both genders are affected equally. The annual incidence rate for all subjects is 0.6 cases per 100,000, but for subjects above age 50 years, the incidence rate is 3 cases per 100,000. The population prevalence of MSA has been estimated at 4 to 16 per 100,000, although with wide confidence intervals. In case series from Europe and North America, MSA-P cases predominate in a ratio of about 4:1; however, in Japan, MSA-C cases predominate [5]. This difference in the proportions of MSA-P and MSA-C cases might contribute to some of the differences in data we report, but likely other factors are also involved.

Two additional factors could possibly contribute to the different outcomes of our studies. First, the criteria for diagnosis of MSA may differ. While we and Sasaki et al. used contemporary guidelines for diagnosis [5] there may be subtle phenotypic differences beyond that of MSA-P and C presentations. Importantly, none of our results suggest a

change in the SHC2 gene copy number when compared to the internal reference (RNaseP gene).

In summary, our data do not support SHC2 gene deletions as a significant genetic susceptibility factor in MSA with respect to North American subjects. All things considered, findings from this study should elicit further exploration of genetic and environmental factors that may convey risk of MSA, and the copy number variation may constitute an important new tool in understanding the nature of MSA.

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References

1. Breheny P, Chalise P, Batzler A, Wang L, Fridley BL. Genetic association studies of copy-number variation: should assignment of copy number states precede testing? PLoS One. 2012; 7:e34262. [PubMed: 22493684]
2. Clayton-Smith J, Giblin C, Smith RA, Dunn C, Willatt L. Familial 3q29 microdeletion syndrome providing further evidence of involvement of the 3q29 region in bipolar disorder. Clinical Dysmorphology. 2010; 19:128–132. [PubMed: 20453639]
3. Combarros O, Infante J, Llorca J, Berciano J. Interleukin-1A (−889) genetic polymorphism increases the risk of multiple system atrophy. Mov Disord. 2003; 18:1385–1386. [PubMed: 14639688]
4. Furiya Y, Hirano M, Kurumatani N, Nakamuro T, Matsumura R, Futamura N, Ueno S. alpha-1-Antichymotrypsin gene polymorphism and susceptibility to multiple system atrophy (MSA). Molecular Brain Research. 2005; 138:178–181. [PubMed: 15907346]
5. Gilman S, Wenning GK, Low PA, Brooks DJ, Mathias CJ, Trojanowski JQ, Wood NW, Colosimo C, Durr A, Fowler CJ, Kaufmann H, Klockgether T, Lees A, Poewe W, Quinn N, Revesz T, Robertson D, Sandroni P, Seppi K, Vidailhet M. Second consensus statement on the diagnosis of multiple system atrophy. Neurology. 2008; 71:670–676. [PubMed: 18725592]
6. Girirajan S, Eichler EE. Phenotypic variability and genetic susceptibility to genomic disorders. Human Molecular Genetics. 2010; 19:R176–R187. [PubMed: 20807775]
7. Heinzen EL, Radtke RA, Urban TJ, Cavalleri GL, Depondt C, Need AC, Walley NM, Nicoletti P, Ge D, Catarino CB, Duncan JS, Kasperaviciute D, Tate SK, Caboclo LO, Sander JW, Clayton L, Linney KN, Shianna KV, Gumbs CE, Smith J, Cronin KD, Maia JM, Doherty CP, Pandolfo M, Leppert D, Middleton LT, Gibson RA, Johnson MR, Matthews PM, Hosford D, Kalviainen R,

Eriksson K, Kantanen AM, Dorn T, Hansen J, Kraemer G, Steinhoff BJ, Wieser HG, Zumsteg D, Ortega M, Wood NW, Huxley-Jones J, Mikati M, Gallentine WB, Husain AM, Buckley PG, Stallings RL, Podgoreanu MV, Delanty N, Sisodiya SM, Goldstein DB. Rare Deletions at 16p13.11 Predispose to a Diverse Spectrum of Sporadic Epilepsy Syndromes. American Journal of Human Genetics. 2010; 86:707–718. [PubMed: 20398883]

8. Infante J, Llorca J, Berciano J, Combarros O. Interleukin-8, intercellular adhesion molecule-1 and tumour necrosis factor-alpha gene polymorphisms and the risk for multiple system atrophy. Journal of the Neurological Sciences. 2005; 228:11–13. [PubMed: 15607204]
9. Ingason A, Rujescu D, Cichon S, Sigurdsson E, Sigmundsson T, Pietilainen O, Buizer-Voskamp J, Strengman E, Francks C, Muglia P, Gylfason A, Gustafsson O, Olason PI, Steinberg S, Hansen T, Jakobsen K, Rasmussen H, Giegling I, Moeller HJ, Hartmann A, Crombie C, Fraser G, Walker N, Lonnqvist J, Suvisaari J, Tuulio-Henriksson A, Bramon E, Kiemeney L, Franke B, Murray R, Vassos E, Toulopoulou T, Muehleisen T, Tosato S, Ruggeri M, Djurovic S, Andreassen O, Zhang Z, Werge T, Ophoff R, Rietschel M, Noethen M, Petursson H, Stefansson H, Peltonen L, Collier D, Stefansson K, St Clair DM. Copy number variations of chromosome 16p13.1 region associated with schizophrenia. Molecular Psychiatry. 2011; 16:17–25. [PubMed: 19786961]
10. Mayo, P.; Hartshorne, T.; Li, K.; McMunn-Gibson, C.; Spencer, K.; Schnetz-Boutaud, N. Current Protocols in Human Genetics. John Wiley & Sons, Inc; 2001. CNV Analysis Using TaqMan Copy Number Assays.
11. Nishimura M, Kawakami H, Komure O, Maruyama H, Morino H, Izumi Y, Nakamura S, Kaji R, Kuno S. Contribution of the interleukin-1 beta gene polymorphism in multiple system atrophy. Mov Disord. 2002; 17:808–811. [PubMed: 12210881]
12. Nishimura M, Kuno S, Kaji R, Kawakami H. Influence of a tumor necrosis factor gene polymorphism in Japanese patients with multiple system atrophy. Neuroscience Letters. 2005; 374:218–221. [PubMed: 15663966]
13. Ozawa T. Pathology and genetics of multiple system atrophy: an approach to determining genetic susceptibility spectrum. Acta Neuropathologica. 2006; 112:531–538. [PubMed: 16855831]
14. Papp MI, Kahn JE, Lantos PL. Glial cytoplasmic inclusions in the CNS of patients with multiple system atrophy (striatonigral degeneration, olivopontocerebellar atrophy and Shy-Drager syndrome). Journal of the Neurological Sciences. 1989; 94:79–100. [PubMed: 2559165]
15. Pollack JR, Perou CM, Alizadeh AA, Eisen MB, Pergamenschikov A, Williams CF, Jeffrey SS, Botstein D, Brown PO. Genome-wide analysis of DNA copy-number changes using cDNA microarrays. Nature Genetics. 1999; 23:41–46. [PubMed: 10471496]
16. Redon R, Ishikawa S, Fitch KR, Feuk L, Perry GH, Andrews T, Fiegler H, Shapero MH, Carson AR, Chen W, Cho EK, Dallaire S, Freeman JL, Gonzalez JR, Gratacos M, Huang J, Kalaitzopoulos D, Komura D, MacDonald JR, Marshall CR, Mei R, Montgomery L, Nishimura K, Okamura K, Shen F, Somerville MJ, Tchinda J, Valsesia A, Woodwark C, Yang F, Zhang J, Zerjal T, Zhang J, Armengol L, Conrad DF, Estivill X, Tyler-Smith C, Carter NP, Aburatani H, Lee C, Jones KW, Scherer SW, Hurles ME. Global variation in copy number in the human genome. Nature. 2006; 444:444–454. [PubMed: 17122850]
17. Sasaki H, Emi M, Iijima H, Ito N, Sato H, Yabe I, Kato T, Utsumi J, Matsubara K. Copy number loss of (src homology 2 domain containing)-transforming protein 2 (SHC2) gene: discordant loss in monozygotic twins and frequent loss in patients with multiple system atrophy. Molecular Brain. 2011; 4:24. [PubMed: 21658278]
18. Scholz SW, Houlden H, Schulte C, Sharma M, Li A, Berg D, Melchers A, Paudel R, Gibbs JR, Simon-Sanchez J, Paisan-Ruiz C, Bras J, Ding J, Chen H, Traynor BJ, Arepalli S, Zonozi RR, Revesz T, Holton J, Wood N, Lees A, Oertel W, Wullner U, Goldwurm S, Pellecchia MT, Illig T, Riess O, Fernandez HH, Rodriguez RL, Okun MS, Poewe W, Wenning GK, Hardy JA, Singleton AB, Del SF, Schneider S, Bhatia KP, Gasser T. SNCA variants are associated with increased risk for multiple system atrophy. Ann Neurol. 2009; 65:610–614. [PubMed: 19475667]
19. Sebat J, Lakshmi B, Malhotra D, Troge J, Lese-Martin C, Walsh T, Yamrom B, Yoon S, Krasnitz A, Kendall J, Leotta A, Pai D, Zhang R, Lee YH, Hicks J, Spence SJ, Lee AT, Puura K, Lehtimaeki T, Ledbetter D, Gregersen PK, Bregman J, Sutcliffe JS, Jobanputra V, Chung W, Warburton D, King MC, Skuse D, Geschwind DH, Gilliam T, Ye K, Wigler M. Strong association

of de novo copy number mutations with autism. Science. 2007; 316:445–449. [PubMed: 17363630]

20. Soma H, Yabe I, Takei A, Fujiki N, Yanagihara T, Sasaki H. Associations between multiple system atrophy and polymorphisms of SLC1A4, SQSTM1, and EIF4EBP1 Genes. Mov Disord. 2008; 23:1161–1167. [PubMed: 18442140]
21. Stemberger S, Scholz SW, Singleton AB, Wenning GK. Genetic players in multiple system atrophy: unfolding the nature of the beast. Neurobiol Aging. 2011; 32:1924–14. [PubMed: 21601954]
22. Wakabayashi K, Yoshimoto M, Tsuji S, Takahashi H. Alpha-synuclein immunoreactivity in glial cytoplasmic inclusions in multiple system atrophy. Neurosci Lett. 1998; 249:180–182. [PubMed: 9682846]
23. Wenning GK, Stefanova N, Jellinger KA, Poewe W, Schlossmacher MG. Multiple system atrophy: A primary oligodendrogliopathy. Ann Neurol. 2008; 64:239–246. [PubMed: 18825660]
24. Zoghbi HY, Warren ST. Neurogenetics: Advancing the “Next-Generation” of Brain Research. Neuron. 2010; 68:165–173. [PubMed: 20955921]
25. The Multiple-System Atropohy Research Collaboration. Mutations in COQ2 in Familial Multiple System Atrophy. N Engl J Med. 2013; 369:233–244. [PubMed: 23758206]

Table 1

Clinical characteristics of the MSA patients

Clinical Characteristics of Patients	
Age (mean)	61.3
Gender (n)	
Male	63
Female	42
Ethnicity (n)	
White	99
Black	5
Hispanic	1