

Columbia University Human Subjects Study Description Data Sheet

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Renewal

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Originating Department: DEPARTMENT OF BIOSTATISTICS (476)
Submitting To: Medical Center
Title: A MULTICENTER STUDY OF IDIOPATHIC GENERALIZED EPILEPSY Phenotypes, Drug Response & Genetic Epidemiology of IGE

Sponsor Protocol Version#:
Abbreviated title: GENETICS OF EPILEPSY
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Principal Investigator: David Greenberg (476)

Study Description

A. Study Purpose and Rationale

Background

We reported the following findings that set the starting point for the specific aims of the current proposal:

1. Completion of a genome scan of adolescent-onset IGE and component syndromes (JME, JAE, EGTCS).
2. Identification of three new major linkage peaks (chromosomes 18, 5p, 5q) in addition to the previously reported peak on chromosome 8. Also, confirmation of the previously reported locus for JME (chromosome 6), and demonstration of both heterogeneity in JME and possible imprinting.
3. The identification of the BRD2 gene as EJM1, the first gene for a common form of epilepsy (JME), using case-control and family-based association studies. Also, the identification of a 6-marker haplotype that conveyed up to a 9-fold risk for JME. Preliminary data show that the causative polymorphism may lie within a highly variable region at the 3' end of intron 2.
4. The identification of malic enzyme 2 (ME2) on chromosome 18, as a susceptibility locus for all the IEs. Linkage and association evidence supports recessive inheritance. We identified a 9-SNP haplotype with an odds ratio of 6 for homozygous carriers. We demonstrated sequence differences between cases and controls in ME2 in intron 1. No such differences were found anywhere else in the gene.
5. Preliminary data show an increase in neuro-excitation when ME2 is inhibited in mouse brain slices.
6. Tentative identification of a SNP association on chromosome 5q specific for juvenile absence seizures and not childhood-onset absence seizures, and evidence for a childhood absence-specific gene on 5p.

Our findings suggest that changes in neuronal metabolism combined with subtle brain structural changes or other insult are involved in IGE susceptibility. This notion represents a possible new direction for research into the causes of

the IGEs.

For the next period, we propose to exploit this new knowledge and determine the mechanisms by which the identified genes affect epilepsy susceptibility.

Specific aims

Our findings suggest that changes in neuronal metabolism combined with subtle brain structural changes or other insult are involved in IGE susceptibility. This notion represents a possible new direction for research into the causes of the IGEs.

We propose to determine the mechanisms by which the identified genes affect epilepsy susceptibility.

We will pursue the following specific aims:

1. Sequencing studies: we have sequenced the entire ME2 gene and identified sequence variations associated with IGE susceptibility. These sequence variations are seen only in intron 1. We will now examine these sequence differences, determining haplotypes and using statistical analysis to identify SNPs or SNP combinations that are related to susceptibility.
2. Expression studies: It is likely that the sequence variants cause changes in mRNA production or processing, leading to changes in enzyme activity levels. We will study transcription using the mini-gene approach, determining the role intron 1 plays in transcription and whether the variants we see in patients affect mRNA processing.
3. Functional studies: We will assay ME2 enzymatic activity from lymphocytes in patients compared to controls and correlate it with genotype. This will establish a functional correlation to the association identified with the ME2 variants. This will be done in previously obtained samples. No new samples will be collected.
4. Fine mapping and gene identification in the Chr. 5 & 8 loci. Our genome scan indicated two regions on chromosome 5 with strong evidence of linkage in families with absence seizures. We had hypothesized that succinate dehydrogenase, at the tip of chromosome 5, may be related to JAE. Preliminary case-control association studies shows strong association of childhood absence within a small region of the most distal part of 5p, perhaps pointing to the gene PDCD6, but little evidence of a juvenile absence (JAE) association at the same locus.
5. We will continue the search in the centromeric direction. JAE seizures show evidence of association with SNPs near marker D5S433 on 5q. We will perform fine mapping studies, and test the GABAA gene, which is a candidate gene located within the 5q linked region. With sufficient evidence, we will begin sequencing studies. We originally reported evidence of a gene on chromosome 8 that was specific for IGE families with JAE and/or EGTCS. We will carry out SNP fine mapping association studies over a 10 mB region around this highest linkage peak at locus D8S1758.
6. Gene-gene interaction studies: From a previous segregation analysis, we had proposed that JME is caused by two epistatically interacting genes, one dominantly and one recessively inherited. We have now identified two genes: BRD2, specific for JME that appears to be dominantly inherited, and ME2, predisposing to JME and other IGEs, that is recessively inherited. Using statistical modeling, we will test for interaction of these genes in our data. When other genes have been identified (e.g. chromosome 5 and chromosome 8), we will also look for interactions among all loci.
7. Ethnicity and IGE susceptibility. Clinically identical IGE syndromes may be influenced by different susceptibility genes in different population groups. Our findings have been based on a European origin population. We will test our identified associations in non-European origin populations. We will collect patients, controls, and family-based association data from African-American and "Hispanic" groups (e.g. Puerto Ricans, Dominicans) in whom ethnic origins can be determined. The idiopathic generalized epilepsies (IGE) are thought to be genetic in origin and occur in

all populations and ethnic groups. There is strong evidence that, although the syndromes may appear clinically identical, the causative genes are different in different populations. Through linkage and association analysis, we have identified 5 areas of the genome containing genes for IGE and have identified three of those genes in a population of uniform ethnic background that was selected for highly specific IGE phenotypes. Using those genes as probes, we will

- Determine the contribution of those genes to the same specific IGE syndromes in African American and "Latino" ethnic groups by ascertaining IGE patients and testing the gene associations in those groups.
- Ascertain other IGE syndromes to test the specificity of those genes in broader diagnostic categories.
- Determine whether IGEs in patients that fit our original diagnostic criteria, but who are refractory to drugs that are efficacious in most of those IGEs, in fact represent a group of patients genetically different from IGE syndrome groups for which the associations were discovered. Our extensive collaborative arrangements with medical centers having large ethnic populations ensure samples large enough for testing association with already-identified genes. We will also continue to collect European-origin patients to confirm associations.

8. Susceptibility to other forms of epilepsy. The associations we observed with ME2, BRD2, and PDCD6 are based on data from highly specific forms of IGE in patients meeting stringent diagnostic criteria. We now ask: Do these genes influence susceptibility to other epilepsies? We will collect DNA from patients with a broader range of phenotypes than we have collected until now. These will include: childhood onset absence epilepsy; Rolandic epilepsy; myoclonic absence; late-onset IGE and photo-convulsive epilepsy.

9. Drug Refractory IGE Patients. 10-20% of IGE patients do not respond to valproic acid (VPA) or Lamictal (LMT), the two most common drugs prescribed for IGE. We will collect data on those (European-origin) patients who are not responsive to VPA and LMT but who otherwise meet our stringent inclusion criteria. We will determine whether this group of patients, in their susceptibility profiles, has the same phenotypes and genotypes as other IGE patients or whether they represent genetic susceptibilities different from the majority of IGE patients at the loci we will examine. As in other areas of common disease genetics, and especially gene association studies, there is controversy and confusion about which associations are real and which spurious, often because of uncertainty about whether diagnostic characteristics and disease classification are the same in different studies. The data collection proposed here will, to our knowledge, be the only one in the world in which different ethnic groups will be collected using the same rigorous diagnostic criteria that led us to successfully identify the first gene for an IGE syndrome (BRD2) to be identified and replicated both in linkage and association studies.

Evidence is accumulating that the genetic background of patients plays a role in their response to drug therapy. In order to design effective therapies, we must understand the causes of diseases. When those causes differ in different populations, it may dictate different treatment approaches. Similarly, we also need to know the limits and overlap or diagnostic categories. This is particularly true for the drug refractory group of IGEs, the group that is most difficult to treat and which causes the greatest burden on society and the health care system.

B. Study Design and Statistical Analysis

Families will be identified through a proband with one of the above epilepsy syndromes.

We will seek families multiplex and/or multigenerational for IGE, irrespective of seizure type (focal seizures will not count as affected but we will classify those families who fit criteria as JME, RGM, JAE and AGM).

We will also include families in which the proband is not easily classifiable into one of the above epilepsy syndromes. For example, families in which the proband has JAE and RGM will be admitted under a new classification.

Non-JME families will be accepted not as JME, but as IGE families to investigate for linkage to the identified loci.

Families with childhood absence epilepsy (CAE) or BRE will be recruited because some of the same loci may contribute to CAE or BRE as well as to the IGEs we study.

Sample size is not a concern for some of the specific aims proposed but for other parts sample size needed is unknown. Therefore we will try to recruit as many families as possible. In order to demonstrate linkage of EJM1 the number of families is not a question. Patients and family members from our study of aging are another source of general population controls.

We will screen healthy control individuals to provide a comparison of marker genes (genes that are at known locations in the genome) in the normal (control) population with those seen in the epilepsy populations. The screening of healthy individuals for these markers will enable us to determine their frequency in the general population.

We will recruit any healthy volunteer that may have an interest in the study. Our recruitment plan is to work by word of mouth mainly collecting subjects by asking colleagues, students in and around the medical center, and social acquaintances or any other individual for their cooperation or to aid us in finding appropriate subjects.

Participants in this research study will be asked to collect 5 ml of saliva in a special container that we will supply. The samples will be used for extracting DNA. The control DNA will be used as a comparison group to perform association analysis to look for linkage disequilibrium to identify the associated gene (s) for epilepsy.

We will not ask volunteers for any identifiable information. Samples will be anonymous; they will be assigned a number and only the gender, age, ethnic group and country of origin of each individual volunteer and his/her parents, grandparents country of origin will be recorded.

Likely duration of entire study and of subject's participation

Participation of patients and relatives will be less than 2 hours and they will be seen once or twice, only if more saliva is needed. Depending on the location, schedules and cooperation of families, it takes an average of 6 months to schedule family members.

Participation of healthy control volunteers should not take more than a few minutes.

Proposed Methods of Statistical Analysis

We have developed the data base management MySQL system and a large number of applications, programs for handling the input, storage, retrieval of data and reformatting of the marker data for linkage analysis, as well as programs to check the data for consistency. Pedigree information, ages, clinical characteristics, as well as marker information are all entered independently and only come together at the time of the analysis.

In addition to available segregation analysis programs and LIPED for linkage and, if appropriate, multipoint linkage analysis programs as LINKAGE, we have developed simulation programs for testing assumptions of linkage and segregation analysis. Linkage analysis attempts to detect genetic linkage between a phenotype (in this case the syndrome JME) and an established genetic marker. We compute the likelihood of there being linkage in a given pedigree or nuclear family.

We use a lod score (logarithm of the odds) for linkage analysis. This method has the advantage that it takes account-confounding factors, such as reduced penetrance, epistasis, or heterogeneity, thus making use of all the data and information available in the data set. The lod score is computed for each pedigree and those scores are summed over all families. A lod score of 3 is considered statistically significant evidence of linkage if the mode of inheritance is known. Similarly, a score of 2 is considered significant evidence of no linkage between a trait and a marker.

C. Study Procedure

Healthy control volunteers:

We will give each volunteer an information sheet. Verbal consent will be obtained from each subject, but that subjects will not be asked to sign an informed consent document. Documentation will be kept in the research records that verbal consent was obtained from each subject.

Patients and relatives

Prior to participating, subjects will have the study explained to them and risks described. Any questions that the subjects have with regard to the study will be answered. Patients will be given the IRB consent form to read and sign before undergoing any procedure.

Patients participating in the study will be seen one or two times only, and the study will not interfere with their normal medical care. The entire procedure will take less than 2 hours.

Medical and family histories will be obtained from patients and relatives. The data will be obtained only for research purposes.

All participants will be asked for a sample of saliva that can be self collected in containers that will be provided to them. Saliva will be used for DNA extraction and analysis, and typed for genetic markers.

It is possible that DNA couldn't be extracted or the amount extracted would not be enough for analysis. If this happens, we may need to ask the subject for another sample.

Healthy control volunteers will never be asked for another sample because no identifying information will be collected.

D. Study Drugs

None

E. Medical Device

None

F. Study Questionnaires

Copy attached

G. Study Subjects

Healthy control volunteers

We will recruit any healthy volunteer that may have an interest in the study

Patients and family members

We accept into the study all qualifying patients and family members without regard to age gender or racial and ethnic variables. As far as is known, epilepsy affects all racial and ethnic groups.

Data from Epidemiology studies suggest that women are affected with IGE slightly more often than men, although JME appears to affect men and women equally.

The probands, by definition, will have epilepsy and family members will usually be healthy. There will be no criteria of specific inclusion or exclusion of any ethnic or racial population or based on gender, but we are investigating ethnic differences and so will make a special effort to recruit non-Caucasian families. Acceptance into the study will be ages between 4 and 70 years old and only with regard to clinical diagnosis. Children will be included. Pregnant women will not be excluded.

Inclusion criteria

The following IGEs with onset between 8 and 20 years of age

1. Juvenile Myoclonic Epilepsy
2. Juvenile Absence Epilepsy
3. Epilepsy with pure idiopathic generalized tonic clonic seizures on awakening or occurring at random
4. Patients with idiopathic generalized epilepsy that have a combination of the above seizure-syndrome types.
5. Childhood Absence Epilepsy with onset at approximately 4 years of age
6. Non-IGE patients with Benign Rolandic Epilepsy with onset at approximately 7 years of age
7. Patients who have no relatives or are adopted may also participate even if they have no genetically known relatives. Because they cannot be compared to their relatives, they can be compared with healthy controls matched by gender, age and ethnic groups.
8. Family members. Participation of subjects without symptoms, such as relatives, allows us to pinpoint the genes by comparing features between people with epilepsy with those who are not affected.
9. Patients with focal seizures or seizures due to trauma or stroke, or who have drop attacks can participate as control groups.

Rejection Criteria

1. Clinical judgment that the epilepsy is the result of metabolic problem or a degenerative disease such as progressive myoclonus epilepsy.
2. Myoclonic or myoclonus absence (absence accompanied by jerking)
3. Alcohol or other substance abuse.
4. Mental retardation
5. History of neuro-degenerative epilepsy in family
6. Severe psychiatric disorders

H- Recruitment of Subjects

We will recruit any healthy volunteer that may have an interest in the study. Our recruitment plan is to work by word of mouth mainly collecting subjects by asking colleagues, students in and around the medical center, and social acquaintances or any other individual for their cooperation or to aid us in finding appropriate subjects. This method has been approved previously by the IRB in protocol AAAA2441

We would also like to recruit controls following the procedures outlined in the attached document: recruitment procedures for healthy volunteers.

A collaborating neurologist and/or a primary care physician will identify potential participants. The collaborating physicians may be at Columbia University or at other Medical Centers. These collaborating physicians may be considered Principal Investigators at their institutions for the project and have obtained IRB approvals for recruitment of subjects. Other investigators may choose to refer patients to the study at Columbia, acting simply as referral centers. These collaborating physicians would not seek formal IRB approval. *

Subjects will be asked by their physician to sign an authorization that will allow a member of the study team to contact them by telephone, or physicians will obtain such consent verbally and note such consent in the patient's chart. This initial consent and contact will allow us to verify that no exclusion criteria exist.

As noted, we will determine if the patient meets initial inclusion criteria, either through a) consented pre-examination of the screened records or b) a patient-authorized brief telephone conversation. If the patient agrees, confirmation of diagnosis is assessed further by examination of his/her medical records, obtained by the potential subject's signed medical records release. Potential subjects also may contact the investigator so that they may discuss the study with a member of the research team. Only those patients, who after discussing the study with the cooperating physician, had agreed to be contacted by the research team will be approached about participation.



Authorized members of our study team will also travel to collaborating sites to a) remind physicians/staff about the goals and requirements of the study; b) obtain from collaborating physicians/staff the signed information sheets or brochures from patients who have agreed to be contacted; c) to prescreen medical records at collaborating sites where such pre-screening has been authorized. Study personnel also will recruit new collaborating sites by visiting such medical institutions at the invitation of cooperating physicians.

After confirmation of diagnosis we will invite the patients to participate in the study. Relatives of the patients will only be contacted with the patient's permission. Minors will be contacted with their parents and their own consent

I. Confidentiality of Study Data

Healthy control volunteers

No identifying information will be collected.

Patients and relatives.

For laboratory and statistical analyses and publication of any results from the study, patients and family members will be assigned a coded number and no personal information will be used. Identifying information and records will be kept in locked files at this institution. All information and data will be entered into a computer and stored using encrypted files. Data will be organized and managed using the data base management system MySQL. Access to the database is restricted to study personnel.

None of the information provided by one member of the family will be released to the participants.

Analysis of the sample for this study might reveal sensitive information about relationships in families (for example, adoptions or non-paternity). If this should occur, neither the participant nor any of the family members would be informed about it. This study does not involve genetic testing. It is aimed at developing such testing for the future, but cannot provide any meaningful information about participants at this time.

Genetic information, which is for research only, will be used only for the study and will not be available to anyone outside the study. Specific genotype information might be shared with other researchers for purposes of determining the epilepsy genes but no identifying information will be disclosed. Genotype information cannot be used for diagnosis of any form of epilepsy that we are studying.

All records connected with this study will be kept confidential to the extent permitted by law. Medical record in connection with this study is subjected to review by agents of OHRP and the National Institute of Health (NIH), the sponsor of this research in accordance with applicable laws and regulations. In addition the CUMC IRB will have access to the data. Information about participating subjects and results from this study will not be placed in their medical records. Neither an insurance company nor employers or potential employers will have access to the results of this study. No insurance company or any other person will be contacted.

We have been granted a Certificate of confidentiality from the National Institute of Neurological Disorders and Stroke for this study to protect the privacy of the participating subjects. This Certificate protects against the involuntary release of information about participants in the study.

The researchers involved in this project cannot be forced to disclose the identity of the participants in the study in any legal proceedings at the federal, state, or local level, regardless of whether they are criminal, administrative or legislative proceedings. However, the participant or the researcher may choose to disclose the protected information under certain circumstances. For example if the subject or guardian request disclosure of participation, the researchers will provide research data.

The Certificate does not protect against that voluntary disclosure. Disclosure will be necessary, however, upon request of DHH for audit or program evaluation purpose.



J. Potential Conflict of Interest

None

K. Location of the Study

Healthy control volunteers will be seen at the place they volunteer.

Patients and/or their relatives will obtain their own samples of saliva at the location of their choice including their own homes and mail them in prepaid pre-addressed envelopes to the researcher. The consent forms and instructions will be included in all packages.

We will phone the participants to ask them if they have any questions about the consent or the procedure for obtaining saliva. We will answer all questions. After we receive the signed consents we will call the participants to ask them questions about their medical and seizures history.

L. Potential Risks

There are no risks involved in collecting saliva.

There are no psychological risks from this genetic research because genetic information that may be obtained from this study does not have clinical diagnostic value. The study is not design to enable researchers to learn whether or not a specific family member carries a gene that raises risk for epilepsy. This is because most of the study's results will pertain to all of the families analyzed as a group, rather than to specific families or individuals.

In addition participants will not receive any medical or genetic information about themselves or their family members.

M. Potential Benefits

There are no specific benefits to the participants. The potential benefits of this study are the identification of human gene markers for IGE and the improved diagnosis of IGE in general. Both are essential steps in understanding the pathogenesis and natural history of the epilepsies. Society in general will benefit, as will the patients and their families, from the increased understanding of the inheritance of JME. The benefit to the individual and society is potentially great in alleviating suffering due to this and other forms of epilepsy.

N. Alternative Therapies

The alternative is not to participate

O. Compensation to Subjects

Compensation to participants will not be provided.

P. Costs to Subjects

All tests are done at no cost to participants; transportation expenses will be reimbursed.

Q. -Minors and Research Subjects

Consent and Assent forms attached

N. Radiation or Radioactive Substances

Not applicable