A Novel Genetic Factor for Corpora Amylacea Deposition in Temporal Lobe Epilepsy with Hippocampal Sclerosis

Thesis submitted in fulfilment of the rules and regulations for DM Degree Examination of Sree Chitra Tirunal Institute for Medical Sciences and Technology, Thiruvananthapuram

By

Dr. Abhijit Das

Resident in Neurology

Month and Year of Submission: September 2009

CERTIFICATE

I, Dr. Abhijit Das hereby declare that I have actually carried out the project under report.

Place: Thiruvananthapuram **Signature:**

Abhijit Das

Date: 30-09-2009 **Dr. Abhijit Das**
Resident in Neurology

Forwarded. He has carried out the project under report.

Signature:

Nair

Prof. M. D. Nair
Senior Professor & Head,
Department of Neurology,
SCTIMST.

Acknowledgement

I take this opportunity to sincerely thank Prof. K. Radhakrishnan, Director, SCTIMST, who was my guide for the study, for his expert guidance, constant review, kind help, and keen interest at each and every step during the completion of this study.

I am indebted to Prof. M. D. Nair, Senior Professor and Head, Department of Neurology for the constant support and encouragement during the period of this study.

I sincerely thank Dr. Moinak Banerjee, PhD, Scientist E-II, Department of Human Molecular Genetics, Rajiv Gandhi Center for Biotechnology, for carrying out the genetic analysis and statistical analysis.

I sincerely thank Dr Anila Mathew and Shabeesh Balan, Rajiv Gandhi Center for Biotechnology, for carrying out the genetic analysis.

I express my gratitude towards the patients who took part in this study.

Dr. Abhijit Das

Contents

Introduction

Introduction

Epilepsy is the most prevalent chronic neurological disorder and a major public health concern, directly affecting an estimated 50 million people worldwide, and involving an additional 500 million people as family members and caregivers of patients.[1,2] About 30—40% of patients with epilepsy do not respond to antiepileptic drugs (AEDs) despite the choice of an appropriate compound or combination thereof and carefully monitored treatment.[3] Furthermore, despite the recent introduction of a range of new AEDs over the last 10 years, the problem of drug resistant or refractory epilepsy has not changed significantly.[4] Owing to remarkable advances in neuroimaging technologies over the past two decades, detection of a variety of brain lesions that are amenable to surgical resection in over 50% of patients with medically refractory focal epilepsies is now possible.[5,6] These lesions include hippocampal sclerosis, malformations of cortical development, benign neoplasms, vascular malformations, and focal gliotic lesions. Mesial temporal lobe epilepsy (MTLE) is the most common surgically remediable human epilepsy syndrome.[7] Hippocampal sclerosis (HS) is the most frequently encountered lesion in subjects with Mesial temporal lobe epilepsy.[8] The abundant premature accumulation of Corpora Amylacea (CoA) in 50 – 60% of subjects is a distinctive marker of Hippocampal sclerosis.[9] Although the extent of accumulation of Corpora Amylacea in the hippocampus has been correlated with seizure duration and interictal psychosis, the etiopathogenesis is unclear.[10] ATP-binding cassette

family gene (ABCB1) polymorphism has been found to be associated with Hippocampal sclerosis in the MTLE subjects.[11] We undertook this study to investigate whether single nucleotide polymorphisms (SNPs) in ATP-binding cassette family gene *(ABCB1)* could be associated with premature accumulation of Corpora Amylacea (CoA) in the hippocampus of subjects with medically refractory mesial temporal lobe epilepsy with hippocampal sclerosis (MTLE-HS).

Review of Literature

Review of Literature

Corpora Amylacea

Historical background

Corpora amylacea (CoA) have a long history of neglect by neuropathologists because of their seeming irrelevance to neurological disease. First noted by J.E. Purkinje in 1837, they were found by Virchow to show the same reactions to iodine and other stains as cellulose. [12] Although they were extensively discussed around the turn of the century, particularly in the German literaturew, Buzzard and Greenfield [13] did not consider them to be of any pathological significance, and this concept of their significance seems to have lingered on into the present day. Nonetheless, Ferraro and Damon[14] gave an extensive review of the early literature and detailed the various ideas as to their nature and origin up to that time. Ford Robertson [12] believed they were products of 'hyaline degeneration of mesoglia', cells demonstrable with his platinum salt impregnation method and now recognised as oligodendroglia. Some considered them to be protein precipitates of lymphatic or haematogenous origin, and others regarded then as merely post mortem artefacts.[12] Ferraro and Damon favoured their origin from oligodendroglia and attempted to demonstrate this using Hortega's metallic impregnation methods. Always found to be very variable in number, there was a general view that they were more prominent in old age and in chronic neurological diseases, and particularly in the spinal cord in tabes dorsalis and in subacute combined

degeneration,[13] but this view was based more on visual impressions than on quantitative assessment. At this period no suggestion was proposed as to their biological role and it was not until the last three decades, with the application of more refined and revealing histochemical, ultrastructural and immunocytochemical methods, that interest in their nature and their relation to certain disease conditions has been awakened. Any role for them in normal circumstances, however, has rarely been suggested and, indeed, some have concluded,[15] somewhat remarkably in view of their almost universal occurrence, that they are a product of an acquired defect in glucose metabolism.

Clarification of terminology

Corpora amylacea is made up mainly of glucose polymers (polyglucosans), hence, are also referred to as polyglucosan Bodies (PB). The terminology of PB was proposed to the group comprising of Lafora bodies (LB), lafora-like bodies, corpora amylacea and Bielschowsky bodies on the basis of their biochemical similitude.[16] A rigorous histochemical differentiation between these different anomalies appears impossible.[16] The terminology used in describing them depends upon the clinical setting at which they occur, i.e. Lafora bodies in Lafora body disease,[17] Bielschowsky bodies in the globus pallidus in choreoathetosis[16,18] and CoA in normal ageing and neurodegenerative conditions.[19] To avoid confusion with polyglucosan body disease, we preferred the term CoA to polyglucosan bodies.

General characteristics of corpora amylacea

Corpora amylacea in man are generally seen with the light microscope to be spherical bodies ranging from less than 2 mm to about 20 mm in diameter although when numbers are great a few may be found larger than this. The mean diameter in paraffin sections is regularly 10–12 mm. While usually spherical, oval or elongated forms may occur. Concentric laminated or target patterns are frequently seen, the centers staining rather more densely than the periphery, but the darkly staining cores with radial striations seen in true Lafora Bodies do not normally occur. Mostly their surface is smooth but not infrequently this may appear to be ragged as if coated by a looser material. In the cortex, in the striatum and in the spinal anterior horn grey matter where they have been seen in terminal neurites or within axons the bodies are usually small, measuring 1.5 mm or less in diameter, and only occasionally reach 10 or 12 mm.[12] However, Mizutani et al.[20] found intra-axonal bodies in the ventral posterolateral nucleus of the thalamus with a mean diameter of between 4 and 18 mm and occasionally up to 24 mm.

Ultrastructural features

Corpora amylacea are found ultrastructurally to consist of tangles of short linear filaments 8–12 nm.in the larger bodies with a whorl-like pattern reminiscent of the concentric lamination seen with the light microscope.[12] The bodies are regularly rounded but are not membrane bound and Ramsey,[21] in a biopsy tissue of the hippocampal and temporal lobe from a 35 year old epileptic subject, consistently

found them within astrocytic processes. They were often surrounded by glial fibrils and occasionally suggestive evidence of fusion of two bodies was noted. Others have also stressed the association of the bodies with astrocytes.[22,23] By contrast, Woodford and Tso[24], who examined optic nerve heads from four subjects, concluded that CoA lay unequivocally within myelinated and unmyelinated axons and saw within them occasional bundles of microtubules and lamellar formations reminiscent of degenerate mitochondria; they saw no similar bodies inside astrocytes in this region. Intra-axonal CoA with the same ultrastructural characters have also been found presynaptically in the striatum, in the thalamus, in human orbital cortex rapidly fixed post mortem, in small myelinated axons in the spinal grey matter in various neurological diseases and in myelinated peripheral nerve axons in cases of Amyotrophic Lateral Sclerosis.[12] Although the amount of evidence associating CoA with axons is thus substantial, it is important to stress that they have not been reported within neuronal perikarya at any time in normal subjects. Moreover, their presence within astrocytes depends much upon where one looks and on the various brain surfaces they seem to be largely related to astroglia. Whether they grow larger within astrocytes as they apparently pass to the surface of the brain is an interesting, though unanswered question. There is, however, no doubt that in the subpial region at least they lie chiefly within astrocyte processes. Sbarbati et al. [23], in biopsy material from the vestibular root entry zone from cases of Meniere's disease, observed them in astroglial processes enveloped by folds of glial cytoplasm. They considered it likely that these folds could split apart, allowing the bodies to escape across the glia limiting lamina into

the pial connective tissue or subpial space where they were seen without any limiting membrane in close juxta-position to subpial collagen fibres. Light microscopists have long noted them within this space and wondered, no doubt, whether this was a natural or an artificial effect. It appears, thus, that this may be a feasible route of escape into the CSF. Whether this is a regular route of disposal, however, is uncertain.

Topography of corpora amylacea in the human nervous system

In elderly subjects CoA may be found in almost all regions of the central nervous system, but are notably concentrated in certain locations, and since this distribution is not random it must be of significance to their life history in a way we do not at present understand. In the cerebrum they are seen congregated in the glial feltwork beneath the ependymal lining of the ventricles, particularly beneath the corpus callosum, in the roof and to a less degree the floor of the third and fourth ventricles, and in the roof of the aqueduct often in very large numbers. On the outer surfaces of the brain they lie most usually in the glial feltwork beneath the pia mater, especially at the base of the brain, on the medial surfaces of the temporal lobes and over the hippocampal formations. They are very common in the surface glial feltwork in the outer part of layer I covering the cortex, but most usually they lie in the depths of sulci, and especially in the insula, rather than on the surface convexity. When present in the white matter they tend to congregate around vessels of medium and large size and are often seen in the Virchow–Robin spaces. In the cerebellum they are also found in the depth of sulci and not uncommonly in

the Purkinje cell line; they are uncommon in the granule cell layer. In the spinal cord they are especially noteworthy in the outer half to one third of the posterior columns and dorsal parts of the lateral columns, lying close to the surface either in the glial feltwork directly under the pia mater or among nearby myelinated nerve fibres. They are also seen clustering in the spaces among the entering spinal sensory root fibres where there is an abundance of astroglial fibres, but they are very rarely found beyond the transitional zone within the peripheral nerve itself. By contrast, they are relatively uncommon in the regions of ventral roots. In the medulla oblongata they also occur in the sensory root entry zones but rarely near motor cranial nerve roots. Nonetheless, it should be noted that they are numerous in the anterior horn grey matter of the spinal cord where they are usually substantially smaller mean 4–6 mm. and they are somewhat less common, and invariably small, in the posterior horns. They have not been reported in spinal ganglia. In view of the growing awareness of the value of CoA in the aging process there is room for a larger survey using PAS stained light microscope samples. [12]

Composition of corpora amylacea

Histochemical reactions indicate that CoA are principally composed of polysaccharides, staining strongly with the Periodic acid-Schiff reagent (PAS) and with Best's carmine. They are also positive with Gomori's methenamine silver method that detects carbohydrates and give a strong reaction with the uranyl nitrate–potassium ferricyanide reagent for phosphate groups.[12] They also stain with the PAS dimedone method, which is more specific for polyglucosans and their acid

esters and does not stain acid mucopolysaccharides, glycoproteins or mucoproteins.[24] With Lugol's iodine and sulphuric acid they turn dark brown and later dark violet indicating their starch-like qualities, and the purple brown colour produced suggests that an admixture of short and long linear polyglucosan chains are present.[24] They are positive with Hale's dialysed iron, they stain with Alcian blue at low pH and the extinction point with methylene blue is at pH-2, all features indicating the acidic nature of some of the components. This may also be responsible for their haematoxyphilia. They are metachromatic with toluidine blue. None of these reactions are affected by prior exposure to diastase or hyaluronidase. Histochemically they react uniformly negatively for lipids, DNA and RNA, but reactions for protein may be weakly positive and they may stain equivocally for iron. The periodic acid-thiocarbohydrazide-osmium reagent, an ultrastructural method for polysaccharides, gives positive reactions in both filaments and granular components of the bodies. X-ray microanalysis techniques indicate the presence of a number of atomic species strongly bound within the bodies, including Na, P, S, Ca, Fe, Cu and Cl.[12]

Biochemical analysis

Corpora amylacea, purified by a centrifugation procedure with final extraction in hot water, yield a clear water soluble fraction containing 87.9% hexose, 4.7% protein and 2.5% phosphate.[24] This protein level was thought probably too low to be detectable by histochemical tests. Stayaert et al.[25] have paid particular attention to the protein component of CA, avoiding the use of proteases in the separation

process. In brain material taken from seven subjects aged from 57 to 87 years, they confirmed a protein content of about 4% of the total weight of isolated bodies. SDS-PAGE analysis consistently produced several polypeptide bands, the most abundant being of molecular weights 133, 94, 43 and 24 kDa. Amino acid sequencing of two of the peaks seen in the HPLC profile, revealed homology with the N-terminal of human ubiquitin. Since the molecular weights of these polypeptides was substantially larger than that of ubiquitin 8.5 kDa., it was considered likely that some proteins in CoA were ubiquitin conjugates.

Immunocytochemical reactions of corpora amylacea

Antibody reactions indicate that CoA probably contain materials derived from several sources.

Positive reactions to anti-tau antibodies and to the extracytoplasmic domain of amypoid precursor protein (APP) suggest a likely but not exclusive neuronal source for some constituents. The presence of proteins produced in response to cell stress, such as ubiquitin and heat shock proteins 28, 60, 70 and 72 as well as heme oxygenase-1 HO-1; HSP 32., could originate from both neurons and glia and it is of interest that this last, HSP 32, which is very sensitive to oxidative stress, is also found in the homologous polyglucosan material of myocardial basophilic degeneration.[26,27] The PGB in both tissues also react with antibodies to advanced glycation end-products (AGEP). These are insoluble and non-degradable products

that result from the interaction between reducing sugars and long lived proteins. They have several rather specific characters and their presence has been detected in a number of age related disease situations. In nervous tissue they are found in CoA and in neuronal lipofuscin granules, but not in Hirano bodies, ubiquitinated intraneuronal bodies or granulovacuolar bodies.[28] Antibodies to proteins of the anion exchanger gene family also react with CoA and this immunoreactivity is especially noteworthy in Alzheimer's disease. These proteins are concerned with facilitation of transport of chloride and bicarbonate ions across cell membranes and regulation of binding sites of cytoskeletal proteins. The anion transporter is one domain of the larger band 3 proteins with many properties, including membrane repair, present in cell membranes of many types. Degradation of the band 3 protein generates senescent cell antigen that marks a cell for death and removal, a process considered important in the process of the immunological removal of effete cells from many tissues, including brain.[29,30] Serum carnosinase is another protein of neuronal origin detectable immunocytologically in CoA. This dipeptidase hydrolyses homocarnosine GABA-L-histidine.and is probably synthesized in brain tissue. Immunostaining shows its presence in a number of nerve fibre tracts, but uncommonly in nerve cells. Purkinje cells, for instance, contain none, but it is abundant in the cerebellar molecular layer. Neurons that contain homocarnosine probably derive GABA by the action of the enzyme.[31] Finally, Cisse and Schipper and Schipper and Cisse have shown the consistent presence of mitochondrial epitopes in CoA, and have drawn attention to the similarity of many features of CoA to the Gomori-positive granules present in a subset of periventricular

astrocytes. Both show the presence of a non-enzymic, iron dependent peroxidase and both react with Gomori's chrome alum-haematoxylin, the only significant difference being that the latter show orange-red autofluorescence with UV light, not seen in CoA. These observations are likely to be of critical relevance to the biogenesis of CoA.[27,32]

Positive reactions in CoA to antibodies to myelin basic protein, proteolipid protein, galactocerebroside and myelinroligodendrocyte glycoprotein[33] indicate that oligodendroglial metabolites also make an important contribution to their composition. Positive reaction also to antiferritin antibodies[33] supports this conclusion, since this cell plays a central role in the CNS in the metabolism of ferritin, transferrin and iron.

Singhrao et al.[34], aware that complement is activated in such chronic conditions as Alzheimer's disease AD., Pick's disease D. and Multiple Sclerosis S., examined CoA immunocytochemically for various complement components. They found positive reactions for both classical and terminal pathway-specific components, and especially well marked reactions occurred in tissues from MS patients, while normal brain tissue showed either less strong or negative responses. They concluded that some protein constituents of CoA were derived from cells previously subjected to complement attack and, since terminal pathway components were also detectable, this attack must have gone to completion. The full cascade of the classical pathway is activated in the amyloid and neurofibrillary tangles of AD, and the same is true for the tissue changes in MS where

complement cascade is considered to be responsible for the destruction of oligodendrocytes.[12]

Variations with age and with neuron loss

The numbers of CoA are almost invariably low in younger age groups, though they may be found if carefully sought. After the age of 30–40 years they become generally larger and increasingly numerous, although there is little systematic quantitative data to support this generally held view. It is of interest to note that the size distributions of CoA in spinal cord suggest two populations, perhaps of different origins. Those in the grey matter of the anterior horns are generally smaller with a size distribution significantly different from those in the white matter of the posterior and lateral columns.[12] Chung and Horoupian [35] found a low incidence in hippocampal and extrahippocampal tissues in a group of 20 control subjects aged 16 to 51 years with various diseases. Of these, when assessed on a 4+ scale, eight had either none or only 'scant' amounts recorded, while the remainder showed 1+ or 2+ abundance. Kubota and Naumann [36] enumerated the CoA in the retina of 51 individuals ranging from 2.5 to 78 years old. The numbers counted per section remained low until after the age of 30, when for the most part thereafter they steadily rose. Individual counts per section, however, varied strikingly and several of those over 75 years old still had counts as low as the 30 year olds. Another study of 64 subjects also showed that, in general, numbers rose in an age-related manner. Busard et al.[37] determined the mean numbers of bodies per microscope field at 200 X magnification in frontal and temporal cortical grey

matter and found that, regardless of sex, after the age of 40 years they were much increased, but exceedingly variable. A similar wide range was also found in an older age group in a study of the total numbers of CoA in sections of lumbar spinal cord from 28 cases of amyotrophic lateral sclerosis aged 54 to 84 years and 21 control subjects aged 64 to 84 years. In the white matter and subpial regions, total counts of bodies per transverse cord section in this elderly series ranged from 130 to more than 5000 in both groups of individuals without any correlation with the disease state, with sex or with age.[38] It was noteworthy, too, that counts of CoA in the white matter of the posterior and lateral columns in these cases did not differ significantly from control values suggesting that the CoA in these two regions were, perhaps, of different origins. In an analogous study Kubota et al. [36] in 48 glaucomatous eyes showing loss of ganglion cells, also found highly significant reductions in counts of CoA per section to about 10% of normal figures in inner plexiform, ganglion cell and nerve fibre layers, while the number in optic nerves was reduced to zero. However, there was no significant reduction in those in the inner nuclear layer. The results of these two studies provide support for the belief that CoA might arise as a product of the metabolic activity of large neurons. Mizutani et al.[20] counted and measured intra-axonal polyglucosan bodies in the ventral posterolateral nucleus of the thalamus in 85 subjects from the first to tenth decade, some with various neurological disorders. The PGB steadily increased in number after the age of 50 years, and were rather more numerous in cases with Parkinson's disease, otherwise their number, again, was very variable and not noticeably influenced by an associated neurological disease. Since the CoA all lay

within myelinated axons these authors concluded they must have arrived from the perikarya of gracile nuclei neurons that project to this part of the thalamic nucleus. In confirmation of this conclusion, in cases where there was a pontine infarction present cutting through the medial lemniscus CoA were difficult to find. While there is, thus, a general relation to increasing age, and in grey matter they are undoubtedly dependant upon neuronal numbers, these are unlikely to be the only factors determining individual or regional variation. To enable a better judgement to be made as to whether or not they are increased in disease states, a search should be made for the reasons for the marked variation amongst apparently healthy people. At present there is a general belief, based largely on anecdotal evidence that chronic neurological disease is associated with an increase in their number, and this may be true for Alzheimer's disease and for Multiple Sclerosis.[34] In a large series of post mortem examinations, however, Leel-O¨ssy[39] found the greatest numbers in vascular encephalopathies. More systematic data are needed since the factors determining these numbers are quite unknown and may be important for our understanding of the aging process.

Putative functional roles of corpora amylacea

Once at subpial and subependymal surfaces the observations of Sbarbati et al.[23] suggest that some CoA at least may be discharged to the subpial space and may thus passively make their way, by means of the regular tissue movements and vascular pulsations, to the subarachnoid space. Whether they can proceed further and pass via arachnoid villi outside the CNS is unknown. They have rarely been

reported in CSF, although anecdotal accounts suggest that they may occasionally find their way there, which may not be surprising in view of the enormous numbers lying so close to CSF pathways. But there may, in fact, be little need for them to escape from the brain for even when numbers are very large indeed, they come lie principally in fairly capacious regions having little more than a structural role, such as the roof of the ventricular system and the subpial zones. In these sequestration sites they are unlikely to disturb neuron systems of any importance. The general conclusions from these and other studies, therefore, is that in normal circumstances polymerized sugar molecules may form the basis for entrapment and sequestration of products of oxidative damage to mitochondria and other proteins, of other potentially damaging materials and of the non-degradable products of the aging process.[33,34] The main burden of this activity is borne, in humans at any rate, by astrocytes that normally have ample stores of glycogen available for the glycation process. The presence of a range of myelin-derived substances clearly originating in oligodendrocytes,[33,34] but the apparent absence of any firm evidence that polyglucosan bodies arise in these cells, suggests that astrocytes might also collect materials from other cells via the extracellular space in addition to the transfer of small axonal bodies. Other sources of potentially harmful materials arise from the production during metabolic processes of free radical intermediates with their capacity to damage proteins, lipids and especially mitochondria. It has been argued that astrocytes plays a pivotal role in dealing with these potentially damaging products employing a range of degradative processes including ubiquitination.[27,32] This is clearly an important function in humans and perhaps in

other primates, but lower species, for no reason apparent at present, except perhaps for their shorter life span, appear not to employ this protective metabolic device on any scale. There is much to learnt about these enigmatic bodies. If a role is being sought for CoA in chronic neurological disease then this important link between CoA, oxidative stress and cellular aging processes in the normal will be playing a large part in major chronic neurological diseases. Singhrao et al. have particularly emphasised, from their studies of the complement cascade, the importance of CoA as repositories of the products of neuronal cell death and myelin breakdown, both in aging individuals and in diseases, such as Temporal lobe epilepsy, Multiple Sclerosis, Alzheimer's disease and Parkinson's disease.[34] The detection of advanced glycation end-products (AGEP) in neurons and in CoA derived from the glycation of proteins such as tau and the interaction of such non-degradable proteins with receptor binding proteins to generate potentially damaging oxygen intermediates leading to oxidative stress are likely to be an important factors in the cell damage in Alzheimer's disease.[40,41] The presence of these and of anti-oxidants, as well as the marked variability in immunological profiles in the greatly increased numbers of CoA in Alzheimer's disease must reflect in their biogenesis the intimate events in progress in these diseases.[42]

Corpora amylacea and oxidative stress

Work from the Schipper laboratory has demonstrated that corpora amylacea are most likely derived from small Gomori-positive granules that are present within astrocytes.[43] Interestingly, the promotion of corpora amylacea formation from

these granules appears to be mediated by the presence of a specialized mitochondrial population and the activity of heme oxygenase.[32,44] As such, corpora amylacea are believed to represent the presence of oxidative stress, mitochondrial abnormalities, and iron imbalances within aging astrocytes. However, the specifics of how iron dysregulation and oxidative stress promote corpora amylacea, and the effects of coropora amylacea on astrocyte homeostasis, are largely unknown. For example, it may be that the development of copora amylacea provides an effective means of cells to deal with sudden or chronic elevations in iron, or allow for cells to cope with the presence of potentially dysfunctional mitochondria. Alternatively, coropora amylacea may promote potentially deleterious iron redox reactions, or obstruct cytoplasmic flow within astrocytes. Clearly, the fact that the development of corpora amylacea is an inevitable consequence of aging, and the ability of these inclusions to promote potentially beneficial as well as deleterious effects, highlights the importance of these pathological features to the aging brain.[19]

Conclusions

CoA is, in fact, part of a cellular system not confined to nervous tissue but an essential part of the metabolism of most, if not all cells. Its functions seem to be directed towards trapping and sequestration of potentially hazardous products of cellular metabolism, principally derived from the aging process, but probably also from any disease state resulting in excessive amounts of potentially harmful metabolic products. It is, thus, another arrangement in parallel with the lysosomal lipofuscin system for the management of metabolic degradation products and for

rendering harmless of potentially damaging metabolites. It is of interest that age-related products of the glycation process of long-lived proteins can be detected in both systems,[28] but whereas the lysosomal lipofuscin system in known primarily to be an enzymic degradation system, with CoA materials become incorporated into a glucose polymer mass, that may grow in size with time and eventually be sequestrated either intracellularly, as in cardiac myocytes and in some axons, or transported by astrocytes to various surfaces of the brain. Here they accumulate, perhaps for many years and even for the lifetime of the individual. Their importance as indicators of the aging process and of age related disease processes is probably linked to the constant generation of potentially tissue damaging active oxygen species during mitochondrial oxidative phosphorylation and other metabolic processes. This implies that their numbers likely to be in part an expression of the amount of metabolic tissue work, and this is also suggested in the heart from their hierarchy of incidence in the various chamber walls, maximum numbers being present in the left ventricular wall where there must be the greatest expenditure of energy. Furthermore, since their numbers vary in different parts of the brain, in a general way they must also reflect variations in the amount and possibly the kind of metabolic activity in each brain region. Second order spinal sensory neurons are noteworthy in this respect for no clear reason and one wonders whether other brains regions might not also be found showing similar features. It has been commented upon by various authors that in Alzheimer's disease and Multiple Sclerosis, both the numbers of CoA are greatly increased, and their immunocytochemical characters are altered, as might, perhaps, be expected in

conditions which are so overtly and selectively destructive of neurons and oligodendroglia respectively. What is especially intriguing, however, is the extraordinary variation in numbers found on the one hand in individuals not subject to any obvious neurological disease and on the other their occasional massive increase, often very locally, in some chronic neurological conditions. The enormously wide variation in numbers found at various ages in the retina and in the spinal cord appears to be unrelated to any disease process and suggests that this may be characteristic of the individual's own aging process. Indeed, there may well be more than one population of individuals, those who show only minimal increases of CoA with age and those who for as yet unknown reasons accumulate and sequester increasing numbers. If in normal health their main role is protection against potentially harmful products of metabolic activity and the effects of aging of long lived proteins, then the numbers of CoA present may be a quantitative and certain reflection of the individual's own aging process. Clearly there is a lot yet to be learnt about these enigmatic bodies.[12]

Temporal lobe epilepsy with Hippocampal Sclerosis

Epileptic disorders due to localized structural brain lesions are referred to as symptomatic (secondary) localization-related epilepsies.[45] The syndrome of mesial temporal lobe epilepsy is the most common, and best-defined, form of symptomatic localization-related epilepsy and is characterized by epileptogenic abnormalities in mesial temporal limbic structures.[46] The associated pathological substrate is usually hippocampal sclerosis, but other discrete structural lesions can also be found alone or in association with hippocampal sclerosis (dual pathology).[47] A recent study at a large epilepsy center in Paris indicated that half of their patients had a diagnosis of temporal lobe epilepsy and that half of these had evidence of hippocampal sclerosis on magnetic resonance imaging (MRI).[48] Because MRI is not 100% accurate in identifying hippocampal sclerosis, it is likely that this population included an even greater percentage of patients with this pathological substrate, confirming that mesial temporal lobe epilepsy with hippocampal sclerosis is likely to be the most common form of human epilepsy.[49] In the same study, the authors found that a diagnosis of hippocampal sclerosis was associated with the worst prognosis in their patient population. Whereas 50% to 80% of patients with epilepsy can expect to become seizure free with adequate medical treatment,[50] only 11% of patients in this series with a diagnosis of hippocampal sclerosis, and only 3% with dual pathology, had been seizure free for the previous year. Consequently, mesial temporal lobe epilepsy also appears to be one of the most medically refractory forms of human epilepsy. On the other hand,

mesial temporal lobe epilepsy has, for many years, been the epileptic syndrome most commonly, and most effectively treated by surgical resection.[51] Mesial temporal lobe epilepsy, therefore, is the prototype of what are now called surgically remediable syndromes.[52] These are disorders with a known pathophysiology and natural history, in which medical intractability can be predicted when first-line pharmacotherapy fails, where 70% to 90% of patients with these disorders can expect to become free of disabling seizures with appropriate surgical treatment. Some evidence suggests that surgical treatment is also able to prevent, or reverse, interictal psychiatric and social disturbances that commonly develop in patients with mesial temporal lobe epilepsy, but only if effective intervention occurs early in the course of the disorder.[53,54]

Historical Perspectives

A hardening of the hippocampus in some patients with epilepsy and odd behavior was first noted in 1829, and the classical microanatomical features of hippocampal sclerosis were described by the end of the nineteenth century. It was about this time when Hughlings Jackson (1898) concluded that epilepsy consisted of more than generalized tonic-clonic convulsions and that seizures characterized by a "dreamy state" were associated with a gross lesion in the mesial temporal area. With the advent of EEG, it eventually became clear that psychomotor seizures originated in the mesial temporal lobe, but hippocampal sclerosis was thought to be a result, rather than a cause, of chronic epilepsy.[55] The EEG also made it possible to localize temporal lobe epileptogenic abnormalities for surgical

resection[56], which in turn provided opportunities to elucidate the role of hippocampal sclerosis. Although many patients with this disorder had a history of complicated febrile convulsions, supporting a conclusion that hippocampal sclerosis is the result of epileptic seizures, it also became apparent that patients usually became seizure free following removal of this lesion, suggesting that it might also be the cause of epilepsy.[57] Hypotheses regarding the epileptogenicity of hippocampal sclerosis were pursued in epilepsy surgery programs, and in-vivo research became possible not only intraoperatively but also chronically with the common use of stereotactically implanted depth electrodes to better localize the epileptogenic region.[56] Paul Crandall was the first to carry out long-term depth electrode recordings of spontaneous seizures[58], in association with EEG telemetry monitoring, and to combine this with a standardized presurgical electrophysiological protocol and detailed investigations of mesial temporal structures that were removed en bloc. As a result of a virtual worldwide explosion in recent years in the number of programs offering surgical treatment for epilepsy, and widespread adaptation of this research strategy, mesial temporal lobe epilepsy has, for many years, been the most studied form of human epilepsy.

Clinical Features

Mesial temporal lobe epilepsy with hippocampal sclerosis has a characteristic clinical presentation.[46] Patients often have a history of complicated febrile seizures or other initial precipitating injuries, such as head trauma or intracerebral infections, within the first 4 or 5 years of life[47], suggesting that brain insults during a critical period of development play a role in initiating epileptogenic hippocampal damage. There is also an increased incidence of family history of epilepsy, perhaps indicative of a genetic predisposition to the characteristic cell loss and neuronal reorganization presumed to be responsible for seizure generation in this condition. Spontaneous afebrile seizures usually begin in childhood with either a complex partial or generalized tonic-clonic ictal event. Typically, these seizures initially respond to antiepileptic drugs, and patients may be seizure free for several years. In the intractable form, however, when seizures recur, usually in adolescence or early adulthood, it then becomes difficult or impossible to bring them under control again with medication. This and the fact that interictal behavioral disturbances, particularly depression, often occur when recurrent disabling seizures persist imply that the underlying neuropathological process is to some extent progressive.[59] The habitual seizures usually begin with an aura, most commonly a sensation of epigastric rising, although a variety of autonomic and emotional signs and symptoms can occur. Initial elementary motor and sensory signs and symptoms are not a feature of the ictal events, with the exception of olfactory and gustatory auras. Auras invariably occur in isolation, as well as immediately preceding

complex partial seizures. This may be a unique feature of hippocampal seizures apparently reflecting seizure-suppressing mechanisms in the hippocampus that are capable of preventing spread of ictal discharge.[60] The complex partial seizure typically begins with motor arrest and staring, followed by oroalimentary automatisms (e.g., lip-smacking, chewing) and other purposeless movements. Automatisms of the upper limb can be unilateral due to dystonic posturing of the hand and arm contralateral to the site of seizure onset. There can be varying degrees of responsiveness to the environment during the seizure, but postictally there is amnesia for the ictal event, and a period of confusion. Reactive automatisms may also occur during the postictal period. The laboratory diagnostic hallmark of mesial temporal lobe epilepsy is interictal anterior temporal spikewave discharges on the EEG, which phase-reverse in basal derivations when recordings are performed with sphenoidal or true temporal electrodes. These may be recorded independently from both mesial temporal areas, even though spontaneous seizures usually are generated from only one side. The typical EEG ictal onset pattern consists of a 5 to 8 Hz rhythmic discharge beginning in one mesial temporal area, either initially or within 30 seconds of a more generalized electrographic change.[61] Depth electrode recordings, however, reveal that these EEG changes are preceded by long periods of ictal discharges in the mesial temporal structures, often associated with the aura, whereas ictal changes on the EEG are usually not observed until after there is sufficient propagation to produce impairment of consciousness and other observable clinical features of the complex partial seizure. Depth electrode-recorded ictal onsets from the hippocampus are more likely to

consist of hypersynchronous discharges than of the buildup of low-voltage fast activity or recruiting rhythm, more commonly seen from the neocortex.[59] Seizures with both types of ictal onset can be seen in individual patients, however. The hypersynchronous discharges are usually restricted to the hippocampus and adjacent structures and either have no clinical correlate or correspond to auras. Transition to a low-voltage fast ictal pattern appears to be necessary for the propagation that results in motor signs and impairment of consciousness. Because EEG-recorded interictal and ictal epileptiform abnormalities can be falsely localizing, confirmation of structural or functional disturbances in the electrophysiologically identified epileptogenic mesial temporal area is important when surgical treatment is considered. Confirmation was initially provided by demonstration of material-specific memory and learning disturbances on neuropsychological testing and inability to support memory with contralateral intracarotid amobarbital injection. Positron emission tomography with fluorodeoxyglucose (FDGPET) was the first modern neuroimaging tool to offer help in identifying hippocampal sclerosis and other mesial temporal lesions, which appeared as interictal temporal hypometabolism. Hippocampal atrophy on T1-weighted images and mesial temporal signal enhancement on T2-weighted images of structural MRI scans now readily indicate the presence of hippocampal sclerosis in most patients with this condition[62], although FDG-PET appears to remain a more sensitive test for hippocampal sclerosis. A typical pattern of temporal hyper- and hypoperfusion with ictal single photon emission computed tomography and a reduction of N-acetyl aspartate with magnetic resonance spectroscopy also aid in

identifying nonepileptic disturbances associated with the epileptogenic mesial temporal lobe. Diagnosis of hippocampal sclerosis and other mesial temporal structural lesions can now be so reliably identified with these neuroimaging techniques that they often constitute the primary diagnostic approach for mesial temporal lobe epilepsy, with EEG being relegated to a confirmatory role. EEG remains necessary, however, to demonstrate that any imaging-identified structural or functional disturbance is epileptogenic.[59]

Pathologic findings

Based on pathological criteria, MTLE with HS can be considered a unique epileptic condition different from other focal temporal lobe epilepsies. Because the specific surgical procedure, available tissue, and methods to assess the tissue differ from center to center, the ILAE Neurosurgery Commission group decided to include minimal criteria for the diagnosis of TLE with HS that can be determined in any center that processes the tissue appropriately after an en bloc resection.

The group proposed the following:

1. Minimal criteria: Neuronal cell loss and gliosis at CA1 and end-folium with relative sparing of transitional cortex measured at the mid-body of the anterior–posterior axis.

2. With quantification, all hippocampal regions may show neuronal cell loss and gliosis to varying degrees, although the extent of cell loss may be variable.

3. Functional and structural glial changes appear.

4. Synaptic reorganization, not limited to the mossy fibers and supragranular layer of the dentate gyrus, often is present. The determination of these changes requires the availability of specialized tests that may not be available to all pathologists.

5. Dentate (granule cell) dispersion is encountered in approx 50% of cases and is defined as greater than 10 cells thick, enlarged granule cell bodies, bilaminar layer, and diffuse upper granule cell boundary. These features can be focal.

6. Extrahippocampal pathology also is found at other mesial temporal lobe structures and/or temporal lobe white matter. Frequent extrahippocampal pathology affects the amygdala, first seen with neuronal cell loss and gliosis in the laterobasal complex (i.e., amygdala sclerosis). Ectopic neurons and perivascular oligodendrocyte-like infiltrates are frequent findings in the temporal lobe white matter. However, these findings have not yet been systematically evaluated with respect to their specificity for MTLE-HS or epileptogenic propensity. The incidence of damage varies depending on the method applied (imaging vs. pathology) and location (white matter vs. limbic structures). Additional studies are warranted to better address extrahippocampal pathology in MTLE .[64-67]

7. When more tissue is available from surgery and more techniques are applied, more extensive and "refined" pathological findings can be demonstrated. Markers for structural analysis may include Timm's stain (mossy fiber sprouting), gliosis [glial fibrillary acidic protein (GFAP) immunohistochemistry]; neuron loss (NeuN; NFP, MAP-2, synaptophysin, etc.); and alterations in myelin, neuropeptides, etc. Markers for functional/metabolic analysis may include neurotransmitter systems, neurotrophic factors (NTFs), calbindin-D28K (CaBP), hormones, abnormal electrophysiology, and so on. Conversely, different techniques may yield different results depending on the "starting point" for morphoanatomic studies (e.g., frozen vs. paraffin sections).

Because pathological analysis is limited to the available tissue and only one region is resected, the extension of focal versus bilateral cell loss cannot be determined from this tissue. The determination of such loss may be best accomplished with neuroimaging because autopsy studies, although important and more precise than neuroimaging, are unlikely to provide significant numbers of cases (even in major epilepsy centers, autopsies on HS patients are rare). The data from Margerison and Corsellis[68] suggests that HS is probably not a unilateral lesion. Hippocampal damage associated with TLE is most often bilateral but asymmetrical, with one side showing severe sclerosis. Along the same lines, additional points must be clarified, including the association of HS with diffuse changes affecting the remaining brain, whether heterotopias are part of the syndrome, and whether the course of the condition is progressive. A precise definition of "dual pathology" is

needed. Although it is likely, doubt still exists that some of the changes may be the consequence of seizures. Finally, some patients have a benign course. These patients obviously do not require surgery for seizure control.

Pathogenesis

No prospective studies have demonstrated the relation of an IPI to the development of MTLE and HS. Retrospective studies have identified several IPIs as probable precipitants. In the currently available histopathological studies, no association was noted with sex/side and HS.[69,70] Concerning the mechanism through which the epileptogenic focus occurs, several theories include the following: Glutamate neurotoxicity and mitochondrial dysfunction can cause cell loss, but the pathways of cell death [including glia, which play a role in excitability in the sclerotic hippocampus[71]] are not identified. Immune factors also may be suggested by some preliminary data worth further study.[72]

One hypothesis indicates that HS may be a developmental disorder based on the persistence of Cajal–Retzius (CR) cells[70] or otherwise compromised reelin signaling pathway or the presence of extrahippocampal white matter neurons and other dual pathologies.[63] These conditions may predispose to the development of HS in the presence of a precipitant. Multiple acquired factors may be important (dual/triple hit). Conversely, the cause of the precipitant also may be responsible for the development of HS, even in the absence of a neurodevelopmental disorder. The evidence for febrile convulsions "causing" HS versus a number of other

potential cerebral insults is quite limited, and it cannot be a singular factor. It is possible that a seizure may allow the persistence of immature features (prolonged maturation[73]), and a subsequent hit may produce the HS phenotype. HS most likely does not result from repeated seizures.[74] However, repeated seizures can produce cell loss but may not be sufficient to produce HS. The contribution of identified pathologic changes in epileptogenesis is difficult to determine and requires further study. The family history of epilepsy implies an association with mendelian features and poor penetrance or other modes of genetic transmission. The latent period is highly variable in humans but may be important in determining how changes lead to epileptogenesis. Animal models will be extremely important to answer these questions.

Genetics of MTLE with HS

Sufficient evidence exists to conclude that a genetic predisposition may be found but that this is not a unitary process. Data from published studies suggest at least three possible scenarios.

1. The genetic predisposition for febrile seizures could be associated with especially severe seizures in some patients to produce HS and MTLE. One example of this could be a form of generalized epilepsy with febrile seizures plus (GEFS+) that leads to partial seizures.[75]

2. Sodium-channel defects in mice can cause HS and it is possible that similar defects could do this in humans, leading to MTLE, with or without febrile seizures.[76]

3. Familial MTLE has been described, and some of these patients are documented to have intractable seizures with HS; in this case, the genetic defect may cause MTLE, which then leads to HS with or without febrile seizures.[77,78]

No evidence suggests that familial partial epilepsies with variable foci, partial epilepsies with auditory features, or temporal lobe variants of benign childhood epilepsy with centrotemporal spikes ever evolve into MTLE with HS. However there is no study looking into genetics of CoA deposition in MTLE with HS.

Corpora Amylacea in Temporal lobe epilepsy

The importance of corpora amylacea in temporal lobe epilepsy has been described only recently. Loiseau et al[79] described numerous polyglucosan bodies in the subcortical white matter of a female subject with intractable partial seizures. In another series from same group, only 4 showed polyglucosan bodies in their 65 temporal lobectomy specimens.[80] In contrast, Mackenzie[81] found excess number of corpora amylacea in 15 out of 40 temporal lobectomy specimens. Chung et al[82] observed increased number of corpora amylacea in 58% of temporal lobectomy specimens. One previous study from thiscenter[9] found that 54 out of 100

specimens with temporal lobe epilepsy contained a significant number of corpora amylacea in the paraffin sections of the hippocampus which were scattered diffusely in the CAI sector and in the dentate gyrus. The mean age of patient group was 26 years (range 3 to 48 years). Furthermore, the distribution of corpora amylacea observed in the specimens with Ammon's horn sclerosis in that study was quite distinctive and could be easily differentiated from those generally regarded as age related. In the paraffin sections of hippocampus from the controls, the number of corpora amylacea were significantly less and they were limited to the subpial and subependymal regions. None of the control hippocampus showed a significant number of corpora amylacea in the pyramidal cell layer CA1 and CA2 sectors as observed in patients with Ammon's horn sclerosis. From a diagnostic standpoint, the presence of large number of corpora amylacea in the pyramidal layer, endfolium, and the dentate gyrus was proposed as a readily identifiable marker for Hippocampal sclerosis. Their occurrence is more helpful in cases where assessment of neuronal loss and gliosis becomes difficult due to technical problems such as tissue fragmentation and piecemeal removal of the hippocampus. The corpora amylacea appear more prominently in the PAS stain. Since routine haematoxylin and eosin stained slides greatly underestimate their number, the study suggested that LFB-PAS combined stain should routinely be undertaken during processing of temporal lobectomy specimens. LFB - PAS stain demonstrates corpora amylacea better because of the bright red colour it imparts and also because of a better appreciation of the pyramidal cell layer in the sections. Subsequent study from thiscenter by Cherian et al. inquired whether there were

differences in the clinical characteristics between MTLE patients who did and who did not have CoA in their hippocampi. [83] The mean age at ATL of the patients in the CoA+ group differed significantly from that of the CoA- group (31.7 + 7.5 years versus 24.8 + 7.0 years, p= 0.02). The mean duration of epilepsy prior to ATL in the CoA+ patients was 19.8 + 10.7 years and in the CoA- patients was 14.0 + 6.2 years (p= 0.055). The postoperative mean Engel score at 2 years of 2.3 + 1.0 of the CoA+ group did not differ from the score of 3.2 + 2.1 of the CoA- group (p= 0.115). Other variables such as gender and past history of febrile seizures also did not differ between the two groups. It was highlighted that the sparse deposition of CoA in the subpial regions seen in aged brains is very much different from the dense deposition seen in the CA1 and CA3 regions of the hippocampi of patients with MTLE. The peculiar pattern of the distribution of CoA correlating with the distribution of the neuronal loss in the hippocampus in MTS with maximal affection of the CA1 and CA3 regions and relative sparing of the CA2 region, suggested a common pathogenetic mechanism. As the selective vulnerability of the CA1 and CA3 regions has been attributed to an excitotoxic mechanism probably related to the high density of glutamate binding sites and relatively small concentrations of calcium buffering proteins like calbindin and chromogranin A in these regions it was hypothesized that patients with MTS and more dense deposition of CoA would have a longer duration of epilepsy as compared to patients with MTS and no CoA. The results showed a trend in that direction, but did not attain statistical significance.

In another study by Castro Rebeiro et al [84] found that CoA were found in 35 patients (49%) whose mean epilepsy duration (28.9 years) was significantly longer than that from the group of patients without CoA in the hippocampus (19. 5 years, p = 0.001). Furthermore, when CoA was found, epilepsy duration was also significantly correlated with their distribution within the hippocampus. Mean duration of epilepsy was 22.7 years in patients with a diffuse distribution of the CoA, while those with exclusively subpial or subpial plus perivascular distribution had seizures for a mean of 15.4 and 17.4 years before operation (p=0.001). In contrast, gender, age at seizure onset, age at operation, seizure frequency, history of status epilepticus and occurrence of secondary generalization did not significantly differ between the groups.

The latest study from this center[10] compared the clinical and EEG characteristics, and post-operative seizure outcome of 373 (mean age 29.4 years, range 7—55 years) surgically treated MTLE-HS patients with (MTLEHS-CoA+, n = 129 [34.5%]) and without (MTLE-HS-CoA−, n = 244 [65.5%]) CoA. Age at surgery was significantly higher and duration of epilepsy before surgery was significantly longer for MTLE-HS-CA+ patients compared to MTLE-HS-CoA− patients. Among the 21 patients with major interictal psychosis detected prior to epilepsy surgery, 19 (90.5%) belonged to MTLE-HS-CoA+ group. Schizophrenia-like psychosis was most prevalent. The post-operative seizure-free outcome was comparable, but significantly more MTLE-HS-CoA− patients were free of antiepileptic drugs. Overall, the observations support the hypothesis that the

pathological process in MTLE-HS is progressive. MTLE-HS-CA+ patients are predisposed to increased psychiatric morbidity.

Genetics of hippocampal sclerosis in TLE

There is hardly any data focusing on the genetics of corpora amylacea deposition in TLE and no study has directly has look into it. Tan et al.[11] studied the genetic polymorphism in ATP-binding cassette subfamily B member 1 transporter (ABCB1) gene in 401 drug-resistant and 208 drug-responsive subjects with epilepsy. Their aim was to replicate the earlier study[85] that found a single nucleotide polymorphism (C3435T) which does not alter the amino acid sequence in the ABCB1 gene was associated with drug resistance; subjects homozygous for the C allele (CC genotype). Their study failed to replicate the association between the CC genotype at the C3435T polymorphism in ABCB1 and drug-resistant epilepsy despite a larger sample size, similar study populations and case-control definitions, and adherence to existing guidelines for genetic association studies. However in their study they found that in the temporal lobe epilepsy (TLE) subgroup, genotype frequencies differed according to the presence of hippocampal sclerosis (HS) ($p = 0.027$). TT genotype was more frequent in TLE with HS compared to TLE without HS (OR - 2.67, 95% CI 1.19 to 5.97, $p = 0.026$). The T allele was also more frequent in TLE with HS compared to TLE without HS ($p = 0.013$). This is the only study that has so far looked directly into genetics of hippocampal sclerosis.

The ATP-binding cassette subfamily B member 1 transporter (ABCB1) gene and its product

The ATP-binding cassette (ABC) genes represent the largest family of transmembrane (TM) proteins. These proteins bind ATP and use the energy to drive the transport of various molecules across all cell membranes. ABCB1 gene, also known as multiple drug resistance (MDR) gene, is the first human ABC transporter cloned and characterized through its ability to confer a MDR phenotype to cancer cells. It is located at chromosome 7q21.12 and codes for ATP-binding cassette (ABC) protein or P-glycoprotein. P-gp is a 1280-residue polypeptide that forms a dimer, resulting in a transmembrane pore.[86] It has been shown to transport a large variety of drugs, including cytotoxic agents, protease inhibitors, immunosuppressants, steroids, statins, calcium channel blockers, beta-blockers, antihistamines, anticonvulsants and antidepressants. P-gp has been reported to be expressed in a wide variety of tissues, including the small and large intestine, adrenal gland, liver, kidney, placenta and capillary endothelial cells of testis and brain. In the blood–brain and blood–testis barriers, it has been shown to transport substrates out of these tissues, whereas in the intestine, P-gp limits the absorption of substrates from the bowel.[86]

A common single-nucleotide polymorphism (SNP), C3435T in exon 26 of ABCB1, has been found to be associated with altered P-glycoprotein level and activity. It has been hypothesized that the 3435 CC genotype, by increasing P-

glycoprotein expression and function at the blood brain barrier, might be associated with drug resistance in epilepsy.[87] However, studies on the association of CC genotype of ABCB1 C3435T polymorphism among patients with pharmacoresistant epilepsies from European, [85,88,89,90] Australian,[11] Japanese,[91] Chinese[92] and Korean[93] and Indian populations[94] have resulted in conflicting data, cause of which may be apparent (due to heterogeneous phenotypes of epilepsy patients studied and variable definition of pharmacoresistant epilepsies used) or real (related to ethnic and racial differences between the populations investigated).

However P-gp has various other roles in human tissues rather than being a simple drug transporter.[86] A role for P-gp-like molecules in removing toxic substances from cells is highly conserved throughout evolution. A physiological efflux role for P-gp has been postulated on the basis of its expression on the apical membranes of gut epithelia, liver cells, kidney tubules and at blood–tissue barrier. P-gp is also expressed in the adrenal gland, on hematopoietic stem cells, natural killer (NK) cells, antigen-presenting dendritic cells (DC), and T and B lymphocytes. Recent works have demonstrated that functional P-gp might play a fundamental role in regulating programmed cell death. Robinson et al. demonstrated that transfected MDR1 P-gp conferred a MDR phenotype and resistance to apoptosis induced by serum starvation, which could be reversed by addition of verapamil, an inhibitor of P-gp, indicating that the transporter function of P-gp is necessary to protect cells from death induced by growth-factor withdrawal.[95] Other studies have further demonstrated that functional P-gp confers resistance to apoptosis induced by a

range of chemotherapeutic drugs, Fas crosslinking, binding of TNF-α to its cell surface receptor and UV irradiation.[96,97] These stimuli induce cell death by activating the common cell-death cascade mediated by a family of cysteine proteases known as caspases. Biochemical analyses have shown that upon Fas ligation, functional P-gp can inhibit the activation of downstream caspases 8 and 3. This inhibitory effect can be completely reversed by addition of anti-P-gp monoclonal antibodies or verapamil (P-gp antagonist). Nevertheless, there is now a growing body of evidence that P-gp can protect cells against a range of different cell-death stimuli.

Another group of data exist that links oxidative stress, apoptosis and P-gp. Thevenod et al. found that Cadmium-mediated toxicity of cultured proximal tubule (PT) cells is associated with increased production of reactive oxygen species (ROS) and apoptosis. They found that cadmium-dependent apoptosis decreased with prolonged $CdCl_2$ (10 mM) application white cell proliferation was not affected. Reduction of apoptosis correlated with a time-dependent up-regulation of the drug efflux pump multidrug resistance P-glycoprotein (P-gp) in cadmium treated cells ('4-fold after 72 h). When P-gp inhibitors (PSC833, cyclosporine A, verapamil) were transiently added to cells with P-gp up-regulation by pretreatment for 72 h with cadmium, cadmium-induced apoptosis increased significantly and to a percentage similar to that obtained in cells with no P-gp up-regulation. Cadmium induced apoptosis and P-gp up-regulation depended on ROS, since co-incubation with the ROS scavengers N-acetylcysteine (15 mM) or pyrrolidine dithiocarbamate

(0.1 mM) abolished both responses. Moreover, cadmium- and ROS-associated P-gp up-regulation was linked to activation of the transcription factor NF-kB; N-acetylcysteine, pyrrolidine dithiocarbamate, and the IkB-a kinase inhibitor Bay 11-7082 prevented both, P-gp overexpression and degradation of the inhibitory NF-kB subunit, IkB-a, induced by cadmium. The data show that 1) cadmium-mediated apoptosis in PT cells is associated with ROS production, 2) ROS increase mdr1 expression by a process involving NF-kB activation, and 3) P-gp overexpression protects PT cells against cadmium-mediated apoptosis. These data suggest that P-gp up-regulation, at least in part, provides anti-apoptotic protection for PT cells against cadmium-mediated stress.[98]

Although no data currently available in human, from the animal data we can surmise that P-gp can play a significant role in corpora amylacea formation.

1) Epileptic state creates an atmosphere of reactive oxygen species in the hippocampus
2) P-gp plays a significant role in protecting the cells from oxidative stress and apoptosis.
3) If for any reason there is derangement of function of P-gp the cells are under an excessive oxidative stress
4) The cells then switches to corpora amylacea formation as a response to the oxidative stress and resultant mitochondrial dysfunction

Thus we hypothesized that patients with reduced P-gp function will harbor higher amount of corpora amylacea.

Single Nucleotide Polymorphism and variation of P-gp function

The human haploid genome contains approximately 3 billion DNA bases, encoding around 25,000 genes, divided among 23 chromosomes. A single-nucleotide polymorphism (SNP, pronounced snip) is a DNA sequence variation occurring when a single nucleotide — A, T, C, or G — in the genome (or other shared sequence) differs between members of a species (or between paired chromosomes in an individual). For example, two sequenced DNA fragments from different individuals, AAGCCTA to AAGCTTA, contain a difference in a single nucleotide. In this case we say that there are two alleles : C and T. Almost all common SNPs have only two alleles.[99]

Within a population, SNPs can be assigned a minor allele frequency — the lowest allele frequency at a locus that is observed in a particular population. This is simply the lesser of the two allele frequencies for single-nucleotide polymorphisms. There are variations between human populations, so a SNP allele that is common in one geographical or ethnic group may be much rarer in another. In the past, SNPs with a minor allele frequency of greater than or equal to 1% (or 0.5%, etc.) were given the title "SNP". Some used "mutation" to refer to variations with low allele frequency. With the advent of a better understanding of evolution,

this definition is no longer necessary, e.g., a database such as dbSNP includes "SNPs" that have lower allele frequency than one percent. Single nucleotide may be changed (substitution), removed (deletions) or added (insertion) to polynucleotide sequence. Ins/del SNP may shift translational frame.[99]

Single-nucleotide polymorphisms may fall within coding sequences of genes, non-coding regions of genes, or in the intergenic regions between genes. SNPs within a coding sequence will not necessarily change the amino acid sequence of the protein that is produced, due to degeneracy of the genetic code. A SNP in which both forms lead to the same polypeptide sequence is termed synonymous (sometimes called a silent mutation) — if a different polypeptide sequence is produced they are nonsynonymous. A nonsynonymous change may either be missense or nonsense, where a missense change results in a different amino acid, while a nonsense change results in a premature stop codon. SNPs that are not in protein-coding regions may still have consequences for gene splicing, transcription factor binding, or the sequence of non-coding RNA.[99]

To date total 28 SNPs have been found in 27 positions on the ABCB1 gene. Hoffmeyer et al[100] first reported that the expression level of P-gp in duodenum was significantly lower in Caucasian population with common synonymous C3435 T polymorphism in exon 26. It was associated with higher plasma levels of digoxin after oral admission. However further studies produced controversial results and suggested that C3435 T polymorphisms might be in linkage disequilibrium (LD) with other functional SNPs. Haplotype analysis in different healthy populations

indeed suggested strong LD of the C3435T SNP with many SNPs across the whole region of ABCB1 gene. Uwai et al found that in renal cell carcinoma downregulation of P-gp mRNA in the kidney cortex was dependent on the T allele at exon 17–76.

Hence SNPs in ABCB1 gene are ideal candidate to look for as a cause of variation in corpora amylacea deposition in temporal lobe epilepsy with hippocampal sclerosis.

Aims & Objectives

Aims & Objectives

This study was designed to investigate whether single nucleotide polymorphisms in ATP-binding cassette family gene *(ABCB1)*, the gene coding for P-glycoprotein, are associated with accumulation of corpora amylacea in hippocampus of subjects having temporal lobe epilepsy with hippocampal sclerosis.

Materials and Methods

Materials & Methods

Study site and subjects

This study was done as a pilot study.

- Recruitment of the human subjects for this study, their clinical screening and blood sample collection for molecular genetic studies was conducted at the R Madhavan Nair Center for Comprehensive Epilepsy Care, Sree Chitra Tirunal Institute of Medical Sciences and Technology, Trivandrum.

- The molecular genetic studies were performed at the Rajiv Gandhi Center for Biotechnology, Trivandrum.

Inclusion criteria

Patients were recruited who fulfilled all the following inclusion criteria

1) Two or more years history of recurrent complex partial seizures of mesial temporal lobe semiology (epigastric aura, behavioral arrest, oro-alimentary and limb automatisms, and amnesia) confirmed by long-term video-EEG monitoring.

2) Unilateral hippocampal sclerosis on MRI confirmed by histopathological examination of the temporal lobectory specimen.

3) Have undergone surgery (anterior temporal lobectory with amygdalo-hippocampectomy) for seizures (≥12 per year) for ≥2 years, unresponsive to at least two monotherapy and one dutherapy trails, each of ≥6 months duration.

4) Remained seizure-free for ≥1 year after epilepsy surgery.

5) Ancestral origin from South India population

Exclusion criteria

1) Secondary hippocampal sclerosis - HS associated with any second pathology seen in temporal lobe like neoplasms either in imaging or histopathology
2) Absence of hippocampal sclerosis or non specific findings in histopathology examination

Surgical procedure

These subjects underwent surgery for medically refractory TLE at the R. Madhavan Nayar Center for Comprehensive Epilepsy Care, Sree Chitra Tirunal Institute for Medical sciences and Technology, Trivandrum, Kerala, South India.

The details of thisnon-invasive presurgical evaluation protocol have been described previously.[12] The demographic and clinical features were abstracted from the elaborate prospective database maintained at the R. Madhavan Nayar Center for Comprehensive Epilepsy care.

For MTLE-HS, an enbloc standard anterior temporal lobectomy is performed with amygdalohippocampectomy under general anesthesia.[6]

Pathological examination

Four-micrometer-thick histological sections of the resected temporal lobe were generated from 10% formalin fixed, paraffin embedded tissue and stained with hematoxylin and eosin.[3] In addition, histological sections were routinely stained with Luxol fast blue-Periodic acid Schiffs' stain to highlight the presence and distribution of CoA in the hippocampus and adjoining temporal structures. HS was defined as the loss of neuronal cell population of ≥30% in the CA1 sector of the hippocampal formation with or without neuronal loss and gliosis involving other mesial temporal lobe structures.[3] Based on the density of CoA in the CA1 sector of the hippocampus, all the specimens were scored on a semi-quantitative scale as:

1. Grade 3: >10 CoA per high power field (HPF)

2. Grade 2: 6—10 CoA per high power field (HPF)

3. Grade 1: ≤5 CoA per high power field (HPF).[3,7]

MTLE-HS-CoA + ve group

Subjects with grade 1, grade 2 and grade 3 CoA deposition in the hippocampi formed MTLE-HS-CoA + ve group.

MTLE-HS-CoA – ve group

Subjects without demonstrable CoA anywhere in the specimen formed the MTLE-HS-CoA – ve group.

For this study, the extent of the neuronal loss or the pattern of distribution of CoA in the hippocampus or extrahippocamal structures was not taken into consideration.

Genotyping:

The genotyping was done at Department of Human Molecular Genetics, Rajiv Gandhi Center for Biotechnology, Trivandrum, India. The study was approved by Institutional Ethics Committee for biomedical subjects as per the Indian Council of Medical Research (ICMR) guidelines.[9]

Peripheral blood was collected in EDTA coated vials and genomic DNA was isolated from lymphocytes as per standard protocol. PCR was carried out in a total volume of 10 µl containing 50ng DNA, 250 µM dNTP (Amersham), 10pm of each primer(Table), 1X PCR buffer and 0.5U Taq polymerase enzyme (Bangalore Genei). PCR was programmed for initial denaturation at 95^0C for 5 minutes, followed by 35 cycles of denaturation at 95^0C for 30seconds, annealing for 30 seconds (at specific temperature depending on the primer-Refer Table 1) and extension at 72^0C for 30 seconds. This was followed by final extension at 95^0C for 10 minutes.(MJ Research). PCR products were digested with restriction enzymes

(depending on the nature of polymorphism) according to manufacturer's protocol (NEB, Inc., USA), separated on 3% agarose gel and stained with ethidium bromide for visualisation.

Selection of SNPs

The study of previously reported C3435T SNP was augmented with the addition of four SNPs (rs1202168, rs1128503, rs1922242 and rs2032562) that together capture the majority of variation across MDR1 (Table 1).

Statistical methods

Allele frequency, genotype frequency and haplotype frequencies were calculated with the software COCAPHASE. Linkage disequilibrium(LD) was analyzed and plotted using Haploview. The significance of genotypic and allelic contingency tables was assessed using the $\chi 2$ distribution and ANOVA. We summarized the quantitative data as mean ± standard deviation (S.D.). $\chi 2$ -tests, Fisher's exact tests and t-tests were used to evaluate the statistical significance of the difference in the clinical characteristics between MTLE-HS-CoA+ and MTLE-HS-CoA− patient groups.

Table 1

Details of methodology used in genotyping

SNP ID	Location	Primer Sequence	Tm (°C)	Enzyme	RFLP Pattern (* wild type; # mutant)
Ex06+139 C/T rs1202168	chr7:87033898	5'-AGGTTTCATTTTGGTGCCTG- 3' 5'-GAACAAAAGGATGCACACGACA-3'	61.5	SspI	299* 275,24#
Ex 12 C1236T rs1128503	chr7:87017537	5'-TACCTGTGTCTGTGAATTGCC-3' 5'-CCTGACTCACCACACCAATG-3'	59.3	HaeIII	269,62,35* 269,97#
Ex 17-76T/A rs1922242	chr7:87011603	5'-TTTGTCAACATTTTTTTGAAGC-3' 5'-TATTATTGCAAATGCTGGTTGC-3'	69.2	ApoI	231,84* 120,111,84#
Ex 21 G2677T/A rs2032582	chr7:86998554	5'-TGCAGGCTATAGGTTCCAGG-3' 5'-TTTAGTTTGACTCACCTTCCC-3'	67.9	BanI	198,26* 224#
Ex26 C3435T rs1045642	chr7:86976581	5'-TGTTTCAGCTGCTTGATGG-3' 5'-AAGGCATGTATGTTGGCCTC-3'	59.4	Sau3AI	158,39* 197#

Results

Results

Patient characteristics

46 subjects with MTLE was recruited who fulfilled the inclusion criteria and whose surgical pathology revealed unequivocal HS and who had remained free of seizures for ≥1 year after surgery. All subjects were self declared south Indian ancestry.

Pathology Findings

Out of 46 MTLE-HS subjects who underwent ATL during the study period, 24 (52.1%) were MTLE-HS-CoA+ and 22 (47.9%) were MTLE-HS-CoA−.

The appearance of HS without CoA is shown in Figure 4.

In the MTLE-HS-CoA+ group 14 patients had grade 1 CoA deposition (Figure 2), 6 patients had grade 2 (Figure 3) and 4 patients had grade 3 CoA deposition (Figure 4). Due to small sample size the groups were analyzed together.

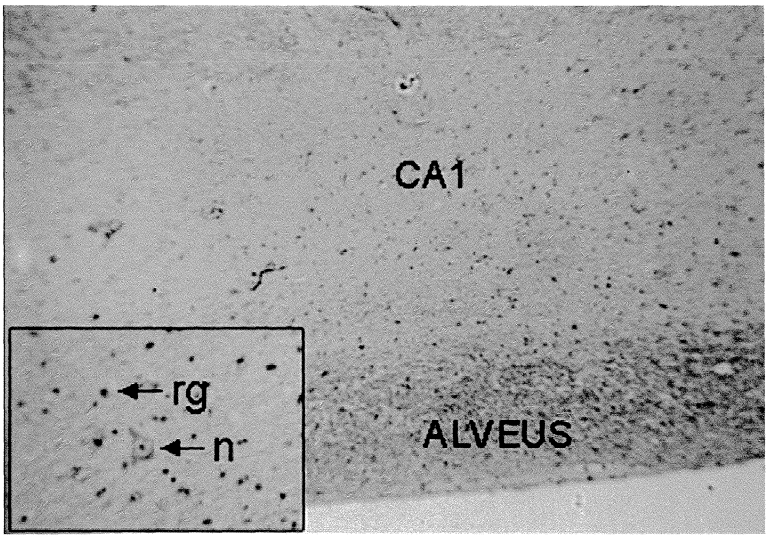

Figure 1 - Photomicrographs of CA1 sector of hippocampus: sparse neurons (n) and reactive gliosis (rg) without corpora amylacea (MTLE-HS-CoA–)

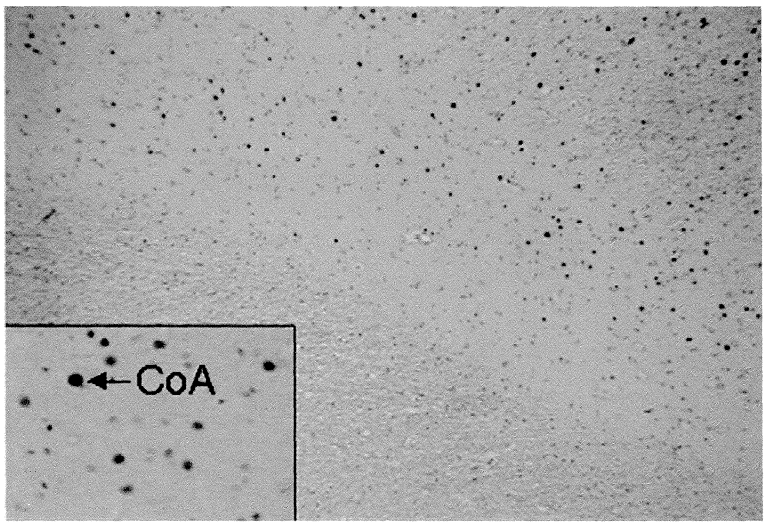

Figure 2 - Photomicrographs of CA1 sector of hippocampus: grade 1, corpora amylacea deposition (MTLE – HS CoA + Grade 1)

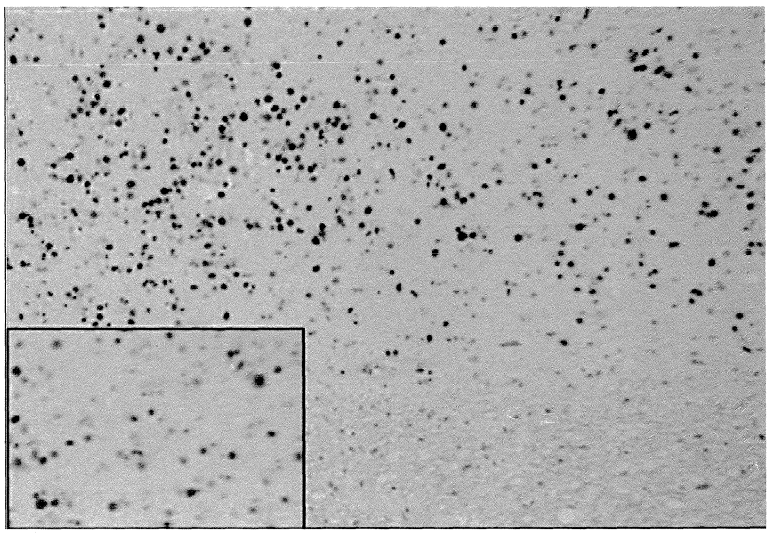

Figure 3 - Photomicrographs of CA1 sector of hippocampus: grade 2, corpora amylacea deposition (MTLE – HS CoA + Grade 2)

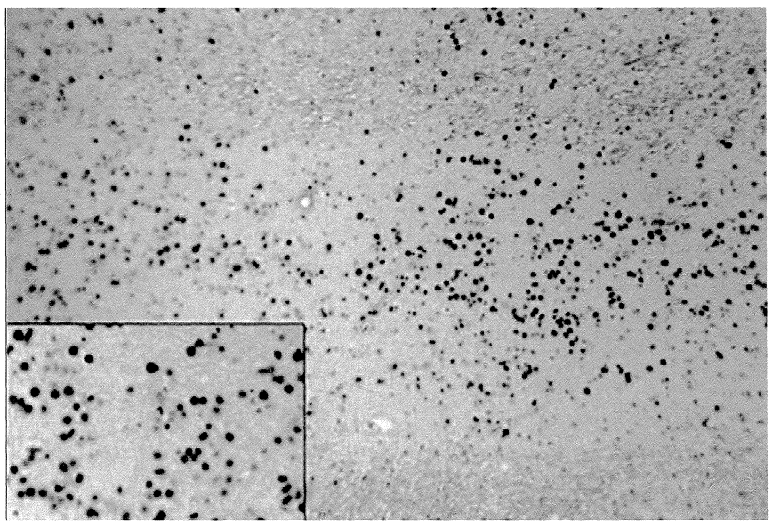

Figure 4 - Photomicrographs of CA1 sector of hippocampus: grade 3, corpora amylacea deposition (MTLE – HS CoA + Grade 3)

Sex

Out of 46 MTLE-HS subjects there were 26 males and 20 females. The sex distribution in two groups is shown in table 2. There was no significant difference between the groups in sex distribution.

Table 2 - Sex distribution in MTLE – HS CoA + and MTLE – HS CoA – groups		
Dignosis	**Febrile Seizure**	**No Febrile seizure**
MTLE-HS-CoA+	13	11
MTLE-HS-CoA –	13	9

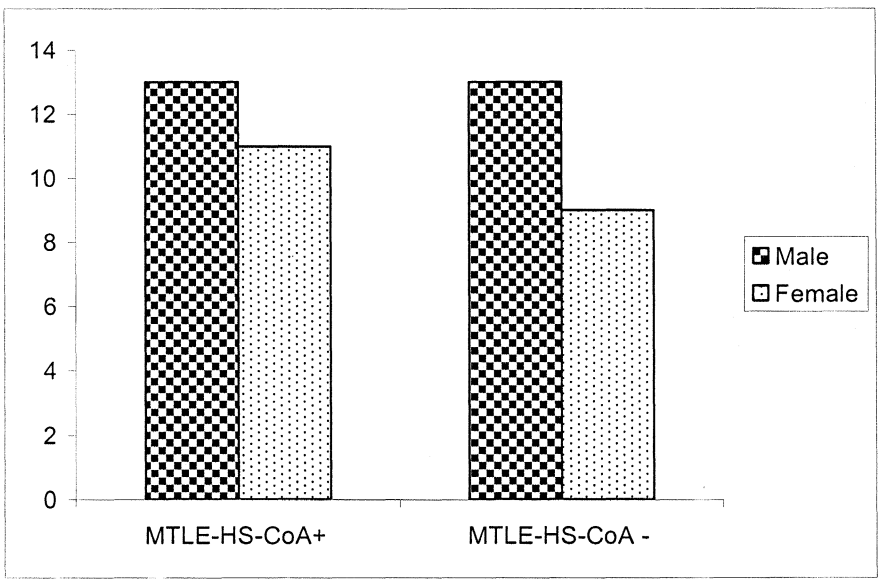

Figure 5 - Graphical representation of Sex distribution in MTLE – HS CoA + and MTLE – HS CoA - groups

Age of Onset of habitual seizures

Age of onset of habitual seizures in the MTLE-HS-CoA+ group was 8.55 ± 5.68 years and in the MTLE-HS-CoA – group was 11.13 ± 7.31 years. However the difference was not statistically significant.

Age at surgery

The mean age at surgery was 32.2 years (range 17—50 years). In the MTLE-HS-CoA+ group the mean age of surgery was 36.42 ± 7.14 years and in the MTLE-HS-CoA – group the mean age of surgery was 27.86 ± 7.64 years. The mean age of surgery was significantly higher in the MTLE-HS-CoA+ group ($p<0.0001$)

Duration of epilepsy before surgery

The mean duration of epilepsy prior to surgery was 22.8 (range 6—47) years. In the MTLE-HS-CoA+ group the mean epilepsy duration before surgery was 25.73 ± 10.63 years. In the MTLE-HS-CoA – group the mean duration of epilepsy before surgery was less 19.56 ± 10.44 years. The MTLE-HS-CoA+ group had borderline significant ($p=0.054$) higher mean duration of epilepsy before surgery.

Febrile seizures

Antecedent history of febrile seizures was present in 29 (63%) subjects. 67% of MTLE-HS-CoA+ group and 59% of MTLE-HS-CoA – group had febrile seizure. However the difference was not statistically significant.

Table 3 - Febrile seizures in MTLE-HS-CoA+ group MTLE-HS-CoA – group		
Dignosis	**Febrile Seizure**	**No Febrile seizure**
MTLE-HS-CoA+	16	8
MTLE-HS-CoA –	13	9

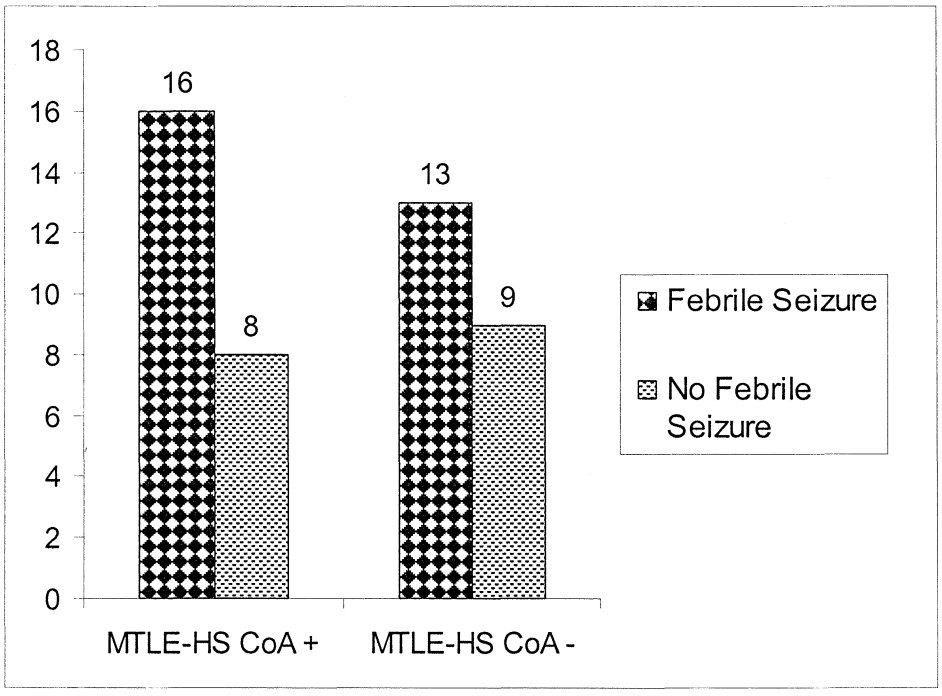

Figure 5 - Graphical representation of Febrile seizure in MTLE – HS CoA + and MTLE – HS CoA - groups

Seizure frequency

Average yearly seizure frequency in the MTLE-HS-CoA+ group was 180.8 ± 92.5 events/yr as reported by the patient and in the MTLE-HS-CoA – group was 123.1 ± 48.8 events/yr as reported by the patient. However the difference was not statistically significant.

Other variables

The seizure types, history of antecedent events, family history of seizures, presence of mental retardation or other co-morbid conditions and antiepileptic use profile did not differ significantly between the groups.

Genetic analysis

Statistical analysis showed a significant association with an intronic SNP (rs1922242) at the *ABCB1* gene (TT genotype) and CoA accumulation (p=0.013); OR = 5.0 (95% CI: 1.34 - 18.55).

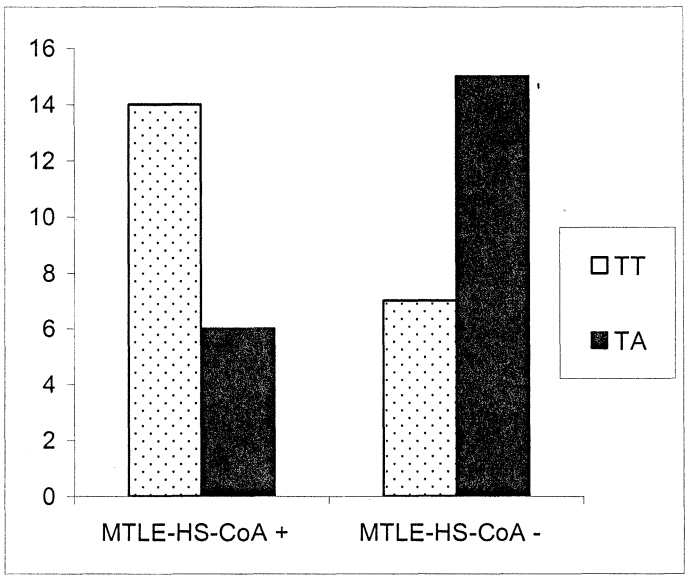

Figure 6 - Graphical representation of Genotype for SNP rs1922242 (Ex 17-76T/A)

Results for other SNPs

The other SNPs did not differ between the MTLE-HS-CoA+ group and the MTLE-HS-CoA – group.

Discussion

Discussion

This study was a pilot study in a strictly defined cohort of epilepsy subjects. All subjects were of self declared south Indian ancestry making it a homogenous population. The diagnosis was confirmed through a detailed presurgical work up and all patients had radiologically and pathologically proven hippocampal sclerosis. All patients underwent similar standard surgical procedures. To further reduce the confounding factors only patients who were seizure free > 1 year after surgery were included. Hence the patient cohort assembled here were very homogenous and with rigorously defined phenotype.

The age at surgery was found to be significantly higher for MTLE-HS-CoA+ subjects compared to MTLE-HS-CoA− subjects (36.42 ± 7.14 years vs 27.86 ± 7.64 years, $P < 0.0001$) The duration of epilepsy before surgery was also borderline significantly longer in MTLE-HS-CoA+ subjects compared to MTLE-HS-CoA − subjects (25.73 ± 10.63 years vs 19.56 ± 10.44 years, $P = 0.054$). However, none of the other variables (e.g. age at onset of habitual seizures, seizure frequency, seizure types, history of antecedent events, family history of seizures, presence of mental retardation or other co-morbid conditions and antiepileptic use profile) differed significantly between the groups.

These results corroborate with the previously done studies. The two earlier series from thiscenter and one by Castro Rebeiro et al also found higher epilepsy duration and increased age at surgery in patients with CoA deposition. However there was

no difference in seizure frequency in between the two groups. Hence we can surmise that corpora amylacea deposition definitely depends on prolonged exposure to epileptic state at the tissue level.

A significant association between an intronic SNP rs1922242 at Ex 17-76T/A (TT genotype) and increased deposition of CoA in hippocampus was found. This is the first study of its kind to have shown any definite genotypic association with corpora amylacea deposition in MTLE – HS. Uwai et al found that in renal cell carcinoma downregulation of P-gp mRNA in the kidney cortex was dependent on the T allele at exon 17–76. Hence we can surmise that because of the TT genotype at intron 16, the cells would have lower level of P-gp expression. Hence they are more prone to oxidative stress because of excitotoxicity induced by epileptic discharges. This results in increased deposition of CoA formation as a response to oxidative stress. Thus subjects with TT genotype will have higher amount of corpora amylacea deposition. This also explains the fact that patient with CoA are of older age and had more mean epilepsy duration compared to patients without CoA deposition. This hypothesis is described in Figure 7.

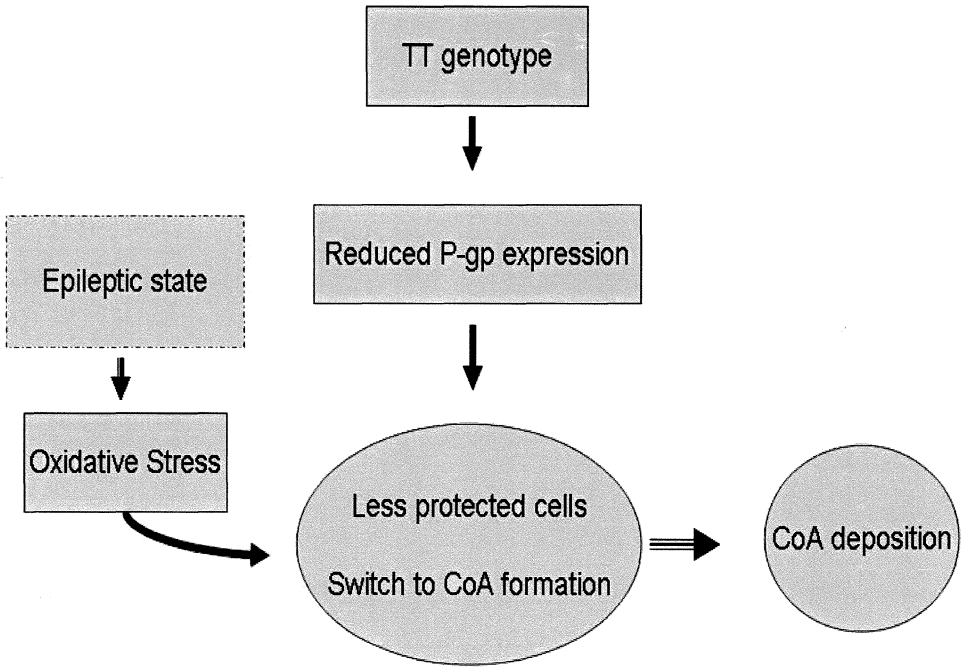

Figure 7 - Graphical representation of hypothetical role of TT genotype at Ex 17-76T/A in Corpora amylacea deposition in MTLE – HS.

The study, being a pilot study, is limited by its small sample size. Additional studies with larger number of subjects will be required to understand the clinical relevance of this association. For want of technology and expertise, CA1 neuronal loss was not quantified and correlated it with the extent of CoA accumulation. However, that was not the objective of this study.

Furthermore, this study provides novel insights into pathogenesis of Corpora amylacea deposition – an intriguing pathophysiological phenomenon. As CoA is also found in other neurodegenerative conditions this study also needs to be replicated in other patient groups – like Alzheimer's disease.

Conclusion

Conclusion

This result suggests new genetic factor for corpora amylacea deposition in subjects having temporal lobe epilepsy with hippocampal sclerosis and provides a novel explanation for this intriguing pathophysiological phenomenon.

References

References

1. Kale, R. Global campaign against epilepsy: the treatment gap. *Epilepsia.* 2002; 43:31–33.
2. World Health Organization. Epilepsy in the WHO Africa Region, Bridging the Gap: the Global Campaign Against Epilepsy "Out of the Shadows." (WHO Press, Geneva, 2004).
3. Regesta, G., Tanganelli, P. Clinical aspects and biological bases of drug-resistant epilepsies. *Epilepsy Res.* 1999;34:109—122.
4. Loscher, W. Current status and future directions in the pharmacotherapy of epilepsy. *Trends Pharmacol. Sci.* 2002;23:113—118.
5. Engel, J. Jr. Etiology as a risk factor for medically refractory epilepsy. A case for early surgical intervention. *Neurology* 51, 1243–1244 (1998).
6. Radhakrishnan, K., Fried, I. & Cascino, G. D. Lesionectomy: management of substratedirected epilepsies. in *Epilepsy. A Comprehensive Textbook* (eds engel, J. J. & Pedley, T. A.), 1891–1905 (Lippincott williams & wilkins, Philadephia, 2008).
7. Engel J Jr, Wiebe S, French J *et al.*. Practice parameter: temporal lobe and localized neocortical resections for epilepsy. Report of the quality Standards Subcommittee of the American Academy of Neurology, in association with the American Epilepsy Society and the American Association of Neurological Surgeons. *Neurology* 2003;60:538—547.

8. ILAE Commission Report. Mesial temporal lobe epilepsy with hippocampal sclerosis. *Epilepsia* 2004;45:695—714.
9. Radhakrishnan VV, Rao MB, Radhakrishnan K et al. Pathology of temporal lobe epilepsy: an analysis of 100 consecutive surgical specimens from subjects with medically refractory epilepsy. *Neurol. India* 1999;47:196—201.
10. Radhakrishnan A, Radhakrishnan K, Radhakrishnan VV et al. Corpora amylacea in mesial temporal lobe epilepsy: clinico-pathological correlations. *Epilepsy Res*. 2007;74:81-90.
11. Tan NCK, Heron SE, Scheffer IE et al. Failure to confirm association of a polymorphism in *ABCB1* with multidrug-resistant epilepsy. *Neurology* 2004;63:1090–1092.
12. Cavanagh, J.B., 1999. Corpora-amylacea and the family of polyglucosan diseases. *Brain Res. Rev*. 29, 265–295.
13. E.F. Buzzard, J.G. Greenfield, Pathology of the Nervous System. Constable and London, 1921.
14. A. Ferraro, L.A. Damon, The histogenesis of amyloid bodies in the central nervous system, *Arch. Pathol.* 1931;12:229–244.
15. E.G. Gray. The fine structure of nerve. Comp. *Biochem. Physiol.* 1970. 419–448.
16. Robitaille Y, Carpenter S, Karpati G, *et al.* A distinct form of adult polyglucosan body disease with massive involvement of central and peripheral neuronal processes and astrocytes. A report of four cases and a

review of the occurrence of polyglucosan bodies in other conditions such as Lafora's disease and normal ageing. *Brain* 1980;103:315-36.

17. Minassian, B.A. Lafora's disease: towards a clinical, pathologic, and molecular synthesis. *Pediatr. Neurol.* 2001;25:21—29.
18. Adler, D., Horoupian, D.S., Towfighi, J. *et al.* Status marmoratus and Bielschowsky bodies. A report of two cases and review of the literature. *Acta Neuropathol. (Berl.)* 1982;56:75—77.
19. Keller J.N. Age-related neuropathology, cognitive decline, and Alzheimer's disease. *Aging Res. Rev.* 2006;5:1–13.
20. T. Mizutani, J. Satoh, Y. Morimatsu. Axonal polyglucosan body in the ventral posterolateral nucleus of the human thalamus in relation to aging. *Acta Neuropathol.* 1987;74:9–12.
21. H. Ramsey. Ultrastructure of corpora amylacea. *J. Neuropathol. Exp. Neurol.* 1965;24:25–39.
22. Palmucci L., Anzil A.P., Luh S. Intra-astrocytic glycogen granules and corpora amylacea stain positively for polyglucosans: a cytochemical contribution on the fine structure polymorphism of particulate polysaccharides. *Acta Neuropathol.* 1982; 57:99–102.
23. Sbarbati A., Carner M., Colletti V *et al.* Extrusion of corpora amylacea from the marginal glia at the vestibular root entry zone. *J. Neuropathol. Exp. Neurol.* 1996; 55:196–201.

24. Sakai M, Austin J, Witmer F *et al.* Studies of corpora amylacea: I. Isolation and preliminary characterization by chemical and histochemical techniques. *Arch. Neurol.* 1969; 21:526–544.

25. Stayaert A., Cisse S., Merhi Y *et al.* Purification and polypeptide composition of corpora amylacea from aged brain. *J. Neurosci. Methods* 1990; 31:59–64.

26. Cisse S., Perry G., Lacoste-Royal G *et al.* Immunochemical identification of ubiquitin and heat-shock proteins in corpora amylacea from normal aged and Alzheimer's disease brains. *Acta Neuropathol.* 1993; 85:233–240.

27. Schipper H.M., Cisse S., Stopa E.G.. Expression of heme oxygenase-1 in the senescent and Alzheimer-diseased brain. *Ann. Neurol.* 1995; 37:758–768.

28. Kimura T., Takamatsu J., Miyata T *et al.* Localization of identified advanced glycation end-product structures, Ne-_carboxylmethyl.lysine and pentosidine, in age-related inclusions in human brain. *Pathol. Int.* 1998; 48:575–579.

29. Kay M.M.B. Isolation of the phagocytosis inducing IGG-binding antigen on senescent somatic cells. *Nature* 1981; 289:491–494.

30. Kay M.M.B. Generation of senescent cell antigen on old cells initiates IGG binding to a neoantigen. *Cell Mol. Biol.* 1993; 39:131–153.

31. Jackson M.C., Scollard D.M., Mack R.J *et al.* Localization of a novel pathway for the liberation of GABA in the human CNS. *Brain Res. Bull.* 1994; 33:379–385.

32. Cisse S., Schipper H.M. Experimental induction of corpora amylacea like inclusions in rat astroglia. *Neuropathol. Appl. Neurobiol.* 1995; 21:423–431.

33. Singhrao S, Neal J, Piddlesden S *et al.* New immunocytochemical evidence for a neuronalroligodendroglial origin for corpora amylacea. *Neuropathol. Appl. Neurobiol.* 1994; 20:66–73.

34. Singhrao S, Morgan B, Neal J *et al.* Functional role for corpora amylacea based on evidence from complement studies. *Neurodegeneration* 1995. 4: 335–345.

35. Chung M, Horoupian D. Corpora amylacea: a marker for mesial temporal sclerosis, J. *Neuropathol. Exp. Neurol.* 1996. 55:403–408.

36. Kubota T., Naumann G. Reduction in number of corpora amylacea with advancing histological changes of glaucoma, *Graefe's Arch. Clin. Exp. Ophthalmol.* 1993; 231:249–253.

37. Busard H, Span J, Renkawek K *et al.* Polyglucosan bodies in brain tissue: a systematic study. *Clin. Neuropathol.* 1994; 13:60–63.

38. Cavanagh J.B. Spinal corpora amylacea and Motor Neuron Disease: a quantitative study. *J. Neurol. Neurosurg. Psychiatry* 1998; 65:488–491.

39. Leel-O¨ssy L. Pathological significance and characteristics of corpus amylaceum. *Neuropatholgy* 1991; 11:105–114.

40. Yan S.D., Chen X., Schmidt A.M., *et al.* Glycated tau protein in Alzheimer's disease: a mechanism for induction of stress, *Proc. Natl. Acad. Sci. U.S.A.* 1994; 91:7787–7791.

41. Yan S.D., Schmidt A.M., Anderson G.M *et al.* Enhanced cellular oxidant stress by interaction of advanced glycation end products with their receptorsrbinding proteins, *J. Biol. Chem.* 1994; 269:9889–9897.

42. Maines M.D. Heme oxygenase: function, multiplicity, regulatory mechanisms and clinical applications, *FASEB J.* 1988;2:2557–2568.

43. Schipper, H.M., Experimental induction of corpora amylacea in adult rat brain. Microsc. *Res. Tech.* 1998; 43, 43–48.

44. Sahlas, D.J., Liberman, A., Schipper, H.M., Role of heme oxygenase-1 in the biogenesis of corpora amylacea. *Biogerontology* 2002; 3, 223–231.

45. Commission on Classification and Terminology of the International League Against Epilepsy. Proposal for revised classification of epilepsies and epileptic syndromes. *Epilepsia* 1989; 30:389–99.

46. Engel J Jr, Williamson PD, Wieser H-G. 1997. Mesial temporal lobe epilepsy. In: Engel J Jr, Pedley TA, editors. Epilepsy: a comprehensive textbook. Philadelphia: Lippincott-Raven. p 2417–2426.

47. Mathern GW, Babb TL, Armstrong DL. 1997. Hippocampal sclerosis. In: Engel J Jr, Pedley TA, editors. Epilepsy: a comprehensive textbook. Philadelphia: Lippincott-Raven. p 133–55.

48. Semah F, Picot M-C, Adam C *et al.* Is the underlying cause of epilepsy a major prognostic factor for recurrence? *Neurology* 1998 ; 51:1256–62.

49. Engel J Jr. Etiology as a risk factor for medically refractory epilepsy: a case for early surgical intervention. *Neurology* 1998;51:1243–44.

50. Hauser WA, Hesdorffer DH. 1990. Epilepsy: frequency, causes and consequences. New York: Demos.

51. Engel J Jr, Shewmon DA. 1993. Overview: who should be considered a surgical candidate? In: Engel J Jr, editor. Surgical treatment of the epilepsies, 2nd ed. New York: Raven. p 23–34.

52. Engel J Jr. Current concepts: surgery for seizures. *N Engl J Med* 1996; 334:647–52.

53. McLachlan RS, Rose KJ, Derry PA *et al.* Health-related quality of life and seizure control in temporal lobe epilepsy. *Ann Neurol* 1997 ; 41:482–9.

54. Sperling MR, O'Connor MJ, Saykin AJ *et al.* Temporal lobectomy for refractory epilepsy. *JAMA* 1996; 276:470–5.

55. Engel J Jr. Mesial Temporal Lobe Epilepsy: What Have We learned? *Neuroscientist* 2001; 7:340–352.

56. Bailey P, Gibbs FA. The surgical treatment of psychomotor epilepsy. *J Am Med Assoc* 1951; 145:365–70.

57. Falconer MA. Genetic and related aetiological factors in temporal lobe epilepsy: a review. *Epilepsia* 1971;12:13–31.

58. Crandall PH, Walter RD, Rand RW. Clinical applications of studies on stereotactically implanted electrodes in temporal lobe epilepsy. *J Neurosurg* 1963; 20:827–40.

59. Engel J Jr. Clinical evidence for the progressive nature of epilepsy. *Epilepsy Res* 1996; (Suppl 12):9–20.

60. Lieb JP, Engel J Jr, Babb TL. Interhemispheric propagation time of human hippocampal seizures: I. Relationship to surgical outcome. *Epilepsia* 1986; 27:286–93.

61. Risinger MW, Engel J Jr, Van Ness PC *et al.* Ictal localization of temporal lobe seizures with scalp/sphenoidal recordings. *Neurology* 1989;39:1288–93.

62. Cascino GD, Jack CR Jr, Parisi JE *et al.* Magnetic resonance imaging-based volume studies in temporal lobe epilepsy: pathological considerations. *Ann Neurol* 1991 ;30:31–6.

63. ILAE Commission Report. Mesial Temporal Lobe Epilepsy with Hippocampal Sclerosis. *Epilepsia* 2004. 45(6):695-714.

64. Bocti C, Robitaille Y, Diadori P, *et al.* The pathological basis of temporal lobe epilepsy in childhood. *Neurology* 2003;60:191–5.

65. Yilmazer-Hanke DM, Wolf HK, Schramm J, *et al.* Subregional pathology of the amygdala complex and entorhinal region in surgical specimens from patients with pharmacoresistant temporal lobe epilepsy. *J Neuropathol Exp Neurol* 2000;59:907–20.

66. Kasper BS, Stefan H, Buchfelder M, *et al.* Temporal lobe microdysgenesis in epilepsy versus control brains. *J Neuropathol Exp Neurol* 1999;58: 22–8.

67. Hermann BP, Seidenberg M, Bell B, *et al.* Extratemporal quantitative MRI volumetrics and neuropsychological function in temporal lobe epilepsy. *J Int Neuropsych Soc* 2003;9: 353–62

68. Margerison JH, Corsellis JA. Epilepsy and the temporal lobes: a clinical, electroencephalographic and neuropathological study of the brain in epilepsy, with particular reference to the temporal lobes. *Brain* 1966;89: 499–530.

69. Mathern GW, Adelson PD, Cahan LD, *et al.* Hippocampal neuron damage in human epilepsy: Meyer's hypothesis revisited.*Prog Brain Res* 2002;135: 237–51.

70. Blümcke I, Thom M, Wiestler OD. Ammon's horn sclerosis: a maldevelopmental disorder associated with temporal lobe epilepsy. *Brain Pathol* 2002;12: 199–211.

71. Kunz WS, Kudin AP, Vielhaber S, *et al.* Mitochondrial complex deficiency in the epileptic focus of patients with temporal lobe epilepsy. *Ann Neurol* 2000;48: 766–773.

72. Crespel A, Coubes P, Rousset MC, *et al.* Inflammatory reactions in human medial temporal lobe epilepsy with hippocampal sclerosis. *Brain Res* 2002;952: 159–69.

73. Blümcke I, Schewe JC, Normann S, *et al.* Increase of nestin-immunoreactive cells in the dentate gyrus of pediatric patients with early onset temporal lobe epilepsy. *Hippocampus* 2001;11: 311–321.

74. Lado FA, Laureta EC, Moshé SL. Seizure-induced hippocampal damage in the mature and immature brain. *Epileptic Disord* 2002;4: 83–97.

75. Abou-Khalil B, Ge Q, Desai R, *et al.* Partial and generalized epilepsy with febrile seizures plus and a novel SCN1A mutation. *Neurology* 2001;57: 2265–72.

76. Kearney JA, Plummer NW, Smith MR, *et al.* A gain-of-function mutation in the sodium channel gene Scn2a results in seizures and behavioral abnormalities. *Neuroscience* 2001;102: 307–17.

77. Cendes F, Lopes-Cendes I, Andermann E, *et al.* Familial temporal lobe epilepsy: a heterogeneous syndrome. *Neurology* 1998;50: 554–557.

78. Kobayashi E, Li LM, Lopes-Cendes I, *et al.* Magnetic resonance imaging evidence of hippocampal sclerosis in asymptomatic, first-degree relatives of patients with familial mesial temporal lobe epilepsy. *Arch Neurol* 2002;59: 1891–1894.

79. Loiseau H, Marchal C., Vital A *et al.* Occurrence of polyglucosan bodies in temporal lobe epilepsy, *J. Neurol. Neurosurg. Psychiatry* 1992; 55:1092–1093.

80. Loiseau H, Marchal C., Vital A *et al.* Polyglucosan bodies: an unusual discovery in a case of temporal epilepsy. Review of the literature, *Rev. Neurol.* 1993; 149:192–197.

81. Mackenzie JM. Polyglucosan bodies are not an unusual finding in temporal lobe epilepsy. *J Neurol Neurosurg Psychiatry* 1993; 56 : 577.

82. Chung MH, Horoupian DS. Corpora amylacea: a marker for mesial temporal sclerosis. *J Neuropathol Exp Neurol* 1996; 55(4): 403-408.

83. Cherian PJ, Radhakrishnan VV, Radhakrishnan K. The significance of corpora amylacea in mesial temporal lobe epilepsy. *Neurol. India* 2003;51:278—279.

84. Ribeiro MC, Barbosa-Coutinho L, Mugnol F *et al.* Corpora amylacea in temporal lobe epilepsy associated with hippocampal sclerosis. *Arq. Neuropsiquiatr.* 2003 ;61:942—945.

85. Siddiqui A, Kerb R, Weale ME, *et al.* Association of multidrug resistance in epilepsy with a polymorphism in the drug-transporter gene. ABCB1. *N Engl J Med* 2003;348:1442–1448.

86. Mizutani T, Masuda M, Nakai E *et al.* Genuine functions of P-glycoprotein (ABCB1). *Curr Drug Metab.* 2008;9(2):167-74.

87. Loscher, W, Potschka H. Drug resistance in brain diseases and the role of drug efflux transporters. *Nat Rev Neurosci* 2005;6:591-602.

88. Zimprich F, Sunder-Plassmann R, Stogmann E, et al. Association of an ABCB1 gene haplotype with pharmacoresistance in temporal lobe epilepsy. *Neurology* 2004;63:1087-9.

89. Sills GJ, Mohanraj R, Butler E, *et al.* Lack of association between the C3435T polymorphism in the human multidrug resistance (MDR1) gene and response to antiepileptic drug treatment. *Epilepsia* 2005;46:643-7.

90. Leschziner G, Jorgensen AL, Andrew T, *et al.* Clinical factors and ABCB1 polymorphisms in prediction of antiepileptic drug response: a prospective cohort study. *Lancet Neurol* 2006;5:668-76.

91. Seo T, Ishitsu T, Ueda N, et al. ABCB1 polymorphism influence the response to antiepileptic drugs in Japanese epilepsy patients. *Pharmacogenomics* 2006;7:551-61.

92. Kwan P, Brodie MJ. Early identification of refractory epilepsy. *N Engl J Med* 2000;342:314-9.

93. Kim DW, Kim M, Lee SK, et al. Lack of association between C3435T nucleotide MDR1 genetic polymorphism and multidrug-resistant epilepsy. *Seizure* 2006;15:344-7.

94. Lakhan R, Mishra U, Kalita J et al. No association of ABCB1 polymorphisms with drug-refractory epilepsy in a north Indian population. *Epilepsy Behav.* 2009; 14:78–82.

95. Robinson, L.J. *et al.* Human MDR1 protein overexpression delays the apoptotic cascade in Chinese hamster ovary fibroblasts. *Biochemistry* 1997; 36:11169–11178.

96. Smyth, M.J. *et al.* The drug efflux protein, P-glycoprotein, additionally protects drug-resistant tumor cells from multiple forms of caspase-dependent apoptosis. *Proc. Natl. Acad. Sci.* 1998; 95:7024–7029.

97. Johnstone, R.W. *et al.* P-glycoprotein protects leukemia cells against caspase-dependent, but not caspase-independent, cell death. *Blood* .1999; 93:1075–1085

98. The´venod F, Friedmann J, Katsen A *et al.* Up-regulation of Multidrug Resistance P-glycoprotein via Nuclear Factor-kB Activation Protects

Kidney Proximal Tubule Cells from Cadmium- and Reactive Oxygen Species-induced Apoptosis. *Journal of Biolog Chem.* 2000; 275:1887-1896.

99. Mullen S, Crompton D, Carney P *et al.* A neurologist's guide to genome-wide association studies. *Neurology* 2009;72:558-565.

100. Hoffmeyer S, Burk O, von Richter O, *et al.* Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. *Proc Natl Acad Sci U S A* 2000;97:3473-8.

101. Uwai Y, Masuda S, Goto M *et al.* Common single nucleotide polymorphisms of the MDR1 gene have no influence on its mRNA expression level of normal kidney cortex and renal cell carcinoma in Japanese nephrectomized subjects. *J Hum Genet* 2004; 49: 40–45.