Alzheimer’s disease amyloid precursor protein gene

Health & Medicine, Disease

Alzheimer’s disease, AD, is a distressing condition that involves the decline in cognition of the mind which results to psychotic disorder, and affective and behavioral disturbances (Bloom 9). It is a progressive central nervous system disorder and the main cause ofdementia(Stavljenic-Rukavina 1). Alois Alzheimer in 1907 reported the case of a 51-year old Frankfurt woman who died in dementia (Bloom 9). He described the neuropathological condition of the woman with neurofibrillary tangles or NFTs and amyloid plaques or NPs (Bloom10). NPs are extracellular beta-amyloid peptide or ASpherical deposits closely related to dendrites, reactive astrocytes, dystrophic axons, and activated microglia (Felician and Sandson 19). Thus, for several decades, collaborative efforts of experts from different scientific and medicinal endeavors were devoted for the neurological and pathophysiological characterization of this disease (Bloom 9). As such, the roles of four specified genes, as well as the mechanism of oxidativestress, tau, inflammation, hormonal changes, and inflammation on the AD’s neurodegeneration have been the central theme of scientific studies conducted on this disease (Felician and Sandson 19).

As experts continuously gained insights on the mechanisms of neurodegeneration, pharmacological strategies are concurrently devised for the development of appropriate drug treatment and interventions (Felician and Sandson 19).

Molecular Mechanism

Early and late-onset ADs are types of familial AD which are genetically heterogeneous. Familial AD is accounted for 10% of AD cases from 30-60

year old patients and ascribed to three types of genes which included APP, presenilin-1 or PSEN1 and presenilin-2 or PSEN2 (Stavljenic-Rukavina, 1).

Nonetheless, the mutations in these genes also cause A-level increase; A is generated by proteolytic APP fragment that was also observed in the brains of AD patients (Stavljenic-Rukavina, 2). However, not all AD cases can be attributed to the three identified genes. Genes are then the most important determinant of AD development (Stavljenic-Rukavina, 2). On the other hand, there is a great chance for children with parents having history of familial AD to inherit the genetic traits and develop either early-onset or late-onset AD (Jayadev et. al. 375). As well, AD development threat in the offspring is directly related to age; the tendency of AD occurrence among children of parents with historical AD background increases as the children gain progress in their growth and development (Jayadev et. al. 375). The pathogenesis of Alzheimer’s disease, on cellular level, has been consistently observed. The pyramidal neurons are the type of cortical cells that are fundamentally deteriorated in AD pathogenesis resulting to the spread of NPs and NFTs in cortex areas (Felician and Sandson 20).

Both NPs and NFTs are normally found in brain areas in the aging process but their concentrations and densities are peculiar in the case of AD (Felician and Sandson 20). Originally, NPs are found at the amygdala and concentrated in parietal association and order temporal cortex parts (Felician and Sandson 20). In the maturity of AD, NPs can also be observed in hippocampus, in other structures of mesial temporolimbic brain, and even in cortical and meningeal blood vessels (Felician and Sandson 20).

Luckily, the areas for sensorimotor and visual are remained unaffected. Meanwhile, in the early stage of AD, NFTs can be found hippocampus, amygdala, and in entorhinal cortex, the association cortex has abundance of NFTs (Felician and Sandson 20). However, NFTs are not exclusively for the cases of AD, these are also detected in the several cerebral troubles like in dementia pugilistica, postencephalitic parkinsonism, and subacute sclerosing panencephalitis (Felician and Sandson 20). The formation of NPs is attributed to the A peptide deposition; A peptide types that only differ in C-terminal are common in cerebrovascular and extracellular plaques (Felician and Sandson 19). A peptide, made up of 39-43 amino acids, is normally generated from APP or amyloid precursor protein. In addition, the series of hydrophobic C-terminal is crucial in its solubility and amyloid formation rate (Felician and Sandson 19). As such, A with 40 amino acids, A 40, as well as A peptide with 42 and 43 amino acids or A 42 and A 43 respectively (Felician and Sandson 19). However, in vitro, the variants of A 42 and A 43 can easily form insoluble fibrils as compared with the A 40 variant (Felician and Sandson 19). Furthermore, the incubation of these A variants can immediately lead to coalescence implying the possible amyloid plaque deposition through these components. In line with this, diffuse plaques have nonfibrillary and A soluble constituents denoting the senile plaques’ early stage (Felician and Sandson 19). Likewise, diffuse plaques have A deposits in the absence of neuritic degeneration (Felician and Sandson 19).

On the other hand, neurofibrillary tangles, comprised of abnormal bundles of intraneuronal filaments, are made up of tau microtubule-associated protein with high degree of phosphorylation (Felician and Sandson 19). The degree

of phosphorylation is largely dependent on the enzymatic activities of kinases that are not yet fully understood (Felician and Sandson 19). Nevertheless, the intraneuronal abnormal filaments arrange themselves in either parallel or helical bundles in perikaryotic cytoplasm that make them in contact with the dentritic processes (Felician and Sandson 19).

The amyloid precursor protein, a membrane glycoprotein, is consisted of 28 A extracellular residues and 12 to 15 putative transmembrane residues (Felician and Sandson 20). It also occurs as 695, 751, and 770-amino acid isoform. While the 695-amino acid isoform occurs mainly in neurons, 770 and 751-amino acid forms are seen on both non-neural and neural cells along with protease inhibitor domains (Felician and Sandson 20). APPs are carried into the cell membrane by secretory vesicles and may undergo proteolytic bond breakage through the action of -secretase (Felician and Sandson 20). Consequently, this cleavage generates -APP, a soluble ectodomain and the precursor for A peptide production through cleavage in A domain. As the generation of soluble APP is, in vitro, ascribed with the activity of protein kinase C, uncleaved APP is inferred to take the proteolytic pathway (Felician and Sandson 20). On the other hand, APP intracerllular recycling and management are done through endocytotic or endosome-lysosome means. The endocytotic route causes proteolytic cleavages by means of

- and -secratases leading to the synthesis of A (Felician and Sandson 20). Moreover, A production is enhanced by intracellular calcium concentration which denoted the significance of calcium-rich proteases in A production (Felician and Sandson 20). In vivo, APP cleavage occurs at N-terminus at the

A -region through the action of -secretase and at the C-terminus by means of -secretase activity (Mohan 1). Also, APP can take a pathway facilitated by -secretase at the A -peptide domain producing soluble -APP (Mohan 1).

Ezymes can also possibly attack APP without A -peptide generation (Stavljenic-Rukavina, 1). Since the putative -secretase, under the control of kinase C, regulates the generation of soluble APP, any agents that supports this metabolism may hinder the A production (Felician and Sandson 21). As well, A deposition may also be lessened by drugs which inhibit APP cleavage into - and -secratases (Felician and Sandson 21). Nonetheless, agents that can impede A coalescence would decrease its neurotoxicity effects (Felician and Sandson 21).

After the formation of amyloid plaques, neurofibrillary tangles and inflammation dictates the death of neurons (Stavljenic-Rukavina 1). In relation to this, microglia and astrocytes cells of the brain are heavily affected by inflammatory process (Stavljenic-Rukavina 1). In AD patients, astrocytes are enlarged and produce prostaglandin which in turn sends signal to activate the inflammation mediated by arachidonic acid (Stavljenic-Rukavina, 1). On the other hand, microglia generates free radicals which cause neurons’ death (Stavljenic-Rukavina 1).

Meanwhile, cell nutrients as well as its regulation components are transported through the microtubules in which structural properties are mainly dependent on tau protein (Stavljenic-Rukavina 1). In AD condition, the tau lessens its capability to bind with microtubules and binds with other tau protein resulting to knots of helical filaments called as neurofibrillary

tangles (Stavljenic-Rukavina 1). APP Duplication is Sufficient to Cause Early Onset Alzheimer’s Dementia with Cerebral Amyloid Angiopathy Studies showed that A encoding through APP gene expression leads to the development of Alzheimer-type dementia (Sleegers et. al. 2977). APP genetic expression results to elevated levels of A 42, a 42-amino acid product of the proteolytic process (Sleegers et. al. 2977). Aside from the cleavage of APP into alpha, beta, and gamma secretases, high APP genetic expression results to elevated levels of A 42 and A deposition (Sleegers et. al. 2977). Meanwhile, it has been long known that APP level triplication in Down’s syndrome patients results to the development of Alzheimer type dementia at early stage; the APP excessive expression leads to neurodegeneration and A deposition (Sleegers et. al. 2977). In relation to this, it was reported that families with cerebral amyloid angiopathy and early onset Alzheimer type dementia had APP genomic duplications which implied that APP over-expression, without full trisomy 21, has triggered Alzheimer-type dementia (Sleegers et. al. 2977). In addition, Alzheimer-type dementia patients have elevated APP mRNA levels in their brains (Sleegers et. al. 2977). Further, the variation on the transcription of APP gene due to genetic factors was believed as the underpinning factor in the pathogenesis of the disease (Sleegers et. al. 2978). In fact, three APP mutations were observed on Alzheimer-type early-onset dementia patients. These mutations, as seen in vitro by means of trisomy 21, caused a two-fold elevation of in APP transcriptions (Sleegers et. al. 2978). With the aforementioned evidences on APP elevation through APP genomic mutations or duplications which resulted to the development of early onset AD, it could logically infer that A has a

crucial role in its aetiology (Sleegers et. al. 2978). Hence, for the evaluation of APP locus duplication on Alzheimer-type dementia cases, Sleegers et. al. conducted a study on Dutch population with early onset Alzheimer-type dementia patients.

Material and Methods

In the approval of the University of Antwerp medical ethical committee, the respondents of this research were recruited form an epidemiological study on early onset AD in several provinces of The Netherlands and in Rotterdam (Sleegers et. al. 2978).

Patients with early-onset dementia diagnosis were enlisted based on the recommendation of medical experts and healthcare providers. As such, the assessment of the patients’ conditions was done in accordance with the standards of the Stroke-Alzheimer’s Disease and Related Disorders Association, and the National Institute of Neurological and Communicative Disorders (Sleegers et. al. 2978). Medical records of the patients and their respective relative with similar trait inheritance were made available for an in-depth examination.

Meanwhile, for the assessment of genetic inheritance, 111 patients with ages 33 to 65 years old of which had 75 respondents with familial background of either late or early-onset of dementia and 10 of which have autosomal dominant inheritance history for several generations of their respective clans were studied (Sleegers et. al. 2978). The genomic DNA or gDNA was derived from lymphocytes and alleles of APP were measured by means of real-time polymerase chain reaction, PCR (Sleegers et. al. 2978).

Also, the PrimerExpress software was utilized for the design of 2-microglubulin or hB2M, exon 5, 11, and 18, ubiquitin C or hUBC, ATP5J, APP, and GABPA (Sleegers et. al. 2978). As the APP alleles were normalized for hB2M and hUBC, 20 nanograms of genomic DNA were combined with the PCR and 400 nanomoles of the respective primers (Sleegers et. al. 2978). Finally, the duplication of the samples was done by means of dosage quotients or DQs calculation through six normal individuals and dementia patients.

Patients with trisonomy 21 were also included as controls (Sleegers et. al. 2978). Fluorescence in situ hybridization, FISH, was utilized to determine APP genomic duplication (Sleegers et. al. 2978). FISH was performed on both interphase nuclei and metaphase chromosomes while the Epstein-Barr virus-transformed patients’ lymphoblasts were taken from the metaphase period by means of 0. 1 microgram/milliliter colcemid treatment and incubated, at 37°C for 25 minutes, in hypotonic solution of 1 molar sodium hydroxide, 30 millimolar glycerol, 0. 8 millimolar magnesium chloride, 2 millimolar HEPES, and 1 millimolar calcium chloride (Sleegers et. al. 2978). This suspension then was used for 106 cells per milliliter as the chromosomes’ mechanical stretching was done through cyto-centrifugation. On the other hand, the Multiplex Amplicon Quantification, MAQ, was applied in the detection of APP locus duplication. MAQ was comprised of multiplex PCR amplification of the reference amplicons and targets which were tainted with fluorescent substance (Sleegers et. al. 2978).

After MAQ, DNA fragment analysis, and comparison target amplicon DQ between control individuals and the patients were done (Sleegers et. al. 2979).

Results and Discussion

Real-time PCR APP measurements of 10 probands showed heterozygous duplication (Sleegers et. al. 2982). Based on the Dutch population sample, APP duplication along with segregation pattern and neuropathology tantamount to autosomal dominant inheritance and AD with excessive CAA were identified with APP duplication in afamily(Sleegers et. al. 2982).

Specifically, the genomic APP locus duplication were observed in five of the 65 family cases with early onset AD autosomal dominance while APP duplication was detected in a single out of ten family cases early-onset AD autosomal dominance (Sleegers et. al. 2982). Even though these numbers are small, the data generated from this study illuminated the significance of genomic APP locus duplication assessment when simple mutations were excluded in AD known genes (Sleegers et. al. 2982). In the 65 patients with familial AD history, a single genomic duplication was identified (Sleegers et. al. 2982).

In addition, the genomic duplications among the Dutch samples have 1. 8% overall frequency and 2. 7% frequency in AD patients and family (Sleegers et. al. 2982). In contrast, duplication was failed to be detected on 36 patients with irregular early-onset AD which denoted that the duplication of de novo genomic APP is a weak cause of early-onset Alzheimer-type dementia (Sleegers et. al. 2982). Moreover, the duplication observed among the Dutch

family samples has only APP which proved that genomic APP duplication, regardless of adjacent genes, has the capacity for AD and CAA mixed phenotype (Sleegers et. al. 2982). As well, duplication size differences signified the non-specific recombination substrate from the genomic attributes of APP locus; APP rather is in increased recombination region as imparted by other factors such as low transcription repeats (Sleegers et. al. 2982). Nevertheless, the mutation that affects APP expression among 4. 5% of the Dutch participants that either genomic APP duplication or APP mutation promoter carrier, are the frequent cause of Alzheimer-type dementia (Sleegers et. al. 2982).

Polymorphism in the Promoter of the Human APP Gene

The cleavage of APP produces A with associated neurotoxicity; hence, genetic studies postulated that abnormal A deposition neuropathologic AD conditions (Athan, Lee, Arriaga, Mayeux, and Tyco1793). The abnormal deposition of A in AD patients has been ascribed to APP gene missence mutations and the proteolytic APP cleavage producing A 42 which in turn triggers the development of early-onset AD (Athan, Lee, Arriaga, Mayeux, and Tyco1793). The most solid proof for this notion is the case on trisomy 21 wherein the duplication of APP gene results to increased A peptide level and aggregation of such in the amyloid plaques of the brain (Athan, Lee, Arriaga, Mayeux, and Tyco1793). While the presenilin enyzymes enhance fibrillogenic APP conversion, the APOE or alipolipoprotein-E elevates A coalescence and deposition (Athan, Lee, Arriaga, Mayeux, and Tyco1793). Since A production is associated with APP concentration and on other factors in both A and APP syntheses, it was hypothesized that the expression of APP gene is a

determinant of AD development (Athan, Lee, Arriaga, Mayeux, and Tyco1793).

Recently, a study reported the weak relation between AD inheritance and microsatellite sequence in the APP first intron and a tetranucleotide non-association with AD (Athan, Lee, Arriaga, Mayeux, and Tyco1794). Hence, to further scrutinize this issue, Athan et. al. anchored their study on APP promoter variant screening in tri-ethnic populations which included white, Caribbean Hipic, and African-American as they intended to determine APP promoter identities.

Methodology

The respondents in this study were Manhattan residents of Washington Heights with ages of more than 65 years (Athan, Lee, Arriaga, Mayeux, and Tyco1794). Personalinterviewand medical background check, neuropsychological, physical and neurological examinations were done on the participants. In addition, individuals with questionable dementia, Parkinson disease, and other types of dementia were excluded in the study. Consequently, a total of 1, 077 participants was successfully enlisted, whereas, 16% of them has family history of stroke (Athan, Lee, Arriaga, Mayeux, and Tyco1794).

For genotyping, DNA from 1, 013 respondents was taken as the panel of neuropsychologists and physicians established the criteria for the identification of AD patients along with the Clinical Dementia Rating Scale (Athan, Lee, Arriaga, Mayeux, and Tyco1794). The oligonucleotide primers used for APP promoter PCR amplification came from GenBank (Athan, Lee,

Arriaga, Mayeux, and Tyco1794). From genomic DNAs and by means of Platinum Taq DNA Polymerase, the fragments were amplified while the product sequence was determined through dye terminators (Athan, Lee, Arriaga, Mayeux, and Tyco1794).

Meanwhile, 15 microliter of the PCR products was introduced into WAVE fragment DNA analyzer (Athan, Lee, Arriaga, Mayeux, and Tyco1794). The haplotypes PCR products were individually cloned through pGL3 vector in between SacI and Bg III sites (Athan, Lee, Arriaga, Mayeux, and Tyco1794). On the other hand, U-87 MG glioma cells were cultured with the solution of Earle’s balanced salt and 2 millimolar L-glutamine with 10% fecal calf serum in EMEM medium (Athan, Lee, Arriaga, Mayeux, and Tyco1794).

At 70% confluence, the cells were transferred by means of FuGene 6 reagent and pGL3 vectors were added to transfected DNA to maintain a constant concentration of about 1 microgram per plate of 35 squared millimeter (Athan, Lee, Arriaga, Mayeux, and Tyco1794). While the isotonic solution of phosphate-buffered sodium chloride was used to wash the U-87 cells, the 250 microliter Reporter Lysis Buffer was applied for cell lysis (Athan, Lee, Arriaga, Mayeux, and Tyco1794).

After this, the centrifugation of the cell extract was done at 10, 000 g for five minutes. From the supernatant, 20-microliter aliquot was taken and combined with 100 microliter Luciferase Assay Buffer for luciferase activity measurement (Athan, Lee, Arriaga, Mayeux, and Tyco1794). Then, with 10-20 microliters of the lysate -galactosidase assays were performed. This -

galactosidase measurement was utilized for the normalization of the luciferase data (Athan, Lee, Arriaga, Mayeux, and Tyco1794).

Each allele was counted and by sample proportion calculation, the frequencies were computed (Athan, Lee, Arriaga, Mayeux, and Tyco1794). For the ethic group comparison of allele frequency, chi square analysis was applied while logistic regression was utilized for APP promoter and AD polymorphisms odd-ratio calculation (Athan, Lee, Arriaga, Mayeux, and Tyco1794). As well, for each ethnic group, logistic regression was employed as the data were classified withrespectto the APOE allele’s occurrence or non-occurrence aseducationand age discrepancies were adjusted.

Finally, Hardy-Weinberg equilibrium was analyzed through chi square analysis while the ethnic comparison of APP promoter and AD polymorphisms odd-ratio calculation as their education, age, and sex were adjusted (Athan, Lee, Arriaga, Mayeux, and Tyco1794). Results and Discussion Two types of APP promoter polymorphisms were detected and identified, with respect to the starting site of the transcription, as G> C at +37 and G> C at -9 variants (Athan, Lee, Arriaga, Mayeux, and Tyco1797).

In connection to this, +37C allele was typically observed among 18% African-American respondents while European and Caribbean-Hipic have 3% and 10% respectively (Athan, Lee, Arriaga, Mayeux, and Tyco1797). Although +37C allele was commonly observed among AD patients, the adjustment of their socio-demographic attributes with respect to this allele produced non-significant observations (Athan, Lee, Arriaga, Mayeux, and Tyco1797). Also, -9C allele was hardly detected for disease association.

On the other hand, even though the adjustment with respect to socio-demographic traits was made, still a strong link was found between APOE allele and AD (Athan, Lee, Arriaga, Mayeux, and Tyco1797). Moreover, the evaluation of both +37C and -9C allele variants in U-87 glioma cells through promoter-reporter assays has resulted to non-significant promoter activity (Athan, Lee, Arriaga, Mayeux, and Tyco1797). The early onset, less than 60 years old, of AD has been ascribed to APP, PSEN1 and PSEN2 while the late stage, greater than 65 years old, AD development has not yet fully explained by the genetic model (Waring and Rosenberg 329).

The development of AD in late age stage was associated with APOE and to other reported genetic variants and alleles, however, they still insufficient to plausibly explain the mechanism involved in the AD occurrence (Waring and Rosenberg 329). Summary Alzheimer ’s disease is a progressive degeneration of the capacity of the mind for cognition thus affecting the psychological and affective attributes of the inflicted individual.

Based on genome-wide study, children of parents with familial Alzheimer’s disease are more prone to inherit and develop this condition either as they take progress in their growth and development or at the senescence stage of their lives (Jayadev et. al. 375). The primary pointed culprit for this cognitive deterioration is the beta-amyloid peptide which is a part of the amyloid precursor protein. APP passes through the fatty membrane of the cells and delineated in the different areas of the brain, even though, the normal function has not yet been fully known.

As APP is attacked by enzymes, fragments are generated including A - peptide with associated neurotoxicity. Sleegers et. al. in 2006 found the coincidence of cerebral amyloid angiopathy with Alzheimer’s disease in a Dutch multigenerational family. This genomic duplication was attributed solely to APP gene expression that was also observed in 65 Dutch families with early-onset of AD cases. However, APP locus duplication was not observed in 36 AD patients that signified the case of de novo mutation. On the other hand, Athan et. al. in 2002 reported the two types of APP promoter polymorphism which involved +37C and –9C alleles. Moreover, they found a strong link between AD inheritance and the apolipoprotein-E role. In this connection, the genetic traits of every individual should be scientifically scrutinized for an accurate determination and identification of the substance involved in the development of the disease in parallel with its molecular mechanisms.

Works Cited

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