

# *DNMT1* mutation hot spot causes varied phenotypes of HSAN1 with dementia and hearing loss

Christopher J. Klein, MD  
Tom Bird, MD  
Nilufer Ertekin-Taner,  
MD, PhD  
Sarah Lincoln, BSc  
Robert Hjorth, MD  
Yanhong Wu, PhD  
John Kwok, PhD  
Georges Mer, PhD  
Peter J. Dyck, MD  
Garth A. Nicholson, MD,  
ME

Correspondence to  
Dr. Klein:  
klein.christopher@mayo.edu

## ABSTRACT

**Background:** Mutations in DNA methyltransferase 1 (*DNMT1*) have been identified in 2 autosomal dominant syndromes: 1) hereditary sensory autonomic neuropathy with dementia and hearing loss (HSAN1E); and 2) cerebellar ataxia, deafness, and narcolepsy. Both syndromes have mutations in targeting sequence (TS) domain (exons 20–21), which is important in mediating DNA substrate binding to the *DNMT1* catalytic domain. Frontal lobe hypometabolism has been documented in an HSAN1E family, but memory loss has been the primary cognitive complaint. The chromosomal location of the *DNMT1* gene at 19p13.2 has been linked to familial late-onset Alzheimer disease.

**Methods:** We sequenced 41 exons of *DNMT1* and their flanking regions in 1) 2 kindreds with HSAN1E; 2) 48 patients with HSAN1 alone without dementia and hearing loss; and 3) 5 probands of familial frontotemporal dementia (FTD) kindreds. We also sequenced exon 20 and 21 in 364 autopsy-confirmed late-onset Alzheimer disease cases.

**Results:** Mutations in *DNMT1* were specific to 2 HSAN1E kindreds with dementia and hearing loss (no narcolepsy). One family carried previously identified mutation Tyr495Cys; the other carried a novel Tyr495His, both in the TS domain. The symptoms of these patients include prominent personality, psychiatric manifestations, and seizures in one and the onset time is later than the previously reported cases.

**Conclusion:** Clinicians should consider *DNMT1* mutations in patients presenting with FTD or primary memory decline who also have sensory neuropathy and hearing loss. Amino acid Tyr495 is a hot spot for HSAN1E, distinct from exon 21 mutations associated with narcolepsy. *Neurology*® 2013;80:824–828

## GLOSSARY

**AD** = Alzheimer disease; **ADCA-DN** = autosomal dominant cerebellar ataxia deafness and narcolepsy; **DNMT1** = DNA methyltransferase 1; **FLOAD** = familial late-onset Alzheimer disease; **FTD** = frontotemporal dementia; **HSAN1** = autosomal dominant hereditary sensory and autonomic neuropathy; **HSAN1E** = hereditary sensory and autonomic neuropathy with dementia and hearing loss; **TS** = targeting sequence.

DNA methyltransferase 1 (*DNMT1*) is the fundamental methylation enzyme in maintaining methylation during DNA replication and DNA repair. Proper epigenetic regulation including DNA methylation is important for development, survival, and connectivity of neural cells.<sup>1</sup> We showed that mutations in *DNMT1* cause hereditary sensory and autonomic neuropathy with dementia and hearing loss (HSAN1E).<sup>2,3</sup> Autosomal dominant hereditary sensory and autonomic neuropathy (HSAN1) includes multiple subtypes (HSAN1A–HSAN1E) based on the specific phenotypes and genetic causes. The affected persons in our previously reported HSAN1E families had normal development and childhood, then developed stereotypic progressive-onset (teens–mid-30s) hearing loss and sensory neuropathy, followed by memory decline in their 30s and 40s.<sup>3</sup> Autopsies in HSAN1E showed diffuse neurodegeneration of brain, spinal cord, and nerve without specific interstitial changes, i.e., no  $\beta$ -amyloid, TDP-43, or  $\alpha$ -synuclein

From the Department of Neurology, Division of Peripheral Nerve Diseases (C.J.K., P.J.D.), Medical Genetics (C.J.K.), Laboratory Medicine and Pathology (Y.W.), and Biochemistry and Molecular Biology (G.M.), Mayo Clinic, Rochester, MN; Department of Neurology (T.B.), University of Washington, Seattle; Department of Neurology (N.E.-T., S.L.), Mayo Clinic, Jacksonville, FL; Doctors Medical Practice (R.H.), Richmond, Victoria; Neuroscience Research Australia (J.K.), Randwick, Australia; and Molecular Medicine Laboratory and Australian and New Zealand Army Corps (ANZAC) Research Institute (G.A.N.), University of Sydney, Australia.

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immunostaining. Nursing home placement and total care requirements were typical by the fifth decade, mainly due to memory loss and sensory ataxia, and foot ulcers or extremity amputations in about half of affected persons. The potential frontal lobe involvement and abnormal behavioral presentations were suggested by SPECT, PET images, and autopsy studies in one Japanese kinship.<sup>4,5</sup> Recently the phenotype spectrum of *DNMT1* mutations has been expanded to include narcolepsy, i.e., autosomal dominant cerebellar ataxia deafness and narcolepsy (ADCA-DN).<sup>6</sup> Patients with ADCA-DN also have neuropathy and frequently unspecified psychosis and dementia with characteristic brain imaging studies.<sup>7</sup> It was speculated that the rare polymorphisms located within the *DNMT1* locus may account for sporadic narcolepsy,<sup>6</sup> since a recent genome-wide association study linked sporadic narcolepsy patients to this chromosomal region.<sup>8</sup>

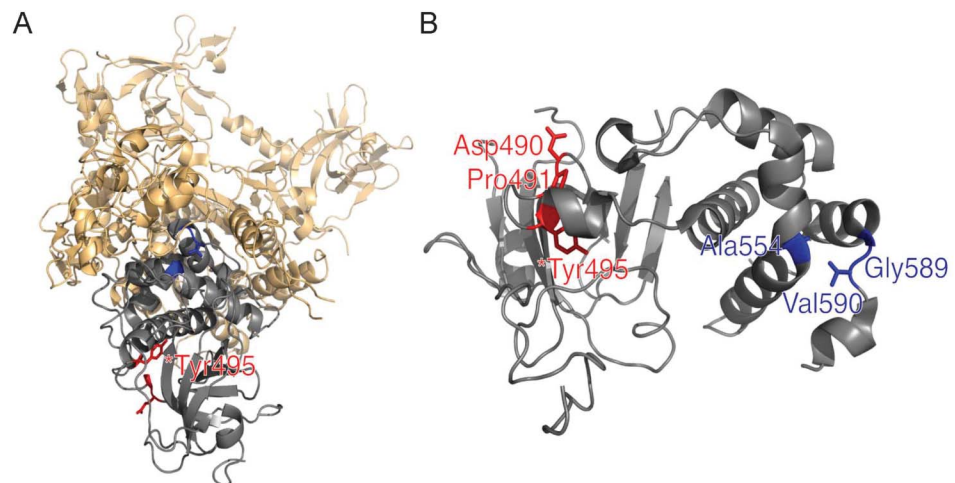
Two mutations causing HSN1E occurred in exon 20 of *DNMT1* (p.Tyr495Cys, p.Asp490Glu-Pro491Tyr) and 3 mutations causing ADCA-DN occurred in exon 21 (p.Ala570Val, p.Gly605Ala, p.Val606Phe) (figure 1). In both disorders, all causal mutations were found within the targeting sequence (TS) domain of *DNMT1*. The TS domain has been demonstrated to be indispensable in mediating

the association of *DNMT1* to heterochromatin during the G2 and M phase of the cell cycle. The interaction between the TS and catalytic domains is crucial for proper methylation maintenance of the genome.<sup>9</sup> Our studied mutations in exon 20 cause premature degradation of the mutant protein, reduced methyltransferase activity of *DNMT1*, and impaired heterochromatin binding during the G2 cell cycle phase. Importantly, we also observed global DNA hypomethylation throughout the genome and site-specific hypermethylation, a common phenomenon in cancer cells, emphasizing the direct link between *DNMT1* and neurodegeneration.<sup>3</sup>

A previous study has linked the chromosomal location of *DNMT1* at 19p13.2 with familial late-onset Alzheimer disease (FLOAD).<sup>10</sup> Herein we investigated whether *DNMT1* mutations may occur in isolated Alzheimer dementia, familial frontotemporal dementia (FTD), or isolated HSN1 without hearing loss or dementia, and whether *DNMT1* mutations may be linked to a wider spectrum of clinical presentations in HSN1E.

**METHODS Patients.** With institutional review board approval and informed patient consent, we searched the Mayo DNA Peripheral Neuropathy database and identified 48 dominantly inherited sensory neuropathy (HSAN1) patients without dementia or hearing loss and without genetic discovery. These patients had been screened for mutations in *SPTLC1* and *RAB7* and

**Figure 1** *DNMT1* protein structure and mutations



(A) *DNMT1* entire protein structure with Tyr495 hot spot for mutation shown. (B) Targeting sequence (TS) domain with mutations. The TS domain (gray) is the site of known mutations for hereditary sensory and autonomic neuropathy with dementia and hearing loss (HSAN1E) (red) in exon 20 for HSN1E. In contrast, all cases of autosomal dominant cerebellar ataxia deafness and narcolepsy arise from mutations in exon 21 (blue), also within the TS domain. Images were created using PyMol (www.pymol.org) and crystal structures of mouse *DNMT1* (amino acids 357–1,608)<sup>17</sup> and human DNA *DNMT1* TS domain.<sup>18</sup>

found negative.<sup>11</sup> Additionally, by utilizing the autopsy-based case series at Mayo Clinic Florida, we identified 364 subjects with Alzheimer disease (AD) who had definite, neuropathologic diagnosis according to National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer’s Disease and Related Disorders Association criteria<sup>12</sup> and had Braak scores of  $\geq 4.0$ . These subjects had mean age at death of  $75.7 \pm 6.1$  (SD) years and 52.2% were female ( $n = 190$ ). Finally, through collaboration we identified 5 kindreds with familial FTD and 2 kindreds with a triad of sensory predominant neuropathy, hearing loss, and some cognitive concern, i.e., possible HSN1E kindreds.

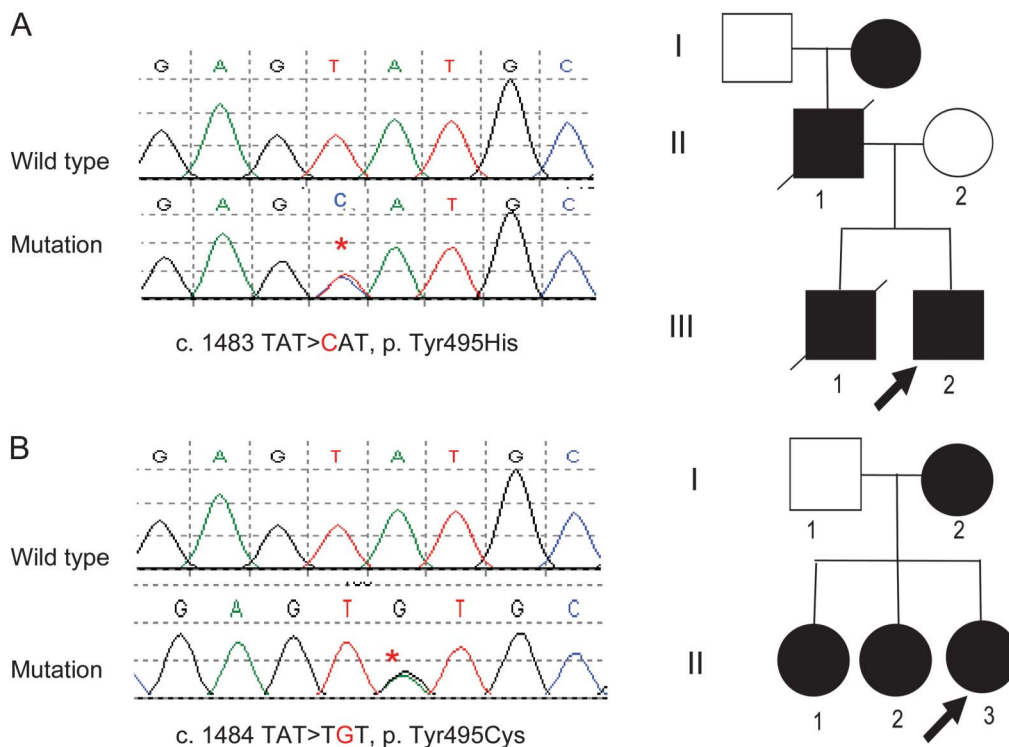
**DNA analysis.** We sequenced all 41 coding exons of *DNMT1* including 30 bps of flanking intron sequence in 48 identified HSN1 patients without dementia and hearing loss, 5 indexed cases with familial FTD, and 2 possible HSN1E kindreds. Focused sequencing of *DNMT1* exon 20 and 21 was performed for AD cases. Identified variants were compared to various Web-based sequence databases, and we further confirmed the novel variants are absent in a set of 1,200 normal controls. Genomic DNA variants were identified using either Sequencher (Gene Codes) or Mutation Surveyor (Soft Genetics) software.

**RESULTS** Only the 2 kindreds affected by HSN1E (with dementia and hearing loss but without any narcolepsy phenotype) had *DNMT1* mutations at Tyr495 located in exon 20 within TS domain. Specifically, a novel mutation c.1483TAT>CAT (p.Tyr495His) was found in one kindred (figure 2A), and the same mutation found in 3 previously described kindreds

c.1484TAT>TGT (p.Tyr495Cys) was found in the other kindred (figure 2B). The identified mutations tracked with clinically affected status of the kinships. No mutations were found in the 48 HSN1 patients who did not have dementia or hearing loss phenotype, nor were they found in 5 FTD probands. Among late-onset AD patients, known polymorphisms (rs75443147, rs2228613, rs721186, rs2114724, and rs74671219) were found with expected minor allele frequencies based on European HapMap data. However, no mutation of exon 20 or exon 21 was found among these 364 AD cases. Thus Tyr495 appears to be a hot spot for mutation specific to HSN1E phenotype.

**HSN1 dementia hearing loss complex kindred 1 (Tyr495His).** The family is of Norwegian descent (figure 2A). The index case in this family had the onset of noticeable hearing loss in his early 40s. He had a generalized seizure at age 45 years without discovered cause at that time. Cognitive difficulties were first noted at age 50 years. MRI brain showed diffuse cerebral and cerebellar atrophy. Mini-Mental State Examination was abnormal, 23 of 30 points (normal >25, consistent with mild cognitive impairment). It was noted on bedside testing that memory was better than judgment and executive functions. He experienced odd behaviors (such as mowing the lawn in the middle of the night)

**Figure 2** The 2 described kinships with *DNMT1* amino acid 495 mutations tracking with hereditary sensory and autonomic neuropathy with dementia and hearing loss phenotype including both with prominent behavioral cognitive components consistent with frontotemporal dementia



(A) Tyr495His mutation pedigree, Norwegian origin (samples II-2, III-1, and III-2 were sequenced); (B) Tyr495Cys mutation pedigree, Scottish origin (samples II-1, II-2, and II-3 were sequenced).

and eventually was given the diagnosis of probable FTD. Peripheral neuropathy was diagnosed at age 55 years. He had tremulousness of his hands, gait ataxia, and vibratory sensory loss. Additional seizures occurred at age 57, thought to be of partial complex type. EEG was diffusely slowed especially in the frontal regions without epileptiform activity. Clinical Dementia Rating score was 1.0 at age 57, 2.0 at age 58, and 3.0 at age 59. He died at age 61 years. Neuropathology was consistent with FTD with selective frontal atrophy without distinct histopathology but including microvascular changes in frontal and temporal lobes, also with cerebellar Purkinje cell loss with Bergmann gliosis. No plaques, tangles, or Lewy bodies were appreciated with negative immunostains for tau,  $\alpha$ -synuclein, and TDP-43. His 47-year-old daughter had developed moderate sensorineural hearing loss and mild peripheral neuropathy.

The brother of the index case is 61 years of age. In his 40s, he had onset of slowly progressive hearing loss, gait ataxia, sensorimotor neuropathy, and mild to moderate cognitive problems. His balance was much worse in the dark. He is presently unable to walk because of his ataxia and neuropathy. He also has profound hearing loss. MRI shows mild diffuse cerebral atrophy. Their father had the onset of dementia and abnormal gait at age 49 and died at age 65; he also had hearing loss. The father's mother and maternal grandmother lived in Norway and by family history were said to have been "confused and disoriented."

**HSAN1 dementia hearing loss complex kindred 2 (Tyr495Cys).** The kindred originates from Scotland, United Kingdom (figure 2B). The mother had deafness and an unspecified dementia and died at age 53 years without neuropathy evaluated. The proband presented at age 47 years with sensory neuropathy, foot ulcers, mild deafness, and cognitive problems. The painless injuries to her foot took months to heal. She had a sensory ataxia and sensory neuropathy to all modalities in the legs extending to proximal calves. Her husband was concerned about her forgetfulness, but she denied memory problems and still works (age 50 years). Neuropsychiatric evaluation on Montreal Cognitive Assessment scored 24 (normal >27, consistent with mild cognitive impairment). MRI was reported as normal. She has 2 sisters who also have deafness and cognitive problems; both have the mutation. One is a hoarder and suffered obsessive-compulsive disorder and was unable to live without support related to her behavioral issues but denied memory problems; she also had sensory neuropathy with Charcot joints of her feet. MRI showed global cortical and cerebellar atrophy consistent with the phenotypes previously found in patients with the same *DNMT1* mutation Tyr495Cys.<sup>3,13</sup>

**DISCUSSION** This study identified that amino acid Tyr495 in *DNMT1* is a hot spot for mutations among patients with autosomal dominant sensory and autonomic neuropathy, dementia, and hearing loss (HSAN1E). The 2 kindreds in this report originate from Norway and Scotland, and the previously reported 4 HSAN1E kindreds with *DNMT1* mutations are also from different geographical regions including Japan, England, Australia, and the United States.<sup>2</sup> Two mutations are found in this study: one has been previously been reported (Tyr495Cys), the other (Tyr495His) is novel. To date, all mutations found in HSAN1E kinships are within exon 20 of TS domain. From our previous and this current study, a total of 6 HSAN1E families have been identified. Five of them have mutations at Tyr495 (4 kindreds with Tyr495Cys, 1 kindred with Tyr495His), and one has mutations 4 amino acids away of Tyr495 (Asp490Glu-Pro491Tyr).<sup>3</sup>

Also emphasized in this report is that *DNMT1* mutations are associated with a wider phenotypic spectrum of HSAN1E, including behavioral psychiatric manifestations prior to prominent memory involvements consistent with FTD. The proband of HSAN1E kindred 1 in this study also had seizures, a recognized associative phenomenon of CNS neurodegeneration.<sup>14</sup> The later and milder clinical onsets compared to the previous reported cases are also noted among the current cases. Both HSAN1E kindreds of this study had individuals presenting after 50 years of age, while the previously reported families all had symptom onsets no later than in their 30s. In the previously reported Japanese family with Tyr495Cys mutation, 2 family members had frontal brain atrophy or functional frontal hypometabolism with behavioral components including paranoia, and also with prominent memory decline.<sup>4</sup> In contrast, the previously reported large American family with Tyr495Cys mutation had 11 clinically examined, affected members with memory decline but no behavioral or frontal executive concern. In this large family, brain autopsy of 3 affected patients, imaging studies, and multiple neuropsychometric data suggested a global process without selective frontal or subcortical involvements.<sup>3,13</sup> It is worth noting that although the cognitive features are highlighted here, all HSAN1E patients have hearing loss and sensory predominant neuropathy when examined. Neither the currently reported 2 kinships nor our previously reported 4 HSAN1E kindreds had narcolepsy or cataplexy as described in ADCA-DN from mutations in exon 21 of *DNMT1*.<sup>6</sup>

The importance of methylation in maintaining the health of the nervous system has been emphasized by the progressive neurodegenerative diseases such as Rett syndrome caused by *MeCP2* mutations<sup>15</sup> and



immunodeficiency, centromere instability, and facial anomaly syndrome caused by *DNMT3b* mutations.<sup>16</sup> The defective genes in these disorders likely have diverse detrimental effects on epigenetic pathways and cellular processes. Similar to *DNMT3b* and *MeCP2*, *DNMT1* is highly expressed in the brain. *DNMT1* expression patterns and its intricate interactions with a series of regulatory molecules are highly complex at the different phases of cell cycle.<sup>8</sup> The phenotypic variability emphasized in this study by the varied cognitive presentations associated with Tyr495 mutations further highlights the complexity of the disease pathogenic mechanisms. Genetic background and environmental influence can also contribute to the clinical variability of HSN1E phenotypes. Based on our current knowledge, *DNMT1* Tyr495 mutations should be considered for patients with dementia, including those with frontotemporal appearance when hearing loss and sensory predominant neuropathy are present.

### AUTHOR CONTRIBUTIONS

C.J.K.: study concept and design, acquisition of the data, analysis and interpretation of data, drafting and revising the manuscript, and obtaining funding. T.B., S.J.L., R.H., Y.W., J.K., P.J.D., G.A.N.: concept and design, acquisition of the data, revising manuscript, analysis and interpretation of data. N.E.-T.: acquisition of the data, revising manuscript, analysis and interpretation of data, and obtaining funding.

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### DISCLOSURE

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