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Polymorphic Ala-allele carriers at residue 1170 of HER2 associated with Parkinson's disease

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ABSTRACT

Objective: HER2, a receptor tyrosine kinase, was originally identified based on its role in cancer research. The protein has subsequently received attention for its role in nerve injury and neurodevelopment. We investigated the polymorphic association of HER2 variants at amino acid residues 655 and 1170 with Parkinson's disease (PD), a neurodegenerative disorder.
Design and methods: Polymerase chain reaction (PCR) was used to amplify DNA samples from PD patients and control subjects. The resulting PCR fragments, which spanned HER2 residues 655 and 1170, were analyzed by restriction fragment length polymorphism and/or direct nucleotide sequencing.
Results: The genetic distribution at residue 655 in PD patients did not differ from that in controls. However, homozygosity for genes encoding Pro at residue 1170 (Pro/Pro) occurred at a significantly lower rate among PD subjects. In other words, Ala-allele carries higher frequency in PD, especially among female PD subjects.
Conclusion: Different signals or potency of the kinase activities resulting from the Ala1170Pro allele of HER2 may be associated with vulnerability to stress on dopaminergic neurons in PD.

1. Introduction

Human epidermal growth factor receptor 2 (HER2) is a transmembrane receptor tyrosine kinase (RTK) that exhibits about 50% homology to EGFR/HER1 and docks the cell membrane [1]. The protein structure of HER2 consists of an extracellular N-terminal cysteine-rich domain required for ligand binding, and an intracellular C-terminal kinase domain with sites of potential tyrosine phosphorylation. Signal transduction is initiated by ligand–receptor binding on the cell membrane, followed by receptor dimerization, autophosphorylation, and recruitment of down-stream cytoplasmic substrates (including members of the MAPK, PI3K, PLCγ and STAT pathways); phosphorylation of these targets results in cell proliferation and cell survival [2]. It is already known that the murine tumorigenesis results from a mutation of the *HER2/neu* gene at residue 664, resulting in a Val to Glu substitution within the HER2 transmembrane domain and enhanced kinase activity. In human cancers, HER2-mediated oncogenesis has been attributed to the overproduction of the HER2 oncoprotein [3], mainly as a result increased *HER2* copy number [4]. Murine HER2 residue 664 corresponds to residue 655 within exon 17 of the human gene; and the polymorphic allele Ile655Val of HER2 protein has been associated with an increased risk of human breast cancer [5,6]. Another polymorphic locus at residue 1170 of the human gene (to encode the Ala1170Pro protein) demonstrates a weaker association with human breast cancer [6–9].

Clinical analyses reveal that changes in members of the EGFR family, including HER2 and HER4, contribute to the aggressive tumor phenotypes, as seen in both somatic cancers and brain tumors [10]. Based on these observations, monoclonal antibodies against the extracellular domain and the competitive kinase inhibitors of these receptors have been developed for use as cancer treatments. However, in neuroscience, EGFR plays a role in thalamocortical navigation in developing brain [11], neurite extension and arborization [12], and guidance of axonal extension with ensheathment by Schwann cells [13]. In dopaminergic neurons, EGFR endocytosis and trafficking by Eps15 may be affected by the proteasome-independent ubiquination pathway of parkin [14]. The proliferation of dopaminergic neurons

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mediated by EGF [15] also implicates EGFR in the dopaminergic status of cells involved in Parkinson's disease (PD).

PD is characterized by the clinical features of tremor, rigidity, bradykinesia and postural imbalance, with pathological findings of Lewy body and depigmentation of substantia nigra due to loss of dopaminergic neurons. A range of exogenous toxins and genetic factors have been proposed to cause PD. Candidate genetic factors have included the genes encoding α-synuclein, parkin, PINK1, DJ-1, and LRRK2; these proteins are known to play individual roles in neuronal cellular functions, and have been investigated for potential roles in the pathogenesis of PD. In addition, subjects harboring mutations in some susceptible genes, such as glucocerebrosidase, have been known to develop early parkinsonian symptoms; carriers have been identified among Ashkenazi subjects and Taiwanese patients [16]. Furthermore, genetic polymorphisms affecting levadopa metabolism may be associated with PD [17–19]. Our recent finding of octarepeat changes of prion protein in PD patients [20] and the published reports [21–23] encouraged us to explore the neurotrophic factors related to this neurodegenerative disease, especially HER2, in PD.

Here, we report a polymorphic association of *HER2* at protein residue 1170, not at residue 655, among PD subjects. To our knowledge, this is the first study suggesting a link between PD and *HER2* polymorphism, suggesting an effect of the C-terminal domain of HER2 in dopaminergic cell survival.

2. Materials and methods

2.1. Patients

From 2003 to 2007, a total of 525 subjects (330 with PD, 195 controls) were enrolled in this study. All subjects provided informed consent prior to enrollment. Idiopathic PD was diagnosed using the criteria of UK Parkinson's Disease Society Brain Bank [24]. Based on the clinical history and the finding of neuroimaging exams, subjects with other neurological diseases (such as hydrocephalus, brain tumor or vascular lesions) or exposure to known drugs or toxins were excluded from the PD group. Clinical evaluations were performed according to the Hoehn and Yahr staging and the Unified Parkinson's Disease Rating Scales. Controls included healthy subjects as well as patients with non-PD disorders (e.g., stroke, hypertension, diabetes mellitus, spinal diseases). For all subjects (PD and control), samples of peripheral blood were obtained by venipuncture.

2.2. Genotyping and sequencing

Leukocytic DNA was extracted from the blood samples using the QIAamp DNA Blood Midi kit (QIAGEN), according to the manufacturer's instructions. Samples of DNA (20–100 ng) were subjected to polymerase chain reaction (PCR) [25]. In brief, to amplify sequences spanning residue 655, we used primers HN-5 (5′-AGA GAG CCA GCC CTC TGA CGT CCA T-3′) and HN-4 (5′-TCC GTT TCC TGC AGC AGT CTC CGC A-3′), generating a product 148 bp in length [26]. For sequences spanning residue 1170, we used primers 1170F (5′-ATG TGA ACC AGC CAG ATG-3′) and 1170R (5′-GTA GGT GTC CCT TTG AAG-3′), generating a product 310 bp in length (NCBI accession number: NM_004448). PCR was performed as forty cycles on the thermocycler (GeneAmp PCR system 9700, Applied Biosystems); for each cycle, samples were denatured for 30 s at 94 °C, annealed for 30 s at 65 °C (for residue 655 amplicon) or 53 °C (for residue 1170 amplicon), and extended for 20 s at 72 °C. The genotype of the residue 655 fragment was determined by taking advantage of a restriction fragment length polymorphism (RFLP), since the residue 655 allele creates a novel *Bsm*AI site. Hence the residue 655 amplicon was subjected to digestion with *Bsm*AI (New England BioLabs) and sized and visualized via agarose gel electrophoresis with ethidium bromide staining. Allelic assignment of the PCR products also was conducted by nucleotide sequencing using the primer HN-6 (5′-TTC CGG ATC TTC TGC TGC CGT CGC T-3′) for the residue 655 amplicons and the amplifying primers (1170F and 1170R, in sense or anti-sense orientations) for the 1170 amplicons.

2.3. Statistics

The Chi-square analysis and Hardy–Weinberg equilibrium were applied to check the allelic polymorphic distribution and compile statistics.

3. Results

This study incorporated samples from a total of 525 recruited subjects, including 230 females and 295 males. The 330 PD subjects (141 females and 189 males) had a mean age of 72.5 years (range, 49 to 91 years) and an average age at onset of PD symptoms of 65.9 years (range, 44 to 89 years). The 195 non-PD controls (89 females and 106 males) had a mean age of 71.4 years (range, 47 to 89 years). Table 1 listed the demography of the PD subjects on enrollment, with 22 subjects scored as Hoehn-and-Yahr stage I; 141 as stage II; 113 as stage III; 45 as stage IV; and 9 as stage V. The grouping by the Hoehn and Yahr stages included 163 subjects as mild (stages I + II), and 167 subjects as moderate-severe group (stages III + IV + V).

Among the PD subjects, the genetic distribution of residue 655 alleles of HER2 included 269 Ile/Ile homozygotes, 54 Ile/Val heterozygotes, and 7 Val/Val homozygotes; the control group comprised 161 subjects with Ile/Ile homozygosity, 30 with Ile/Val heterozygosity, and 4 with Val/Val homozygosity. Thus, the Ile-allele (as homozygote or heterozygote) carriage rate was 89.7% and 90.3% in the PD and control groups, respectively (Table 2); these Ile-allele carrying distributions did not differ significantly ($\chi^2 = 0.086$, df = 1, $p = 0.771$; odds ratio, 0.94). In addition, there was no significant difference between the distribution of Val-allele carriers (as homozygote or heterozygote) and the Ile/Ile homozygotes ($\chi^2 = 0.091$, df = 1, $p = 0.763$).

Of the original 525 subjects, we obtained genotypes for residue 1170 from 481 samples (91.6%). The remaining samples failed to provide genotypes by either sense or antisense sequencing. Among the 298 PD subjects, the genetic distribution of residue 1170 alleles included 108 subjects with Pro/Pro homozygosity, 146 Pro/Ala heterozygotes, and 44 Ala/Ala homozygotes; the 183 members of the control group comprised 87 with Pro/Pro homozygosity, 76 with Pro/Ala heterozygosity, and 20 with Ala/Ala homozygosity. The Ala-allele carriage rate was 39.3% and 31.7% in the PD and control groups, respectively (Table 3). That is, the PD subjects had significantly higher carriage rate of the Ala allele at residue 1170, no matter whether the Pro/Ala heterozygosity or Ala/Ala homozygosity, as compared with controls ($\chi^2 = 5.610$, df = 1, $p = 0.018$), with an odds ratio of 1.39 for the Ala-allele carriage among PD subjects. Furthermore, we found a lower distribution of Pro/Pro homozygosity in the PD group compared to controls ($\chi^2 = 6.005$, df = 1, $p = 0.014$). That is, PD diagnosis was associated with an elevated frequency of the Ala1170-allele and a reduced frequency of Pro/Pro homozygosity at residue 1170. This significant distribution was mainly contributed by the genotypes

Table 1
Demographic list of PD subjects.

	Age at onset (mean), years	Mild	Moderate–severe	Cancer history	
				Breast	Others
PD, female (n = 141)	52–86 (64.9)	65	76	3	12
PD, male (n = 189)	44–89 (67.7)	98	91	1	18

PD, Parkinson's disease.
Mild: Hoehn and Yahr stages I–II, Moderate–severe: Hoehn and Yahr stages III–V.