Towards identifying a potentially causal gene for hot water epilepsy

A submission for the partial fulfillment of MS of
the Integrated-PhD programme

By

Mariyam Abdullah

Molecular Biology and Genetics Unit
Jawaharlal Nehru Centre for Advanced Scientific Research
(A Deemed University)
Bangalore 560 064, India

April 2014

DECLARATION

I hereby declare that this thesis entitled "Towards identifying a potentially causal gene for hot water epilepsy" is an authentic record of research work carried out by me under the guidance of Prof. Anuranjan Anand in Molecular Biology and Genetics Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore. This work has not been submitted elsewhere for the award of any other degree.

In keeping the norm of reporting scientific observations, due acknowledgements have been made wherever the work described here has been based on the findings of other investigators. Any omission, which might have occurred by oversight or misjudgment, is regretted.

Mariyam Abdullah

Place: JNCASR, Bangalore

Date:

CERTIFICATE

This is to certify that the work described in this thesis entitled "Towards identifying a potentially causal gene for hot water epilepsy" is the result of the investigations carried out by Ms. Mariyam Abdullah in the Molecular Biology and Genetics Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, under my guidance.

The results presented in this thesis have not previously formed the basis for the award of any other diploma, degree or fellowship.

Anuranjan Anand

JNCASR, Bangalore

Date:

ACKNOWLEDGEMENTS

I have always felt that “Thanks” is a very small word for people who mean the world to me. This feeling is even more prominent as I attempt to express my gratitude to a few very special people.

I am deeply grateful to my guide, Prof. Anuranjan Anand. His valuable guidance and deep concern for his students has touched me more times than I can remember and his advice and approval has meant a lot to me over the months. All our discussions - some scientific and some a little less scientific - have been thought provoking and enlightening.

I would also like to acknowledge Professors Tapas Kundu, Kaustuv Sanyal, Namita Surolia, Hemalatha Balaram, Maneesha Inamdar, Ravi Manjithaya, Jayanta Haldar and G Ramesh for the interesting course work which gave a lot of insights in different avenues of biology. Professors Balasubramanian, Amitabh Joshi, Chandrabhas Narayana, Shivaprasad, Umesh Waghmare and Ms. Nabonita Guha deserve a special mention for their diverse and interesting thoughts on scientific communication.

I am deeply grateful to Professor Ranga UdayKumar for his valuable guidance during my lab rotation project. His dedication and approach to science is very inspiring and his rock solid knowledge about basic techniques and concepts in science has motivated me to strive for the same.

My time here has been educational and pleasant made memorable by all my labmates. I am particularly indebted to Praveen, Manpreet, Kalpita, Shalini, Shveta, Rammurthy, Sourav, Somya, Mohan and Chandru for their help, guidance and advice at all times. I would also like to acknowledge Anitha for all the help in DNA sequencing.

I would like to thank my friends Lakshmeesha, Shreyas, Sundar, Shilpee, Anjali, Malini, Dr. Anand, Prabhu, Shambhu, Rebu and Rishi for all the wonderful times we’ve had together. I am deeply indebted to my very close friends – Pooja, Sutanuka and Kirthana – for their love and concern at all times.

I now come to the people without whom any success in my life would not have been possible – my parents, my grandparents and my siblings, whose unwavering confidence in me kept me going even at the most difficult times; my husband, who gave me love, friendship and respect, whose confidence in me was at times more than my own, and who was an enthusiastic partner in all my undertakings; my parents and sister-in-law, whose love, support and encouragement have been deeply touching.

I would like to thank JNCASR for the fellowship. I thank hostel, mess, comp-lab, library, security, academic and administrative staff for all the help.

Finally, I thank God for leading me through every situation, for nothing is possible without his will.

Abbreviations

°C	Degree Celsius
ABI	Applied Biosystems
ADLTE	Autosomal Dominant Lateral Temporal lobe Epilepsy
AED	Anti-Epileptic Drugs
BDNF	Brain-Derived Neurotrophic Factor
BFNC	Benign Familial Neonatal Convulsions
CEPH	Centre D'Etudes Du Polymorphisme Humain
cM	Centimorgan
CT	Computed Tomography
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
EEG	Electroencephalogram
EIG	Epilepsy, Idiopathic Generalized, Susceptibility to
EtBr	Ethidium bromide
EVS	Exome Variant Server
FCMTE	Familial Cortical Myoclonic Tremor with Epilepsy
GABA	Gamma amino butyric acid
GABRG2	$GABA_A$ receptor gamma 1 subunit gene
GEPD	Generalized Epilepsy with Paroxysmal Dyskinesia
HGP	Human Genome Project
HWE	Hot water epilepsy
ILAE	International league against epilepsy
KCNA1	Potassium voltage-gated channel subfamily A member 1
KCNC2	Potassium voltage-gated channel subfamily C member 2
KCNMA1	Potassium large conductance calcium-activated channel, subfamily M, α member 1
LGL1	Late gestation lung protein 1
LOD	Logarithm of odds

Mb	Megabases
µg	Microgram
µl	Microliter
µM	Micromolar
ml	Milliliter
mM	Millimolar
min	Minutes
MGR1	Mitochondrial inner membrane i-AAA protease supercomplex subunit MGR1
MRI	Magnetic resonance imaging
mTOR	Mammalian targets of Rapamycin
NCBI	National Centre for Biotechnology Information
NEB	New England Biolabs
ng	Nanogram
NIMHANS	National Institute of Mental Health and Neurosciences
PCR	Polymerase Chain Reaction
REST	Repressor Element 1-Silencing Transcription factor
s	Seconds
SCN1A	Sodium channel protein type 1 subunit alpha
Taq	*Thermus aquaticus*
TRKB	Tropomyosin-Related Kinase B
U	Units
UK	United Kingdom
USA	United States of America
UTR	Untranslated regions
WHO	World Health Organization

Contents

1. Introduction

1.1 Epilepsy

Epilepsy is a chronic and non-communicable disorder of the brain. It is not a single disorder but a group of about 40 different neurological conditions. The most common type of epilepsy in adults is temporal lobe epilepsy and it usually follows a traumatic brain injury, although it can even be genetic (Goldberg and Coulter, 2013).

According to the World Health Organization, epilepsy is the most common serious brain disorder in the world. It usually affects adolescents or older people and is rarely seen in adults. Epilepsy is known to affect 3-40 people per 1000 worldwide. It is estimated that 60 million people in the world are living with epilepsy, 85% of which live in developing countries, and it accounts for about 45,000 deaths each year. However, for 80% of the patients diagnosed with epilepsy, the symptoms can be effectively controlled with the available medication. For the remaining 20%, surgical methods might be helpful.

Epilepsy is characterized by seizures which are a result of excessive and abnormal electrical discharges in the brain. The way in which a seizure manifests itself depends on a number of factors such as age of the individual, cause of the seizure and part of the brain affected. The diagnosis of the type of epilepsy and seizures is made with the help of cues from Electroencephalogram (EEG), Magnetic Resonance Imaging (MRI) and Computed Tomography (CT) scans.

Although epilepsy as a disorder can occur by itself, it is a common symptom of other disorders such as tumors, brain injury or infection. Genetically, epilepsy usually occurs due to mutation in a single gene thus affecting the function of the corresponding protein product. Ion channel genes like SCN1A, KCNA1 and KCNC2 have shown involvement in the manifestation of epilepsy (Goldberg and Coulter, 2013). In seven out of ten cases of epilepsy, the causes are unknown.

Current research in the field of epilepsy focusses on the identification of molecular signaling pathways, the disruption of which could lead to epileptic seizures. Pathways which have shown to have some link are the mTOR (mammalian Target of Rapamycin) pathway, the REST (Repressor Element 1-Silencing Transcription factor) pathway and brain-derived neurotrophic factor (BDNF)-tropomyosin-related Kinase B (TRKB) signaling (Goldberg and Coulter, 2013).

1.2 **Seizures**

Seizures occur when the normal activity of the brain is briefly disrupted. During a seizure, the neurons fire at about four times their normal rate, temporarily affecting the movements and thought processes of the individual. The sensations felt by the patient during a seizure depend on which part of the brain is affected and to what extent.

According to the ILAE (International League Against Epilepsy), seizures are broadly classified as generalized and partial (Figure 1.1). Generalized seizures can further be absence, myoclonic, tonic, clonic, tonic-clonic and atonic seizures. Partial seizures can be either simple partial or complex partial based on whether the patient retains awareness during the seizure or loses consciousness. Sometimes the partial seizures can proceed to secondary generalization.

Seizures can turn life threatening during Status Epilepticus, when the seizures last for more than 30 minutes. In these cases, the patient needs immediate medical attention.

1.3 Reflex/Sensory epilepsy

Seizures which occur in response to specific sensory stimuli constitute reflex or sensory epilepsies. In the 1989 ILAE classification, reflex epilepsies were described as 'Epilepsies characterized by seizures with specific modes of precipitation'. According to the new ILAE diagnostic scheme, 'Reflex' is the preferred name for such epilepsies (Panayiotopoulos CP, 2005) (Table 1.1).

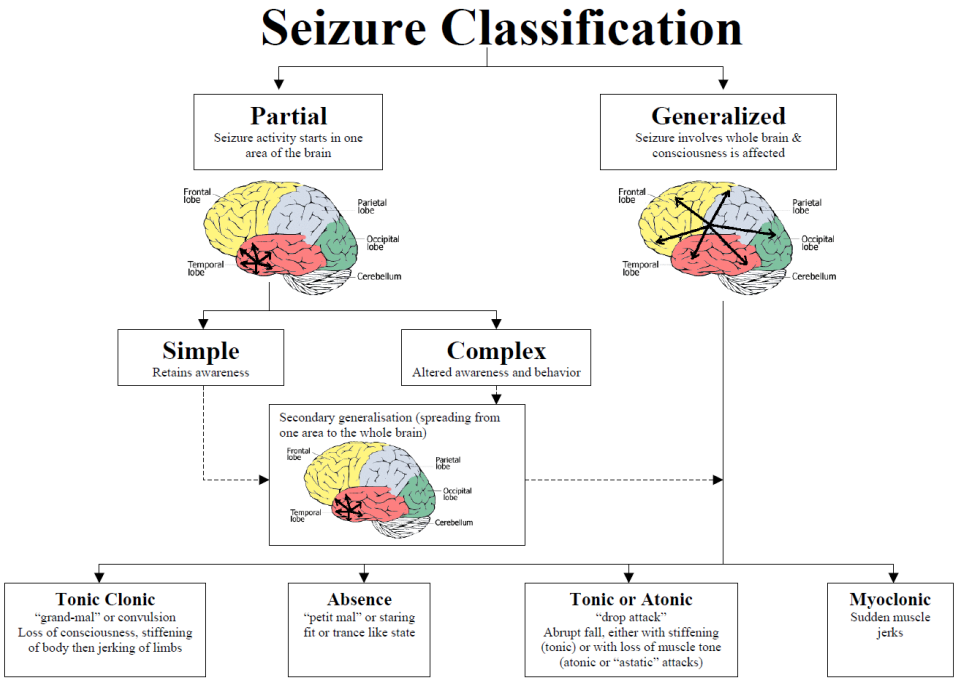

Figure 1.1: This figure shows the classification of seizures based on the part of the brain being affected and its symptoms (http://neurology.ufl.edu/patient-care/for-professionals/epilepsy/seizure-classification/).

Reflex seizures and its types have been known from centuries. Even before the invention of EEG, seizures induced by flashing lights were identified. A Greek gynecologist, obstetrician and pediatrician, Soranus, was one of the first to describe the stimulus of photosensitive epilepsy in his book, 'On Acute and Chronic Diseases', which was published in the second century A.D. Reflex epilepsy was first observed in animals in the 1920's and 1930's. The first medical observation by Gowers in 1881 recorded occipital seizures induced by light (Panayiotopoulos and Engel, 2004).

Stimuli for these epilepsies can be external or less often, internal mental processes (Striano et al, 2012). Triggers might include listening to music, reading, thinking, eating, playing chess, calculating, concentrating or playing piano. A number of studies have been done in case of visual or audiogenic stimuli (Reflex Epilepsy, 1975). This class of epilepsies is very useful in contributing to the understanding of how a normal brain rapidly transforms itself to an epileptic brain.

I. Somatosensory stimuli

1. ***Exteroceptive somatosensory stimuli***

a. Tapping epilepsy and benign childhood epilepsy with somatosensory evoked spikes
b. Sensory (tactile) evoked idiopathic myoclonic seizures in infancy
c. Tooth brushing epilepsy

2. ***Complex exteroceptive somatosensory stimuli***

a. Hot water epilepsy

3. *Proprioceptive somatosensory stimuli*

a. Seizures induced by movements
b. Seizures induced by eye closure and/or eye movements
c. Paroxysmal kinesiogenic choreoathetosis
d. Seizures induced by urination

4. Complex proprioceptive stimuli

a. Eating epilepsy

II. Visual stimuli

1. *Simple visual stimuli*

a. Photosensitive epilepsies (including self-induced photosensitive epilepsy)
b. Pattern-sensitive epilepsies (including self-induced pattern-sensitive epilepsy)
c. Fixation-off sensitive epilepsies
d. Scotogenic epilepsy

2. Complex visual stimuli and language processing (language-induced seizures)

a. Reading epilepsy
b. Graphogenic epilepsy

III. Auditory, vestibular, olfactory and gustatory stimuli

a. Seizures induced by pure sounds or words
b. Audiogenic seizures
c. Musicogenic epilepsy (and singing epilepsy)
d. Telephone-induced seizures
e. Olfacto-rhinoencephalic epilepsy
f. Eating epilepsy triggered by tastes
g. Seizures triggered by vestibular and auditory stimuli

IV. High-level process induced seizures (cognitive, emotional, decision-making tasks and other complex stimuli)

a. Thinking (noogenic) epilepsy
b. Reflex decision-making epilepsy
c. Epilepsia arithmetica (mathematica)
d. Emotional epilepsies
e. Startle epilepsy

Table 1.1: Reflex seizures, related reflex epilepsies and the precipitating stimuli (Adapted from Panayiotopoulos and Engel, 2004)

Photosensitive epilepsy is the most common type of reflex epilepsy, the trigger being flickering lights. It affects about three in every hundred people diagnosed with epilepsy. Seizures might involve impairment of consciousness, jerks in the arms or the whole body or absence seizures. These can later become generalized (Ferlazzo et al, 2005).

One type of reflex epilepsy is 'Rub epilepsy' wherein seizures are precipitated by prolonged rubbing of a delineated area of the body. Usually, the trigger zone is present only on one side of the body (Kanemoto et al, 2001). Another unusual type of reflex seizures occurs upon playing oriental card or board games. Its prevalence is higher among males and the seizures are mostly generalized (Lee et al, 2006). An astonishing report has described a patient developing seizures whenever he lied for business reasons: generalized seizures with loss of consciousness occurred in the patient (Sellal et al, 1993).

Rarely, reflex seizures might also be acquired. The most common causes are stroke, encephalitis and cortical dysplasia. They usually manifest in the form of startle seizures, as sudden contractions of the extremities (Yang et al, 2010).

1.3.1 Pathophysiology

Reflex seizures usually occur as a reproducible and time-dependent response to specific stimuli. Reflex seizures may be generalized such as absence seizures, myoclonic seizures or generalized tonic-clonic seizures, or focal such as visual, motor or sensory. The same patient may have either one or a combination of the above mentioned seizures. Usually, absence or myoclonic seizures might occur before proceeding to generalized tonic-clonic seizures (Panayiotopoulos, 2010).

In individuals where seizures are triggered by reading, the symptoms usually are jerking of the jaw or eyelids, generalized seizures or focal seizures which might progress to generalized tonic-clonic seizures (Bickford et al, 1956).

1.3.2 **Genetics of reflex epilepsies**

Although acquired lesions are known to play a role in reflex epilepsies, genetic predisposition is a more important and likely cause of reflex seizures.

Genetics of photosensitive epilepsy has been very well established. Family studies reveal a sibling risk of 20-50%. It shows an autosomal dominant pattern of inheritance; however, in some rare cases, it can also be autosomal recessive (Panayiotopoulos CP, 2005). Loci found till date in photosensitive epilepsy are 7q32 (Pinto et al, 2005), 16p13 (Pinto et al, 2007), 6p21 and 13q31 (Tauer et al, 2005). Also, chromosomal abnormalities in children are linked to photosensitive epilepsy (Grosso et al, 2006). There may also be complex inheritance with interaction of genetic and environmental factors (Ferlazzo et al, 2005).

A single gene identified in case of audiogenic epilepsy so far is *MGR1*, which maps to chromosome 5q14. This gene is also linked to febrile seizures. Although it is well established that malfunctioning of ion channel proteins might be involved in epilepsies, the identification of *MGR1* shows that this is not true in all cases (Ptacek and Fu, 2003). Animal models of audiogenic epilepsy also point to a genetic predisposition to develop seizures upon audiogenic stimuli (Midzyanovskaya et al, 2006, Faingold et al, 2010).

Mutations in the *LGl1* gene in 10q22-q24 might have consequences in cases of seizures provoked by speech or auditory stimuli (Brodtkorb et al, 2005).

1.4 **Hot water epilepsy**

Hot water epilepsy is a very interesting type of reflex epilepsy wherein seizures are triggered by pouring hot water over the head, face or trunk (Syed, 2010, Midi et al, 2005). Less often, seizures might occur during tub bathing or in a shower, or in very rare cases, upon pouring cold water (Panayiotopoulos and Engel, 2004). This type of epilepsy is also known as bathing epilepsy or water immersion epilepsy (Satishchandra, 2003). It is a benign type of reflex epilepsy. The mean age of onset is 13±11 years.

Hot water epilepsy was first reported in New Zealand in 1945, followed by reports of sporadic cases from Australia, USA, Canada, UK and Japan. However, the genetic basis of the phenotype has been established by studying families affected with hot water epilepsy from the southern parts of India. There have been reports of Indian families where hot water epilepsy shows an autosomal dominant inheritance. It is estimated that hot water epilepsy accounts for about 7% of all epilepsies in south India with a prevalence rate of 60-250 per 100,000 (Satishchandra, 2003).

A common practice among south Indians is to wash the head once every 3-15 days. The temperature of the water is usually 40–50 °C. Hot water epilepsy might occur in people bathing with a mug, shower or tub. Children are more frequently affected although adults are also affected. This is more prevalent among males than females in a ratio of 2:1. Upto about 10% of the patients develop seizures even upon pouring hot water over their body. The seizures may be generalized tonic-clonic (in 33% cases) or complex partial (in 67% cases) with or without secondary generalization. About 10% of these patients have self-induced seizures i.e. they enjoy the sensation of the seizure so much that they keep pouring hot water to keep experiencing the sensation. The duration of the seizure may be 0.5-3 minutes. Some of the patients may also go on to develop spontaneous seizures i.e. seizures occurring without any trigger (Satishchandra, 2003).

The probable reasons for this type of epilepsy are genetic and environmental factors, consanguineous marriages and the typical method of taking bath by pouring hot water over the head in these regions (Meghana et al, 2012). Family history of hot water epilepsy is seen in about 18% of HWE cases in south India. Apart from genetic reasons, epileptic seizures might also occur in cases of developmental brain abnormalities, brain injuries, birth traumas, brain tumors, strokes or brain infections. These factors might or might not be combined with a genetic pre-disposition.

1.4.1 **Electroencephalography (EEG)**

An EEG is a recording of the electrical activity of the brain. Seizures are induced in the patient and the EEG taken during the seizure reveals the brain wave pattern and helps in the identification of the type of brain disorder.

In most cases, the interictal scalp EEG (EEG taken between seizures) turns out normal, but a few cases might show some abnormalities. A few cases also show lateralized or localized spikes in the anterior temporal regions in EEG. As EEG also demonstrates focal spikes in frontal or temporal lobe, it further indicates that complex partial seizures are common in these cases (Satishchandra, 2003).

1.4.2 **Animal models of hot water epilepsy**

Experimental seizures have been induced in adult rats by exposing their heads to hot water. This is also called hyperthermic kindling. This is similar to seizures induced by exposure to sub-threshold electrical current. This might explain the mechanism of induction of seizures in humans (Satishchandra, 2003).

It has been seen that the hippocampus is very vulnerable to hyperthermic stimulation. So, hippocampal EEG of rats was taken to probe into the mechanisms of seizure induction. The rats were found to develop generalized clonic seizures with no tonic phase. A study of anti-epileptic medication in these rats revealed that Nifedipine, which is a calcium channel blocker, was able to curb seizures induced hyperthermically (Ullal et al, 1996).

1.4.3 **Case reports of hot water epilepsy**

Case report 1: A 32-year old, three months pregnant woman had started having seizures during bathing since her first month of pregnancy. Her features included auras, epigastric sensations and oral automatisms accompanied by a loss of consciousness. Seizures occurred upon pouring

hot water over the head and occurred about twice a month. There was no family history or past history of seizures (Milanlioglu et al, 2010).

Case report 2: A 5-year old Indian male child presented with seizures upon a warm water head bath. The child would lose consciousness and then sleep for one hour. In this case there was perhaps a genetic link: a female first cousin who had a history of *petit mal* epilepsy (Menon et al, 1989).

Case report 3: A six year old male child from North India had started having seizures since the age of three upon bathing with hot water. The symptoms were activity arrest, blank stare, uprolling of eyeballs, frothing from mouth, cyanosis and tonic-clonic movements. There was no history of febrile seizures or other neurologic abnormalities. Upon examination, the child was found to have seizures upon bathing with water at 38°C, but not with water at temperatures below 37°C (Sharma et al, 2002).

Case report 4: A 10 month old Belgian boy presented with activity arrest, unresponsiveness, hypotonia and cyanosis, 1 minute after contact with hot water (37 °C) and the symptoms lasted for 1 minute. There was no clonic phase and there was drowsiness after the attack. Bathing in lukewarm water (32°C) or showering did not help in prevention. There was no family history of epilepsy or febrile seizures (Keyzer et al, 2005).

Case report 5: A hot water epilepsy family from Turkey, in which seven individuals were affected across six generations has been reported. There was a consanguineous marriage in the family. Three of the affected patients had other types of seizures and two patients had a history of febrile seizures. The age of onset ranged from 6 to 14 years. In addition to seizures, three of the patients also had cerebral lesions, a possible effect of hot water seizures (Kaplan et al, 2009).

1.4.4 **Management of hot water epilepsy**

Management of hot water epilepsy constitutes more of prevention than cure. Seizures can be avoided by lowering the temperature of water used during bathing, showering, sponging rather

than pouring hot water over the head and reducing the duration of the bath (Panayiotopoulos and Engel, 2004). Conventional anti-epileptic drugs (AEDs) such as phenytoin or carbamazepine also help (Satishchandra, 2003). A single dose of clobazam can also be taken immediately before bathing to prevent seizures (Meghana et al, 2012).

2. Genetics of Family HWE 307

2.1 Background of the current study

Hot water epilepsy is a benign type of reflex epilepsy which is triggered by pouring hot water over the head, face or trunk during bathing. Thus, seizures are triggered in this type of reflex epilepsy by a specific thermal cutaneous stimulus. Very rarely, entry of water into the mouth might also trigger seizures (Panayiotopoulos, 2010). Hot water has been known to precipitate severe myoclonic seizures in infants, though hot water epilepsy is unique in that the seizures are generalized or partial tonic or clonic seizures. The exact mechanism of precipitation of seizures in hot water epilepsy is unknown, and sometimes seizures can also occur when hot water is not poured over the head (Syed, 2010).

Hot water epilepsy is apparently more common in India than in several other countries. This difference in prevalence may be due to environmental factors, bathing habits and genetic susceptibilities in the population (Syed, 2010). Hot water epilepsy is usually diagnosed in adolescence and the types of seizures are, more often, complex partial seizures with or without secondary generalization. Onset includes a dazed look, a sense of fear, incoherent speech and visual and auditory hallucinations (Satishchandra, 2003). A small group of patients have a history of febrile seizures in early childhood. Some patients might even go on to develop spontaneous seizures at a later stage. Hot water epilepsy is a geographically specific epilepsy syndrome where genetic factors might be combined with environmental factors to produce the symptoms (Syed, 2010). Family history of hot water epilepsy has been reported in 18% of patients from India and in 10% of patients from Turkey. Autosomal recessive inheritance in these regions is likely as the rate of consanguineous marriages is quite high (Panayiotopoulos, 2010).

In sporadic cases, the reasons might be abnormalities of the brain such as cortical malformations, which might lead to the presentation of hot water seizures in childhood. However, in cases where hot water epilepsy affects two or more members in a family, a genetic basis can usually be

uncovered (Hizem et al, 2012). Almost all people affected with hot water epilepsy are otherwise healthy with normal brain and neurologic examination (Panayiotopoulos and Engel, 2004).

There are no genes reported so far for hot water epilepsy. However, two loci are known to map at 4q24-q28 (Ratnapriya et al, 2009) and 10q21-q22 (Ratnapriya et al, 2009).

As the temperature of water is high (45 °C) in these cases, warmth receptors are known to be stimulated. Thus, a sudden exposure of a part of body to an increase in temperature in the form of hot water may stimulate an aberrant thermoregulatory centre in the hypothalamus and lead to seizures (Syed, 2010). It is also seen that in these patients, the time taken for the body temperature to return to normal after hot water bathing is higher compared to controls (Pavlidou et al, 2013). Seizures could also be precipitated due to a decreased ability of the body to inhibit afferent responses to somatosensory stimuli. Altered autonomic regulations or some form of channelopathies could also be the cause. Mutations in ion channel genes like SCN1A, chloride channels and calcium channel blockers have been implicated. Malfunctioning of blood brain barrier might also play a role in epileptic seizures. Another mechanism, called a 'domino effect', is also assumed to be a possible pathogenic cause of hot water epilepsy. Here, the rapid increase in body temperature might lead to hyperactivity of neurons which might in turn, activate the sympathetic nervous system. This might lead to an increase in vascular resistance and arterial blood pressure which can further lead to a seizure (Pavlidou et al, 2013). In these cases, usually, the cerebral cortex becomes hyperexcitable, and this could be due to the presence of an underlying lesion, mostly cortical dysplasia (Sharma et al, 2002).

Understanding the mechanisms of epileptogenesis of hot water epilepsy is important for therapeutic applications which might help to either reduce the seizure intensity or make the patient seizure free.

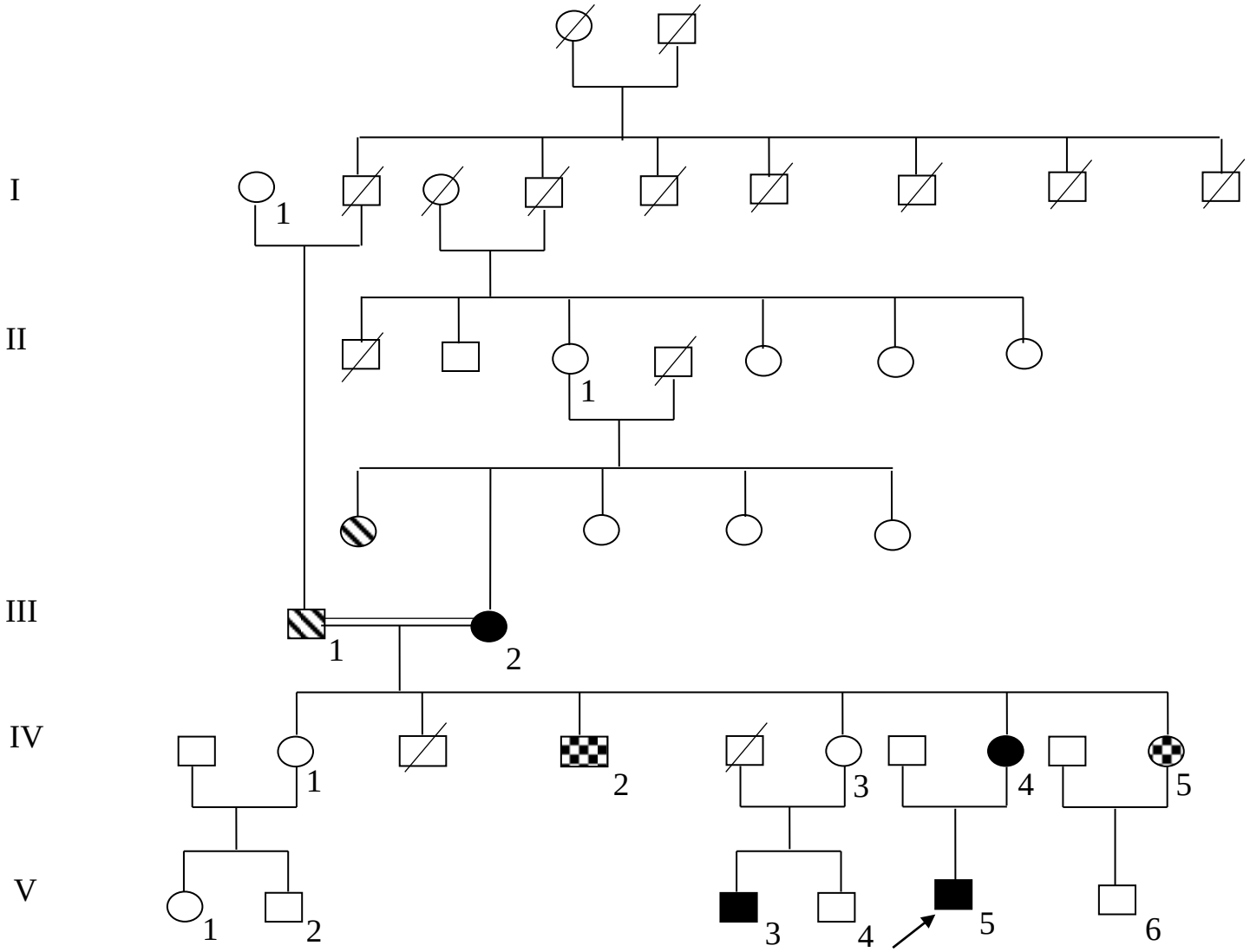

Figure 2.1: Pedigree chart of Family 307. The filled symbols indicate affected individuals and empty symbols, unaffected individuals. The solid symbols indicate individuals having the classic symptoms of HWE, i.e. GTCS. The symbols filled with lines indicate individuals with complex partial seizures and the symbols filled with checks indicate individuals having complex partial seizures with secondary generalization. The Roman numerals to the left of the pedigree denote generations and Arabic numerals beside the symbols denote individuals. The proband V:5 is indicated with an arrow.

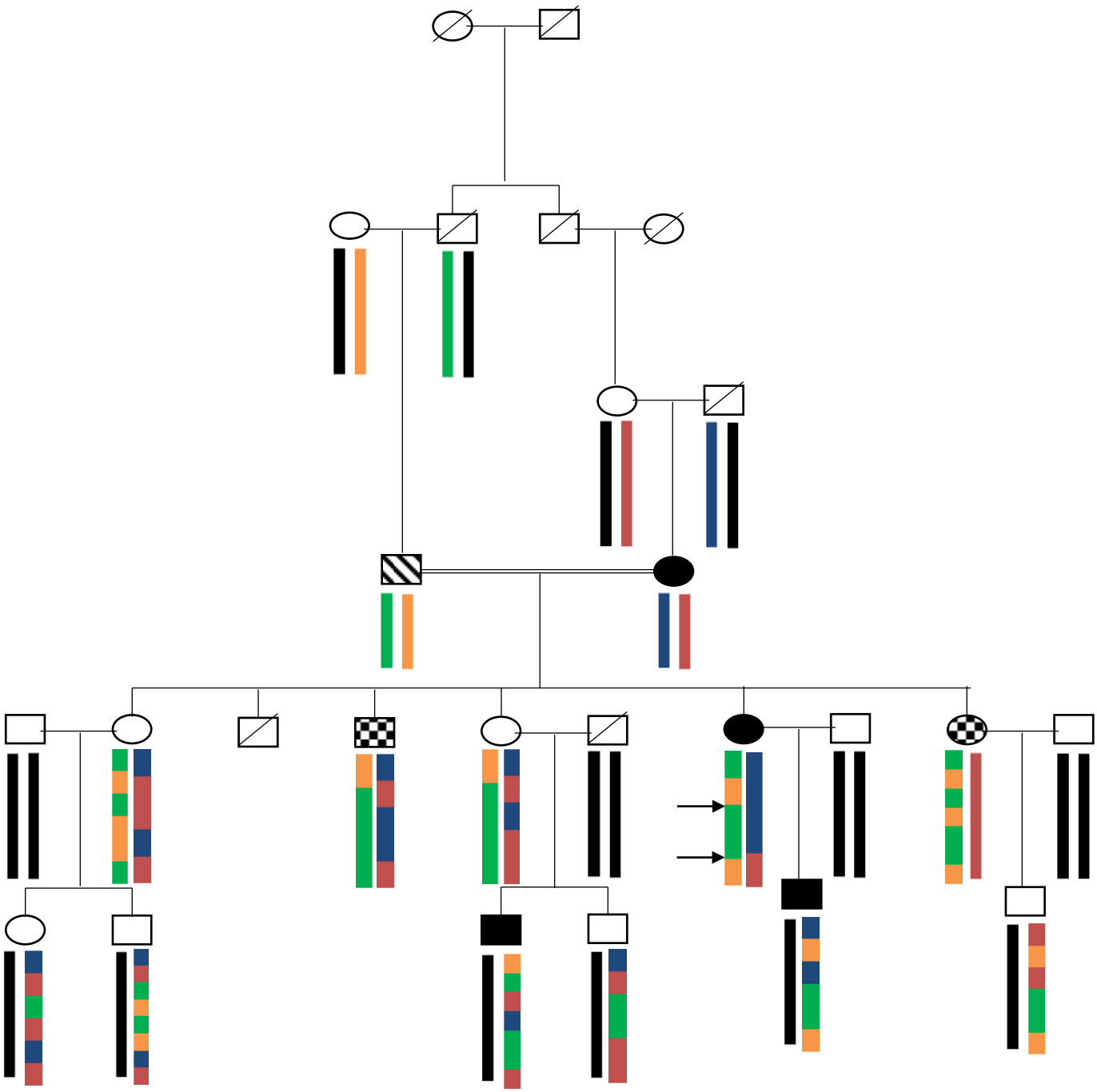

Figure 2.5: Haplotype of chromosome 5. The region of the green chromosome was found to segregate in the family with the disease phenotype. The arrows on the haplotype of individual IV:4 indicate the centromere-proximal and centromere-distal boundaries respectively.

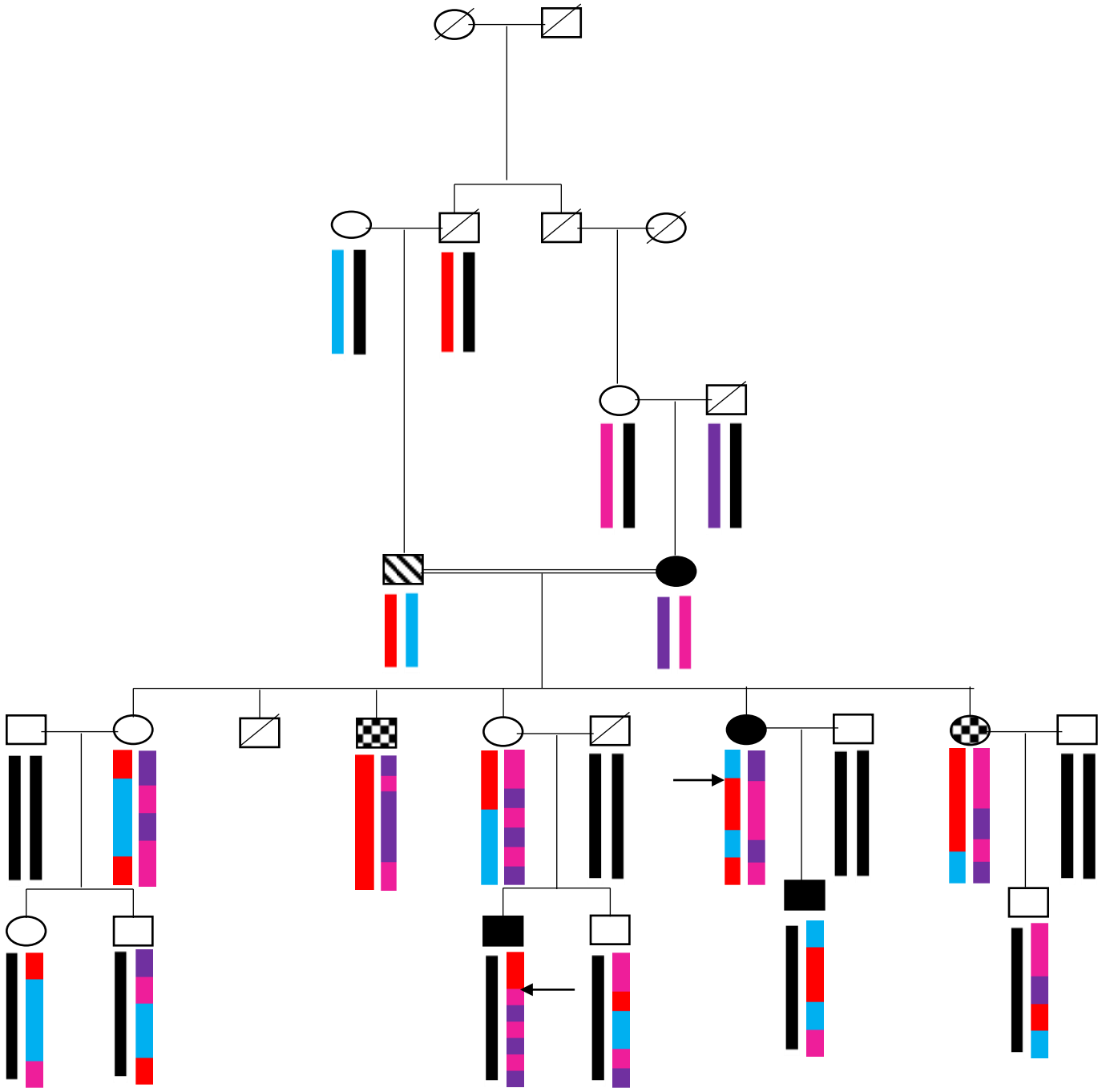

Figure 2.3: Haplotype of chromosome 10. The region of the red chromosome is found to segregate in the family with the disease phenotype. The arrows on the haplotypes of individuals IV:4 and V:3 indicate the centromere-distal and centromere-proximal boundaries respectively.

2.2 Aims and objectives

This study was taken up to examine the genetics of family HWE 307, in which hot water epilepsy shows an autosomal dominant pattern of inheritance with incomplete penetrance. The aim was to identify the locus which carries the causative mutation leading to the symptoms of hot water epilepsy in this family and eventually isolate the gene which carries the deleterious mutation.

2.3 Family pedigree

The HWE307 (Figure 2.1) has been ascertained at the Department of Neurology, National Institute of Mental Health and Neurosciences (NIMHANS), Bangalore. Each affected patient has been shown to experience seizures precipitated by a hot water bath. Information regarding the type of seizures, age of onset, temperature of water used for bathing and family history of epilepsy was obtained from the family members.

A 10 ml blood sample was collected from all available family members and DNA was isolated from peripheral white blood cells using a standard protocol (Sambrook and Russell, 2001).

The HWE307 has three generations of members affected with hot water epilepsy showing an autosomal dominant inheritance with incomplete penetrance. There are eight affected members in this family with a consanguineous marriage between III:1 and III:2. This family was ascertained by the proband, V:5 who began to exhibit symptoms of hot water epilepsy at the age of 8 years.

2.4 Clinical features of Family HWE307

Four members out of fifteen in the family show the classic symptoms of hot water epilepsy which is generalized tonic-clonic seizures. Two members show complex partial seizures and two members show complex partial seizures with secondary generalization. The average age of onset in this family is 21 years.

2.5 Genome wide linkage analysis

Whole genome wide linkage analysis was carried out using ABI Prism MD-10 linkage mapping set v2.5 (Applied Biosystems). This analysis consisted of 382 fluorescently labelled microsatellite markers spaced at a distance of 10 cM throughout the genome. Information about the markers was obtained from Genethon linkage maps (1996 Genethon Microsatellite Maps at Genlink Database; Dib et al, 1996). Following the protocol in the ABI user's manual, the individual DNA samples were used to amplify all the markers using the thermal cycler, GeneAmp® PCR System 9700 (Applied Biosystems). In addition to the DNA samples of members of family 307, DNA of a reference individual (CEPH 1347-02) with known genotype was amplified for each marker. The amplified products were pooled and mixed with Hi-Di formamide (Applied Biosystems) and LIZ-500 internal size standard (Applied Biosystems) and denatured at 95 °C for 5 min. The products were loaded onto an ABI3100 Genetic Analyzer (Applied Biosystems) and electrophoresed. Allele were called using Genemapper v3.7 (Applied Biosystems) and the inheritance pattern of the alleles was checked for Mendelian inconsistencies in the family.

Seventeen additional microsatellite markers - D5S2098, D5S1984, D5S500, D5S2017, D5S2090, D5S2014, D5S2112, D5S2066, D5S415, D5S671, D5S625, D5S429, D5S2069, D5S498, D5S2034, D5S2030 and D5S2006 - were analyzed for the region on chromosome 5q31.3-q35.3 to confirm linkage and to refine the recombination boundaries. These additional markers spanned about 62 cM between 135 – 197 cM genetic positions, with an average spacing of 3.6 cM.

The amplification of each primer was carried out in a 10 µl reaction containing 50 ng of genomic DNA, 0.5 µl of each marker-specific primer (5 µM) and 4.5 µl of ABI Prism® True Allele® PCR premix containing an optimized mixture of AmpliTaq Gold® DNA Polymerase, buffer, magnesium chloride and dNTPs. The reaction mix was made up to 7.5 µl with the help of autoclaved MilliQ. The amplification cycle was as follows: initial denaturation at 94 °C for 12 min, followed by 10 cycles of denaturation at 94 °C for 15 s, annealing at 55 °C for 15 s,

extension at 72 °C for 30 s, followed by 20 cycles of denaturation at 89 °C for 15 s, annealing at 55 °C for 15 s, extension at 72 °C for 30 s and a final extension at 72 °C for 10 min.

2.6 Haplotyping and LOD score analysis

Linkage analysis was performed using MLINK of LINKAGE v5.2, considering autosomal dominant inheritance with 50-90% penetrance, 1-5% phenocopy, a disease allele frequency of 0.0001 and equal male and female recombination rates. Haplotypes were constructed manually for all chromosomes following the marker order of the Genethon linkage map.

2.7 Candidate gene screening – Chromosome 10

The total number of protein coding genes found in the desired region are 100. All the genes in this region were searched in databases like National Centre for Biotechnology Information (NCBI), Uniprot and Pubmed, and a list of 20 genes was drawn up based on their involvement in neurological phenotypes, their expression and function in the brain (Table 2.1). These genes were analyzed by direct sequencing of the DNA sample of the proband V:5.

GAD2	Epilepsy - auto antibodies found
NMT2	Epilepsy in fowl, human brain tumors
PLXDC2	Sotos syndrome
OPTN	Amyotrophic Lateral Sclerosis, Huntington's disease
RSU1	Glioblastoma, glioma, oligodendroglioma, Alzheimer's disease
YME1L1	Glioma - seizures in 50 - 80% cases
ZEB1	Glioblastoma - seizures in 30 - 40% cases
MLLT10	Meningioma - seizures in 30 - 40% cases
CUBN	Alzheimer's disease, Neural tube defects, Spina Bifida
MPP7	Alzheimer's disease
FRMD4A	Alzheimer's disease
ARL5B	Alzheimer's disease
CELF2	Late onset Alzheimer's disease
SVIL	Multiple sclerosis
ZNF438	Multiple sclerosis risk
RAB18	Warburg microsyndrome Type 3

MTPAP	Ataxia - seizures might occur
ITGA8	Schizophrenia, Parkinson's disease
PIP4K2A	Schizophrenia
SLC39A12	Schizophrenia

Table 2.1: List of genes screened in chromosome 10

Primers were designed for all candidate genes using PRIMER3, covering all coding regions, adjacent exon-intron boundaries and parts of 5'- and 3'-UTR regions. The primers were designed based on the reference sequence obtained from NCBI. The PCR conditions were standardized for each primer set and PCR was done using the thermal cycler, GeneAmp® PCR System 9700 (Applied Biosystems). The PCR reaction mix contained 1X *Taq* Polymerase Buffer, 0.8 mM dNTPs, 1.5 mM $MgCl_2$, 0.25 µM of each primer, 0.05 U/µl *Taq* Polymerase (NEB) and 5 ng/µl DNA in a 20 µl reaction. The PCR products were electrophoresed on 1.5% agarose gel containing 2 µl EtBr (2 µg/ml), and purified using a Millipore vacuum manifold plate. The sequencing reactions were performed using 0.8 µl of BigDye® Terminator v3.1 Cycle Sequencing reaction mix, 1X sequencing buffer, 0.25 µM primer and 2 µl of purified PCR product in a 20 µl reaction mix. The following cycling conditions were used: initial denaturation at 95 °C for 1 minute, followed by 25 cycles of denaturation at 96 °C for 10 seconds, annealing at 50 °C for 5 seconds and extension at 60 °C for 4 minutes, and a final hold at 4 °C. Sequencing was done using an automated DNA sequencer.

Novel variations obtained in the sequences were confirmed by sequencing using both forward and reverse primers. Variants obtained in the sequence were detected by comparing the sequences of the candidate genes in the proband sample with the reference sequence from NCBI using SeqMan v5.01.

All variations obtained were checked in databases like NCBI, 1000 Genomes and Exome Variant Server for allele frequencies. Variations that were novel or were present at very low frequency in the population were analyzed by sequencing all members of the family to check for segregation. Variants which were found to be segregating in the family were analyzed for allele frequencies by sequencing control samples.

2.8 Candidate gene screening – Chromosome 5

The same experimental and analysis approach was followed for the region on chromosome 5. The total number of protein coding genes found here were 239. All the genes in the list were searched in databases like NCBI, Uniprot and Pubmed and a list of 28 genes was drawn up based on their involvement in neurological phenotypes and their expression and function in the brain (Table 2.2). These genes were analyzed by direct sequencing of the DNA sample of the proband V:5.

GM2A	GM2-gangliosidosis AB
TCERG1	Autism, Huntington's disease
PPARGC1B	Huntington's disease
FBXW11	Holoprosencephaly
NSD1	Sotos syndrome, Weaver syndrome, Beckwith-Wiedemann syndrome
PDGFRB	Fahr's syndrome
PTTG1	Systemic Lupus Erythematosus - seizures develop in 9 to 58% cases, Brain tumors, Glioma
TNIP1	Systemic Lupus Erythematosus - seizures develop in 9 to 58% cases
HMP19	Glioma
SH3RF2	Autism - seizures in 20-40% cases
SAP30L	Alzheimer's disease - seizures in 17% cases
DBN1	Alzheimer's disease - seizures in 17% cases
CPLX2	Behcet's disease - Epileptic seizures seen in about 5% of cases, Schizophrenia
CAMK2A	Wilson's disease - low prevalence of seizures
KCNIP1	Multiple sclerosis - sezures occur in a minority of cases, Schizophrenia related features
IL12B	Multiple sclerosis - sezures occur in a minority of cases
LOC285626	Multiple sclerosis - sezures occur in a minority of cases
RGS14	Multiple Sclerosis
SGCD	Multiple sclerosis
BNIP1	Down syndrome - mental retardation, high chance of developing seizures and epilepsy
GABRP	Schizophrenia
GPRIN1	Schizophrenia
NMUR2	Schizophrenia - risk factor for epilepsy
GRIA1	Schizophrenia - risk factor for epilepsy, Bipolar disorder
ADAM19	Schizophrenia

CLINT1	Schizophrenia
B4GALT7	Ehler's Danlos syndrome - may be accompanied by epilepsy
CSF1R	Gliosis - might/might not develop seizures

Table 2.2: List of genes selected in chromosome 5. The genes highlighted in red indicate ones that have been sequenced in the proband.

Primers were designed for all candidate genes using PRIMER3, covering all coding regions, adjacent exon-intron boundaries and parts of 5'- and 3'-UTR regions as mentioned above. The primers were designed based on the reference sequence obtained from NCBI. The PCR conditions were standardized for each primer set and PCR was done according to the above mentioned reaction conditions. The PCR products were purified using a Millipore vacuum manifold plate and the sequencing reactions were performed as mentioned above.

Novel variations obtained in the sequences were confirmed by sequencing using both forward and reverse primers. Variants obtained in the sequence were detected by comparing the sequences of the candidate genes in the proband sample with the reference sequence from NCBI using SeqMan v5.01.

All variations obtained were checked in databases like NCBI, 1000 Genomes and Exome Variant Server for allele frequencies.

2.9 Results

Whole genome wide linkage analysis of Family307 revealed three regions, which could possibly be linked to the disease phenotype. They were on chromosomes 3, 5 and 10 based on a suggestive LOD score obtained for markers in these regions (Tables 2.3, 2.4 and 2.5). Maximum LOD scores were obtained at 90% penetrance value, 1% phenocopy and recombination faction (Θ) = 0 for markers D3S1601 at 1.14, D5S400 at 1.22 and D10S1653 at 1.39. These regions were analyzed further for a segregating haplotype i.e. a region of chromosome that would be present in all affected members and absent in all unaffected members implying the presence of a possible causative mutation in this region. No such haplotype was seen in case of chromosome

3, despite having a positive LOD score and hence it was excluded from further analysis. Segregating haplotypes were seen in chromosome 10 and 5 and hence, were carried forward for further analysis.

MARKERS	Recombination Faction (Θ)				
	0	**0.1**	**0.2**	**0.3**	**0.4**
D3S1297	-0.57	-0.32	-0.14	-0.05	-0.01
D3S1304	-0.33	-0.18	-0.08	-0.02	0.00
D3S1263	-0.57	-0.32	-0.14	-0.05	-0.01
D3S2338	-0.31	-0.18	-0.09	-0.03	-0.01
D3S1277	-0.35	-0.16	-0.05	0.00	0.00
D3S1289	0.40	0.30	0.18	0.08	0.02
D3S1300	-0.37	-0.20	-0.10	-0.04	-0.01
D3S1285	-0.05	-0.01	0.03	0.03	0.01
D3S1566	-0.61	-0.18	-0.06	-0.01	0.00
D3S3681	0.05	0.07	0.07	0.03	0.03
D3S1271	0.19	0.16	0.11	0.11	0.11
D3S1278	-0.25	-0.10	-0.03	0.00	0.00
D3S1267	-0.25	-0.11	-0.03	0.00	0.00
D3S1292	-1.10	-0.44	-0.17	-0.05	-0.01
D3S1569	-0.70	-0.07	0.01	0.02	0.01
D3S1279	0.24	0.26	0.19	0.11	0.03
D3S1614	0.64	0.55	0.39	0.20	0.05
D3S1565	-0.86	-0.12	0.00	0.02	0.01
D3S1262	-1.10	-0.41	-0.20	-0.08	-0.02
D3S1580	-0.54	0.10	0.06	0.01	0.00
D3S1601	1.14	0.72	0.34	0.10	0.01
D3S1311	-0.51	-0.27	-0.13	-0.05	-0.01

Table 2.3: LOD scores on chromosome 3. The highlighted row indicates the highest LOD score obtained for marker D3S1601.

MARKERS	Recombination faction (Θ)				
	0	**0.1**	**0.2**	**0.3**	**0.4**
D5S1981	0.00	-0.02	-0.01	0.00	0.01
D5S406	-0.31	-0.21	-0.12	-0.05	-0.01

D5S630	-1.48	-0.36	-0.10	-0.01	0.01
D5S416	-0.18	-0.06	-0.02	0.00	0.00
D5S419	0.28	0.16	0.08	0.03	0.01
D5S426	-0.27	-0.03	0.04	0.05	0.02
D5S418	-0.56	-0.21	-0.07	-0.02	0.00
D5S407	-0.89	-0.36	-0.14	-0.03	0.00
D5S647	0.39	0.32	0.16	0.04	0.00
D5S424	-0.17	-0.09	-0.05	-0.02	-0.01
D5S641	-0.75	-0.21	-0.03	0.02	0.01
D5S428	-0.32	0.18	0.15	0.06	0.01
D5S644	0.04	0.29	0.24	0.14	0.04
D5S433	0.59	0.37	0.19	0.07	0.02
D5S2027	-0.66	-0.28	-0.12	-0.04	-0.01
D5S471	-1.25	-0.34	-0.11	-0.02	0.00
D5S2115	0.00	0.00	0.00	0.00	0.00
D5S436	-0.31	-0.08	0.01	0.03	0.01
D5S410	-0.12	0.06	0.08	0.05	0.02
D5S422	0.16	0.22	0.18	0.10	0.03
D5S400	1.22	0.89	0.55	0.25	0.06
D5S408	-0.47	-0.23	-0.12	-0.05	-0.02

Table 2.4: LOD scores on chromosome 5. The highlighted row indicates the highest LOD score obtained for marker D5S400.

	Recombination Faction (Θ)				
MARKERS	**0**	**0.1**	**0.2**	**0.3**	**0.4**
D10S249	-0.86	-0.39	-0.14	-0.04	0.00
D10S591	-0.24	-0.19	-0.13	-0.07	-0.02
D10S189	-1.34	-0.36	-0.15	-0.06	-0.01
D10S547	-1.07	-0.18	-0.04	0.00	0.01
D10S1653	1.39	1.01	0.63	0.29	0.06
D10S548	-0.21	-0.07	-0.02	0.00	0.00
D10S197	0.23	0.19	0.12	0.05	0.00
D10S208	-1.47	-0.27	-0.06	0.01	0.01
D10S196	-0.77	0.05	0.09	0.06	0.02
D10S1652	0.27	0.32	0.20	0.09	0.02
D10S537	0.22	0.21	0.15	0.07	0.02
D10S1686	-1.52	-0.30	-0.10	-0.03	-0.01
D10S185	-1.22	-0.18	-0.05	-0.02	-0.01

D10S192	-0.23	-0.05	0.01	0.02	0.01
D10S597	-0.21	-0.12	-0.06	-0.03	-0.01
D10S1693	-1.65	-0.16	0.00	0.02	0.00
D10S587	0.43	0.46	0.34	0.17	0.04
D10S217	0.43	0.46	0.34	0.17	0.04
D10S1651	-0.68	-0.36	-0.15	-0.04	0.00
D10S212	-1.74	-0.69	-0.33	-0.14	-0.03

Table 2.5: LOD scores on chromosome 10. The highlighted row indicates the highest LOD score obtained for marker D10S1653.

The region on chromosome 10 which segregated with the disease phenotype in the family was a 3-marker region between markers D10S1653 and D10S197 (Figure 2.2). The centromere-distal boundary was determined by recombination between markers D10S547 and D10S1653 in individual IV-4. The centromere-proximal boundary was determined by recombination between markers D10S197 and D10S208 in individual V-3 (Figure 2.3). The region of interest spans 21 Mb on chromosome 10p12.1-p15.1 and corresponds to a 32 cM genetic interval. The total number of protein coding genes in this region is 100.

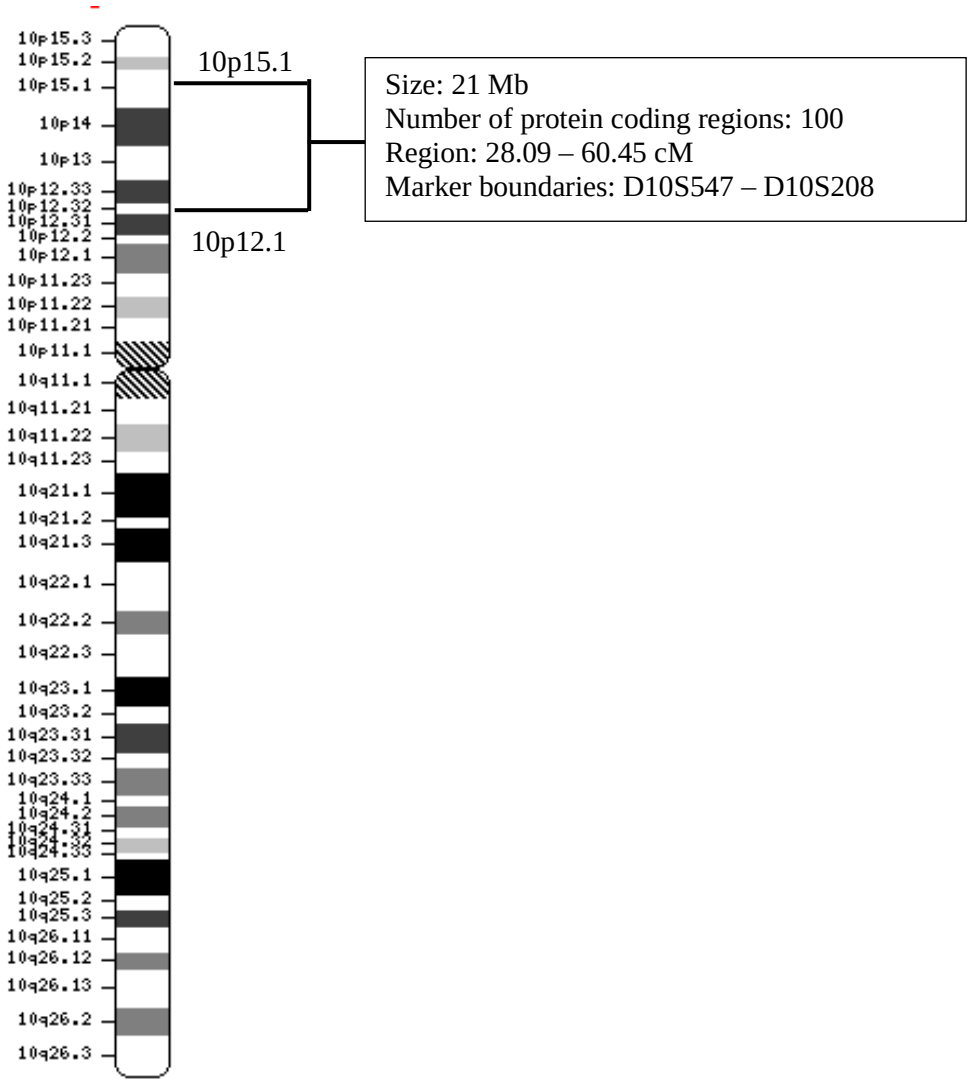

Figure 2.2: Ideogram and features of the critical region on chromosome 10. Adapted from NCBI Mapviewer.

The region on chromosome 5 which segregated with the disease phenotype was a 4-marker region between markers D5S436 and D5S400 (Figure 2.4). Additional markers analyzed in this region – D5S2098, D5S1984, D5S500, D5S2017, D5S2090, D5S2014, D5S2112, D5S2066, D5S415, D5S671, D5S625, D5S429, D5S2069, D5S498, D5S2034, D5S2030 and D5S2006 – further helped in refining the recombination boundaries. The centromere-proximal boundary was determined by recombination between markers D5S2017 and D5S436 in individual III-5. The centromere-distal boundary was determined by recombination between markers D5S2034 and D5S2030 in individual III-4 (Figure 2.5). This region spans 36 Mb on chromosome 5q31.3-

q35.3 and corresponds to a 51 cM genetic interval. The total number of protein coding genes in this region is 239.

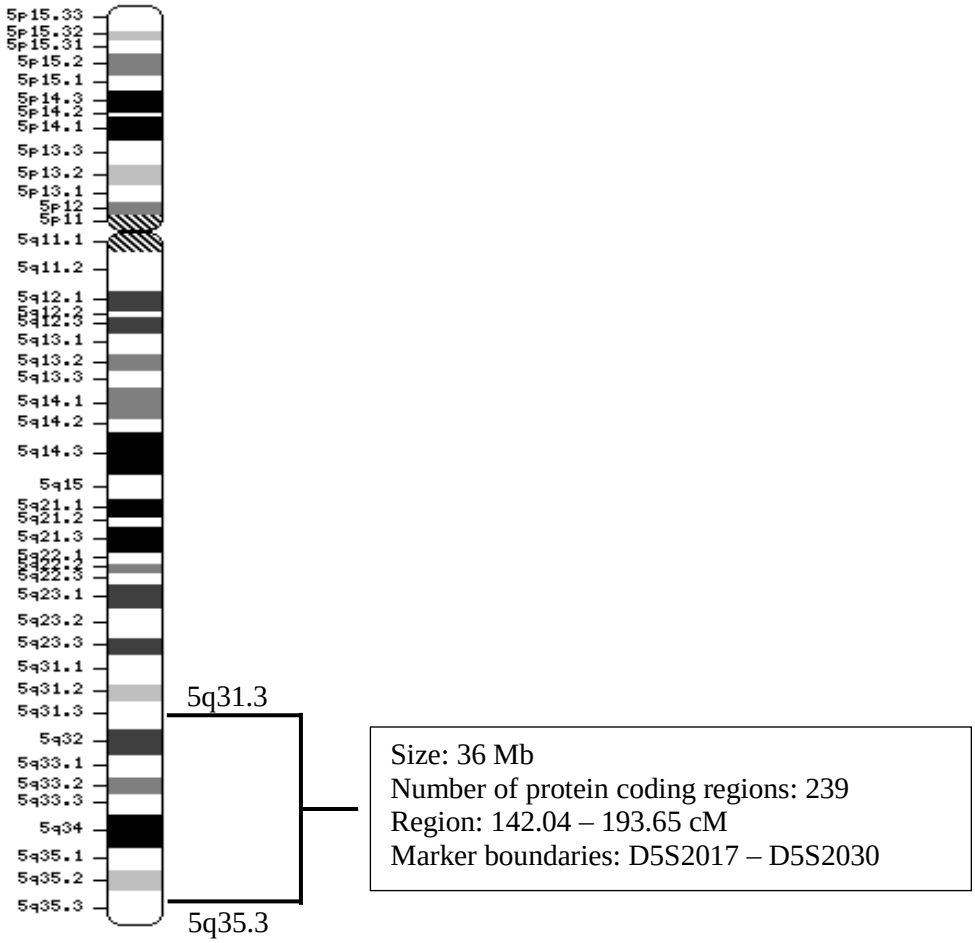

Figure 2.4: Ideogram and features of the critical region on chromosome 5. Adapted from NCBI Mapviewer.

The next step was to take a look at the genes in these regions to identify a possible player in a neurological pathway, the disruption of which could lead to seizures upon contact with hot water. Due to the extensive sequencing done during the Human Genome Project, substantial information is available about the human genome and both the regions of interest on chromosomes 10 and 5 are known to harbor genes with important functions in the human brain. The list of genes was downloaded from NCBI Mapviewer and an extensive literature search was done. Based on information obtained from databases like NCBI, Uniprot and Pubmed regarding

involvement of these genes in neurological phenotypes and/or pathways, a small number of genes were selected from both regions. These genes were sequenced in the proband's DNA sample to look for any possible sequence variants that could prove causative.

The list of all variations that were found to be reported in NCBI, 1000genomes or Exome Variant Server are listed in Table 2.6. These variations were found to be quite common in the control population according to the above mentioned databases and hence were not analyzed further.

Gene	Gene sequence variant	Predicted effect on protein	SNP id	Frequency of minor allele in control population
GAD2	NM_000818.2:c.-525G>C		rs2236417	0.25
GAD2	NM_000818.2:c.-243A>G		rs2236418	0.40
GAD2	NM_000818.2:c.287-125C>G		rs7071922	0.46
GAD2	NM_000818.2:c.1158-183A>G		rs8190748	0.45
GAD2	NM_000818.2:c.1387-73G>C		rs3824694	0.36
GAD2	NM_000818.2:c.*597A>G		rs4749110	0.25
NMT2	NM_004808.2:c.510+39T>C		rs41284465	0.01
NMT2	NM_004808.2:c.719+97C>T		rs142602430	0.01
NMT2	NM_004808.2:c.1170+68T>G		rs10906832	0.25
NMT2	NM_004808.2:c.1171-12T>C		rs7072269	0.04
PLXDC2	NM_032812.7:c.-73T>C		rs989767	0.31
PLXDC2	NM_032812.7:c.541+15A>G		rs7072650	0.23
PLXDC2	NM_032812.7:c.541+99A>G		rs75861169	0.03
PLXDC2	NM_032812.7:c.665-16T>C		rs41277354	0.02
PLXDC2	NM_032812.7:c.1122+85T>G		rs10827997	0.31
PLXDC2	NM_032812.7:c.1123-29T>C		rs12783379	0.39
PLXDC2	NM_032812.7:c.1186G>A	p.Val396Ile	rs3817405	0.44
PLXDC2	NM_032812.7:c.1372A>G	p.Ile458Val	rs2778979	0.21
PLXDC2	NM_032812.7:c.1474-108T>C		rs2681949	0.21
OPTN	NM_001008212.1:c.-36G>A		rs41291309	0.03
OPTN	NM_001008211.1:c.102G>A	p.Thr34=	rs2234968	0.18
OPTN	NM_001008211.1:c.553-5C>T		rs2244380	0.21
OPTN	NM_001008211.1:c.780-53T>C		rs765884	0.19
OPTN	NM_001008211.1:c.882+109A>G		rs489040	0.48
OPTN	NM_001008211.1:c.964G>C	p.Glu322Gln	rs523747	0.01

OPTN	NM_001008211.1:c.1532+72G>A		rs77873111	0.02
OPTN	NM_001008211.1:c.1612+101A>C		rs7086894	0.33
OPTN	NM_001008211.1:c.1613-48C>A		rs10906310	0.27
RSU1	NM_012425.3:c.-25G>C		rs1130684	0.24
YME1L1	NM_001253866.1:c.34-25G>A		rs10764675	0.34
YME1L1	NM_001253866.1:c.168+1540T>G		rs3780926	0.43
YME1L1	NM_139312.2:c.339+132T>G		rs3780926	0.43
YME1L1	NM_001253866.1:c.169-155G>A		rs2274635	0.34
YME1L1	NM_001253866.1:c.205C>T	p.Leu69=	rs2274634	0.34
YME1L1	NM_001253866.1:c.592+16A>G		rs2275752	0.28
YME1L1	NM_001253866.1:c.851-41T>A		rs2274743	0.34
YME1L1	NM_001253866.1:c.1621-57G>A		rs7901976	0.42
YME1L1	NM_001253866.1:c.1822-144C>G		rs10829187	0.09
YME1L1	NM_001253866.1:c.1909-7G>A		rs11015538	0.34
ZEB1	NM_001128128.2:c.637-15G>A		rs220060	0.06
CUBN	NM_001081.3:c.387+145A>G		rs2295812	0.18
CUBN	NM_001081.3:c.489+35A>C		rs2273737	0.06
CUBN	NM_001081.3:c.489+42C>T		rs41289315	0.06
CUBN	NM_001081.3:c.594-34G>A		rs2295813	0.10
CUBN	NM_001081.3:c.618C>T	p.Tyr206=	rs41289313	0.05
CUBN	NM_001081.3:c.720+68C>T		rs45498199	0.06
CUBN	NM_001081.3:c.758T>C	p.Phe253Ser	rs1801222	0.23
CUBN	NM_001081.3:c.1165C>A	p.Pro389Ala	rs1801224	0.44
CUBN	NM_001081.3:c.1765+52G>T		rs1033765	0.29
CUBN	NM_001081.3:c.1911C>T	p.Leu637=	rs41289311	0.03
CUBN	NM_001081.3:c.2791+9C>T		rs10795440	0.11
CUBN	NM_001081.3:c.2791+130G>A		rs4747297	0.11
CUBN	NM_001081.3:c.3417A>G	p.Leu1139=	rs1801228	0.03
CUBN	NM_001081.3:c.4168+151T>C		rs3740164	0.32
CUBN	NM_001081.3:c.4350+52G>A		rs2291521	0.17
CUBN	NM_001081.3:c.4563T>A	p.Ile1521=	rs1801229	0.37
CUBN	NM_001081.3:c.4675C>T	p.Pro1559Ser	rs1801231	0.37
CUBN	NM_001081.3:c.4695+79G>A		rs3012484	0.37
CUBN	NM_001081.3:c.4695+108T>A		rs2932895	0.37
CUBN	NM_001081.3:c.6463-22A>C		rs1276713	0.14
CUBN	NM_001081.3:c.7705+60T>G		rs780804	0.32
CUBN	NM_001081.3:c.7705+106C>T		rs780805	0.43
CUBN	NM_001081.3:c.7912+25T>C		rs780807	0.25
CUBN	NM_001081.3:c.8062+59delC		rs5783520	0.42
CUBN	NM_001081.3:c.8905+137T>A		rs780847	0.24
CUBN	NM_001081.3:c.8906-127T>C		rs780843	0.45

CUBN	NM_001081.3:c.9843A>G	p.Thr3281=	rs703064	0.32
CUBN	NM_001081.3:c.10363-131C>T		rs3740169	0.31
MPP7	NM_173496.3:c.37+70_37+71insT		rs56069980	0.15
MPP7	NM_173496.3:c.235-79T>C		rs41306320	0.03
MPP7	NM_173496.3:c.315+157A>G		rs17684826	0.03
MPP7	NM_003174.3:c.827+13T>C		rs10159964	0.31
MPP7	NM_173496.3:c.887+69G>A		rs10763644	0.41
MPP7	NM_173496.3:c.952+37C>T		rs78800124	0.04
MPP7	NM_173496.3:c.952+619C>T		rs57575764	0.06
MPP7	NM_173496.3:c.952+1050G>A		rs10826402	0.41
MPP7	NM_173496.3:c.965A>G	p.Lys322Arg	rs2997211	0.08
MPP7	NM_173496.3:c.1205-173G>A		rs79866904	0.05
MPP7	NM_173496.3:c.1205-143G>A		rs1361922	0.26
MPP7	NM_003174.3:c.1663C>A	p.Arg555=	rs7070678	0.36
MPP7	NM_173496.3:c.1299-83C>T		rs12570424	0.40
MPP7	NM_173496.3:c.1407+908T>G		rs7921181	0.40
MPP7	NM_173496.3:c.1542A>G	p.Lys514=	rs75802203	0.05
MPP7	NM_003174.3:c.2238+4259T>C		rs16930338	0.04
MPP7	NM_173496.3:c.*254C>A		rs12249305	0.12
FRMD4A	NM_018027.3:c.46-31962G>A		rs2027551	0.04
FRMD4A	NM_018027.3:c.46-31715A>G		rs1544021	0.02
FRMD4A	NM_018027.3:c.46-31617G>T		rs2257199	0.01
FRMD4A	NM_018027.3:c.46-113042G>T		rs4750440	0.34
FRMD4A	NM_018027.3:c.207-71A>G		rs7079138	0.05
FRMD4A	NM_018027.3:c.837-39C>T		rs1541004	0.21
FRMD4A	NM_018027.3:c.1117+295C>G		rs943090	0.20
FRMD4A	NM_018027.3:c.1117+311C>T		rs737376	0.36
FRMD4A	NM_018027.3:c.1117+493C>T		rs11258531	0.23
FRMD4A	NM_018027.3:c.1117+496T>C		rs943089	0.27
FRMD4A	NM_018027.3:c.1118-32G>C		rs3814663	0.29
FRMD4A	NM_018027.3:c.1660+144_1660+145delAG		rs55876188	0.20
FRMD4A	NM_018027.3:c.1661-115A>G		rs10796105	0.20
FRMD4A	NM_018027.3:c.2954-58T>C		rs10906442	0.48
ARL5B	NM_178815.3:c.-268delG		rs10711928	0.17
CELF2	NM_001025077.2:c.-144C>G		rs2773499	0.24
CELF2	NM_001025077.2:c.509A>T	p.Val139=	rs2277212	0.25
CELF2	NM_001025077.2:c.748+163insT		rs3215825	0.45
CELF2	NM_001025077.2:c.748+174T>C		rs2134361	0.14
CELF2	NM_006561.3:c.976+10G>A		rs2277214	0.50
CELF2	NM_006561.3:c.976+33G>A		rs2277215	0.41
CELF2	NM_006561.3:c.976+61C>T		rs2277216	0.39

SVIL	NM_003174.3:c.-400+96G>A		rs1891668	0.18
SVIL	NM_003174.3:c.-200-6837A>G		rs973286	0.49
SVIL	NM_003174.3:c.-200-6500T>C		rs7903861	0.30
SVIL	NM_003174.3:c.-50-30A>G		rs1547170	0.31
SVIL	NM_003174.3:c.39G>A	p.Gly13=	rs1547169	0.27
SVIL	NM_003174.3:c.189T>C	p.Ser63=	rs3740002	0.27
SVIL	NM_003174.3:c.315T>C	p.Ile105=	rs3740003	0.31
SVIL	NM_003174.3:c.489T>G	p.Ala163=	rs1270874	0.26
SVIL	NM_003174.3:c.566T>C	p.Val189Ala	rs10160013	0.31
SVIL	NM_003174.3:c.827+13T>C		rs10159964	0.30
SVIL	NM_021738.2:c.1264G>A	p.Val422Ile	rs1247696	0.01
SVIL	NM_003174.3:c.925-54G>A		rs3780843	0.27
SVIL	NM_003174.3:c.1064+30T>G		rs913035	0.27
SVIL	NM_003174.3:c.1663C>A	p.Arg555=	rs7070678	0.36
SVIL	NM_003174.3:c.2112-58C>T		rs12775779	0.23
SVIL	NM_003174.3:c.2238+4259T>C		rs16930338	0.04
SVIL	NM_003174.3:c.2238+4844C>T		rs61849293	0.25
SVIL	NM_003174.3:c.2741+77T>C		rs4749439	0.48
SVIL	NM_003174.3:c.2741+115C>A		rs4749438	0.20
SVIL	NM_003174.3:c.3071-98delTinsTT		rs34472178	0.09
SVIL	NM_003174.3:c.3784T>C	p.Ser1262Pro	rs11007612	0.07
SVIL	NM_003174.3:c.3976G>A	p.Val1326Ile	rs61737920	0.06
SVIL	NM_003174.3:c.4252-37G>A		rs2274494	0.43
SVIL	NM_003174.3:c.4566-232G>A		rs4749434	0.35
SVIL	NM_003174.3:c.4704T>C	p.Pro1568=	rs56248456	0.05
SVIL	NM_003174.3:c.4735A>G	p.Ile1579Val	rs7921306	0.20
SVIL	NM_003174.3:c.4770A>G	p.Thr1590=	rs1057952	0.43
SVIL	NM_021738.2:c.*1091A>C		rs7916641	0.41
ZNF438	NM_001143766.1:c.-584C>T		rs1690622	0.44
ZNF438	NM_001143766.1:c.-379+81C>G		rs1771646	0.33
ZNF438	NM_001143766.1:c.-115+186T>C		rs2934644	0.16
ZNF438	NM_001143766.1:c.-114-1279T>C		rs1494118	0.30
ZNF438	NM_001143766.1:c.-31-212T>C		rs10763849	0.44
ZNF438	NM_001143766.1:c.-31-94C>T		rs10763848	0.37
ZNF438	NM_001143770.1:c.-28A>G		rs4237394	0.38
ZNF438	NM_001143766.1:c.517C>T	p.Pro173Ser	rs10160116	0.38
ZNF438	NM_001143766.1:c.*189T>C		rs117045459	0.02
RAB18	NM_001256410.1:c.-297C>T		rs2505302	0.01
RAB18	NM_001256410.1:c.69-130T>A		rs11015829	0.35
RAB18	NM_001256410.1:c.347-44_347-43delTC		rs72264716	0.35
MTPAP	NM_018109.3:c.555+73G>A		rs2689215	0.05

MTPAP	NM_018109.3:c.781-74T>C		rs1029172	0.05
MTPAP	NM_018109.3:c.992+163G>A		rs2484286	0.07
ITGA8	NM_003638.1:c.1001+157T>C		rs2275617	0.50
ITGA8	NM_003638.1:c.1399+46T>A		rs4748191	0.10
ITGA8	NM_003638.1:c.1902+41G>A		rs2282383	0.30
ITGA8	NM_003638.1:c.2880+82C>A		rs9333229	0.10
ITGA8	NM_003638.1:c.*339T>C		rs2296511	0.22
ITGA8	NM_003638.1:c.*1425A>G		rs7916029	0.50
PIP4K2A	NM_005028.4:c.1036+14C>G		rs943192	0.07
SLC39A12	NM_001145195.1:c.544-95A>G		rs483822	0.26
SLC39A12	NM_001145195.1:c.544-58T>C		rs966720	0.31
SLC39A12	NM_001145195.1:c.751+36C>T		rs11011754	0.11
SLC39A12	NM_001145195.1:c.738C>A	p.Thr246=	rs691513	0.37
SLC39A12	NM_001145195.1:c.910G>A	p.Val304Ile	rs2478568	0.34
SLC39A12	NM_001145195.1:c.925-33G>A		rs11011850	0.04
SLC39A12	NM_001145195.1:c.1096+10G>A		rs1926739	0.49
SLC39A12	NM_001145195.1:c.1760-65G>A		rs11012030	0.03
SLC39A12	NM_152725.3:c.*350A>G		rs1935502	0.24
PTTG1	NM_004219.2:c.277-33G>C		rs2910203	0.14
PTTG1	NM_004219.2:c.371-58A>G		rs73316398	0.11
KCNIP1	NM_001034837.1:c.*284G>T		rs1363713	0.38
KCNIP1	NM_001034837.1:c.*561T>C		rs1055381	0.22
SGCD	NM_000337.5:c.-94C>G		rs13170573	0.48
SGCD	NM_000337.5:c.84T>C	p.Tyr28=	rs1801193	0.49
ADAM19	NM_033274.4:c.407+31G>T		rs3734032	0.11
ADAM19	NM_033274.4:c.407+74G>A		rs3822696	0.49
ADAM19	NM_033274.4:c.600+44G>T		rs11465284	0.08
ADAM19	NM_033274.4:c.850A>G	p.Ser284Gly	rs1422795	0.38
ADAM19	NM_033274.4:c.991-43A>T		rs11466770	0.05
ADAM19	NM_033274.4:c.1398+47G>A		rs11466776	0.06

Table 2.6: List of known variations in proband sample.

There were a few variations which were not reported in any of the databases and they are listed in Table 2.7. The next step was to check for segregation of these novel variants by sequencing all family members to see if the variation was also present in all members who carried the haplotype. Out of all novel variants, there was only one variation that was found to be segregating in the family. A variation can be considered as a possible cause if it is rare in the control population. Hence, the segregating variation was checked by sequencing 48 controls out

of which it was found to be present in 4 samples: present at about 8% in the population. Due to its high frequency in the control population, this variation was not analyzed further.

S.No.	Gene	Gene sequence variant	Position	Segregation	Allele frequency among controls
1	MLLT10	NM_001195627.1:c.241-15delT	Intron	Non-segregating	
2	MLLT10	NM_001195626.1:c.241-5delT	Intron	Non-segregating	
3	**CUBN**	**NM_001081.3:c.387+146C>T**	**Intron**	**Segregating**	**8.3% (4/48)**
4	CUBN	NM_001081.3:c.388-24T>A	Intron	Non-segregating	
5	CUBN	NM_001081.3:c.2791+26delT	Intron	Non-segregating	
6	CUBN	NM_001081.3:c.5345-1G>C	Intron	Non-segregating	
7	CUBN	NM_001081.3:c.9663+102A>G	Intron	Non-segregating	
8	CUBN	NM_001081.3:c.*446delA	3'UTR	Non-segregating	
9	ARL5B	NM_178815.3:c.*181delT	3'UTR	Non-segregating	
10	CELF2	NM_001025077.2:c.261+127C>A	Intron	Non-segregating	
11	SVIL	NM_021738.2:c.-200-201insA	Intron	Non-segregating	
12	RAB18	NM_001256410.1:c.124+278A>G	Intron	Non-segregating	
13	MTPAP	NM_08109.3:c.993-233T>C	Intron	Non-segregating	
14	ADAM19	NM_033274.4:c.600+71T>G	Intron	?	

Table 2.7: List of novel variations. The highlighted variant was the only one found to be segregating with the disease phenotype and hence it was checked in 48 controls. However, due to its high frequency in controls, the variation was not analyzed further.

Apart from these novel variations, there were some variations that were reported to be present at a very low frequency in the control population (Table 2.8). There were also some variations for which the frequency was not mentioned in databases (Table 2.9). Segregation and control frequency check for these variations is in progress.

Gene	Gene sequence variant	Predicted effect on protein	Frequency of minor allele in control population	rsID	Segregation
CUBN	NM_001081.3:c.8150C>G	p.Ser2717Trp	0.000	rs2796835	Non-segregating
FRMD4A	NM_018027.3:c.1117+9104T>C		0.000	rs1544303	?

SVIL	NM_003174.3:c.3786G>A	p.Ser1262=	0.000	rs2505302	?
MTPAP	NM_018109.3:c.993-68G>A		0.002	rs2484284	Non-segregating

Table 2.8: List of variations reported to be present at low frequencies in the control population.

S.No.	Gene	Gene sequence variant	rsID	Segregation
1	GAD2	NM_000818.2:c.*35-48delCinsCTCTCT	rs8190817	
2	NMT2	NM_004808.2:c.391+82_391+83delAA	rs112834963	
3	PLXDC2	NM_032812.7:c.1273+44_1273+48delTTTT	rs150262505	
4	PLXDC2	NM_032812.7:c.*331delA	rs199670298	
5	OPTN	NM_001008211.1:c.369+38T>G	rs75617318	
6	OPTN	NM_001008211.1:c.369+29delT	rs34617995	Non-segregating
7	RSU1	NM_012425.3:c.-4+75_-4+76insCCTGCCCTACCCC	rs111960598	Non-segregating
8	RSU1	NM_012425.3:c.599-14_599-16AAA>GGGG	rs377555512	
9	ZEB1	NM_001128128.2:c.2734+90delT	rs59206948	
10	MLLT10	NM_001195627.1:c.241-47delA	rs146959127	Non-segregating
11	MLLT10	NM_001195627.1:c.241-48T>A	rs76428544	
12	MLLT10	NM_001195626.1:c.406-21	rs369797804	Non-segregating
13	MLLT10	NM_001195626.1:c.1052-31delT	rs35452885	Non-segregating
14	MLLT10	NM_001195626.1:c.1700-5200delT	rs368073882	Non-segregating
15	CUBN	NM_001081.3:c.9454+78delC	rs59702069	Non-segregating
16	CUBN	NM_001081.3:c.10764+82delT	rs370322118	Non-segregating
17	FRMD4A	NM_018027.3:c.46-149251_46-149250insCACACACACACA	rs36042908	Non-segregating
18	CELF2	NM_001025076.2:c.525+165_525+166delTC	rs72509391	
19	SVIL	NM_003174.3:c.160+39G>C	rs371927595	
20	SVIL	NM_003174.3:c.3678-129C>T	rs10763721	
21	SVIL	NM_003174.3:c.3771G>A	rs61733423	
22	RAB18	NM_001256410.1:c.347-180delA	rs5784015	
23	MTPAP	NM_018109.3:c.1220-31delA	rs371479504	
24	ITGA8	NM_003638.1:c.3192+1537delT	rs35079226	
25	ITGA8	NM_003638.1:c.3192+1547delT	rs11297384	

Table 2.9: List of variations for which the allele frequencies are not available in databases examined.

3. Discussion

Hot water epilepsy is an unusual form of reflex epilepsy wherein seizures are precipitated by contact with hot water. A literature review of case reports of hot water epilepsy reveals that the trigger might vary between individuals based on the part of the body exposed to hot water, the temperature of the water or the time taken for the seizures to occur. This observation is interesting as it points to the fact that hot water epilepsy is phenotypically heterogeneous. The types of seizures also varies, the most common being complex partial seizures with or without secondary generalization followed by generalized tonic-clonic seizures. The age of onset varies from two months to 58 years. This condition is also geographically specific as the majority of cases are reported from India and Turkey and is less common in Europe, Japan and USA.

Hot water epilepsy has been clinically diagnosed and reported for the past 65 years, starting from the first report from New Zealand in 1945, of a 10 year old boy who presented with seizures upon contact with hot water during bathing (Allen, 1945). In spite of numerous case reports from various countries like Japan, USA, Saudi Arabia, Turkey and India, no genetic analysis was reported till recently, for this phenotype. Whole genome wide linkage analysis of a few families from southern parts of India revealed two loci at chromosome 10q21.3-q22.3 (Ratnapriya et al, 2009) and chromosome 4q24-q28 (Ratnapriya et al, 2009) to be linked to hot water epilepsy. Candidate gene screening in these two regions have not yet revealed any possibly deleterious mutation that could be linked to the phenotype.

The mechanisms leading to seizures occurring upon temperature dependent stimuli remain an unexplored field. Also, the mechanisms for the differences in the types of seizures, triggers, age of onset and prevalence are not well understood.

This study was undertaken to identify the genetic locus responsible for the symptoms of hot water epilepsy in family 307. Whole genome wide linkage analysis and LOD score analysis brought two regions into focus, on chromosomes 10 and 5. As it was not possible to eliminate either of the regions on the basis of LOD scores or haplotype segregation, both regions were examined for a possible causative mutation.

There are a number of candidate genes in both regions, the disruption of which might lead to hot water seizures in these patients. Additionally, there are many regions on chromosome 10 which have been reported in different types of epilepsy. A locus for idiopathic generalized epilepsy, EIG5, has been reported at 10p11.22 (Kinirons et al, 2008). Another locus for generalized epilepsy with paroxysmal dyskinesia (GEPD) has been reported at 10q22.3 with the causative mutation in KCNMA1, which is a calcium-dependent potassium channel (Du et al, 2005). There are four loci reported for frontal lobe epilepsies: 10q22-q24 reported for a partial epilepsy (Ottman et al, 1995), 10q22-q24, for an autosomal dominant lateral temporal lobe epilepsy (ADLTE) (Poza et al, 1999), 10q23-q25, for a partial epilepsy (Mautner et al, 2000), and 10q22-q24, for familial temporal lobe epilepsy with aphasic seizures (Brodtkorb et al, 2002). Also, a susceptibility locus for generalized tonic-clonic seizures, EIG4, has been reported at 10q25-q26 (Puranam et al, 2005).

A genome wide linkage analysis study has implicated a region on chromosome 5q14-q15 for onset of childhood febrile seizures (Nakayama et al, 2000). In one family, a pericentric inversion of chromosome 5 has been found to segregate with an autosomal dominant form of epilepsy called benign familial neonatal convulsions (BFNC) (Concolino et al, 2002). A region on chromosome 5p15.31-p15 has been linked to familial cortical myoclonic tremor with epilepsy (FCMTE) (Depienne et al, 2010). Patients with duplication of small regions on chromosome 5p have also found to develop epilepsy (Stulpnagel et al, 2012). A very important group of genes present in our region of interest is the GABR cluster. The GABR family of receptors are very important candidate genes for causing epilepsy and mutations in the $GABA_A$ receptor gamma-1 subunit gene (GABRG2) on chromosome 5 have been linked to febrile seizures and childhood absence epilepsy (Feucht et al, 1999). GABA receptors are ligand gated channels which are expressed largely in the dendritic regions (Poolos and Johnston, 2012). Hence, our first step was sequencing these genes to identify potentially causative mutations. However, no significant variations were identified in any of these genes clearly indicating that the cause for hot water epilepsy in this family lies elsewhere.

Maximum LOD scores for both the regions were obtained at 90% penetrance value and 1% phenocopy indicating an autosomal dominant inheritance with incomplete penetrance which is

very common in these cases. With the help of literature available on known genes responsible for various types of epilepsy, candidate genes on both these regions were examined by sequencing the proband sample. Some of the genes included GAD2 (glutamate decarboxylase 2), GM2A (GM2 ganglioside activator), GRIA1 (glutamate receptor, ionotropic, AMPA 1), PTTG1 (pituitary tumor-transforming 1) and KCNIP1 (Kv channel interacting protein 1).

All variants obtained were systematically eliminated on the basis of segregation with the disease phenotype and allele frequencies in the control population. Although a few variations were promising, they were eliminated upon further examination. So far, no significant variation has been found which could be a potential cause for hot water epilepsy in family 307. The search for such a mutation continues.

4. Appendix

4.1 Buffers used

Tris-acetate EDTA (TAE) buffer (50X):

Tris base – 242 g

Glacial acetic acid – 57.1 ml

EDTA – 18.61 g

Distilled water – to 1000 ml

Sterilized by autoclaving

Tris-EDTA (TE) buffer:

Tris-HCl (1M, pH – 8.0) - 1 ml (final concentration = 10 mM)

EDTA (0.5 M) – 200 µl (final concentration = 1 mM)

Distilled water – to 100 ml

Sterilized by autoclaving

6X DNA loading buffer:

Bromophenol blue – 0.2%

Xylene cyanol – 0.2%

Glycerol – 60%

4.2 Primer sequences

Primer name	Primer sequence	Length of the primer	Primer name	Primer sequence	Length of the primer
GAD2-EX1A-F	acctgcttggaggaaaacg	19	GAD2-EX12-R	ttaggcaccagaccttcacc	20
GAD2-EX1A-R	gtttagggacgtggcaacc	19	GAD2-EX13-F	catttgaccctgagccattg	20
GAD2-EX1B-F	ctttccctcaaatgctctgg	20	GAD2-EX13-R	gaaagcaaatcttgggtaagg	21
GAD2-EX1B-R	ccgaagaagtttcctgttgc	20	GAD2-EX14-F	aaagggaacaaaagcacctg	20
GAD2-EX2-F	cctcgggaaacagataaagg	20	GAD2-EX14-R	ggaccactttgagagtttcgtc	22
GAD2-EX2-R	gtagagcagggctgggtaag	20	GAD2-EX15-F	ctctcaaagtggtccatataacg	23
GAD2-EX3-F	aacaaactgtgcggtgagtg	20	GAD2-EX15-R	caacctggaagacacagtgag	21
GAD2-EX3-R	tctgaagattctggcaggtc	20	GAD2-EX16-F	gcttgctgttgggttctttg	20
GAD2-EX4-F	tctcattcattcccttcacc	20	GAD2-EX16-R	ggcaatttggcactattcag	20
GAD2-EX4-R	gcagtggaaggtgctttg	18	GAD2-3'UTR1-F	cctcagattggggttgtgc	19
GAD2-EX5-F	tcttcgtggttagaggaaacg	21	GAD2-3'UTR1-R	gttgggggaatgttgatgtc	20
GAD2-EX5-R	tctggtcccatttgtctttg	20	GAD2-3'UTR2-F	aggaacctcaaactgggtatc	21
GAD2-EX6-F	tcgtgcattatgtcagtagttgg	23	GAD2-3'UTR2-R	tttccctacaaaagacccaaag	22
GAD2-EX6-R	ttggaggcatgttcaatgg	19	NMT2-EX1-F	ggggttagagagctggattg	20
GAD2-EX7-F	gtagcgcctttgagtctgag	20	NMT2-EX1-R	gcaagtgacagtagcgtctcc	21
GAD2-EX7-R	ctatattgcccaggctgctc	20	NMT2-EX2-F	ttcctcacctacatcctaacacag	24
GAD2-EX8-F	caggctttggaggaacagac	20	NMT2-EX2-R	tggcattcaacacacagtcac	21
GAD2-EX8-R	catggcatcaaggaaccac	19	NMT2-EX3-F	ttggaggaggattgttagtgtttag	25
GAD2-EX9-F	ctggaagcagtcaaggagttg	21	NMT2-EX3-R	ggttatcaaaagccaatgtcac	22
GAD2-EX9-R	acctgggagattgaggcttg	20	NMT2-EX4+5-F	accactgcctagctttcctg	20
GAD2-EX10-F	gctgctgcttcctcaaattc	20	NMT2-EX4+5-R	acaggcaccagttattgtcc	20
GAD2-EX10-R	tataatcagccttgggatgc	20	NMT2-EX6-F	attcacccgagttcctgttg	20
GAD2-EX11-F	gcccttcggtgtaaactctg	20	NMT2-EX6-R	ttacaggtgcctaccaccac	20
GAD2-EX11-R	cataaggcaaggaacacaagc	21	NMT2-EX7-F	cccagcctgcattcttattc	20
GAD2-EX12-F	ggccagacttcaccactacg	20	NMT2-EX7-R	taatcaccaagtggcaaatg	20

NMT2-EX8-F	ctggacggataaaatgatgac	21	PLXDC2-EX10-F	gcttctccagttgcgagttc	20
NMT2-EX8-R	ggcatttccaaacaaactcc	20	PLXDC2-EX10-R	tgcatctattaggggttgtagc	22
NMT2-EX9-F	tcttgttgagttgcagattcg	21	PLXDC2-EX11-F	aatgccttgttccatccatc	20
NMT2-EX9-R	ctctctacccttttagtacacatctttg	27	PLXDC2-EX11-R	caagaagccagaatcaatgg	20
NMT2-EX10-F	gggcaagggagagaacagag	20	PLXDC2-EX12-F	ctagtcatgttccgaccaagg	21
NMT2-EX10-R	gtctcgcataaattccacctg	21	PLXDC2-EX12-R	agaagcccgtattgcctttc	20
NMT2-EX11-F	ataggcatgagccacagc	18	PLXDC2-EX13-F	gcagaaaagttgtccaggaag	21
NMT2-EX11-R	caggcatggtggtggatg	18	PLXDC2-EX13-R	catccatgtgtgtgaaatcag	21
NMT2-EX12-F	ggattacaggtgtgagctgag	21	PLXDC2-EX14A-F	ggcttggagggttgataagg	20
NMT2-EX12-R	gattccataaatgttcccagtg	22	PLXDC2-EX14A-R	cagataagtccacgggagatg	21
PLXDC2-EX1A-F	ccttccctcctcaaagtgtg	20	PLXDC2-EX14B-F	gctgctgtagcctgaagaagac	22
PLXDC2-EX1A-R	gcaaactgtcggttccactc	20	PLXDC2-EX14B-R	tttgttctcactcttgcatgg	21
PLXDC2-EX1B-F	gcgctggctgtggaattag	19	OPTN-5'UTR1-F	gtgacgccttagagcagtcc	20
PLXDC2-EX1B-R	acgagagtgccagagaggtc	20	OPTN-5'UTR1-R	cccaccctgtgttcactacc	20
PLXDC2-EX2-F	tccaatcagagatgttcgtacc	22	OPTN-5'UTR2-F	ggtggttggtaacacagaagc	21
PLXDC2-EX2-R	accaagcctgtgtttgtctg	20	OPTN-5'UTR2-R	gagggagacgagggtatgac	20
PLXDC2-EX3-F	tttcttacagttcgtatgtgtgtg	24	OPTN-EX1-F	ccctttttatacacccatacacac	23
PLXDC2-EX3-R	cacttgaaaagcgattaccac	21	OPTN-EX1-R	cccaccagctaccacctatg	20
PLXDC2-EX4-F	atattctttagtacgaccgaggac	24	OPTN-EX2-F	caaggctaagcatggcatc	19
PLXDC2-EX4-R	aagaaaagcagaggtgatctagg	23	OPTN-EX2-R	ggcaaaacaccaatccagac	20
PLXDC2-EX5-F	ccagctaccatgacattttattttc	25	OPTN-EX3-F	tgtaaagatgggggtcttgc	20
PLXDC2-EX5-R	tcagaccatttgcatcaagc	20	OPTN-EX3-R	aatgttcaatttgccaggac	20
PLXDC2-EX6-F	cagatttgaaggccagatgc	20	OPTN-EX4-F	ttccttgggttgcatgtc	18
PLXDC2-EX6-R	ccctttcttgacatttggag	20	OPTN-EX4-R	agtgtgagccaaacaggaac	20
PLXDC2-EX7-F	ggaactccctgtctcttctattc	23	OPTN-EX5-F	gcattgtaagctggcctctc	20
PLXDC2-EX7-R	agttagctcaagggtgacagag	22	OPTN-EX5-R	ttggaggagcagacagtgag	20
PLXDC2-EX8+9-F	tcagtcttctcacaggatgagg	22	OPTN-EX6-F	gagtttcacttgccttttacctc	23

PLXDC2-EX8+9-R	caagcatccctcctactttctc	22	OPTN-EX6-R	aattgacacagagcaggacaag	22
OPTN-EX7-F	ttggggtattgtcaaagttgg	21	RSU1-EX7-R	tgttccctgcttttggtaatg	21
OPTN-EX7-R	ttcatgctcacacattaactgg	22	YME1L1-EX1-F	gcgctgctccttgtaaatg	19
OPTN-EX8-F	gcgtaagggttcagaaatatgg	22	YME1L1-EX1-R	cctgatccactcctcaatcc	20
OPTN-EX8-R	agacagagtgcaaacaatcagc	22	YME1L1-EX2-F	agttttcgtctcaccagcag	20
OPTN-EX9-F	cgtggggtgataaaggtagg	20	YME1L1-EX2-R	ggagggacaatatccttaatcc	22
OPTN-EX9-R	tcagaagttacaaaccctagatgc	24	YME1L1-EX3-F	cagggtttggtgaaactgc	19
OPTN-EX10-F	ccacctcagcctctcaattc	20	YME1L1-EX3-R	agcctggtgaagaaagcaag	20
OPTN-EX10-R	aagatccactgagcactttcc	21	YME1L1-EX4-F	ggacagtagtggtttgggtttg	22
OPTN-EX11-F	agcaggattgtgcatctgtg	20	YME1L1-EX4-R	cagcccacaataaacgctac	20
OPTN-EX11-R	ggcgcgaacacagctattc	19	YME1L1-EX5-F	gagaatggcgtgaacctg	18
OPTN-EX12-F	tgaaccttggcagtgtagtttg	22	YME1L1-EX5-R	ccaaatactctttaggtcctgtg	23
OPTN-EX12-R	gattcggtgggtaatggatg	20	YME1L1-EX6-F	tgtcaggcaaattagatagactagg	25
OPTN-EX13-F	tgtgctcatgtcccactacg	20	YME1L1-EX6-R	tggtgcccagaagagttagag	21
OPTN-EX13-R	gccacttcctgggttcaag	19	YME1L1-EX7-F	tgcaggaatgggatctagtg	20
RSU1-5'UTR-F	ctcgaagccgaataacgaac	20	YME1L1-EX7-R	gaaagggaaaccatcagcag	20
RSU1-5'UTR-R	gatgacagcaagcgcagag	19	YME1L1-EX8-F	tgagaaagaatgccagattcc	21
RSU1-EX1-F	gaacccttcccactccatc	19	YME1L1-EX8-R	tctgggaaactaaccactactagg	24
RSU1-EX1-R	tcctactgcaaaccctctgc	20	YME1L1-EX9-F	tttctaggctttgctttcttgc	22
RSU1-EX2-F	ttggtgagcgatagtgcttc	20	YME1L1-EX9-R	cactgtagaatcaaatatcgacctc	25
RSU1-EX2-R	agaccaacctgggtaacacg	20	YME1L1-EX10-F	aaggggccatttttgtatgc	20
RSU1-EX3-F	ggatcatggcactgtcttacac	22	YME1L1-EX10-R	aaagggtatgaggagcattctatg	24
RSU1-EX3-R	tgcacatactgctgagctacg	21	YME1L1-EX11-F	gcagagacaagtacggtttgc	21
RSU1-EX4+5-F	aaagctgactccctcgtattg	21	YME1L1-EX11-R	agcctccaacttcatcttgc	20
RSU1-EX4+5-R	aggtggagagtagttgccaag	21	YME1L1-EX12-F	atgaatgcccaactgttttg	20
RSU1-EX6-F	ccaagaggcacttagggtttg	21	YME1L1-EX12-R	gggactactctgtttcctatacacatc	27

RSU1-EX6-R	ctgccatcaataggatactgtcac	24	YME1L1-EX13-F	ctaggccacaaagcgagtcc	20
RSU1-EX7-F	aacacagcgtagcacccagag	21	YME1L1-EX13-R	tccccattctccctaacctc	20
YME1L1-EX14-F	aaaactccatctgcgctctg	20	ZEB1-EX5-F	acatttgaaagggatactgtgag	23
YME1L1-EX14-R	acctttctttagactcagggttc	23	ZEB1-EX5-R	aggcacctgtaataaagtttggtc	24
YME1L1-EX15-F	gcacaatcttggcttactgc	20	ZEB1-EX6-F	tgggtagcacaatatctggaac	22
YME1L1-EX15-R	cttgaaaacccagacactgaag	22	ZEB1-EX6-R	gtggtttttaatctcaggaatgacc	25
YME1L1-EX16-F	cctcttccccatagttttaatcc	23	ZEB1-EX7-F	ttctgtccccactatcactatcc	23
YME1L1-EX16-R	agaattaggcagggcatgg	19	ZEB1-EX7-R	ttccattcccctgctaattg	20
YME1L1-EX17+18-F	ttgggattatagccatgcac	20	ZEB1-EX8A-F	ccgcttgttttagggaaatg	20
YME1L1-EX17+18-R	aatcagaaccagctctcaaatc	22	ZEB1-EX8A-R	tggcttgattattctccaacac	22
YME1L1-EX19-F	cgtttctccgtgtttccag	19	ZEB1-EX8B-F	catggtgcaagctgttgttc	20
YME1L1-EX19-R	actgttcttagcccgtgtcc	20	ZEB1-EX8B-R	caaattgccatctccagtagc	21
YME1L1-EX20-F	tgccttctcaggtgtttattctc	23	ZEB1-EX8C-F	ctgtgtgatgattgtccaggag	22
YME1L1-EX20-R	gagctgttttcaatcctgatagttc	25	ZEB1-EX8C-R	acttgtggctcttcttgtgc	20
ZEB1-EX1-F	agggtacagggagaatcagc	20	ZEB1-EX8D-F	ggttccagaagtagtacaccatcc	24
ZEB1-EX1-R	actttcccactccactttgc	20	ZEB1-EX8D-R	tagttggctctacgggactg	20
ZEB1-EX2-F	tcgagaaaacgaggaaatacg	21	ZEB1-EX9-F	tcagtgtgcttgctttggtc	20
ZEB1-EX2-R	tcgtcagcaacaagaaaagc	20	ZEB1-EX9-R	tgagccgagattgagattgc	20
ZEB1-5UTR1-F	aggaggcaaatcatattcacg	21	ZEB1-EX10A-F	aaccctcccctttctacaac	20
ZEB1-5UTR1-R	aaattcaaacacttgtcattatgtcc	26	ZEB1-EX10A-R	ccctcattctctgcctcttc	20
ZEB1-5UTR2-F	agtgatgggggtggatgttg	20	ZEB1-EX10B-F	tcggacgagagagagagtttg	21
ZEB1-5UTR2-R	cccaggacatgcttcacttc	20	ZEB1-EX10B-R	cagcttcctttaacctcttgtattg	25
ZEB1-5UTR3-F	tcccagtcatagtaaaaccttcc	23	MLLT10-5'UTR1-F	tctgtctaagcagcgcattg	20
ZEB1-5UTR3-R	cagaatttggaagtaaagcacagac	25	MLLT10-5'UTR1-R	agcgaatcccttcccaag	18
ZEB1-EX3-F	gtcattttggtcatgtaatatctagtg	27	MLLT10-5'UTR2-F	tcttcccgacgctcacac	18

ZEB1-EX3-R	agcttgatcctggttaataaattc	24	MLLT10-5'UTR2-R	cacatgcaagccgattagtg	20
ZEB1-EX4-F	tttcgggaagttaaaatgtttg	22	MLLT10-EX1-F	gagtgactgagcggcaaag	19
ZEB1-EX4-R	tcacaaatgcgaaatcagtg	20	MLLT10-EX1-R	cgcactttctcccacatgc	19
MLLT10-EX2-F	ggatgtgaactgttttacttctctg	25	MLLT10-EX13B-R	gccgaacatctttcccaaac	20
MLLT10-EX2-R	agccagtgtaaccttcaaacttc	23	MLLT10-EX14-F	cattccacacactcccattaag	22
MLLT10-EX3-F	gcataagggcaggaatgaag	20	MLLT10-EX14-R	gcagtacattatctttgcaggag	23
MLLT10-EX3-R	aggttgaggtgggaggatg	19	MLLT10-EX15-F	agatttgcatcctacagtgactc	23
MLLT10-EX4A-F	cctttagccagatggatgttg	21	MLLT10-EX15-R	gatgaaccatattgacctatcagc	24
MLLT10-EX4A-R	ccttcttgaaacgccaaatg	20	MLLT10-EX16-F	tggggattttaaccctgatg	20
MLLT10-EX4B-F	agtttctggtgctctgatgc	20	MLLT10-EX16-R	cctaccaagtctgaagcaatatg	23
MLLT10-EX4B-R	gtcactgaagaacaaaatggacag	24	MLLT10-EX17-F	cctccctatcctgaacagatg	21
MLLT10-EX5-F	ctaatccgcctgcctcag	18	MLLT10-EX17-R	aagctctagcaatgcctaccac	22
MLLT10-EX5-R	catactgcataaaatacccaatgc	24	MLLT10-EX18-F	ggtgtaggttcttttagacaactgc	25
MLLT10-EX6-F	gcaatggaagtaagacagcaatg	23	MLLT10-EX18-R	ttggtgcccatacactcatc	20
MLLT10-EX6-R	aaggtaacacagttttgcttgc	22	MLLT10-EX19-F	aatttgcttttcagaaggtcac	22
MLLT10-EX7-F	gactgatgaagataatgcctttgtg	25	MLLT10-EX19-R	gacattgctattttgcagcac	21
MLLT10-EX7-R	tcagaaccagagtccacttattg	23	MLLT10-EX20-F	tgggaactgcaaagatagtgg	21
MLLT10-EX8-F	tgcaggttggggtgaaatac	20	MLLT10-EX20-R	gagctaaaactggaacccaaac	22
MLLT10-EX8-R	ctccagccaaaaactcaagg	20	MLLT10-EX21-F	ggaacctgatacttctggcatag	23
MLLT10-EX9-F	cggcattttctctttgtattgaaac	25	MLLT10-EX21-R	caggattcaagtccaagcag	20
MLLT10-EX9-R	attcacaaatatggcattaaacc	23	MLLT10-EX22-F	aggaacactttgcctttgtg	20
MLLT10-EX10-F	cagaggaagtgatttttgacagaag	25	MLLT10-EX22-R	tttctacatcttcatataactggtgac	27
MLLT10-EX10-R	accaggtaagtggaggttgc	20	MLLT10-EX23-F	ggttttgatacgggcttcag	20
MLLT10-EX11-F	gattgtcatgtggaagtgaatagc	24	MLLT10-EX23-R	actcctgagccttgttgagc	20
MLLT10-EX11-R	tgtcaccctgttgccatc	18	MLLT10-EX24-F	ttccttgaacagcagtaagagc	22
MLLT10-EX12-F	ggatgattttcttaggtgaactgac	25	MLLT10-EX24-R	acacattgaaagccccaatc	20

MLLT10-EX12-R	ttcaactctggaagcacaaatc	22	MLLT10-EX25-F	ctgttttcagagtgtttggattcag	25
MLLT10-EX13A-F	gctgtggcaaaaggtaggtg	20	MLLT10-EX25-R	acgcaccacatctcagcac	19
MLLT10-EX13A-R	caggcccatgcttactttg	19	MLLT10-EX26-F	tctcctatgtgtgtcctgttttg	23
MLLT10-EX13B-F	accttaattggcctcccttc	20	MLLT10-EX26-R	ggggacgtataaattggcttag	22
MLLT10-EX27A-F	agtagccttgccaacttgc	19	CUBN-EX10-R	tttctcctgcctgcttgttg	20
MLLT10-EX27A-R	actgtaacccaaacaaaagagc	22	CUBN-EX11-F	ctgcccgtcaaaatacacag	20
MLLT10-EX27B-F	gctttgttgcactgaaatgg	20	CUBN-EX11-R	cttgatctcctggctcgtg	19
MLLT10-EX27B-R	tgtcccaccctgctgtctac	20	CUBN-EX12-F	tgcaaaaagttagccaggtg	20
MLLT10-EX27C-F	ggaaccagatcaattcaaagc	21	CUBN-EX12-R	gaaagtgggttcagcagagg	20
MLLT10-EX27C-R	cagaaatgaggtaagaactgtgc	23	CUBN-EX13-F	gaatggcagggcttttatctc	21
MLLT10-EX27D-F	tgtgacagtaccgcagagtg	20	CUBN-EX13-R	ccagcccctgtgacttatttc	21
MLLT10-EX27D-R	aaaacacaggccaagactcc	20	CUBN-EX14-F	ataccttggtctggtggacttg	22
CUBN-EX1-F	tttgacctctcacaagttgaag	22	CUBN-EX14-R	tcttcagatcccaaaacatctc	22
CUBN-EX1-R	tgtttttccctgtttgcttctc	22	CUBN-EX15-F	gcacagagcttgacacatgg	20
CUBN-EX2-F	agatgagcaggggcagttg	19	CUBN-EX15-R	gaagcaacaaacaggcacag	20
CUBN-EX2-R	gggaggctgaagacaggttg	20	CUBN-EX16-F	cagacagagagaaggcattgg	21
CUBN-EX3-F	ccagtgtttattacgcatggtg	22	CUBN-EX16-R	gggatcaaagggtattgcag	20
CUBN-EX3-R	tttcctgccttctgtccatc	20	CUBN-EX17-F	tcccacaacacatcaaactg	20
CUBN-EX4-F	aggggagaggaatgaagagg	20	CUBN-EX17-R	tcagccttatttgcctgcac	20
CUBN-EX4-R	gcaaactcagggtcagaagc	20	CUBN-EX18-F	ttgcagatttggctaacacg	20
CUBN-EX5-F	cacaagagaggtgatttgaagaag	24	CUBN-EX18-R	gcctgaggagataaccgaatataac	25
CUBN-EX5-R	atttgggaggccacagaatc	20	CUBN-EX19-F	ttcagagctgtttatcaagtcg	22
CUBN-EX6-F	attcctctcctgtgactgctg	21	CUBN-EX19-R	aattctcgggacaaatctcac	21
CUBN-EX6-R	gctatgtagacgttaagcaagagc	24	CUBN-EX20-F	gggggtgactaaaatgtgc	19
CUBN-EX7-F	attgctggctgacgattc	18	CUBN-EX20-R	gactttgaatggtccctgttg	21
CUBN-EX7-R	tcctcgcctatgtcctacttac	22	CUBN-EX21-F	cctcctcttgggcttgtatc	20

CUBN-EX8-F	ctctttccctggcatctttg	20	CUBN-EX21-R	cagatgtcaaaacttaatgcctac	24
CUBN-EX8-R	agatcgtcgagcagaaccag	20	CUBN-EX22-F	gtgttttaaggttgatgagaggtagag	27
CUBN-EX9-F	tttgcccactttgttgtcag	20	CUBN-EX22-R	cacaggtttgcggatgatg	19
CUBN-EX9-R	atgtgatctgcccacctcag	20	CUBN-EX23-F	ctccatagcttagatttagtgttgg	25
CUBN-EX10-F	gaaacaacccagaacattgc	20	CUBN-EX23-R	tcaggctccaaatgacaaac	20
CUBN-EX24-F	aacatattctctcagtttgtgattgg	26	CUBN-EX37-R	ttgcccttattgagccattc	20
CUBN-EX24-R	tgttaaaaagaaatggctgctg	20	CUBN-EX38-F	tccttttcaacacccttgc	20
CUBN-EX25-F	aagatcatgccgttgcattc	20	CUBN-EX38-R	cagactatcccacatgatttgc	22
CUBN-EX25-R	tcgaagatggtcactttcacc	21	CUBN-EX39-F	catcttatgagtgattcccacag	23
CUBN-EX26-F	ttcatggctattcttttgtgatg	23	CUBN-EX39-R	atatggtggaagggctttg	19
CUBN-EX26-R	aatttgaaggcccactctagg	21	CUBN-EX40-F	attgcagctttccgaatcag	20
CUBN-EX27-F	ctgctctccacaaattcaagg	21	CUBN-EX40-R	aattccaaaccaaacggatg	20
CUBN-EX27-R	tatagatgcgtggggcagag	20	CUBN-EX41-F	ggtaaagaaaagggcaggaag	21
CUBN-EX28-F	cccacctcagcagatttacc	20	CUBN-EX41-R	ctgaaggcagcacacttttg	20
CUBN-EX28-R	tccactttgatgtcacttcctc	22	CUBN-EX42-F	ccaccacaaactgagtagcc	20
CUBN-EX29-F	tttcggtgagttgaatttgg	20	CUBN-EX42-R	gtcccaagggtggagaacag	20
CUBN-EX29-R	ttcggaatcttggcagag	18	CUBN-EX43-F	cttggtggtaggtcttgctg	20
CUBN-EX30-F	ttcctaaggcacagggagtg	20	CUBN-EX43-R	ggtatgtggccttggaaaag	20
CUBN-EX30-R	tctcagtaggctgcccttg	20	CUBN-EX44-F	acagatgaaaggaaatggagac	22
CUBN-EX31-F	gggataaatggcttaatgtgg	21	CUBN-EX44-R	tggccgactgtgttttcag	19
CUBN-EX31-R	tagcgcagaaactgacttgc	20	CUBN-EX45-F	tgacatggcttatgctgattc	21
CUBN-EX32-F	ccctagaaatgaccaaagacttg	23	CUBN-EX45-R	aattttggggagcagtatgg	20
CUBN-EX32-R	gagttgggataattgtaagtcacag	25	CUBN-EX46-F	tggacttgtggtatcatttctg	22
CUBN-EX33-F	gtgctcctgttttctcatgg	20	CUBN-EX46-R	aattgagggagagaatcaccag	22
CUBN-EX33-R	gcaactgtagaaatgacttccctatc	26	CUBN-EX47-F	tcttcataggcagggtcttg	20
CUBN-EX34-F	tcattaaccatccccacctc	20	CUBN-EX47-R	ggtgggcattacagtataactctc	24

CUBN-EX34-R	ttggcttaggctgctctttc	20	CUBN-EX48-F	ctaaattgctgcctgtggac	20
CUBN-EX35-F	tgaccctgaatgaagtggttc	21	CUBN-EX48-R	tgatgcacagagtgatagctg	21
CUBN-EX35-R	ttccaaccaatcctccacac	20	CUBN-EX49-F	gggtaacagtggcttgtgg	19
CUBN-EX36-F	ggcccttggtcttcagtttg	20	CUBN-EX49-R	cagtgcgtttttcatctgc	19
CUBN-EX36-R	tggtcgagagaaacttcatgc	21	CUBN-EX50-F	catgccttttacccataagc	20
CUBN-EX37-F	gagacagccaaataatgttgtg	22	CUBN-EX50-R	actgaggcaagaggatcacc	20
CUBN-EX51-F	caaatattgtgcaatgtctgctac	24	CUBN-EX64-R	aatttccggtgtttggactg	20
CUBN-EX51-R	aaatttctagatatcagccacaacc	25	CUBN-EX65-F	ctgcctggtgttttaattgc	20
CUBN-EX52-F	ggtgatttgcccacttcg	18	CUBN-EX65-R	tggatgacacagcgagattc	20
CUBN-EX52-R	gccaatgtatgtgctctagtgag	23	CUBN-EX66-F	ggtgtagcccaaagaagagg	20
CUBN-EX53-F	gttgccatcccaaaccag	18	CUBN-EX66-R	acatggcaaaaccctgtttc	20
CUBN-EX53-R	atcaacccatgcttcctcag	20	CUBN-EX67A-F	cttccccattttccctcaac	20
CUBN-EX54-F	atggagagagcttgctgtgc	20	CUBN-EX67A-R	gaattacaggcacccaccac	20
CUBN-EX54-R	aattttgcctgtggtagtgc	20	CUBN-EX67B-F	ccccctgtattctcagcac	19
CUBN-EX55-F	aagtcaagaatgggacatgc	20	CUBN-EX67B-R	cctcctgtgtagctggaacc	20
CUBN-EX55-R	tgactcatcctccccgtagac	21	CUBN-EX67C-F	aataatagccaggggcagtg	20
CUBN-EX56-F	gggatttgcagtagatttgtgg	22	CUBN-EX67C-R	gcttggctccagaaatgaag	20
CUBN-EX56-R	tgccaatagcgaataacaagg	21	MPP7-5'UTR1-F	tatcagcaggcccctcttac	20
CUBN-EX57-F	cctgttttatcatacctgtgcttg	24	MPP7-5'UTR1-R	agtttttcctccacctgtcg	20
CUBN-EX57-R	ccatgaacctcactgacaatc	21	MPP7-5'UTR2-F	agaacattcccagatccactg	21
CUBN-EX58-F	ccctcttgctgtctttcatc	20	MPP7-5'UTR2-R	cggtccacattcttaaccattag	23
CUBN-EX58-R	ttcccaggttgaactgtcatc	21	MPP7-5'UTR3-F	tggtatcgggagacttttcg	20
CUBN-EX59-F	tgagttttgaatgctctactgg	22	MPP7-5'UTR3-R	aatggggcaaccacttagg	19
CUBN-EX59-R	aaaaccaagcccttacatgg	20	MPP7-5'UTR4-F	gtgcatacatttagagaacaacagg	25
CUBN-EX60-F	cccaggagggaatgatctg	19	MPP7-5'UTR4-R	tcaaggaatccaagatttgc	20
CUBN-EX60-R	aatgttggaagccagtcacac	21	MPP7-EX1-F	ttgtcaagatcagaaactctcacag	25

CUBN-EX61-F	ggcgtcataacaggtttttcc	21	MPP7-EX1-R	acccagagaaagaggcaatg	20
CUBN-EX61-R	tcatgtctcatctcaggcaatc	22	MPP7-EX2-F	ccctttggttctttggagaag	21
CUBN-EX62-F	aggcagggaagacagaattg	20	MPP7-EX2-R	cgtcggtgaatggaacaag	19
CUBN-EX62-R	tttcatcatggcttcattacttc	23	MPP7-EX3-F	gttgtaaacactgggccatag	21
CUBN-EX63-F	ttgcagagcacttccaacag	20	MPP7-EX3-R	cgaaagcagaacaagagagtagc	23
CUBN-EX63-R	gctaaattgggctgggttc	19	MPP7-EX4-F	ggatctggactaccgaaggtc	21
CUBN-EX64-F	cctgtaggcgtgtttgtgtg	20	MPP7-EX4-R	gagcttcccaataagagtattttgc	25
MPP7-EX5-F	ggtcaggaggcgtggaac	18	MPP7-EX18-R	gcgctgaactcccatacatac	21
MPP7-EX5-R	cactggtaacctatcccttgg	21	FRMD4A-5'UTR1-F	tggagagatgggagggaag	19
MPP7-EX6-F	ggcatgatgtctatttttcctg	22	FRMD4A-5'UTR1-R	cacggaatgtttaactgctacc	22
MPP7-EX6-R	aggctctcacatttcaacacag	22	FRMD4A-EX1-F	tcctccctaagcacagttcc	20
MPP7-EX7-F	ggcatagaggctgaaacctg	20	FRMD4A-EX1-R	aaagagcctgcgggataaag	20
MPP7-EX7-R	tgcaaatgaaaacactgagg	20	FRMD4A-EX2-F	ctccagatggtgatattccaaatag	25
MPP7-EX8+9-F	gtttcctccgtttagtctatctaagag	27	FRMD4A-EX2-R	ccaactgttcaggaaacgtg	20
MPP7-EX8+9-R	cagaaatccaaacccacagg	20	FRMD4A-EX3A-F	cgggataggttgactttacctc	22
MPP7-EX10-F	atttgaaagtgggcgatttg	20	FRMD4A-EX3A-R	tcaccaccaccctcactaatc	21
MPP7-EX10-R	ctgagaactgatgatcccaaag	22	FRMD4A-EX3B-F	gccgttatctgcacactgag	20
MPP7-EX11A-F	cctttagcctatccctttgg	20	FRMD4A-EX3B-R	catggcacctagaagatgagg	21
MPP7-EX11A-R	gccagtgaaaagtcatcacg	20	FRMD4A-5'UTR2-F	gcattgttgtccaggatcg	19
MPP7-EX11B-F	tgcttgtccttgctacattaagtc	24	FRMD4A-5'UTR2-R	agggttgcagaagaaagcac	20
MPP7-EX11B-R	ggtattcaagaggcagaaaaag	22	FRMD4A-5'UTR3-F	cttggtgccttttctccag	20
MPP7-EX12-F	attaaccagccgtggtgatg	20	FRMD4A-5'UTR3-R	tcccaggatgaagtcagcag	20
MPP7-EX12-R	tccccacttgacaaaggttc	20	FRMD4A-5'UTR4-F	tgtgaaggaaactgaagtggag	22
MPP7-EX13-F	ttgaggaaaaggtgaggatttc	22	FRMD4A-5'UTR4-R	ttgcctactcgttgacatcc	20
MPP7-EX13-R	aactcacatgccccataaatac	22	FRMD4A-EX4-F	gaactgatccttccaactcctg	22
MPP7-EX14-F	ggttagagagccttcaaaatagc	23	FRMD4A-EX4-R	tggtgggtcctagtcattctc	21

MPP7-EX14-R	tcattctgcaaagcagtgtc	20	FRMD4A-EX5-F	ccttgacttggctgtgtgc	19
MPP7-EX15-F	gggggtgatggaaaacattag	21	FRMD4A-EX5-R	gtaccaggcatgaccacaag	20
MPP7-EX15-R	gagcttcctccacttctctttc	22	FRMD4A-EX6-F	tgggtcacagagcaagacttag	22
MPP7-EX16-F	cctgtcatcctggaaactcc	20	FRMD4A-EX6-R	tttatccctggcaatgaacc	20
MPP7-EX16-R	aaccctcctcctttgctttc	20	FRMD4A-EX7-F	tggtagacccagttctttgg	20
MPP7-EX17-F	attccccagcttcttttcag	20	FRMD4A-EX7-R	tggtgccgttagcttcataac	21
MPP7-EX17-R	atcatgcctggctaatttcc	20	FRMD4A-EX8-F	gaggcagcatctcattatgttg	22
MPP7-EX18-F	tctggctgaggggaaagac	19	FRMD4A-EX8-R	tgctatggtttccgtcagtg	20
FRMD4A-EX9-F	ggcaaaggtgtgcattcag	19	FRMD4A-EX21-F	agcataagcgttgacctgtatc	22
FRMD4A-EX9-R	tgtttcagtggacaagttgagag	23	FRMD4A-EX21-R	taccgaggggaaccacatac	20
FRMD4A-EX10-F	ggtgaaaggcggttactgg	19	FRMD4A-EX22-F	tgggtgcagattttaggagtg	21
FRMD4A-EX10-R	tggtttcaggaagacacagg	20	FRMD4A-EX22-R	aaggcagcggacagttagg	19
FRMD4A-EX11+12-F	aggagctgttggtgccttg	19	FRMD4A-EX23-F	caaaagaaacacccaacagc	20
FRMD4A-EX11+12-R	aagatcgcaccctgcacac	19	FRMD4A-EX23-R	cactcccaaccaaagaggtg	20
FRMD4A-EX13-F	gctccatccattccaagg	18	FRMD4A-EX24-F	tgacttgtcttggcaactgg	20
FRMD4A-EX13-R	agggaactcagatgggattg	20	FRMD4A-EX24-R	aacagtgtcggcagcagag	19
FRMD4A-EX14-F	ccgaaattcccagtatgtgg	20	FRMD4A-EX25-F	ctggggaaaggctgagaac	19
FRMD4A-EX14-R	cagacttttcccttccaggag	21	FRMD4A-EX25-R	gcatgggtctcttggctaac	20
FRMD4A-EX15-F	ttcccatttcagcactggag	20	FRMD4A-EX26A-R	tgagtgagactctggcttgc	20
FRMD4A-EX15-R	tgaccatgaacaacgacagc	20	FRMD4A-EX26A-R	tgagccttgacgctgtagtg	20
FRMD4A-EX16-F	atcagaaggtggcctttgc	19	FRMD4A-EX26B-F	tgtacctgcacagccagagc	20
FRMD4A-EX16-R	tgtagccctaagaggcaagc	20	FRMD4A-EX26B-R	atgattcccgttcctcactg	20
FRMD4A-EX17-F	atgatgaggccaagggatg	19	FRMD4A-EX27-F	gatgctgagaccctgctctg	20
FRMD4A-EX17-R	ttcctgggagcagttttctc	20	FRMD4A-EX27-R	acacctcccagtgatgaacc	20
FRMD4A-EX18-F	gtagaaccacgcagtgcaag	20	FRMD4A-EX28-F	gcatgacttgtatgatctgttgc	23

FRMD4A-EX18-R	gaccttggaactcctctgtcc	21	FRMD4A-EX28-R	agttccagttccccatgtatc	21
FRMD4A-EX19A-F	aagggaaccaggaccaaatg	20	FRMD4A-EX29-F	ctccgaacaggaaagactcg	20
FRMD4A-EX19A-R	aaccaagcacctttgatgg	20	FRMD4A-EX29-R	ggtcaccctgtaactcaagtcc	22
FRMD4A-EX19B-F	gctgggtattttactggatgc	21	ARL5B-EX1A-F	ccgtttgggactctcctacc	20
FRMD4A-EX19B-R	aggaggggaaacttcaaagc	20	ARL5B-EX1A-R	aacgctctgtcccctgtctc	20
FRMD4A-EX19C-F	tgggaatataccgccttcttc	21	ARL5B-EX1B-F	tgatggggctgatcttcg	18
FRMD4A-EX19C-R	ttcactaataatctggccaacg	22	ARL5B-EX1B-R	tttctgggcggacacttatc	20
FRMD4A-EX20-F	tcacactcccttgattcctg	20	ARL5B-EX2-F	ctcccacattcttttacatgagaac	25
FRMD4A-EX20-R	atgcggttctgctctttctc	20	ARL5B-EX2-R	aagtgtttgtgaagggtttagc	22
ARL5B-EX3-F	aattcacaccacactgtttcc	21	CELF2-EX9-R	gtcatgcacagcgaaggtg	19
ARL5B-EX3-R	gaatggcttgaactcagcag	20	CELF2-EX10-F	ttgcatcagagagaactgaagg	22
ARL5B-EX4-F	tggggcacagtttttatttg	20	CELF2-EX10-R	gaatgtgtgagaacggcttg	20
ARL5B-EX4-R	tttacctcaagactttcatcagtttag	27	CELF2-EX11-F	caagcacagctcctcagctc	20
ARL5B-EX5-F	ttttcagtttgaccagttcgac	22	CELF2-EX11-R	gtggttggggattttctgag	20
ARL5B-EX5-R	gtttttaagttgccagtgattttac	25	CELF2-EX12-F	caccatttcctcatcacacg	20
ARL5B-EX6-F	tgtgacttgaatgttgacactg	22	CELF2-EX12-R	agcacacaattacactgacagg	22
ARL5B-EX6-R	ggagtgctggtcaagaattg	20	CELF2-EX13-F	cattttcccccattatcacg	20
CELF2-EX1-F	aaaaggcaactgggaaattg	20	CELF2-EX13-R	gatcctcccatctcaacctg	20
CELF2-EX1-R	tgggtctcgatttcatctcc	20	CELF2-EX14-F	aaggcactccagcacttgac	20
CELF2-EX2-F	gggtggccctaaacctactc	20	CELF2-EX14-R	gcccaaatctgcttacaagg	20
CELF2-EX2-R	ctggtcctcacccggact	18	CELF2-EX15-F	acggtggactcacgatcag	19
CELF2-EX3A-F	acccgcatcttgcattagtc	20	CELF2-EX15-R	aaactgggtaggggaagtgg	20
CELF2-EX3A-R	agctccgttcatcttgttgg	20	SVIL-5'UTR1-F	tctcagggctccagggtaag	20
CELF2-EX3B-F	gcctccgctttgttttagttc	21	SVIL-5'UTR1-R	tgaactcttccagcaggttg	20
CELF2-EX3B-R	tgggctgcctagaaagtgac	20	SVIL-5'UTR2-F	caggggtgcttgtgcttg	18
CELF2-EX4-F	gcttgagatgttgttattgctg	22	SVIL-5'UTR2-R	cagccagagcgagactgac	19

CELF2-EX4-R	tttctaaccaggggatgcac	20	SVIL-5'UTR3-F	ttcaaatcctactgtgctggtc	22
CELF2-EX5-F	aaagggtttgattcccgaac	20	SVIL-5'UTR3-R	gctttgatggtaacaccttaatcc	24
CELF2-EX5-R	ggccacagagaagattccag	20	SVIL-5'UTR4-F	agctctttggaggcagcag	19
CELF2-EX6-F	acgtgcttggtctcacatgg	20	SVIL-5'UTR4-R	ctatggttccacaccgcatc	20
CELF2-EX6-R	ctgttggcttcacacctgac	20	SVIL-5'UTR5A-F	gctgaaagacagtgtggacaac	22
CELF2-EX7-F	ttggtcagattagcaagtcagc	22	SVIL-5'UTR5A-R	acgatgtcagcaagcaactg	20
CELF2-EX7-R	actggagagcaagtggaagg	20	SVIL-5'UTR5B-F	ggcgttctttttggagagg	19
CELF2-EX8-F	agatcccccaaagaaaccac	20	SVIL-5'UTR5B-R	tgcccaagggtatctacagg	20
CELF2-EX8-R	ctttgcccctcctacatcag	20	SVIL-EXON1-F	aattttccctcttgcagatcc	21
CELF2-EX9-F	ggatttacagccccagctc	19	SVIL-EXON1-R	gacacggttttctcaataactgac	24
SVIL-5'UTR6-F	gcctggaccaaaattgcttag	21	SVIL-EXON12-R	tctgggtgaaacacagttgg	20
SVIL-5'UTR6-R	gcaatgacaagagacagcaag	21	SVIL-EXON13A-F	tgaaactgcatcaaatcgag	20
SVIL-5'UTR7-F	cgactcccagttcagcaatg	20	SVIL-EXON13A-R	ccttatggctgtctccttcc	20
SVIL-5'UTR7-R	ggccgaaaggagactgtaag	20	SVIL-EXON13B-F	gcgagaacaagggaatgttg	20
SVIL-EXON2+3-F	ttggtcagttttgcatgtcac	21	SVIL-EXON13B-R	ccatgaagatgtgtgaaagctc	22
SVIL-EXON2+3-R	cttctgtggaatatctcatgttctg	25	SVIL-EXON14-F	tcacagagtgtagcgtgctg	20
SVIL-EXON4A-F	cggtgagaaactggtccttc	20	SVIL-EXON14-R	catgagcaaaggcagttcac	20
SVIL-EXON4A-R	gctcttgacctcgtctttgg	20	SVIL-EXON15-F	cggctgtggtcatatcttgg	20
SVIL-EXON4B-F	ccggtgaatccaaggactatg	21	SVIL-EXON15-R	cccaattttagctgctctgtg	21
SVIL-EXON4B-R	gagtgggaccctgtcttcag	20	SVIL-EXON16-F	aatgagttggcctgaggttc	20
SVIL-EXON5-F	ctctctcctccctcaagtgg	20	SVIL-EXON16-R	gaggaaccatgactttcattgac	23
SVIL-EXON5-R	atggattagggcatgtggag	20	SVIL-EXON17-F	atatttaccgcgcttgtgtg	20
SVIL-EXON6A-F	tcaggtcagtttccattcactg	22	SVIL-EXON17-R	cactcatagatacacactctcatgg	25
SVIL-EXON6A-R	ccacgttttccagttccttc	20	SVIL-EXON18-F	gccaagcccatttcagatag	20
SVIL-EXON6B-F	tcgagcaaagcaagaaaacc	20	SVIL-EXON18-R	catgactgagccactgcac	19
SVIL-EXON6B-R	gcacgcacttgtgagctaag	20	SVIL-EXON19+20-F	cagttggttgtcccacagtc	20

SVIL-EXON7-F	ctccttccttacgtgcatgg	20	SVIL-EXON19+20-R	ggagtccccaatttagtacgttatc	25
SVIL-EXON7-R	acaattatggaatcgccaac	20	SVIL-EXON21-F	tcaggtgggaagtccttacg	20
SVIL-EXON8-F	tacttggctgggattgtgtg	20	SVIL-EXON21-R	acatctggtctgccgtctg	19
SVIL-EXON8-R	ggtcactgtatgattctctaactgc	25	SVIL-EXON22-F	ggtgtggctgagatttgaac	20
SVIL-EXON9-F	ctgcaaggggaaactaccac	20	SVIL-EXON22-R	tgaacaggtgatacacactttgag	24
SVIL-EXON9-R	aggctgggtccacaagattc	20	SVIL-EXON23-F	tggtccacgtcatcactcag	20
SVIL-EXON10-F	cttgaactcttggcctccag	20	SVIL-EXON23-R	agcttgagagacaggaggttg	21
SVIL-EXON10-R	gctaaatcacctccccaatg	20	SVIL-EXON24-F	agcacaaaccaggaaagcag	20
SVIL-EXON11-F	tcttaccccatttgctttgg	20	SVIL-EXON24-R	ctatcccttctcacttgaatgc	22
SVIL-EXON11-R	cgcattctggaacaactcac	20	SVIL-EXON25+26-F	ttgcaggcacactgtttctc	20
SVIL-EXON12-F	ctgcctctgtcctagacgtg	20	SVIL-EXON25+26-R	tggataccgacactggctaac	21
SVIL-EXON27-F	cccgttaggatttctgtcttc	21	ZNF438-5'UTR1-R	actccccaaggaactgacg	19
SVIL-EXON27-R	gactacgacatccatatttctgc	23	ZNF438-5'UTR2-F	caggcagtcttctgaccttaac	22
SVIL-EXON28-F	tcaggcacatcaaaaactcc	20	ZNF438-5'UTR2-R	gccagtccataaaagccttg	20
SVIL-EXON28-R	atctggggtgacagccatac	20	ZNF438-5'UTR3-F	gcgccctgaatatgaatcac	20
SVIL-EXON29-F	tgttcagacctcccagagtc	20	ZNF438-5'UTR3-R	ctgtctactttgccaggaacc	21
SVIL-EXON29-R	tctcaaaggcgcatgactg	19	ZNF438-5'UTR4-F	agtctttgagcacagcttgag	21
SVIL-EXON30-F	aagttctgggtcaggagctg	20	ZNF438-5'UTR4-R	tttcccaagaccagtgattc	20
SVIL-EXON30-R	gaaagaaatgggaggcacag	20	ZNF438-5'UTR5-F	atggtgctgaggcaatagag	20
SVIL-EXON31-F	agaccctccctgagtaacacc	21	ZNF438-5'UTR5-R	aagtgatttccagtcctttgc	21
SVIL-EXON31-R	catgagcacccatagagctg	20	ZNF438-5'UTR6-F	tgtgtctccgttcttcatgc	20
SVIL-EXON32-F	ctttttaggctggcaggaag	20	ZNF438-5'UTR6-R	tggctgcacaaagacttcag	20
SVIL-EXON32-R	tcctctctgctcggaatgtc	20	ZNF438-5'UTR7-F	agatgtggccttaatgatatgc	22
SVIL-EXON33-F	gactaaggatgggggaaatg	20	ZNF438-5'UTR7-R	tctgtgaagtagtatccatttgcag	25
SVIL-EXON33-R	aaaacatgaatggggcacag	20	ZNF438-EXON1-F	cccatgagagcagaagtgtg	20
SVIL-EXON34-F	tttctccaccttgaagctcag	21	ZNF438-EXON1-R	tgtgagttttgggtggaatg	20

SVIL-EXON34-R	cttttggtgtaggggtgagg	20	ZNF438-5'UTR8-F	agaaaggagatgggcaaacc	20
SVIL-EXON35-F	cttctccccgttgaactgtc	20	ZNF438-5'UTR8-R	ggtcaatggaagtatccaagc	21
SVIL-EXON35-R	ctgccaggctttatggatg	19	ZNF438-EXON2-F	gttctgccaatggacatcag	20
SVIL-EXON36-F	tcaggttgattataggtcactatgg	25	ZNF438-EXON2-R	aggtatgagccaccccatc	19
SVIL-EXON36-R	catgattcaactcacctttgc	21	ZNF438-EXON3A-F	tggaggtaggtcaaggaatctg	22
SVIL-EXON37-F	agggctgtgtgtctgtaagc	20	ZNF438-EXON3A-R	tggtcacttccattggtcag	20
SVIL-EXON37-R	tctgataacctcctgaatggtg	22	ZNF438-EXON3B-F	ctcaccaccctgaactcctg	20
SVIL-EXON38-F	actgcaccagggaaatgg	18	ZNF438-EXON3B-R	ttctctttgctgctccactg	20
SVIL-EXON38-R	tccagagtccatgccattaag	21	ZNF438-EXON3C-F	ctttaatgcagccaccaagg	20
SVIL-3'UTR-F	gaattagctctgccaccaatatg	23	ZNF438-EXON3C-R	gcgacaaatccgacaactg	19
SVIL-3'UTR-R	gggcattgagatcctaaaattg	22	ZNF438-EXON3D-F	aagaagccttggcacagatg	20
ZNF438-5'UTR1-F	aggctttggtagagcagtcg	20	ZNF438-EXON3D-R	tggttagggtaattccaagtatgg	24
ZNF438-EXON4A-F	ttttagatccctgaaggctgac	22	MTPAP-EXON5-R	ggaacaccaaattatcaacagg	22
ZNF438-EXON4A-R	ttatgctggtgcttccagtg	20	MTPAP-EXON6-F	ctcgttgtccttgaactcctg	21
ZNF438-EXON4B-F	acaagccaagggaaacctg	19	MTPAP-EXON6-R	gggactttggtaacacatgg	20
ZNF438-EXON4B-R	tcagcatttaccatctaacttgatac	26	MTPAP-EXON7+8-F	agctgagatcatgccgctac	20
RAB18-EXON1-F	ctgccgaaataaaacagcttg	21	MTPAP-EXON7+8-R	ctctggacatcaacctccttg	21
RAB18-EXON1-R	agagcgacccagagaacttg	20	MTPAP-EXON9-F	agtacacacacccaccatgc	20
RAB18-EXON2-F	tgggggattgtgtaggagag	20	MTPAP-EXON9-R	taaggggggccaatgagaag	19
RAB18-EXON2-R	ttagtagcagtccctggcatac	22	ITGA8-EXON1A-F	gttagctctgccaggaggtg	20
RAB18-EXON3-F	gaagttggggtgtgaattgg	20	ITGA8-EXON1A-R	gtggctgctacccaggag	18
RAB18-EXON3-R	cctcctccccttaaaaattgtc	22	ITGA8-EXON1B-F	tagaccagccgcgaggag	18
RAB18-EXON4-F	atcctccctactgtcttgctg	21	ITGA8-EXON1B-R	atggcaggggagagaagg	18
RAB18-EXON4-R	gcagttgctcagaatttgttg	21	ITGA8-EXON2-F	aatacgttcatgccctccac	20
RAB18-EXON5-F	cttgtcagtaagcgaacacatc	22	ITGA8-EXON2-R	taaacgagcagcacaccaag	20
RAB18-EXON5-R	gcccagtttctgtaatactccaag	24	ITGA8-EXON3-F	acaggcatgagccaccatac	20

RAB18-EXON6+7-F	gctgttgttttgctttaatgg	21	ITGA8-EXON3-R	aaatgccaacagccttatcg	20
RAB18-EXON6+7-R	cacaaggcaactaggaacaatac	23	ITGA8-EXON4-F	tgaccaaggcactcattagc	20
RAB18-EXON8-F	gggaaaaattgctgatttgg	20	ITGA8-EXON4-R	cacgcttttgacttgctgtc	20
RAB18-EXON8-R	cgtggtgctaggagttctaaatac	24	ITGA8-EXON5-F	gaatcacaacgctatttggtg	21
MTPAP-EXON1-F	ttggtaaattgtgtgactgtgg	22	ITGA8-EXON5-R	tttaataggccgctatgtgc	20
MTPAP-EXON1-R	acatctcacttctgcctcagc	21	ITGA8-EXON6-F	ccatgtctgcaaaactgcac	20
MTPAP-EXON2-F	ttggatcataacccctgtgg	20	ITGA8-EXON6-R	aacgcttcatttgagaaactatg	23
MTPAP-EXON2-R	acatagttggaccccgtctc	20	ITGA8-EXON7-F	ctctgccttggagtttgagc	20
MTPAP-EXON3-F	gcccagtgttccttttcttc	20	ITGA8-EXON7-R	gcggctggaaaaatagcag	19
MTPAP-EXON3-R	ccacgattcatctccgtaac	20	ITGA8-EXON8-F	ttaaaattgacctcagaatatctcc	25
MTPAP-EXON4-F	gtcttcaacgccaccaaatc	20	ITGA8-EXON8-R	ttcattcataacatgggattatgg	24
MTPAP-EXON4-R	caatacaaagaaagacttcaccaaag	26	ITGA8-EXON9-F	cagacaggtgctggttgatg	20
MTPAP-EXON5-F	actggggatcttttaagtttgc	22	ITGA8-EXON9-R	tgatgttcggtggattaggtg	21
ITGA8-EXON10-F	agatcattccgtgggctttc	20	ITGA8-EXON23-R	gggtttactctgctttccaatg	22
ITGA8-EXON10-R	agctggatgggatctgtctg	20	ITGA8-EXON24-F	gctggagagcctacactagcag	22
ITGA8-EXON11-F	ttcactgggtcaggggttag	20	ITGA8-EXON24-R	tttgagcccagaaagtcgag	20
ITGA8-EXON11-R	gcagcagatgtggaaaatctc	21	ITGA8-EXON25-F	agcccatgatgctttcagag	20
ITGA8-EXON12-F	aagcagcgaaagcaatctg	19	ITGA8-EXON25-R	tttggatgtgctgctaattg	20
ITGA8-EXON12-R	aaacgtaaagctgccttgag	20	ITGA8-EXON26-F	cacactgctggtaatcacagg	21
ITGA8-EXON13-F	acttgagtccgggagtgtg	19	ITGA8-EXON26-R	gcaagtgctccttaattcttacag	24
ITGA8-EXON13-R	tgaaagtcgctctggaatcg	20	ITGA8-EXON27-F	ttcatcctcgccattaaactg	21
ITGA8-EXON14-F	gaagctccaacatcctttcc	20	ITGA8-EXON27-R	tggcctcaagtaatccatctg	21
ITGA8-EXON14-R	ctggaagtttggtcatgcag	20	ITGA8-EXON28-F	acgcttagatggatggatgc	20
ITGA8-EXON15-F	ttggcacaaatgctgagaac	20	ITGA8-EXON28-R	gaaattggtgagctgaagagg	21
ITGA8-EXON15-R	ctcaaacttcggccacagac	20	ITGA8-EXON29-F	ttcatgttcgagtgctacctc	21
ITGA8-EXON16-F	cctcctgtactattttccagtattacc	27	ITGA8-EXON29-R	tgccaaagcaaacaagacac	20

ITGA8-EXON16-R	ccaagcatgataaatgagctg	21	ITGA8-EXON30A-F	tggggaatagagagcgagac	20
ITGA8-EXON17-F	cagctcatttatcatgcttgg	21	ITGA8-EXON30A-R	agggaggtgtcaaatgttcc	20
ITGA8-EXON17-R	tgaagtcagggtcaagaggag	21	ITGA8-EXON30B-F	aaggtgaactaaggtgaaatgactg	25
ITGA8-EXON18-F	ccacataaatcagcctccatc	21	ITGA8-EXON30B-R	ccatgacttttaaacactccaaag	24
ITGA8-EXON18-R	tgcacaagaggaaaggcttc	20	ITGA8-EXON30C-F	tgctaaggaaaggaagattgg	21
ITGA8-EXON19-F	cctgtattgggaggtcactg	20	ITGA8-EXON30C-R	ttcaagatgatgggaaaatagc	22
ITGA8-EXON19-R	gacccaaaccacaggctaac	20	ITGA8-EXON30D-F	tacaggcatgagccaaactg	20
ITGA8-EXON20-F	tgcctcttcctaccatctcttc	22	ITGA8-EXON30D-R	tcaggacacaaagttttccaac	22
ITGA8-EXON20-R	ttgtggctcattgctgttatg	21	PIP4K2A-EX1-F	cgcagcctaacggtccag	18
ITGA8-EXON21-F	tttctcttctgagttaagggtcatc	25	PIP4K2A-EX1-R	gaggaggaggggaacgag	18
ITGA8-EXON21-R	gttgttgctgctaggtgctg	20	PIP4K2A-EX2-F	agattatgatggcagagggaag	22
ITGA8-EXON22-F	gcagaacaaaggaaagagttacag	24	PIP4K2A-EX2-R	gacagatggttatgtttatcacagg	25
ITGA8-EXON22-R	ggtgagcttttaaggccaag	20	PIP4K2A-EX3-F	ctttctgcgggttgtacctg	20
ITGA8-EXON23-F	ttagtaacgtgtgttctcctgtg	23	PIP4K2A-EX3-R	gaggtccccaatcaagagaac	21
PIP4K2A-EX4-F	ttatctgggaatcggaaagc	20	SLC39A12-EX5-F	gtcgtaccggcttcatgtg	19
PIP4K2A-EX4-R	gccttggtgagaaatcgctac	21	SLC39A12-EX5-R	ttgaatggcaactccatcac	20
PIP4K2A-EX5-F	tggtaagcagtacaaatgtggtc	23	SLC39A12-EX6-F	tccatatccctactggctgttac	23
PIP4K2A-EX5-R	ttgcctcacaggaaggaatac	21	SLC39A12-EX6-R	atgtggcgagcctaatgttg	20
PIP4K2A-EX6-F	aatcgccctgagaaacattg	20	SLC39A12-EX7-F	catacccaaaagcaaactgg	20
PIP4K2A-EX6-R	ggctggagaagaatcacagg	20	SLC39A12-EX7-R	gaaggagctgtgtattcttgaaatc	25
PIP4K2A-EX7-F	gagggatgagaagcccattc	20	SLC39A12-EX8-F	tgtggcaactaccattttgg	20
PIP4K2A-EX7-R	caggaagaaccacaatagcag	21	SLC39A12-EX8-R	aacagctttcaggtgcttgg	20
PIP4K2A-EX8-F	ccggtggtattcaagccttc	20	SLC39A12-EX9-F	cattgtgtgtgtctatgaaacctg	24
PIP4K2A-EX8-R	tgagagggctgctaaactgg	20	SLC39A12-EX9-R	atttgagagctggataggaattag	24
PIP4K2A-EX9-F	ccaggagtgtaagagcatcg	20	SLC39A12-EX10-F	ttgcggaaacaaagtgatg	19
PIP4K2A-EX9-R	ggagtttggaggatgagtgc	20	SLC39A12-EX10-R	ggattttattccaaggtacaggag	24

PIP4K2A-EX10-F	tctctggactccttccatcc	20	SLC39A12-EX11-F	cagccaaaggaatggaactc	20
PIP4K2A-EX10-R	agcagctcagtggcagaaag	20	SLC39A12-EX11-F	gcaactgctatcacacacctg	21
PIP4K2A-EX11-F	gatgtttttcagagcagagactg	23	SLC39A12-EX12A-F	aaagacatggtagagtaggtattcagc	27
PIP4K2A-EX11-R	cactcattcattcggccatag	21	SLC39A12-EX12A-R	aaagcaagagcttcatcatgg	21
SLC39A12-5'UTR-F	ctattctgcgaggtgaaatcc	21	SLC39A12-EX12B-F	tcttaggcaaagtgtgtctctttc	24
SLC39A12-5'UTR-R	tgactgtgggctagtaacaaagg	23	SLC39A12-EX12B-R	aggtctcccaaaatggcttg	20
SLC39A12-EX1-F	tcctgccatttggggttag	19	PTTG-EX-1F	cctggctgcttaggtccttt	20
SLC39A12-EX1-R	agatttgcctgtgattttgc	20	PTTG-EX-1R	gctcggagccatctaagc	18
SLC39A12-EX2-F	ctgcctgcttttcctttctg	20	PTTG-EX-2F	cgtgactgttccgctgttta	20
SLC39A12-EX2-R	acttctggcgaaaactgctc	20	PTTG-EX-2R	gactccaattgcccaaaaag	20
SLC39A12-EX3-F	ggaccagtgagatgagggttag	22	PTTG-EX-3F	gggggtgagaggtcaagttt	20
SLC39A12-EX3-R	ttgaaaggccacataagctg	20	PTTG-EX-3R	ctcagagggaagaaagctacg	21
SLC39A12-EX4-F	atgtccattctgtcatctctagg	23	PTTG-EX-4F	tgctgacaggtgctggtact	20
SLC39A12-EX4-R	tcactcaatctgaacccaagg	21	PTTG-EX-4R	gtccaaagcaacccagattc	20
PTTG-EX-5F	caacccagtggaaaaaggtg	20	ADAM19-EX-12R	tttttcatggggtattggaca	21
PTTG-EX-5R	acctcccgcacaactcttc	19	ADAM19-EX-13F	ttgctagagagctggggttc	20
PTTG-EX-6F	aaatgtgtcaggaggggatg	20	ADAM19-EX-13R	ccttttccgctctgtgtttc	20
PTTG-EX-6R	gactctttgttaaacacctcagc	23	ADAM19-EX-14F	tggtttgggctaatgaggtc	20
ADAM19-EX-1F	agcctcccctcctccatc	18	ADAM19-EX-14R	cctgagtcaaggggatgaaa	20
ADAM19-EX-1R	gcagaacgtgggaacaaag	19	ADAM19-EX-15F	tggagggaacaggaagaaga	20
ADAM19-EX-2F	gcagctcacagaaagtgcat	20	ADAM19-EX-15R	gggccgagaatagatgactg	20
ADAM19-EX-2R	agagtgggccagaaacagaa	20	ADAM19-EX-16F	gggttccaaagcacatgact	20
ADAM19-EX-3F	agcgctgtgctagatcctgt	20	ADAM19-EX-16R	ccaccaaggctcagaggtt	19
ADAM19-EX-3R	ctgcttccttccagaacagc	20	ADAM19-EX-17-18F	gccatgggtcctcatatgtt	20
ADAM19-EX-4F	ggatagtcctgcctggttca	20	ADAM19-EX-17-18R	caatgtggatgctctgcaac	20
ADAM19-EX-4R	tcccttcagaggagagacca	20	ADAM19-EX-19F	tgcaaatcaaatgcctgaag	20

ADAM19-EX-5F	ggaaagctctttgcaggttg	20	ADAM19-EX-19R	cagtcatagcaggggagagg	20
ADAM19-EX-5R	tcaatggactccttcccaag	20	ADAM19-EX-20F	ccagagcagacttgggaaag	20
ADAM19-EX-6F	gaaccaatgatcaaaacccact	22	ADAM19-EX-20R	gggcaagactccatctcaaa	20
ADAM19-EX-6R	cctggattcaaagtaagcaacc	22	ADAM19-EX-21F	tgggctctggtttatctcaaa	21
ADAM19-EX-7F	agtgccgaatgacaaaatca	20	ADAM19-EX-21R	agtttcaccttccccacctt	20
ADAM19-EX-7R	gagaaagatgaatgggcaaca	21	ADAM19-EX-22F	ccagcttgctctcctgactt	20
ADAM19-EX-8F	ggtcctaccctccaaagctc	20	ADAM19-EX-22R	gggcaccaagaaacatgaat	20
ADAM19-EX-8R	tgctcattccctttcagaaga	21	ADAM19-EX-23F	gttctcagtgggtggtccat	20
ADAM19-EX-9F	tcaagctccaccagaatgaa	20	ADAM19-EX-23R	agagcagaagcaatgggaag	20
ADAM19-EX-9R	ctctggaccagggagtcaga	20	KCNIP1-EX-1aF	ccgggtctgtctcttaggg	19
ADAM19-EX-10F	cgtgatggccacttactcc	19	KCNIP1-EX-1aR	gaggcttctggcttccttct	20
ADAM19-EX-10R	attgcccttcctgatgtcaa	20	KCNIP1-EX-1bF	cttcagggcaccgtcctc	18
ADAM19-EX-11F	gtggaattggtggagacctg	20	KCNIP1-EX-1bR	ctccctcctctgggtgttc	19
ADAM19-EX-11R	ttggccccatcagaagttta	20	KCNIP1-EX-2F	cactcttttgacagcccaga	20
ADAM19-EX-12F	aggagtgacagcacgagtga	20	KCNIP1-EX-2R	ttatgagctcccttggcatt	20
KCNIP1-EX-3F	cttgggagaagtggaaatgc	20	KCNIP1-EX-8aR	gggcacgtaagcagcatatt	20
KCNIP1-EX-3R	agcaaatggcaaggaagaga	20	KCNIP1-EX-8bF	atggaaggtccctctgctta	20
KCNIP1-EX-4F	tcctctctttcttgtcgaagc	21	KCNIP1-EX-8bR	tccctttcacagcttacctc	21
KCNIP1-EX-4R	ccccaatgccttgagattta	20			
KCNIP1-EX-5F	cccaaagacacagcttcaca	20			
KCNIP1-EX-5R	tgcctcattcagacagtgct	20			
KCNIP1-EX-6F	tccccttctgcttgcttcta	20			
KCNIP1-EX-6R	acagggttggacacaaatca	20			
KCNIP1-EX-7F	gagctccagcaagaaagcag	20			
KCNIP1-EX-7R	tgtgcaggtttctcatcacc	20			
KCNIP1-EX-8aF	ggaaacccatgcttgacatt	20			

5. References

Allen IM (1945) Observations on cases of reflex epilepsy. *New Zealand Med J* 44:135-142

Bickford RG, Whean JL, Klass DW, Corbin KB (1956) Reading epilepsy: clinical and electroencephalographic studies of a new syndrome. *Trans Am Neurol Assoc* 81st meeting:100-102

Brodtkorb E, Gu W, Nakken KO, Fischer C, Steinlein OK (2002) Familial temporal lobe epilepsy with aphasic seizures and linkage to chromosome 10q22-q24. *Epilepsia* 43:228-235

Brodtkorb E, Michler RP, Gu W, Steinlein OK (2005) Speech-induced aphasic seizures in epilepsy caused by LGl1 mutation. *Epilepsia* 46:963-966

Concolino D, Iembo MA, Rossi E, Giglio S, Coppola G, Miraglia Del Giudice E, Strisciuglio P (2002) Familial pericentric inversion of chromosome 5 in a family with benign neonatal convulsions. *J Med Genet* 39:214-216

De Keyzer K, Corthouts I, Van Coster R, Verhelst H (2005) Hot-water epilepsy: a new Caucasian case. *Eur J Pediatr* 164:184-185

Depienne C, Magnin E, Bouteiller D, Stevanin G, Saint-Martin C, Vidailhet M, Apartis E, Hirsch E, LeGuern E, Labauge P, Rumbach L (2010) Familial cortical myoclonic tremor with epilepsy: the third locus (FCMTE3) maps to 5q. *Neurology* 74:2000-2003

Dib C, Faure S, Fizames C, Samson D, Drouot N, Vignal A, Millasseau P, Marc S, Hazan J, Seboun E, Lathrop M, Gyapay G, Morissette J, Weissenbach J (1996) A comprehensive genetic map of the human genome based on 5,264 microsatellites. *Nature* 380:152-154

Du W, Bautista JF, Yang H, Diez-Sampedro A, You SA, Wang L, Kotagal P, Luders HO, Shi J, Cui J, Richerson GB, Wang QK (2005) Calcium-sensitive potassium channelopathyin human epilepsy and paroxysmal movement disorder. *Nat Genet* 37:733-738

Faingold CL, Randall M, Tupal S (2010) DBA/1 mice exhibit chronic susceptibility to audiogenic seizures followed by sudden death associated with respiratory arrest. *Epilepsy Behav* 17:436-440

Ferlazzo E, Zifkin BG, Andermann E, Andermann F (2005) Cortical triggers in generalized reflex seizures and epilepsies. *Brain* 128:700-710

Feucht M, Fuchs K, Pichlbauer E, Hornik K, Scharfetter J, Goessler R, Fureder T, Cvetkovic N, Sieghart W, Kasper S, Aschauer H (1999) Possible association between childhood absence epilepsy and the gene encoding GABRB3. *Biol Psychiatry* 46:997-1002

Goldberg EM, Coulter DA (2013) Mechanisms of epileptogenesis: a convergence on neural circuit dysfunction. *Nat Rev Neurosci* 14:337-349

Grosso S, Pucci L, Bartalini G, Anichini C, Di Bartolo RM, Bazzotti S, Morgese G, Balestri P (2006) Photoparoxysmal responses in children with chromosomal aberrations. *Epilepsy Res* 72:164-170

Hizem Y, Gargouri A, Ben Djebara M, Kacem I, Abdelkefi I, Jemli F, Gouider R (2012) Hot water epilepsy with pachygyria. *Neurol Sci* 33:631-633

Kanemoto K, Watanabe Y, Tsuji T, Fukami M, Kawasaki J (2001) Rub epilepsy: a somatosensory evoked reflex epilepsy induced by prolonged cutaneous stimulation. *J Neurol Neurosurg Psychiatry* 70:541-543

Kaplan Y, Kurt SG, Karaer H, Sarikaya B, Bebek N (2009) Intra-familial incidence and characteristics of hot water epilepsy. *Can J Neurol Sci* 36:575-581

Kinirons P, Verlaan DJ, Dube MP, Poirier J, Deacon C, Lortie A, Clement JF, Desbiens R, Carmant L, Cieuta-Walti C, Shevell M, Rouleau GA, Cossette P (2008) A novel locus for idiopathic generalized epilepsy in French-Canadian families maps to 10p11. *Am J Med Genet A* 146A:578-584

Kluger G, Koehler U, Neuhann TM, Pieper T, Staudt M, von Stulpnagel C (2013) Generalized epilepsy in two patients with 5p duplication. *Neuropediatrics* 44:225-229

Lee SA, Choi EJ, Kang JK (2006) Reflex epilepsy induced by playing oriental card or board games. *J Clin Neurol* 2:262-267

Mautner VF, Lindenau M, Gottesleben A, Goetze G, Kluwe L (2000) Supporting evidence of a gene for partial epilepsy on 10q. *Neurogenetics* 3:31-34

Meghana A, Sinha S, Sathyaprabha TN, Subbakrishna DK, Satishchandra P (2012) Hot water epilepsy clinical profile and treatment – a prospective study. *Epilepsy Res* 102:160-166

Menon R, Ryan S, Congdon P (1989) Water induced epilepsy. *J R Soc Med* 82:301

Midi I, Agan K, Aykut-Bingol C (2005) Hot water epilepsy (HWE) familial and non-familial case reports. *Marmara Med J* 18:131-134

Midzyanovskaya IS, Kuznetsova GD, van Luijtelaar EL, van Rijn CM, Tuomisto L, Macdonald E (2006) The brain 5HTergic response to an acute sound stress in rats with generalized (absence and audiogenic) epilepsy. *Brain Res Bull* 69:631-638

Milanlioglu A, Tombul T, Sayin R (2010) Hot water epilepsy in a pregnant woman: a case report. *Case Rep Med* 2010:134578

Nakayama J, Hamano K, Iwasaki N, Nakahara S, Horigome Y, Saitoh H, Aoki T, Maki T, Kikuchi M, Migita T, Ohto T, Yokouchi Y, Tanaka R, Hasegawa M, Matsui A, Hamaguchi H, Arinami T (2000) Significant evidence for linkage of febrile seizures to chromosome 5q14-q15. *Hum Mol Genet* 9:87-91

Ottman R, Risch N, Hauser WA, Pedley TA, Lee JH, Barker-Cummings C, Lustenberger A, Nagle KJ, Lee KS, Scheuer ML, Neystat M, Susser M, Wilhelmsen KC (1995) Localization of a gene for partial epilepsy to chromosome 10q. *Nat Genet* 10:56-60

Panayiotopoulos CP (2005) The Epilepsies: Seizures, Syndromes and Management. *Bladon Medical Publishing*, Oxfordshore (UK)

Panayiotopoulos CP (2010) A clinical guide to epileptic syndromes and their treatment. *Springer*

Panayiotopoulos CP, Engel J (2004) Reflex Seizures. *MedLink Neurology*

Pavlidou E, Gkampeta A, Dosiadi E, Pavlov E (2013) Hot water epilepsy: case report and literature review. *J of Pediatr Sci* 5:e197

Pinto D, Kasteleijn-Nolst Trenite DG, Cordell HJ, Mattheisen M, Strauch K, Lindhout D, Koeleman BP (2007) Explorative two-locus linkage analysis suggests a multiplicative interaction between the 7q32 and 16p13 myoclonic seizures-related photosensitive loci. *Genet Epidemiol* 31:42-50

Pinto D, Westland B, de Haan GJ, Rudolf G, da Silva BM, Hirsch E, Lindhout D, Trenite DG, Koeleman BP (2005) Genome-wide linkage scan of epilepsy-related photoparoxysmal electroencephalographic response: evidence for linkage on chromosomes 7q32 and 16p13. *Hum Mol Genet* 14:171-178

Poolos NP, Johnston D (2012) Dendritic ion channelopathy in acquired epilepsy. *Epilepsia* 9:32-40

Poza JJ, Saenz A, Martinez-Gil A, Cheron N, Cobo AM, Urtasun M, Marti-Masso JF, Grid D, Beckmann JS, Prudhomme JF, Lopez de Munain A (1999) Autosomal dominant lateral temporal epilepsy: clinical and genetic study of a large Basque pedigree linked to chromosome 10q. *Ann Neurol* 45:182-188

Ptacek LJ, Fu YH (2003) What's new in epilepsy genetics? *Mol Psychiatry* 8:463-465

Puranam RS, Jain S, Kleindienst AM, Saxena S, Kim MK, Kelly Changizi B, Padma MV, Andrews I, Elston RC, Tiwari HK, McNamara JO (2005) A locus for generalized tonic-clonic seizure susceptibility maps to chromosome 10q25-q26. *Ann Neurol* 58:449-458

Ratnapriya R, Satishchandra P, Dilip S, Gadre G, Anand A (2009) Familial autosomal dominant reflex epilepsy triggered by hot water maps to 4q24-q28. *Hum Genet* 126:677-683

Ratnapriya R, Satishchandra P, Kumar SD, Gadre G, Reddy R, Anand A (2009) A locus for autosomal dominant reflex epilepsy precipitated by hot water maps at chromosome 10q21.3-q22.3. *Hum Genet* 125:541-549

Reflex Epilepsy [Editorial] (1975). *Br Med J* p.338

Sambook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual. *Cold Spring Harbour Laboratory*, Cold Spring Harbour, New York

Satishchandra P (2003) Hot-water epilepsy. *Epilepsia* 44:29-32

Sellal S, Chevalier Y, Collard M (1993) 'Pinocchio syndrome': a peculiar form of reflex epilepsy? *J Neurol Neurosurg Psychiatry* 56:936

Sharma M, Sharma VK, Kaushal RK, Chaudhary S (2002) Hot water epilepsy. *Indian Pediatr* 39:879-880

Striano S, Coppola A, del Gaudio L, Striano P (2012) Reflex seizures and reflex epilepsies: old models for understanding mechanisms of epileptogenesis. *Epilepsy Res* 100:1-11

Syed R (2010) Hot water epilepsy: a rare form of reflex epilepsy. *J Neurosci Rural Pract* 1:99-101

Tauer U, Lorenz S, Lenzen KP, Heils A, Muhle H, Gresch M, Neubauer BA, Waltz S, Rudolf G, Mattheisen M, Strauch K, Nurnberg P, Schmitz B, Stephani U, Sander T (2005) Genetic dissection of photosensitivity and its relation to idiopathic generalized epilepsy. *Ann Neurol* 57:866-873

Ullal GR, Satishchandra P, Shankar SK (1996) Effect of antiepileptic drugs and calcium channel blocker on hyperthermic seizures in rats: animal model for hot water epilepsy. *Indian J Physiol Pharmacol* 40:303-308

Yang Z, Liu X, Qin J, Zhang Y, Bao X, Wang S (2010) Clinical and electrophysiological characteristics of startle epilepsy in childhood. *Clin Neurophysiol* 121:658-664

Websites used for bioinformatics analysis

1000genomes – http://www.browser.1000genomes.org/index.html

Ensembl – http://www.asia.ensembl.org/index.html

Entrez gene, SNP and Genbank database: http://www.ncbi.nlm.nih.gov/

Exome Variant Server – http://www.evs.gs.washington.edu/EVS/

NCBI Mapviewer – http://www.ncbi.nlm.nih.gov/mapview/

OligoCalc: http://www.basic.northwestern.edu/biotools/OligoCalc.html

Primer3: http://frodo.wi.mit.edu/

Uniprot: http://www.uniprot.org

Softwares used for data analysis

Genemapper v3.7

MLINK – LINKAGE5.1

SeqMan^{TM} II – DNA Star