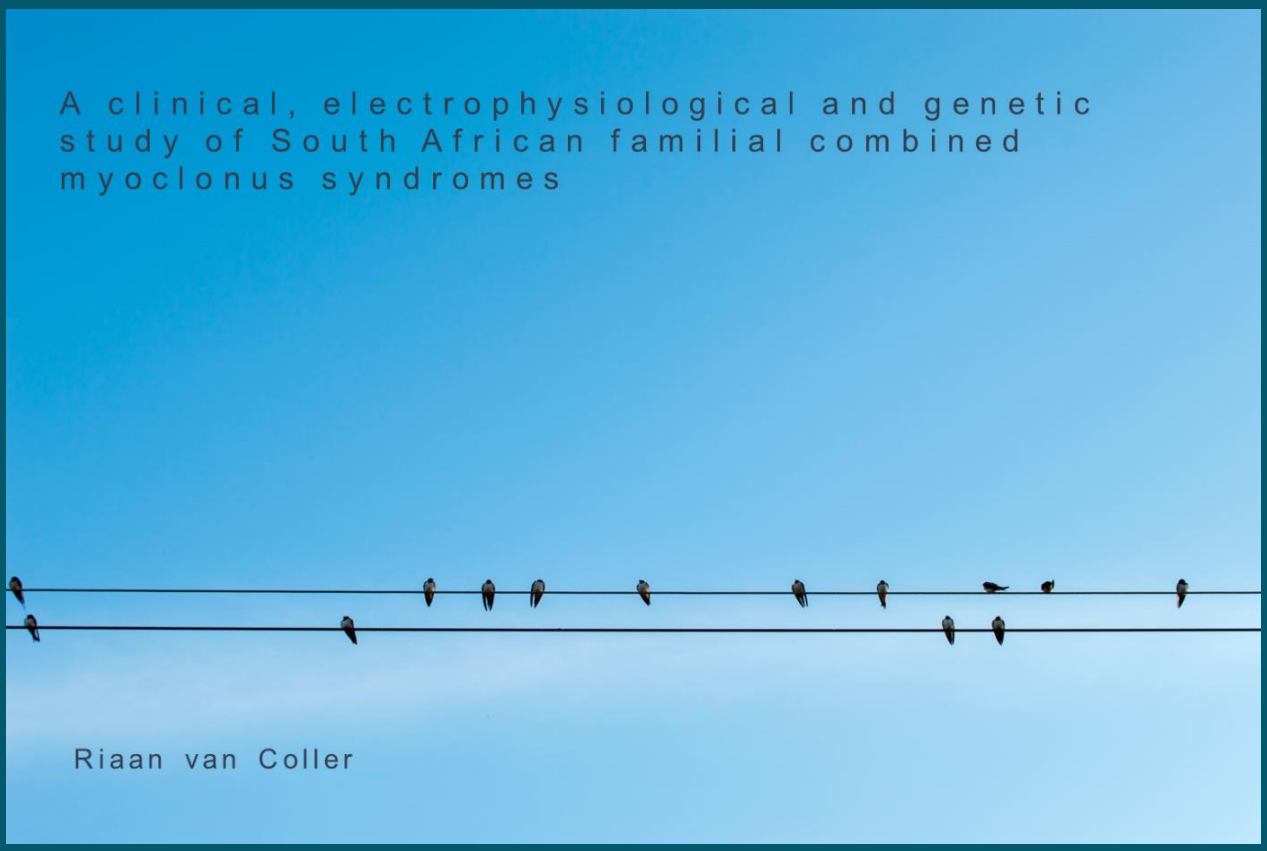
A clinical, electrophysiological and genetic study of South African familial combined myoclonus syndromes



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A clinical, neurophysiological and genetic study of South African familial combined myoclonic syndromes

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Table of Contents

Index of tables:

Index of figures:

Ethics statement

The study: A Clinical, Electrodiagnostic and Imaging Study of the First South African Family with Familial Cortical Myoclonic Tremor With was approved by the Clinical Ethics Committee, University of Pretoria, number: 152/2011. The publications *Familial cortical myoclonic tremor and epilepsy: Description of a new South African pedigree with 30 year follow up* and *Successful Treatment of Disabling Paroxysmal Nonkinesigenic Dyskinesia with Deep Brain Stimulation of the Globus Pallidus Internus* were published from this study. All participants signed written informed consent before participation in the study. Additional written consent was obtained for the online publication and dissemination of the video material as required by the publication and is on file.

The study: The outcome of Deep Brain Stimulation in South Africa, was approved by the Clinical Ethics Committee, University of Pretoria, number 36/2015. The publication: *A South African family with myoclonus-dystonia syndrome with a novel mutation in the SGCE gene responding to deep brain stimulation* was a product of this study. All patients signed written informed consent to participate in the study. Additional written consent was obtained for the online publication and dissemination of the video material; and is on file.

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Summary

Myoclonus is a complex disorder of rapid repetitive muscle jerks that can occur in proximal or distal appendicular or axial muscles. It can be of cortical, sub-cortical or spinal cord origin; part of progressive and severely disabling epilepsy syndromes, basal ganglia conditions, and physiological or even functional (psychogenic)[1].

A systematic review of the literature shows the knowledge gap of the genetic causes of myoclonus in South Africa with 25 identified publications from Africa of which eleven were from South Africa. Publications varied from case studies to case series and included four publications with cortical myoclonic tremor (CMT) and two with North Sea Progressive Myoclonic Epilepsy, two with subcortical myoclonus and case studies with rare cases of individuals with myoclonic disorders.

In this publication the study of myoclonus in three different settings is presented. In the first: cortical myoclonic tremor (CMT), a rapid distal form of myoclonus, resembling tremor, with neurophysiological evidence of cortical origin. The study researched a South African family with Familial Cortical Myoclonic Tremor with Epilepsy (FCMTE). The first part of this study showed the median onset of cortical tremor 16 was and that of epilepsy was 42 years; patients were stable with long term follow up after 30 years without evidence of progressive ataxia or cognitive impairment. The second part of the study presents the discovery of the genetic mutation causing this condition: a pentanucleotide repeat expansion in the intronic region of the *STARD7* gene. This mutation was also found in families with FCMTE2 with a similar phenotype and followed on work showing pentanucleotide repeat expansion mutations in other forms of FCMTE in different genetic locations.

The second setting proved a new mutation, a premature stop mutation p.L275X, in the epsilon-sarcoglycan gene causing subcortical origin, Myoclonus Dystonia Syndrome (MDS) in a three generation South African family with mild phenotype differences in the clinical presentation: myoclonus and dystonia in the same appendicular body part as well as truncal. Two of the affected individuals studied underwent Deep Brain Stimulation surgery of the Globus Pallidum with significant sustained improvement in the motor and non-motor features of MDS recorded and confirmed by a blinded rater.

In the third setting, two patients with sporadic Paroxysmal Non-kinesigenic dyskinesia (PNKD) with the complex phenomenology of episodic dystonia, myoclonus and chorea of South African origin is presented. Both patients underwent successful DBS of the pallidum with long-term outcomes presented. Although these two individuals were not tested for the known myofibrillogenesis regulator-1 (*MR-1*) mutation they represent two cases of this rare disorder from South African setting and prove the successful use of DBS treatment.

Myoclonic Syndromes: A review of genetic causes in South Africa

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Myoclonus can be defined as sudden, brief, involuntary muscle jerks that can affect a single muscle or group of muscles. A muscle contraction or a sudden loss of muscle tone differentiates between positive or negative myoclonus, respectively. The contractions are caused by synchronous bursts of agonist and antagonist muscle groups and generally characterised by the duration of contraction and the latency of contraction recruitment. Myoclonus is often associated with other movement disorders, ataxia or epilepsy[2].

Classification

The clinical field of myoclonus is vast and poorly defined. Anatomical, clinical and etiological classification approaches have been described[2,3].

The anatomical classification approach is most often used as an initial classification option and allocates the origin of myoclonus to the cortex, subcortex, spinal cord or peripheral nerve[1]. A further sub-classification of cortical-subcortical myoclonus is sometimes included[4]. This approach relies on the neurophysiological evidence of cortical hyperexcitability shown with electro-encephalography (EEG) -back averaging, coherence analysis, the presence of giant-somato-sensory evoked potentials (g-SEP) and a positive c-reflex on long-loop reflex stimulation[1,5–7]. Examples of cortical myoclonus include Progressive Myoclonic Epilepsy (PME), Familial Cortical Myoclonic Tremor with Epilepsy (FCMTE) and Progressive Myoclonic Ataxia (PMA); subcortical myoclonus is seen most commonly in Myoclonus Dystonia Syndrome (MDS), whereas propriospinal myoclonus occurs in spinal cord and myoclonus due to peripheral nerve involvement in conditions like clonic hemifacial spasm.

The clinical classification of myoclonus is limited by the wide range of causes and clinical scenarios in which myoclonus can occur. It is also biased by the

approach from the different subspecialities of movement disorders and epilepsy that are involved in treating patients with myoclonus[3]. This is demonstrated best by the borderline area between tremor and myoclonus. Cortical myoclonic tremor (CMT) is a myoclonic phenomenon that is often clinically indistinguishable from tremor. Polymyography is necessary for the differentiation and typically shows pseudo-rhythmical irregular short (less than 50ms) 8 – 13Hz bursts affecting the hands[2,6,8]. CMT occurs in Familial Cortical Myoclonic Tremor with Epilepsy (also known as Familial Adult Myoclonic Epilepsy (FAME)) and in various other conditions including Huntington's disease like-2 (HDL-2)[9] and Angelman syndrome[10]. Identification of myoclonus co-existing with other movement disorders (Parkinson's disease, Cortical Basal Syndrome, Creutzfeldt Jacob disease) is often difficult, especially when myoclonus is of low amplitude and stimulus induced[8].

The etiological classification is divided into four groups: physiological myoclonus, essential myoclonus, epileptic and symptomatic myoclonus[4].

Epidemiology

Due to the wide range of conditions in which myoclonus can be a clinical feature and the multitude of possible causes, little is known about the epidemiology. Only one community-based study has been published, showing the average annual incidence of myoclonus as 1.3 cases per 100 000 in Olmsted county, Minnesota between 1976 and 1990[11].

Despite this lack of data, myoclonus appears to be a common condition: myoclonus was the reason for Emergency Room visits in 30% of patients who presented with a movement disorder for acute care in a study from an urban centre in South Korea[12]. In this setting, metabolic causes and drug reactions were the most common causes.

In a hospital-based retrospective chart analysis done in Cameroon in a geographical area including a large urban and smaller rural population, 3.2% of cases had problems relating to the sub-speciality of movement disorders. Of these, the most common presentations were grouped as hyperkinetic disorders including tremor and myoclonus; the second most common was found to be Parkinson's disease[13].

Causes

As mentioned in the discussion of the different forms of classification of myoclonus, a wide spectrum of disorders and causes for this condition exist. The etiological classification is divided into four groups by Caviness: physiological myoclonus (exercise induced, hiccoughs), essential myoclonus (sporadic, inherited), epileptic myoclonus (seizures dominate) and symptomatic myoclonus (secondary, progressive or static encephalopathy dominates)[4]. It is important to note that before inherited causes of myoclonus are considered, metabolic, pharmacological, infectious and immunological causes should be excluded[1,4]. Inherited causes can be found in the essential, epileptic and symptomatic classification groups[4]. Zutt classifies the genetic causes of myoclonus in four groups: as myoclonus with epilepsy (including Progressive Myoclonic Epilepsy (PME)), myoclonus with ataxia (Progressive Myoclonic Ataxia (PMA)), myoclonus caused by inherited metabolic conditions (mitochondrial conditions, lysosomal storage diseases, inborn errors of metabolism) and progressive myoclonic encephalopathies[2].

PME is characterised by an association with epilepsy and progressive neurological deficits, which often include cognitive impairment and ataxia. Table 1 summarises the genetic classification of PME (www.omim.org). In PMA, progressive and early onset ataxia with myoclonus in the absence of cognitive decline are the main features[1].

Newly identified genetic causes of myoclonus will increase with the availability of Next Generation Sequencing (NGS) techniques and identification of patients in specific geographical and ethnic settings. NGS has enabled a shift from targeted single gene mutation analysis to parallel sequencing of multiple genes in a single assay. It has certain limitations - for example, in finding nucleotide repeat mutations and mutations in the intronic areas of genes[14]. A diagnostic approach for patients with myoclonus is reviewed by Zutt[1] and Caviness[4], offering a detailed algorithmic approach to finding a specific cause.

EPM# **(Epilepsy Progressive Myoclonus)**	**Name**	**Gene (omim designation)**	**Inheritance pattern**	**Geographic distribution**	**Specific features**
1A	Unverricht-Lundborg disease	Cystatin B	AR	Northern European Italy North Africa	Progressive myoclonus stabilises after adolescence Minimal cognitive impairment
1B		*PRICKLE1*	AR	Middle East: Jordanian-Palestinian	
2A	Lafora disease	*EPM2A*	AR		Visual hallucinations Intractable seizures Cognitive decline

2B		*NHLRC1*	AR		
3	Neuronal Ceroid Lipofuscinosis	*KTCD7*	AR	Morocco Mexico Turkey	Developmental regression
4	Action myoclonus-Renal failure syndrome	*SCARB2*	AR	Canada, USA, Australia, Cuba, Germany	
6	North Sea PME	*GOSR2*	AR	The Netherlands Germany Australia	Early onset ataxia Variable course
7	MEAK	*KCMC1*	AD		Severe cognitive impairment
8	EPM8	*CERS1*	AR	Algeria	Severe progressive course with dementia
9	EPM9	*LMNB2*	AR	Palestine	Severe progressive
10	EPM10	*PRDM8*	AR	Pakistan	Severe and multi-system involvement
11	EPM11	*SEMA6B*	De novo mutations	Japan Israel Malaysia	Developmental regression with severe impairment in 1st decade

Table 1: A review of the genetic causes of Progressive Myoclonic Epilepsy; referenced from www.omim.org.

Abbreviations: AD: Autosomal dominant; AR: Autosomal recessive; EPM: Epilepsy Progressive Myoclonic; PME: Progressive Myoclonic Epilepsy.

Review of genetic causes of myoclonus in Africa and South Africa

Rationale for this research article

Phenotype-genotype knowledge is influenced by geographical and ethnic factors. This is classically described in Huntington's disease where patients with the typical phenotype in South Africa often do not harbour the well-known CAG-trinucleotide repeat expansion mutation in the Huntingtin gene on chromosome 4 but a repeat mutation in the junctophilin-3 gene[15]. This changed the approach in local South African laboratories to include testing the junctophilin-3 CTG/CAG expansion on chromosome 16q24.3 in patients with a negative CAG repeat expansion mutation on chromosome 4 as a standard procedure. In the adult onset Spino-Cerebellar Ataxias (SCA) the possible genetic causes are wide-ranging and although guided by certain phenotypical features, knowledge of the prevalence of certain mutations in a geographical area enhances testing with reduced cost. In South Africa, data collected over the last decade indicates that *SCA1* and *SCA7* are the most frequently found mutations, with *SCA2* and *SCA7* most frequent among patients of black African ancestry. This knowledge is applied in the selection of SCA variants included in first line genetic testing when a diagnosis of SCA is considered in clinical practice in South Africa.

When evaluating a patient with myoclonus, it would therefore be very advantageous to know which genetic causes are predominantly encountered in South Africa. With this in mind, a literature search for genetic causes of myoclonus in Africa and specifically in South Africa was performed.

Aim

The aim of this study was to identify the genetic causes of myoclonus and myoclonic disorders in South Africa, in comparison to causes described in the rest of the African continent.

Methods

To identify relevant articles investigating the genetic causes of myoclonus in South Africa, we performed a search on the National Library of Medicine (PubMed), Embase and Medline databases. Key words for the search were "myoclonus", "myoclonic epilepsy", "cortical myoclonic tremor" and "Africa"; the flow diagram of the search strategy is provided in figure 1. Additional records were identified by searching the literature list of the references cited in relevant studies. The search was updated to 22 October 2020 and was not restricted by year or language of publication. All causes of myoclonus were included in the original search; inflammatory, metabolic and infectious causes were excluded on the initial assessment. Duplicate articles or publications of the same study population were included but listed in the same line. General review articles on myoclonus, originating in Africa, were excluded. To be included, the studies had to report the clinical description of the syndrome and possible cause and geographical area studied or where the study population originated from. The study design identified clinical syndrome and/or cause and geographical origin of each study are reported in Table 2.

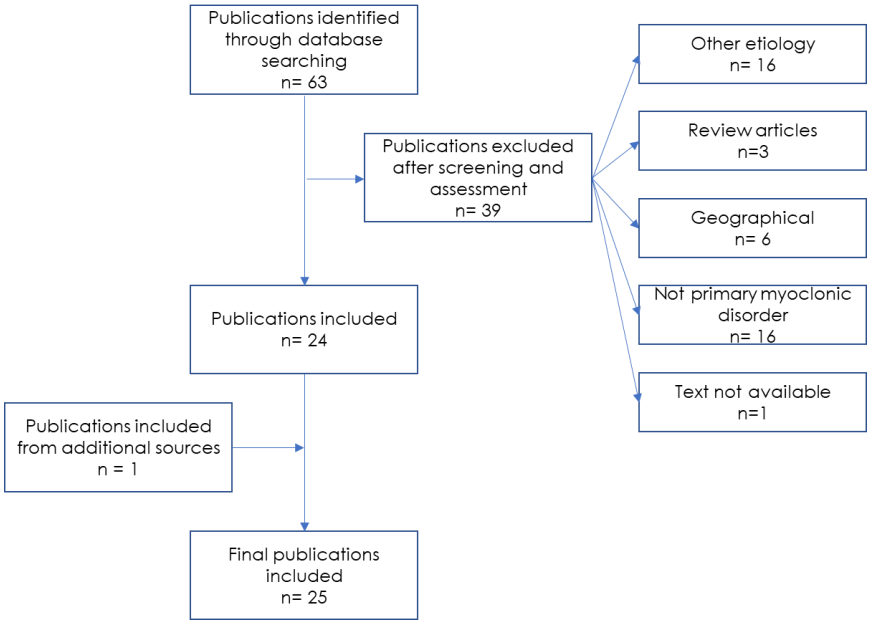

Figure 1: Process of literature review – myoclonus in South Africa.

Results

We identified 25 publications, excluding three review articles[3,13,16]. Three publications were case studies and 22 case series. Thirteen studies were either from North Africa or included North African patients, twelve were from sub-Saharan Africa of which eleven were from South Africa including one that included South African patients, but the publication came from The Netherlands. The clinical syndrome, diagnosis, genetic findings and geographical data are summarised in table 2. Geographical data from South Africa includes regional information.

First author	Year of publication	Type of publication	Region	Origin of publication	Clinical syndrome	Diagnosis	Genetic cause
De Graaf[17]	1989	Case study n=1	Cape Town, South Africa	South Africa	Severe myoclonic epilepsy, cardiac failure with optic atrophy	Lafora disease	Not done
Genton[18]	1990	Case series n=43	Algeria n=24 Tunisia n=1 Morocco n=1 Réunion n=2	Italy France	Moderate course, myoclonus responded well to treatment, no dementia	Mediterranean myoclonus	Not done
Stübgen[19]	1991	Case Study n=1	Pretoria, South Africa	South Africa	Unilateral episodic myoclonus	Segmental ballistic overflow myoclonus	Not done

De Graaf[20]	1995	Case series n=4	Cape Town, South Africa	South Africa	Early onset ataxia with distal limb wasting; telangiectasis; intractable myoclonus	Ataxia Telangiectasis	Linkage to the ATM gene on chromo-some 11q22.3 not proven
Lalioti[21]	1997	Case series n=29	North Africa n=18	France, Morocco	Young onset progressive seizures, stimulus induced myoclonus	Unverricht-Lundborg (EPM1A)	Cystatin B
Gouider[22]	1998	Case Series n=44	Morocco Algeria Tunisia	Tunisia	Typical ULD	Unverricht-Lundborg	Linkage confirmed to 21q22.3 (Cystatin B)
Moulard[23]	2002	Case series n=95	North Africa n=47	Morocco France Swiss Austria	PME	Unverricht-Lundborg	Cystatin B
Magaudda[24]	2006	Case Series n=20	North Africa n=9	France	Progressive myoclonic epilepsy	Unverricht-Lundborg	Cystatin B
Carr[25]	2007	Case series n=17	Cape Town, South Africa	South Africa	Progressive epilepsy, ataxia, and cognitive impairment	South African Adult Myoclonic Epilepsy	Excluded *FCMTE1* and *FCMTE2* by linkage analysis
Bardien[9]	2007	Case series n=5	Cape Town, South Africa	South Africa	Progressive chorea, cortical myoclonic tremor and cognitive impairment	Chorea – Huntington's chorea like -2	*JPH3*

Von Bogaert[26]	2007	Case series n=3	Morocco	Belgium	Young childhood onset myoclonus	Neuronal Ceroid lipofuscinosis	*KCTD7*
Traoré[27]	2009	Case series n=2 (8)	Mali	Mali UK USA	Typical course LD	Lafora disease	*NHLRC1 / EPMA2B* (novel mutation)
Khiari[28]	2011	Case study n=1	Tunisia	Tunisia France Swiss	Typical course LD	Lafora disease	*EPM2A* (Novel mutation)
Bouhouche[29]	2013	Case series n=3	Morocco	Morocco	Myoclonus and epilepsy; mental deterioration; death	Neuronal ceroid lipofuscinosis	*CLN6*
Stogman[30]	2013	Case series n=5	Egypt	Austria Egypt France Italy Germany	AR cortical myoclonic tremor with epilepsy	FCMTE5	*CNTN2*
Ferlazzo[31] **Ferlazzo**[32] **Vanni**[33]	2016 2009 2014	Case series n=4	Algeria	France	Severe progressive epilepsy, myoclonus and dementia	EPM8	*CERS1*
Van Coller[34]	2014	Case series n=2	Pretoria, South Africa	South Africa	Episodic dystonia with myoclonus and chorea	PNKD	Not done
Van Coller[35] **Corbett**[36]	2017 2019	Case Series n=23 (DNA n=5)	Bloemfontein and Pretoria, South Africa	South Africa Australia	Cortical myoclonic tremor and epilepsy; no cognitive impairment	FCMTE	*STARD7* – pentanucleotide repeat in intron of gene
Van Coller[37]	2019	Case series n=2	Pretoria, South Africa	South Africa	Myoclonus and dystonia	Myoclonus Dystonia	*SGCE* (new mutation)

Rachad[38]	2019	Case series n=12	Morocco	Morocco	Myoclonus and dystonia	Myoclonus Dystonia	*SGCE* mutations
Polet [39] **Anderson**[40]	2020 2017	Case series N=4	Johannesburg, South Africa	South Africa The Netherlands	Progressive early onset ataxia with variable myoclonus and epilepsy	North Sea PME	*GOSR2*

Table 2: A comprehensive summary of all publications on the genetic causes of myoclonus in Africa.

Abbreviations: Ceroid-lipofuscinosis neuronal protein 6; CNTN2: Contactin-2; EPM: Epilepsy Progressive Myoclonus; FCMTE: Familial Cortical Myoclonic Tremor with Epilepsy; GOSR2: Golgi SNAP receptor complex member 2; JPH3: junctophilin-3; KCTD7: Potassium channel tetramerisation domain containing 7; n: number of patients reported; NA: North Africa; NHLRC1: NHL repeat-containing protein 1; PME: Progressive Myoclonic Epilepsy; SGCE: Epsilon sarcoglycan; STARD7: StAR-related lipid transfer domain protein 7.

Cortical myoclonic tremor

Four publications from South Africa describe families with CMT[9,25,35,36]. Two reference the same family with Familial Cortical Myoclonic Tremor with Epilepsy with a pentanucleotide repeat expansion mutation in the intronic region of the *STARD7* gene on chromosome 2[35,36]. This family showed the typical early age at onset of the cortical myoclonic tremor with the development of generalised epilepsy later in life, without ataxia or cognitive impairment. The family was followed up over a period of 30 years and no progression of disability or features of neurodegeneration were reported. Two other families from the Western Cape were also reported with CMT and epilepsy but with a progressive course and prominent ataxia and cognitive impairment[25]. Linkage analysis did not find mutations in the known FCMTE1 or FCMTE2 regions and further investigations were not reported. In a third family from the Western Cape CMT was described in a patient with Huntington's disease like-2 with a proven mutation in the junctophilin-3 gene[9]. Although myoclonus has been reported in Huntington's disease, this seems to be an atypical clinical finding as other publications from South Africa examining the phenotype of HDL2 did not report CMT in their patients[41].

One Egyptian family with CMT and severe epilepsy with autosomal recessive inheritance and a mutation in the contactin 2 (*CNTN2*) gene was described. Although this condition was classified as FCMTE5, several authors were critical of this designation due to the progressive nature of the condition[30,42].

Progressive myoclonic epilepsy

From the Western Cape, one case study of Lafora disease with atypical features was reported[17]. Genetic studies were not performed, but the diagnosis was based on the typical skin biopsy findings. Lafora disease was further described in a Malian family with a novel homozygous single-nucleotide variant in the *NHLRC1* gene[27] and in a Moroccan patient with a novel mutation in the *EPM2A* gene[28].

Several families of North African origin with Unverricht-Lundborg disease (ULD) have been described. In a study of 44 ULD patients from 19 families originating in Tunisia, Algeria and Morocco, linkage to chromosome 21q22.3 was confirmed in 11 families[22]. In another haplotype study of 47 ULD North African patients, a founder effect of the haplotype A variant of the cystatin B gene was discovered[23]. No reported cases of ULD were found from South Africa or sub-Saharan Africa.

North Sea Progressive Myoclonic Epilepsy (NSPME) was described in four South African patients from the same family; all were treated with Deep Brain Stimulation of the posterior subthalamic area[40]. These four patients were also referred to in a large Dutch cohort describing the clinical features of patients with NSPME[39]. All four patients were shown to be homozygous for the founder missense mutation (c.430G>T.p.Gly144Trp) of the golgi Qb-SNARE gene (*GOSR2*).

Subcortical myoclonus

One South African family from Pretoria with Myoclonus Dystonia Syndrome with a novel mutation in the *SGCE* gene (a heterozygous c.824T>A nucleotide substitution in exon 6) was described[37]. This family with an autosomal dominant inheritance pattern showed some unique clinical features: co-occurrence of myoclonus and dystonia in the same body region and truncal myoclonus.

In a large study from Morocco the entire coding region of the *SGCE* gene was sequenced in 12 patients with sporadic Myoclonus Dystonia Syndrome. Two different heterozygous *SGCE* mutations (c.769A>C;c.391-3T> C) were identified in the *SGCE* gene. This finding confirmed that MDS with mutations in the *SGCE* gene occurs in North Africa and that *SGCE* mutations can be found in sporadic cases when the phenotype is consistent with MDS[38].

Discussion

Understanding the genetic basis of neurological disorders has rapidly expanded over the last three decades, initially driven by focussed research with linkage analysis and more recently by an explosion of data from next generation sequencing (NGS) techniques[14].

From Africa, representing a diverse population of around 1.2 billion people, published data on the genetic causes of neurological disease were from 17 out of 58 countries[16]. Regarding the genetic cause of myoclonus, publications originated from seven countries only (fig 2): four countries in North Africa (Egypt, Tunisia, Algeria and Morocco) and three in sub-Saharan Africa (South Africa, Réunion and Mali). Remarkably, most of these studies used linkage analysis to find the genetic cause and the utilisation of NGS seemed underused and was employed in only two publications[30,36]. The increased availability of NGS in Africa in research centres and large genetic consortiums should play

an important positive role in expanding the knowledge of genetic cause of disease in this population[16].

Figure 2: Map of Africa showing countries where study populations originated from in red dots. (Map from www.thegaudian.com)

Eleven of the published studies (two case studies and nine case series) regarding genetic causes of myoclonus were from South Africa. In two case studies and two case series the genetic cause was not identified: the diagnosis of Lafora disease was confirmed with skin biopsy in one case, and in the other two publications on segmental ballistic overflow myoclonus and Paroxysmal Non-Kinesigenic Dyskinesia (PNKD) respectively, the diagnosis was based on clinical grounds. Unfortunately, in the two families with a form of CMT with epilepsy and ataxia from the Western Cape, a genetic diagnosis could also not be made; linkage analysis only excluded localisation to two known loci associated with FCMTE on chromosome 2 and 8.

The lack of a definitive genetic diagnosis is a limitation that could be addressed by NGS testing with focussed NGS panel testing techniques in the case of Lafora disease and PNKD, but it is likely that more extensive whole genome sequencing (WGS) in the CMT families will be needed. This opens the question of funding and logistics in resource poor countries. Partaking in large international collaborations seems essential in this environment, but the formation of African-based consortiums offers a more practical and sustainable option which should be pursued.

In the spectrum of myoclonus associated with PME, only one case of biopsy proven Lafora disease was published from South Africa and one family of Afrikaner ancestry with *GOSR2* mutations was described. Thus, the causes of PME in the South African population remain indefinable and research which includes genetic data is important. However, achieving a publication with small case series in rare conditions has become increasingly difficult in the era of big data and large studies, thus impacting on the motivation of researchers in this field and subsequently hampering the distribution of essential knowledge. In this setting, knowledge distribution would be extremely important, since information of local etiological patterns would improve the diagnostic yield, treatment and genetic counselling that can be provided.

Myoclonus Dystonia Syndrome (MDS) is the most common cause for sub-cortical myoclonus[2]. It is most often caused by mutations in the epsilon sarcoglycan gene (*SGCE*, DYT-*SGCE*) with autosomal dominant inheritance showing maternal imprinting[43]. The Moroccan study of *SGCE* mutations in sporadic cases of MDS is an example showing genetic causes of a clinical condition in a local environment. In South Africa, MDS was reported in one case series of a single South African family with a unique mutation in the *SGCE* gene. The genetic causes of dystonia, the third most common movement disorder, are far less known with only two other publications from the continent.

The prognosis and treatment outcome in Isolated and combined forms of dystonia is influenced by the genetic cause and geographical data will aid in treatment decision making.

Importantly two new mutations were described in NCL and FCMTE5, and four novel mutations in known locations: one in *EPM2A*, one in *EPM2B* and two in the *SGCE* gene. All the other published mutations were confirmations of already known mutations. Although publications of new locations and novel mutations in known genes are certainly important, confirmation of traditional previously described mutations in a new geographical setting is of significant clinical value not only for the practicing neurologist but also for the study of the pathogenesis and epigenetic influences on expressions of disease.

A limitation of this study is the lack of information on the ethnic diversity of the populations studied. In some of the North African publications reference is made to the Maghreb lineage of the study population[22] and in the South African publications three reference Afrikaner[35,37,40] and two Mixed-ancestry origin[9,25]. African populations have a broad heterogeneous genomic diversity with widespread ethnic influences. Specific data on the ethnicity of study populations can be culturally sensitive but do contribute to etiological patterns and possible prognostic models.

Conclusion

25 studies – a combination of case series and case studies – were found regarding causes of inherited forms of myoclonus in Africa. With regard to cortical myoclonus there were nine publications from South Africa confirming the existence of Lafora disease, North Sea Progressive Myoclonic Epilepsy and Familial Cortical Myoclonic Tremor with Epilepsy in South Africa. The small number of publications raises questions on the prevalence of other more

common causes of Progressive Myoclonic Epilepsy, like Unverricht-Lundborg disease and Neuronal ceroid lipofuscinosis, in South Africa. Two publications on subcortical forms of myoclonus confirmed the presence of *SGCE* mutation positive Myoclonus Dystonia Syndrome.

This review shows the paucity of knowledge about genetic causes of myoclonus and other movement disorders in the large heterogeneous population of Africa. The expected high burden of neurological disease and genomic heterogeneity of African populations offers a unique opportunity to identify and understand novel genes and molecular pathways with improved detection, prevention and treatment of people of African ancestry. By extension this will benefit populations from around the world in the new paradigm of personalised and precision medicine.

Investing in infrastructure and collaboration of genomic research is crucial even in the current difficult economic and socio-political climate. Funding support from private and government agencies and collaboration efforts of research groups on the continent is needed to answer many questions on the causes of neurological disease in Africa. In an era of big data, publications of small but important case series and studies on the causes of rare disorders should be supported and encouraged[44].

Familial Cortical Myoclonic Tremor with Epilepsy: A South African family with a rare disorder bridging the sub-disciplines of Epilepsy and Movement Disorders

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Familial cortical myoclonic tremor and epilepsy: Description of a new South African pedigree with 30 year follow up

https://www.prd-journal.com/article/S1353-8020(17)30066-4/fulltext

Intronic ATTTC repeat expansions in STARD7 in familial adult myoclonic epilepsy linked to chromosome 2.

https://www.nature.com/articles/s41467-019-12671-y

Review: Familial Cortical Myoclonic Tremor with Epilepsy

1. *Many different names; one disorder*
2. *Geographical lines and Founder effects*
3. *Review – Clinical features*
   - 3.1. *Cortical Myoclonic Tremor*
   - 3.2. *Epilepsy*
   - 3.3. *Neurophysiology and Imaging*
4. *Many different mutations; one disorder*
   - 4.1. *Linkage analysis*
   - 4.2. *Intronic repeat mutations*
5. *South African Myoclonic Epilepsy (SAME)*

Cortical Myoclonic Tremor (CMT) was first described at the end of the twentieth century when small amplitude distal hand and finger tremor-like movements were recognised as myoclonus (polyminimyoclonus) of cortical origin[45]. Japanese investigators described a familial disorder of patients with Cortical Myoclonic Tremor, myoclonus and epilepsy with onset at different life stages[45].

Van Rootselaar identified a large Dutch kindred with a similar clinical course: autosomal dominant inheritance, young onset cortical myoclonic tremor with myoclonic and generalised epilepsy occurring later in life without evidence of neurodegeneration (cognitive impairment or ataxia) - Familial Cortical Myoclonic Tremor with Epilepsy (FCMTE)[46]. Since then more than 100 families from around the world were identified with this autosomal dominantly inherited clinical syndrome - with some heterogeneity around age at onset of CMT and epilepsy, but no features of cerebral or cerebellar degeneration[10].

Genetic localisation initially identified four different loci corresponding to geographical distribution but could not indicate a pathologic mutation at any location; since then, six main chromosomal domains where pathological intronic pentanucleotide repeat mutations result in FCMTE in different populations and geographical areas have been identified but the molecular pathophysiological disease-causing mechanism at present is still unknown.

Interest in this South African family with FCMTE originated in a clinical discussion of the index patient (IV:3) during an academic ward round at Universitas Hospital in Bloemfontein in 2010. This led to the retrospective analysis of patients who were seen by Prof C H van der Meyden, a South African neurologist, in clinical practice in Bloemfontein, in 1977 to 1979. This analysis was used to compile the pedigree and prospectively identify affected and unaffected

family members, together with recent patient evaluations, to describe the phenotype[35] and find the genotype[35,36].

Many different names; one clinical disorder

From the world literature many different names and acronyms were used to name the disorder: Autosomal Dominant Cortical Myoclonus and Epilepsy (ADCME), Benign Adult Familial Myoclonic Epilepsy (BAFME), Familial Adult Myoclonic Epilepsy (FAME) and Familial Cortical Myoclonic Tremor with Epilepsy (FCMTE). Van Rootselaar grouped these reports in 2005 and suggested the name Familial Cortical Myoclonic Tremor with Epilepsy[10]. By comparing the 50, by then, published families, she showed that all of them represented one disease spectrum with minimal phenotypical heterogeneity. It was proposed that this term, Familial Cortical Myoclonic Tremor with Epilepsy (FCMTE), should be used to describe the condition[47] and the designated gene name by the HUGO Gene Nomenclature Committee (HGNC; www.genenames.org).

More recently, with new advances in genetic diagnosis, the term Familial Adult Myoclonic Epilepsy (FAME) is used more frequently in world literature and as the primary OMIM designation. Although this term is easy to use, it denies the complexity of the clinical picture, especially the cortical tremor, and the pathophysiological origin of the disorder. FCMTE is considered an overlap between a movement disorder and an epileptic syndrome and using FCMTE instead of FAME reflects this complexity better.

For the purpose of this publication the term Familial Cortical Myoclonic Tremor with Epilepsy (FCMTE) will be primarily used with Familial Adult Myoclonic Epilepsy (FAME) as synonym.

Geographical lines and founder effects

Since the original descriptions of FCTME it was clear that clinical heterogeneity was reflected in the geographic origin of the kindred[48,49]. When the first genetic loci were identified, this confirmed the geographic-genetic classification with patients of Japanese and Chinese ancestry localising to a mutation on chromosome 8 (FCMTE1/FAME1), Italian patients to chromosome 2 (FCMTE2/FAME2), French patients to chromosome 5 (FCMTE3/FAME3) and Thai patients to chromosome 3 (FCMTE4/FAME4)(table 6). After extensive search for the mutation in the chromosome locations identified in these families, Ishiura[50] found a pentanucleotide repeat mutation in the *SAMD12* gene on chromosome 8 in a Japanese family. In this study they found similar mutations in two other locations in one (FCMTE6/FAME6) and another (FCMTE7/FAME7) Japanese families with typical FCMTE. This discovery led on to the identification of similar pentanucleotide repeat mutations in the intronic regions of the *STARD7*(FCMTE2)[36], *MARCH6*(FCTME3)[36] and *YEATS2*(FCMTE4)[51] genes in known families with FCMTE (table 7).

The identification of this novel TTTTA/TTTCA pentanucleotide repeat mutation in Japanese families with FCTME in three different gene locations led to the discovery of an intronic pentanucleotide repeat mutation on chromosome 2 in the *STARD7* gene, in an Australian/New Zealand family[52]. This mutation was then also found in the South African FCMTE family.

Two other families of African origin with CMT were described. The first was a family from the Western Cape, South Africa, described by Carr[25] in 2007. The clinical picture in this family was different to the originally described families, with refractory epilepsy and progressive ataxia and the authors suggested naming it SAME (South African Myoclonic Epilepsy) to differentiate it from the

FCMTE designation[25]. The other African family with CMT was from Egypt – a family with myoclonus and epilepsy with autosomal recessive inheritance and a genetic mutation on the contactin-2 (*CNTN2*) gene situated on chromosome 1q32.1 which was described by Stogmann in 2013[30]. The clinical course in this family was also progressive with early onset of seizures and cortical myoclonic tremor that developed only later in life. Although this was categorised as FCMTE5 in OMIM, there was similar criticism to this, progressive and treatment refractory condition, being designated FCMTE[42].

Review of the clinical features of FCMTE

FCMTE is a unique and rare condition of young onset Cortical Myoclonic Tremor and myoclonus with epilepsy that develops later in life; the inheritance is autosomal dominant with high penetrance and possibly anticipation explained by expansion of the intronic pentanucleotide repeat mutations found in different gene locations. The toxic mechanism or gene product has not been identified yet.

The standard and generally presenting feature of FCMTE is the cortical myoclonic tremor (CMT). It is characterised by postural tremulous movements of the fingers that resemble myoclonus rather than tremor[45]. Cortical myoclonic tremor has also been described in patients with Angelman syndrome, Progressive Myoclonic Epilepsy and after stroke.

FCMTE has a benign course and does not have features of the Progressive Myoclonic Epilepsies: refractory epilepsy, axial myoclonus, cognitive impairment and cerebellar degeneration are typically absent. Cognitive involvement is not a key feature in the described families and patients

generally do not have a progressive course with major deficit or early mortality[10,47,48]

Complex involvement of different neuroanatomical networks involving the cerebellum, spinal cord and basal ganglia, is also not a feature of FCMTE[53]. This differentiates it well from the autosomal dominant cerebellar ataxias. The Spino-Cerebellar Ataxias (SCA) are complex heterogeneous disorders with multiple genetic loci that vary in clinical presentation according to the genetic mutation. There is usually prominent progressive cerebellar ataxia and a progressive course, unlike FCMTE, which does not feature progression or central nervous system degeneration.

Cortical Myoclonic Tremor

Tremor is characterised by a rhythmical or pseudo rhythmical oscillation of a body part around an axis and the pathophysiology is thought to be from the exposure of centrally located neuronal oscillators[54–56].

Essential tremor (ET) is a common movement disorder characterised by a postural tremor with an agonist-antagonist nature that has an autosomal dominant family pedigree in 50% of patients. Its prevalence varies widely from 5/100 000 in Ethiopia to 41/100 000 in Tanzania and 12/1000 in Nigeria in population-based studies[57,58]. Some association with cerebellar ataxia, parkinsonism, dementia and myoclonus has been described, although these associations are uncommon and difficult to validate[59].

Previously, Essential tremor was considered a monosymptomatic disease entity characterised only by kinetic limb and infrequently axial tremor. In recent years, however, the monosymptomatic nature has been contested with several reports of axial balance disturbance, dementia and even parkinsonism described in patients with ET[60]. The pathophysiology is thought to be from oscillators located in the cerebellum and inferior olivary nucleus creating a 7 to 12 Hz oscillation. These abnormal cerebellar pathways underlie ET which has been shown in several functional imaging studies. The tremor generally improves with alcohol intake and with beta-blockers, especially propranolol. Electromyographic studies (EMG) show synchronous agonist -antagonist action in the affected limb muscles indicating the rhythmical postural contractions causing the tremor[55].

The two patients described by Ikeda had a movement disorder resembling essential tremor: the movements were postural, pseudo-rhythmical and affected mostly the hands. Neurophysiologic investigations did not show the typical alternating agonist-antagonist pattern seen in ET, but showed features in-keeping with cortical myoclonus: giant somato-sensory evoked potentials and enhanced long loop reflexes (c-reflexes)[45].

Like ET, Cortical myoclonic tremor is also a postural movement disorder, but the nature differs from ET: in CMT, there are distinct tremulous, jerky and pseudo rhythmical movements of the fingers representing distal myoclonic jerks. Cortical myoclonic tremor is therefore defined as fine distal, involuntary and abnormal high frequency myoclonic movements that occur in action and posture and affect mostly the hands. It resembles the fast jerk-like movements of myoclonus in an irregular pattern, pseudo-rhythmical, and has a cortical origin.

Tremor recordings typically show irregular contractions of the fingers and hands with a mean frequency of 9 to 10 Hz (range 6 to 20 Hz) and no definite synchronization in agonist-antagonist muscle groups. Surface EMG shows brief bursts of approximately 50 microseconds. Cortical origin is supported by giant Somato-Sensory potentials (g-SEP) and the presence of long-loop reflexes (c-waves)[6,61]. A pre-movement cortical spike is often found patients with back-averaging electro-encephalogram (EEG)[5] although back-averaging can be difficult to demonstrate due to the fast nature of the jerks. Coherence analysis has been shown to be a reliable examination to distinguish ET from CMT[6].

In FCMTE, cortical myoclonic tremor is the first noted symptom in 86% of patients and can start within a range of 10 to 60 years. Most patients develop tremor before the age of 30 years[48].

The severity of cortical myoclonic tremor is described as varying from no impairment of normal activity to severely incapacitating up to a point where patients are unable to use their hands. It is progressive over years, especially in European families and does not respond to propranolol. The data regarding alcohol responsiveness is conflicting, but the tremor generally responds well to anti-epileptic drugs used for the treatment of myoclonus: valproate, clonazepam and primidone. Some reports also mention successful use of levetiracetam[53]. In a follow up analysis of patients from three Italian families with the FCMTE2 haplotype, the cortical myoclonic tremor was shown to be the most disabling symptom with progression matched to age and duration of disease[47].

Epilepsy

Generalised epilepsy is a feature of all the families with FCMTE that have been described and expresses clinical heterogeneity between families. All patients with seizures have features of generalised epilepsy. Atypical absence seizures – stare episodes without automatisms - were also noted in some pedigrees[62,63]. However, some families were also noted to have complex partial seizures (focal epilepsy with impaired awareness), some with secondary generalisation[48].

The general features of seizures, epilepsy subtype and response to treatment can be divided into four groups, following the genetic mutation locations (table 3):

1. In FCMTE1, described in Japanese families, all patients with epilepsy had generalised tonic-clonic seizures. The mean age among the 40 families for the onset of epilepsy was 30 years and seizure frequency varied from rare to frequent.
2. In FCMTE2, initially described in Italian pedigrees, all patients had generalised tonic-clonic seizures but some were described with partial seizures with secondary generalisation. The published data does not differentiate the type of partial seizures further. The mean age of onset was 26.6 years, which is younger than in the other European families. Seizures were described as rare in all families. In most families the epilepsy was mild and not a cause of disability[47]. Seizure frequency did not correlate with severity of cortical myoclonic tremor or myoclonus.
3. In the French FCMTE3 family, age of seizure onset was also younger, at a mean age of 28 years and both generalised tonic-clonic seizures and complex partial with secondary generalisation were described[62]. All seizures were controlled on one or two drugs.

4. In the other FCMTE3 family from the Netherlands, seizures were exclusively generalised, with a mean age of onset older and seizure frequency higher than in the other groups[46].
5. The Thai family with FCTME4 had early onset generalised seizures – 19 to 33 years – but with a mild course.

	Publication	Country of origin	Mean AAO	Semiology
FCMTE1	Hitomi[64]	Japanese	33.7	G
	Cen[65]	Chinese	36	G
FCMTE2	De Falco[66]	Italy	42	G
	Striano[67]	Italy	31.5	G
	Suppa[68]	Italy	26.5	FIA/G
	Gardella[69]	Italy	N/A – 16*	G
	Lebauge[70]	Spain	44.6	G
	Crompton[52]	Australian/New Zealand	44	G an FIA
FCMTE3	Van Rootselaar[46]	Netherlands	43	G
	Depienne[62]	French	30	G/FIA
FCMTE4	Yeetong[71]	Thai	25	G

Summary of Epilepsy – mean age at onset and epilepsy classification of the major families with FCTME where mutation location and information are available.

Abbreviations: AAO- age at onset; FIA – focal epilepsy with impaired awareness; G- generalised

Table 3: Summary of epilepsy in the major known families with FCMTE.

Neurophysiology and Imaging

The pathophysiological origin of cortical myoclonic tremor remains uncertain. Although neurophysiological studies and clinical evaluation indicate a cortical origin, pathology and imaging studies point toward cerebellar pathology.

Standard electrophysiology suggests a cortical origin of the tremor similar to cortical myoclonus, including giant somato-sensory evoked potentials and enhanced long loop reflexes (LLR) or c-reflexes. Coherence analysis shows strong cortico-intramuscular coherence in the 8 to 30 Hz range in patients with cortical myoclonic tremor as opposed to normal controls and patients with ET[6]. This indicates cortical hyperexcitability.

However, post-mortem studies of patients with FCMTE show specific cerebellar Purkinje cell changes[61], which are supported by functional imaging studies. This conflicting evidence is possibly explained by abnormal cortical oscillatory activity in the central motor networks modulated by excessive cortical hyperexcitability due to dysfunction of the cerebello-thalamo-cortical circuitry[47].

Several of the available published studies in FCMTE refer to standard imaging – MRI and CT scan of the brain – as normal. Imaging evidence of cerebellar atrophy was not described in the published studies or in the latest updated review[48]. However, two studies on functional imaging, one with an fMRI study and one with MRI spectroscopy (MRI-S), indicated cerebellar involvement in the pathophysiology of the disorder[72].

The fMRI study was a simultaneous original design EMG-fMRI study[73] using EMG linked fMRI to identify abnormal regional brain activity directly involved in involuntary movement. By subtracting the mean EMG from the raw EMG data, the remaining EMG (r-EMG) reflects the involuntary movement (tremor) as opposed to the involuntary movement combined with the voluntary movement when changing posture. This study showed cerebellar dysfunction during postural cortical myoclonic tremor of the hands by conventional fMRI imaging in keeping with the known cerebellar pathology. It also showed bilateral signal change in the FCMTE patients in the secondary sensory areas. To assess whether hand movements alone could activate sensory areas, r-EMG activation was compared between healthy controls and FCMTE patients. With rapid hand movements recording of r-EMG-fMRI, patients with FCMTE showed activity in the inferior parietal gyrus that was absent in controls. This novel fMRI-EMG study adds to the difficult explanation of the pathophysiology of FCMTE. In this study, objective abnormalities were shown in the secondary sensory areas that could not be explained by normal afferent sensory input.

The MRI-S study which examined the findings of brain spectroscopy in Italian patients with FCMTE2 also showed cerebellar abnormalities[72]. The authors were able to demonstrate spectroscopic abnormalities of the cerebellum in patients with recent onset illness. Unfortunately, MRI-S is only able to study targeted areas, and in this study only a frontal pre-motor area and the

cerebellum were targeted. The parietal sensory areas were not studied and therefore they could not confirm the findings of the r-EMG-fMRI study.

Post-mortem pathology findings were reported in a Dutch patient with FCMTE showing cerebellar Purkinje cell loss with normal cortical areas. The cerebellar cortex and vermis demonstrated severe and diffuse loss of Purkinje cells and some Bergmann gliosis. The remaining Purkinje cells had abnormal morphology consisting of a stellate configuration with a poorly developed dendritic tree, short peri- somatic dendrites and torpedoes, hyperchromatic cell nuclei, and sometimes two nuclei. Heterotopic Purkinje cells were found in the molecular layer. Empty basket cells were observed. The sensory-motor cortex and basal ganglia were normal[61].

Neurophysiological evidence indicates cortical origin of the CMT, myoclonus and seizures. However, MRI-spectroscopy, f-MRI studies and pathological findings indicate abnormalities in the cerebellum. It is therefore likely that a complicated pathological interaction between different anatomical areas and networks exists, but at present, there is no evidence to explain how this relates to the clinical syndrome of CMT, myoclonus and epilepsy[74].

Many different mutations; single clinical syndrome

Genetic heterogeneity has been demonstrated in FCMTE. Linkage to chromosomes 8q24 (in five Japanese families, FCMTE1/FAME1) and 2p11.1-q12.2 (in five Italian families, a Spanish family and an Australian/New Zealand family, FCMTE2/FAME2) has been described. Linkage to chromosome 8q24 was excluded in the Spanish[70] and Italian families and both mutations were excluded in a Dutch[46], French[63] and Chinese[75] family. These unlinked families

argued for a third locus that was reported in a large French pedigree mapped to chromosome 5p15.31-p15[62]. A fourth locus was found on chromosome 3 (3q26.32-3q28) in a Thai family (FCMTE4/FAME4)[71]. Gene identification was fruitless until Van Rootselaar reported a mutation in the contactin-2 gene in the Dutch FCTME3 family; unfortunately, this finding was not repeated in the French family with the same locus[76]. With the identification of an intronic pentanucleotide repeat mutation in a Japanese family, mutation discovery was accelerated with pentanucleotide mutations discovered on all the previously found locations.

Linkage analysis

Genome-wide linkage analysis is used to identify regions that might be implicated in families with a shared clinical phenotype. This allows for identification of smaller regions that can be studied with micro-satellite markers to identify a specific region that segregates in the affected (and potentially affected) members. Statistical analysis of these regions should be significant and a logarithmic score is calculated to signify relevance. This LOD (Logarithm of Odds) score is regarded as significant if the score is greater than three. This signifies a 1000 to 1 odds that the linkage being observed did not occur by chance. Linkage can be decisively excluded if the LOD score is negative (<-2)[77].

Gene candidates for FCMTE should be able to cause both focal and generalised hyperexcitability in the brain of affected patients and possibly disinhibition of the motor and sensory cortex. As with the primary generalised epileptic (PGE) syndromes ion channels were thought to be ideal candidates. Several ion channel mutations have been identified in PGE. This includes mutations of sodium channels – Generalised Epilepsy with Febrile Seizures Plus

(GEFS+), potassium channels – Benign Familial Neonatal Convulsions (BFNC) and calcium channels – Juvenile Myoclonic Epilepsy. The channelopathy hypothesis was supported by the finding that there are similarities in eye movement abnormalities between Dutch FCMTE members and patients with Spino-Cerebellar Ataxia-6 (SCA-6). SCA-6 is an autosomal dominant cerebellar syndrome with associated extrapyramidal symptoms caused by a CAG repeat mutation in the voltage gated calcium channel gene *CACNA1A* on chromosome 19[78].

The first families with cortical myoclonic tremor, myoclonus and epilepsy were described in Japan as Familial Adult Myoclonic Epilepsy (FAME)[79] and Benign Adult Familial Myoclonic Epilepsy (BAFME)[80]. The first positive linkage analysis was published in a Japanese kindred with the classical clinical phenotype of FCMTE – tremulous finger movements with associated myoclonus and generalised epilepsy with a non-progressive neurological course. Genome wide linkage analysis localised to chromosome 8q24 with maximum multipoint LOD score of 5.42 for the region between markers D8S555 to D8S1779. This area was confirmed with genome-wide linkage analysis of 30 members of four different Japanese families; 21 affected with FCMTE; and defined to a 4.6 centi-Morgan(cM) region on chromosome 8q24 with maximum LOD score of 4.86 at no recombination[81].

Several genetic abnormalities implicated in autosomal dominant generalised epilepsy syndromes localise to chromosome 8: Benign Familial Neonatal Convulsions 2 (*BFNC2*) to 8q24, familial febrile convulsions to 8q13-21 and childhood absence epilepsy to 8q24. These disorders are all due to mutations in genes encoding voltage gated ion channels. These known genetic aberrations on chromosome 8 are all several cM in either the centromeric or telomeric directions of the identified region for FCMTE1[80].

This locus was confirmed in an independent genome-wide linkage analysis[82]. Genome wide linkage analysis using 10K single-nucleotide polymorphism arrays and followed up by additional micro-satellite markers confirmed the location of the abnormal sequence to chromosome 8q23.3 to 8q24.13 in affected Japanese family members. Sequential analysis and copy number variant analysis of all 38 genes localised in the region could not identify any pathological mutation. The aetiology remained unsolved and further study including analysis of the non-coding regions of the gene was suggested.

Phenotypical anticipation could be demonstrated in three FCMTE1- families [83]and confirmed in a follow up study[64] that investigated anticipation in four families. Only two or three patients per family could be included making the outcome less robust. Tremor and epilepsy were of greater severity in older generations, but severity of cortical myoclonic tremor increases with age and the finding of greater severity in older generations possibly reflects this, rather than reversed anticipation.

The locus on chromosome 8q24 was excluded in a Spanish[70] and large Italian[84] pedigree with cortical myoclonic tremor, myoclonus and epilepsy. By performing genome wide linkage analysis, a region on chromosome 2 was identified and examined with microsatellite markers. A region of 12.4 cM on 2p11.1-2q12.2 was shown to be significant with a two-point LOD score of 3.46 at no recombination. Linkage analysis demonstrated significant linkage to this region on chromosome 2 in two further Italian families with LOD scores > 3 [68,85]. A fourth Italian family also demonstrated positive linkage but with a lower LOD score of 1.55 at no recombination[86]. This locus was confirmed in the Spanish family[70] by performing genome wide linkage and demonstrating significant linkage for a region in the centromeric area of chromosome 2 overlapping the described area and refining it to 9.42 cM between markers D2S2161 and D2S2264[87]. Linkage to this region on chromosome 2 was further confirmed in 53

affected individuals from Naples, Italy, from five families. Four of the Italian families mentioned above and a newly described family with the typical phenotype. Significant linkage was found on chromosome 2p11.1-2q12.2 with a maximum accumulative LOD score of 18.5 for markers D2S2161 and D2S388[88]. Haplotype analysis done on the affected individuals and checked in 50 non-affected people originating from Naples confirmed a common founder mutation within these five families.

In a Dutch family with the typical phenotype, linkage analysis excluded the 8q24[46] and 2p loci as candidate regions in ten affected patients with consistent negative LOD scores for all markers to the identified areas.

Exclusion of both regions was also found in a French family with typical phenotypic features[63]. A genome wide scan was then performed on this family which pointed to a possible location on chromosome 5 that was refined by using microsatellite markers to identify a common 9.31-Mb haplotype with highly significant LOD scores[62].

141 genes are found in the described area on chromosome 2 but none could be demonstrated as a possibility in FCMTE[87]. Two possible gene candidates were also identified on chromosome 5 (*SEMA5A* and *CTNND2*) – both involved with axonal function and integrity. Pathological mutations in both were, however, excluded in the French pedigree[62]. A mutation was found in *CTNND2* gene on chromosome 5p15 in the Dutch family with FCMTE3. Exome sequencing done on three affected patients revealed an anomaly that segregated in 15/16 affected patients. One patient with the mutation was not affected and thought to represent reduced penetrance and one patient who was affected did not have the mutation. This patient (IV-9) was examined when he was 16 years old and had cortical myoclonic tremor with

neurophysiological findings in keeping with cortical origin myoclonus; he was unfortunately lost to follow up and the authors concluded that he might be a possible phenocopy. Four in silico bio-information algorithms indicated a high likelihood of this mutation being pathological. *CTNND2* is highly expressed in the cerebellum and in a mouse model the pathophysiology explained the clinical presentation of cortical myoclonus[76]. A mutation in this gene was not found in the French FCTME-3 family with localisation to chromosome 5 and no data was available from the Chinese FCTME-3 family[62,89].

Geographical distribution characterises the four identified genetic loci with minor clinical phenotype differences; this supports the heterogeneity that is generally seen and makes an argument for a founder effect in each of the three regions - as was demonstrated in Naples, Italy[88,90].

Intronic pentanucleotide repeat mutations

The discovery of a TTTTA pentanucleotide repeat expansion in families with FCMTE1 was a milestone in the search for the pathogenesis of this condition[50].

This discovery and the subsequent discoveries of the same TTTTA/TTTCA pentanucleotide repeat expansion in other locations on the genome will be discussed in chapter 2.4.

South African (Progressive) Myoclonic Epilepsy (SAME)

Genetic heterogeneity in FCMTE is supported by some differences in the clinical phenotype. The clinical differences are generally related to age at onset of tremor and epilepsy, frequency of seizure and influence of myoclonus on activities of daily living. The main features of the disorder are homogenous in all affected families[48]:

1. Cortical myoclonic tremor with neurophysiological evidence of cortical origin
2. Generalised (tonic clonic) seizures
3. Non-progressive course
4. No ataxia or evidence on imaging of progressive cerebellar atrophy.

In 2007 Carr described two South African families with cortical myoclonic tremor and epilepsy[25]. The patients presented with refractory generalised tonic-clonic epilepsy and were noted to have distal tremulous movements – cortical myoclonic tremor – and both positive and negative myoclonus. It was proposed to name this disorder FAME3 (Familial Adult Myoclonic Epilepsy) and therefore to group it as part of the spectrum of FCMTE.

In this South African study, sixteen affected family members, from two families of mixed ancestry, were examined. All the examined patients had cortical myoclonic tremor, myoclonus and epilepsy. Eleven of the patients had prominent ataxia and eleven had hyperreflexia with extensor plantar responses indicating pyramidal tract involvement. Eight of the eleven patients had both ataxia and hyperreflexia.

Early cognitive decline was a common feature with bedside cognitive testing. A Mini Mental State Examination (MMSE) score median of 18/30 (range: from unable to perform the test to 30/30) was recorded. The patients with marked cognitive involvement (<18/30) at the point of examination were severely disabled with marked ataxia and lower limb spasticity. Three patients died in the fourth decade (at 31, 35 and 39 years), probably of seizure related causes.

Neurophysiological testing was done on most of the sixteen affected family members. All patients had abnormal EEG's, ranging from slowed background activity to focal epileptiform features. Cortical origin of myoclonus was confirmed with enlarged SEP's and c-reflex recordings. MRI scans were done on nine of the 12 affected patients and showed cerebellar atrophy in all 9/12 patients.

One of the three patients who died during the study was examined post-mortem. The following findings were made:

- Focal Purkinje cell loss in the cerebellum
- Early Bergman gliosis and the presence of torpedoes
- Neuronal loss and atrophy of the dentate nucleus
- Atrophy of the superior cerebellar peduncles
- Neuronal loss with gliosis of the olive nuclei

- There was also mild neuronal loss noted in the basal ganglia with normal substantia nigra and subthalamic nucleus
- The cerebral cortex was reported as normal

These findings accentuated that the two South African families had important features that differed significantly from the published data on FCMTE:

- Progressive course with marked neurological disability, including progressive cognitive impairment
- Ataxia and pyramidal involvement
- Abnormal EEG's with significant findings as opposed to paroxysms of generalised spike and wave activity with normal background features
- Cerebellar atrophy on MRI

Other pathological descriptions of patients with FCMTE are rare; on an affected patient from a Dutch pedigree, a post mortem examination was performed, showing some cerebellar Purkinje cell degeneration[61]. It is noted that the post-mortem abnormalities in this case seem less extensive than the South African case.

An Italian family with genetic localisation to 2p11.1-2p12.2 also differed phenotypically from the already described FCMTE phenotype with 3/8 patients with mild mental retardation and complex partial epilepsy with secondary generalisation. 6/8 patients had focal abnormalities on EEG – uni- or bilateral temporal lobe epileptiform foci with normal background. The course was however, still described as non-progressive and all affected members had cortical myoclonic tremor with electrophysiological evidence of cortical origin. The condition was therefore still considered as part of the FCMTE spectrum as was later confirmed with genetic allelism to the other Italian families[84].

Localisation to the chr2 and chr8 FCMTE loci were excluded in the South African family with negative LOD scores in linkage analysis to all the markers stretching the known regions.

In the journal's rapid online response section, Striano subsequently pointed out that FCMTE (FAME) should be applied to a familial nonprogressive form of cortical myoclonic tremor and epilepsy with a benign course[25]. Therefore, it was suggested that these two South African families described by Carr do not form part of the spectrum of FCMTE. Although the severity of disability does not allow inclusion in the PME disorders, the described families probably resort under this group of conditions. Carr proposed this condition to form a unique subtype of the myoclonic epilepsy syndromes and proposed it be called South African Myoclonic Epilepsy (SAME)[25].

Familial cortical myoclonic tremor and epilepsy: Description of a new South African pedigree with 30 year follow up

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Familial Cortical Myoclonic Tremor with Epilepsy (FCMTE) (ADCME/BAFME/FAME[49]) with autosomal dominant inheritance, has been reported in more than sixty families since its first description in 1990[45]. Four genetic loci have now been found and these can possibly be divided into geographical patterns – FCMTE1 (Japanese[8082] and one Chinese family[65]), FCMTE2 (Italian[84]), FCMTE3 (French[62] and Chinese family[89]), and FCMTE4 (Thai[71]), as well as unclassified Chinese[75] and Dutch families[46]. The condition is characterized by fine distal myoclonic jerks with cortical origin - termed Cortical Myoclonic Tremor, found in most affected patients, and epilepsy that usually occurs later during the disease. FCMTE appears to be mildly progressive and features of neuro-degeneration, i.e. progressive ataxia, spasticity or cognitive decline are usually absent in the affected patients [9110]. This study describes the first South African family with FCMTE that was initially identified in 1977/8 and followed up in 2011-2013.

Aim

The aims of this study are to report the index case with cortical myoclonic tremor and epilepsy, to fully describe the pedigree with the clinical findings and results of additional investigations, and to report the follow-up evaluation of the patients after 30 years. The findings will be compared to other described pedigrees and used to comment on the natural history of this disorder.

Patients and Methods

The index case (IV-3) (fig 3) was seen in clinical practice in 2011 and investigated for cortical myoclonus. It was then discovered that this patient was part of a large, four generation pedigree with cortical myoclonic tremor and epilepsy that had first come under the attention of a neurologist (CH vd M) in 1977/8 at a university hospital in Bloemfontein, South Africa. At the time, the identified affected family members were considered novel with essential

tremor-like distal movements, myoclonus and generalized epilepsy. Affected and unaffected family members were examined and their clinical and neurophysiological findings were well documented. The clinical notes of patients belonging to this pedigree were retrieved.

After approval of the medical ethics committee of the University of Pretoria, affected and unaffected family members were contacted in order to compile the pedigree (Fig 3) and delineate the clinical characteristics and course of the condition (summarized in table 4). Signed informed consent was obtained in all patients. From those patients who had been seen in 1977/8, the previous clinical information, including history of tremor and epilepsy, severity of tremor, treatment and electro-encephalography recordings, was retrieved from patient files. The following parameters were recorded on patients available for follow up and newly identified patients: full neurological examination, clinical assessment of tremulous hand movements, analysis of seizure frequency and control on anti-epileptic medication, as well as eventual patient outcome (Table 4). Follow up neurophysiological recordings were performed: a standard electroencephalogram (EEG) using the international 20/10 method; tremor recording with surface electromyography (EMG) recordings of the first dorsal interosseous, extensor indices and deltoid muscles with off-line analysis to assess duration, frequency and rhythm of muscle contraction; somatosensory evoked potentials to investigate the presence of a giant potential (g-SEP); and long loop reflex analysis (LLR/C-reflex) [92].

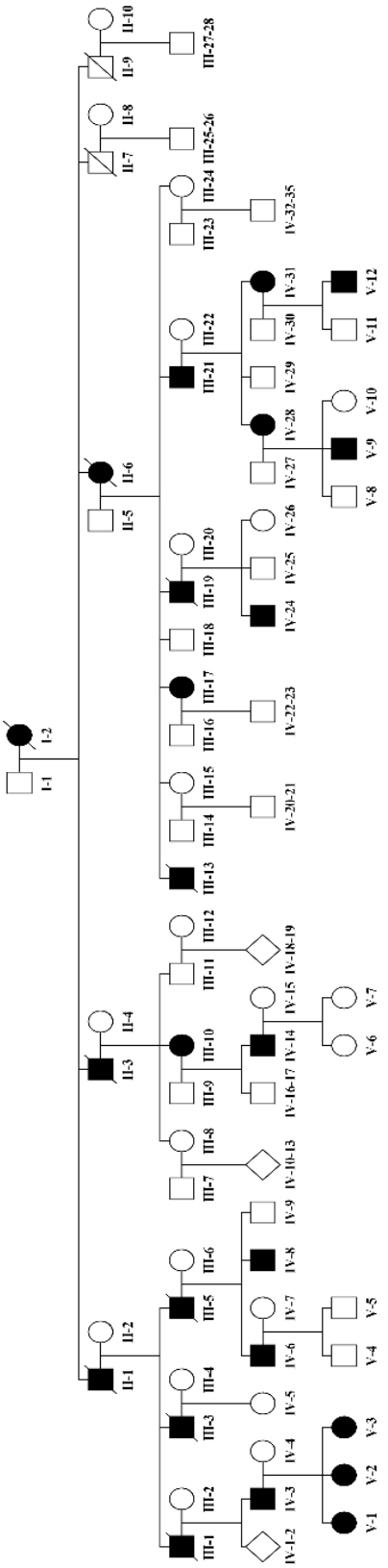


Figure 3: Pedigree of South African Family with Cortical Myoclonic Tremor with Epilepsy

Pedigree number	Sex, Age	Age at Onset		AED's used	Neurophysiology		
		Cortical Tremor	Epilepsy		EEG	g-SEP	LLR
III:1	M, #	17	37	PHB, MYS	G	ND	ND
III:2	M, #	20	32	#	ND	ND	ND
III:3	M, #	*	*	MYS	ND	ND	ND
III:5	F, 79	16	42	PHB	G	ND	ND
III:9	F, 92	15	40	PHY; CLN	ND	ND	ND
III:12	M,82	16	50	PHY	TLE	ND	ND
IV:3	M,52	16	30	VPA; MYS	G	+	+
IV:5	M,44	15	No seizures	NONE	NOR	ND	ND
IV:6	M,40	17	No seizures	MYS	NOR	+	NEG
IV:12	M,52	14	45	VPA, LTG, LEV	G	+	+
IV:34	F,59	18	No seizures	NONE	TLE	NEG	NEG
IV:36	F, 52	15	No seizures	NONE	ND	ND	ND
V:4	F, 25	12	No seizures	MYS	NOR	+	+
V:5	F, 24	15	No seizures	MYS	NOR	+	+
V:6	F,13	13	No seizures	NONE	NOR	NEG	+

Table 4: Summary of affected family members with known data

Abbreviations: NOR – Normal; ND – Not Done; + - positive; PHY – phenytoin; VPA – valproate; MYS – primidone (Mysoline); LTG – lamotrigine; LEV – levetiracetam; G – Generalised epilepsy; TLE – temporal lobe epilepsy.

The Fahn-Tolosa-Marin Tremor Rating Scale (FTMTRS) [93] was completed at last follow up with all the patients who could be contacted from the previous cohort as well as all newly identified patients. This was used to assess the severity of tremulous hand movements, and the subset on activities of daily living (ADL) was used to assess the impact of cortical myoclonic tremor on ADL. The impact of myoclonus on ADL was also investigated by a retrospective myoclonus rating scale– the Myoclonus Rating Scale (MRS) - that was utilized before in a similar study[47]. The epilepsy severity was classified according to the number of seizures per year and number of anti-epileptic drugs used to secure seizure control. The data sets were compared with an ANOVA regression analysis model to assess the correlation of cortical myoclonic tremor with activities of daily living and the myoclonus rating scale, as well as the correlation of age with cortical myoclonic tremor and the myoclonus rating scale.

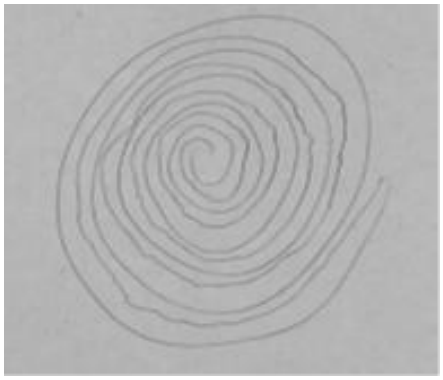
Results

Index case

The index case of this South African family with cortical myoclonic tremor and epilepsy was a 52-year-old male of European descent (case IV:3). The tremulous hand movements started at the age of 16 years and were characterized by jerky distal movements resembling postural tremor and distal myoclonus. These jerky movements were more pronounced with sleep deprivation and emotional stress; later, the patient noticed that alcohol reduced the intensity but caused a rebound effect the following day with exacerbation of the tremulous hand movements. Treatment with propranolol was ineffective, but valproic acid, started at the age of 30, reduced the movements. When the patient was 18 years old, an EEG had shown paroxysms of generalized spike and wave activity, with normal background activity and normal photic stimulation (fig 4). At that stage, the patient had never had a convulsion. During the next three decades, progression consisted of more

frequent proximal myoclonic jerks and more severe tremulous movements in his fingers. Epilepsy was diagnosed when the patient had his first seizure at the age of 30 years. Another EEG was performed at age 53, which showed similar features as before (fig 4). C-reflexes with latencies of 45 ms and g-SEP with P25-N33 amplitudes of 9,4uV were recorded, consistent with cortical myoclonus.

A



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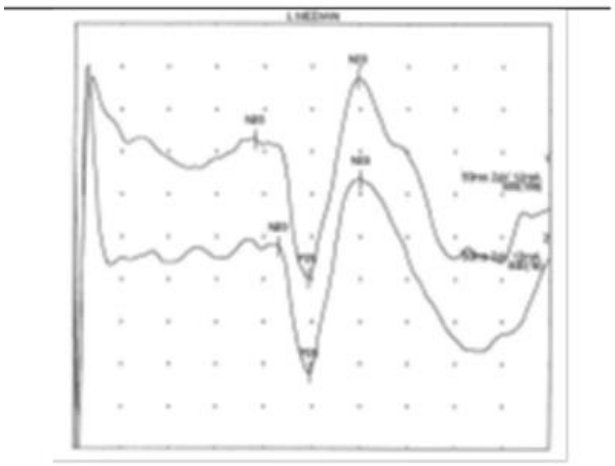

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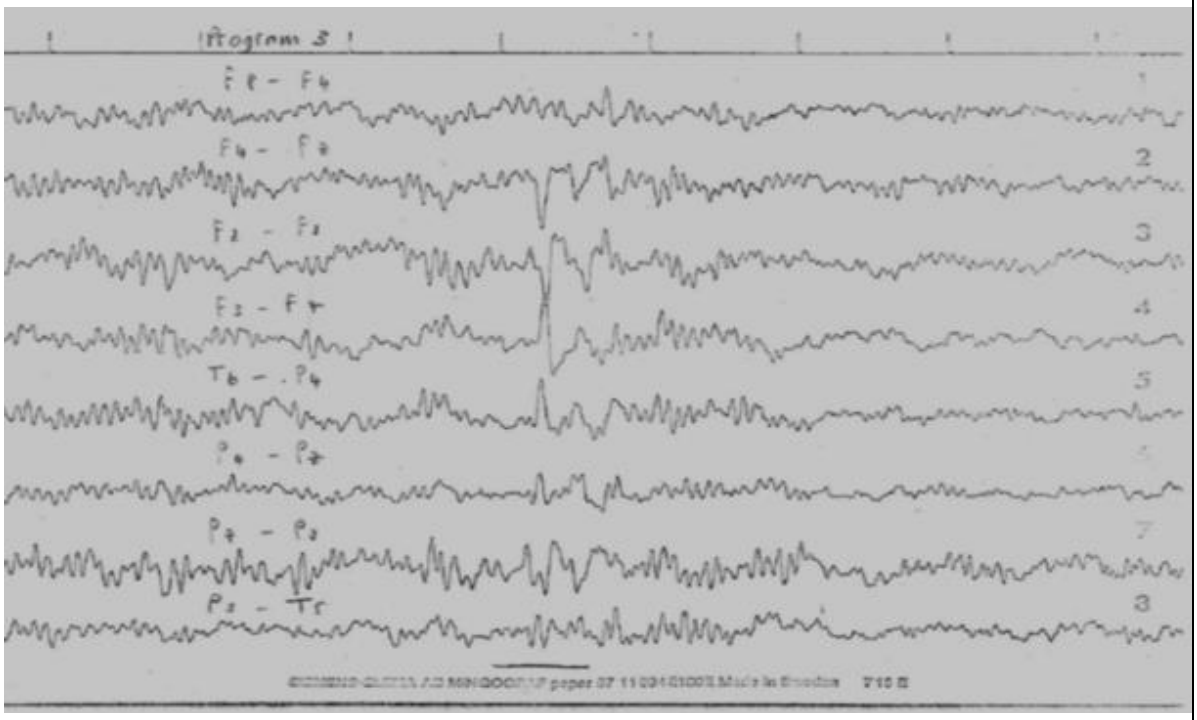

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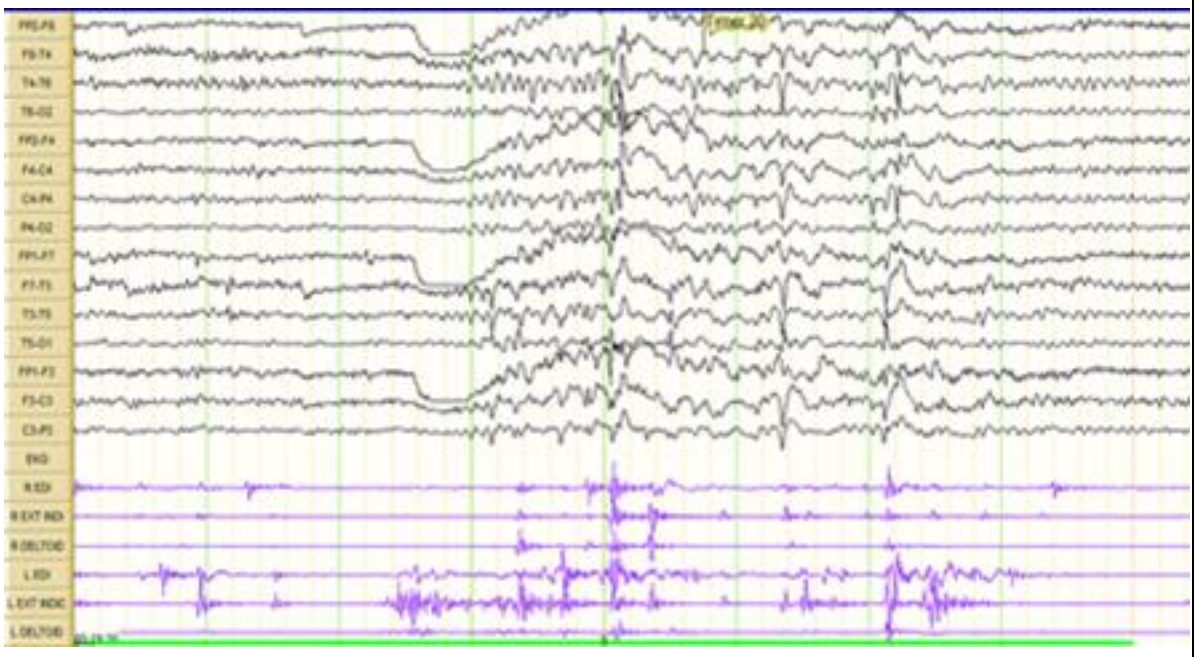

Spiral drawing of patient IV:3 in 1978 (a) and 2012 (b). Somato-sensory evoked potential done in 2012 with giant-SEP over contralateral scalp (c). EEG done in 1978 showing generalised spike and wave discharges (d) and repeated in 2012 with similar findings (e). Polymyography findings shown on (e) with generalised myoclonic jerk.

Figure 4: Composite figure showing the Archimedes spiral drawing and neurophysiological findings in the index patient, IV:3, in 1978 compared to 2012.

Clinical description of the pedigree

The South-African FCMTE pedigree currently spans five generations. Of the 23 family members of whom information was available, 19 had cortical myoclonic tremor and 8 of them had epilepsy. Fourteen patients (including the index patient) were seen by one of the investigators (CH vd M) in 1977/8. Eleven of the original patients and six additional patients were seen in 2011-13 (RvC). The median age of cortical myoclonic tremor onset was 16 years (average 15,8; range 11-20); the cortical myoclonic tremor appeared to be progressive, causing mild disability and generally responding with moderate reduction in the cortical myoclonic tremor to alcohol and anticonvulsants (valproic acid (average dosage 1000mg daily), primidone (250mg bd) and levetiracetam (750mg bd)).

The Fahn-Tolosa-Marin tremor rating scale showed a median value of 24 (average 24,6, range 11- 43). The myoclonus rating revealed a mean rating of 2,5 (average 2,4, range 1-4) on activities of daily living. Increased age showed a clear correlation to worsening of cortical myoclonic tremor and subsequent impact on ADL. Increase in the FTMTRS score showed significant correlation with impairment of ADL as reflected in the ADL sub-score of the FTMTRS and the MRS (fig 5). In addition, increased age correlated with worsening of cortical myoclonic tremor as well as increased impact on ADL as reflected in the ADL sub-score and the MRS. We compared the marginal effect of cortical myoclonic tremor and increase in age on the MRS and activities of daily living sub-score of the FTMTRS individually and combined. We were able to show that the activities of daily living sub-score and MRS were significantly affected by the severity of the cortical myoclonic tremor (p-value 0.001) (Fig 5 and fig 6), age (p-value 0.036) (Fig 7), and cortical myoclonic tremor and age combined (p-value 0.005) (supplementary data supplied at end of chapter.)

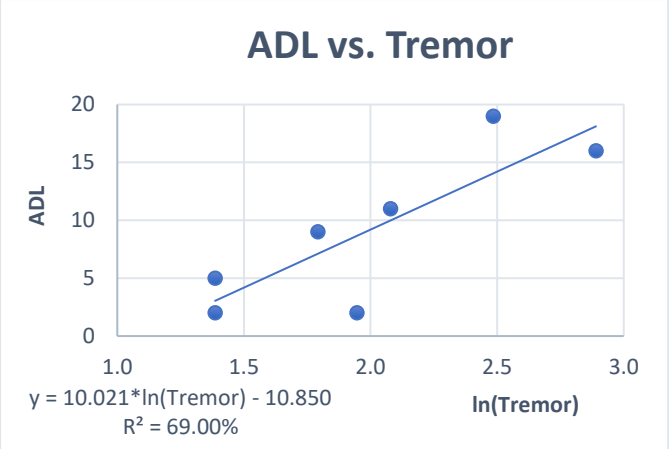

Figure 5: Comparison of Cortical Myoclonic Tremor to the Activities of Daily Living sub-scale of the FTMTRS

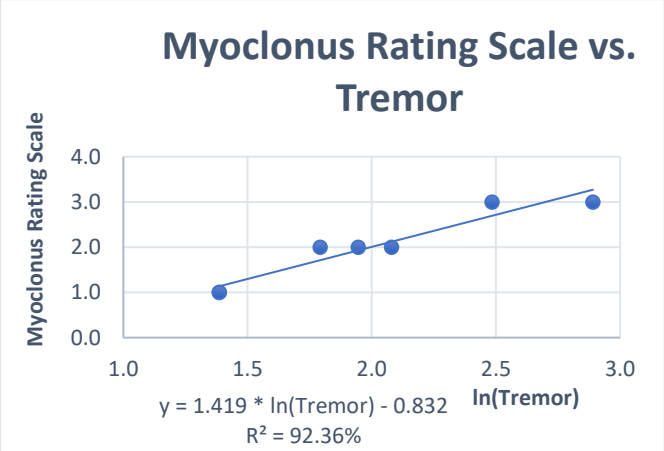

Figure 6: Comparison of the Myoclonus Rating Scale to the Tremor sub-scale of the FTMTRS

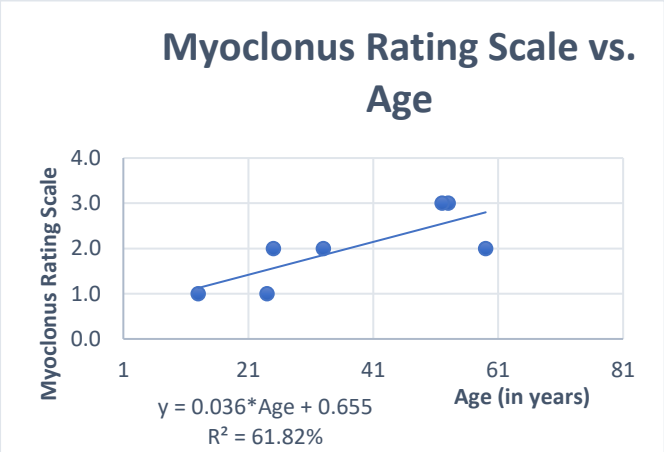

Figure 7: Comparison of the Myoclonus Rating Scale to Age at Onset

The median age of seizure onset was 42 years (average 41; range 30 - 50). Seizures were generalized with tonic-clonic convulsions without aura or prodrome in all cases. The frequency of seizures was generally less than two per year. All patients with cortical myoclonic tremor who were above 35 years had epilepsy, except one patient (IV:34) who had never had a clinically apparent seizure, and had not received anti-epileptic medication for cortical myoclonic tremor but showed epileptiform abnormalities on EEG.

Other neurologic symptoms and signs included myoclonic leg movements, causing mild gait dysfunction in one case, and facial myoclonic movements in two patients. Progressive neurological deficit (ataxia, dementia, spasticity) was not noted.

The cortical origin of the distal tremulous finger movements (myoclonic tremor/ distal myoclonus) was confirmed by increased amplitudes of the cortical g-SEP and LLR in 7 patients (Table 4). Simultaneous EEG and surface EMG recordings to assess tremor were performed in 7 patients. All 7 patients showed irregular, short duration distal jerks that were not time-locked to the cortical activity.

Clinical findings at follow up after 30 years

Of the fourteen patients with complete clinical notes available from 1977/8, eleven patients were followed up by the investigator in 2011-2013 while three had already passed away (aged 54 years, liver disease complications; aged 54 lung cancer; and 50 years, gunshot accident). Treatment with propranolol had been ineffective and primidone or phenobarbitone as standard second line treatment for essential tremor at the time had been used, while phenytoin or phenobarbitone had been used to treat the convulsions.

Of these eleven patients, five were from the 3rd generation of the pedigree and five from the 4th generation. Three patients of the 3rd generation had shown cortical myoclonic tremor and epilepsy at the first visit, persisting at follow-up after more than 30 years. Cortical myoclonic tremor severity appeared to be more in older patients without development of ataxia or cognitive impairment. One patient, however, complained of significant impairment of mobility due to lower limb myoclonus. Impairment of mobility was noted in one other patient (93-year-old female with cortical myoclonic tremor and epilepsy), but this was ascribed to complications of age and not to ataxia or myoclonus. Two patients of this group subsequently died from natural and unrelated causes at the ages of 76 and 79 years respectively (Table 5).

Three patients who were followed up from the fourth generation of the pedigree initially had presented with tremulous fingers movements. The cortical myoclonic tremor was still present in all of them at follow-up, and in addition, two patients had developed seizures. No other neurological features had developed over a period of more than 30 years. The last subject of the fourth generation had initially been seen as part of the family when he was 16 years old but had no symptoms in 1978. At follow-up in 2011, now aged 49, he had developed cortical myoclonic tremor, but no seizures (Table 5).

Pedigree number	Patients from the 1977-1979 cohort								Patients from the 2010-2013 follow up							
	Clinical picture			Neurophysiology			Treatment		Clinical picture			Neurophysiology			Treatment	
	Age	Tremor	Epilepsy	EEG	g-SEP	c-reflex			Age	Tremor	Epilepsy	EEG	g-SEP	c-reflex		
III:4	49	-	-	N	ND	ND	None		82	-	-	ND	ND	ND	None	
III:5	46	+	+	G	ND	ND	PHB		79	+	+	ND	ND	ND	PHB	
III:6		-	-	ND	ND	ND	None		79	-	-	ND	ND	ND	None	
III:9	60	+	+	ND	ND	ND	PRI		93	+	+	ND	ND	ND	PRI,CLN	
III:12	49	+	+	ND	ND	ND	PHB		79	+	+	TLE	ND	ND	PHY	
IV:3	18	+	-	G	ND	ND	PRI		53	+	+	G	+	+	VPA, PRI	
IV:5	15	+	-	ND	ND	ND	None		44	+	-	ND	ND	ND		
IV:6	11	-	-	ND	ND	ND	None		40	+	-	N	+	+	PRI	
IV:9	18	-	-	N	ND	ND	None		51	-	-	ND	ND	ND	None	
IV:11	13	-	-	N	ND	ND	None		52	-	-	ND	ND	ND	None	
IV:12	17	+	-	G	ND	ND	PRI		49	+	+	G	+	+	LTG, LEV, VPA	

Table 5 Comparison of clinical findings and neurophysiological data in eight patients that were available for follow up in 2009 – 2011.

"+" indicates feature being present and "-" feature is absent. ND= not done; G= generalized epileptiform findings; N=normal; PHB=phenobarbitone; PRI= primidone; CLN clonazepam; PHY=phenytoin; VPA= valproate; LTG=lamotrigine; LEV= levetiracetam.

EEG's were recorded in 10 subjects in 1977/8 – seven patients were clinically affected and three were not affected. These three subjects showed normal EEG's. Of the seven affected patients, five had abnormal EEG's- four with generalized spike and wave paroxysms and one with a left temporal lobe epileptiform focus. Of the five patients with abnormal EEG's three were affected with cortical myoclonic tremor and epilepsy and two only with cortical myoclonic tremor. These two patients both eventually developed epilepsy. Four EEG's were performed in newly diagnosed patients with cortical myoclonic tremor and epilepsy in 2011, showing a left temporal epileptiform focus in one patient and three normal recordings. All four of these patients were affected with cortical myoclonic tremor only.

Of the 1977/78 group, EEG's were repeated in three patients in 2011. Two patients were initially clinically only affected by cortical myoclonic tremor but had had abnormal EEG's. With follow up their EEG's still showed generalized paroxysmal spike and wave activity and clinically they were both now affected with cortical myoclonic tremor and epilepsy. The third subject, also mentioned above, had not been clinically affected and had had a normal EEG initially. With follow up he had now developed cortical myoclonic tremor, but the EEG remained normal.

Epilepsy frequency and control

Eight patients were affected with epilepsy. Of these, useful information on epilepsy control was available in seven. Judging epilepsy severity according to number of seizures per year and anti-epileptic drugs (AED) used, six were rated as having mild epilepsy (one or less seizures/year) and one as moderate (2 to 5 seizures per year). The patient with moderate epilepsy had more than five seizures per year but was only on a single AED at follow up, possibly indicating sub-optimal seizure control. Two patients developed epilepsy in the period between the first and the second follow up.

Discussion

Our patients' clinical and electrophysiological findings are compatible with a diagnosis of FCMTE with symptoms and signs as described in several publications related to this condition[10]. The disorder is autosomal dominantly inherited and the cortical myoclonic tremor appears early during the disease while generalized epilepsy has a later onset. On EEG's, epileptiform dysfunction is seen but no other features of neurodegenerative conditions are apparent; anti-epileptic medication is effective to control the seizures in most subjects.

The cortical myoclonic tremor is the main feature of FCTME and present in all affected family members described. Initially starting as fine tremulous movements in the fingers, this cortical myoclonic tremor progressed over the follow-up period to become the most disabling component of this disorder. The cortical myoclonic tremor onset in our family with FCTME was in the second decade of life in all individuals, with the mean age of onset of 15,8 years. This was earlier than in the Dutch (mean 23.5)[46], French (mean 30.8)[63] and Italian (older than 20)[8486] series but possibly in keeping with the Japanese (range 15-45). Lower limb myoclonus with effect on gait stability was also reported in the French family[62] but not in the Japanese or other European pedigrees[10].

The patients in our study developed epilepsy at an average age of 41 years; this compared well with the mean age of epilepsy onset in the Dutch pedigree (43 – range 13-44)[46] but was older than in the French (mean 29.1)[63], Italian (mean 26.6)[84]and Japanese families (older than 30)[49] (fig 8). All our patients had generalized tonic-clonic seizures and epileptiform dysfunctions on EEG. All patients developing epilepsy were on treatment with phenobarbitone or primidone for the tremor at the onset of seizures, which might have influenced

the age at onset and frequency of seizures. The seizures were generally controlled on standard anti-epileptic medication.

Our patients with FCTME have been followed up for a period of more than 30 years, enabling us to document the progression of this disorder in detail. To our knowledge, there is only one other report of long-term follow-up in a group of patients with FCTME[47]. This is a study from Italy that investigated the long-term outcome of cortical myoclonic tremor and epilepsy in 14 patients from three families affected with FCMTE and localized to chromosome 2p11.1-q12.2. In their study, the mean disease duration was 27.5 years (mean follow up 14.0 years) and cortical myoclonic tremor was minor in all patients at onset but progressed to significant effect on activities of daily living in later years. This was also noted in our group of patients. As also found in our patients, disease duration was the only risk factor identified as a predictor of cortical myoclonic tremor severity in the Italian pedigree. Seizures were rare and seizure frequency was not associated with cortical myoclonic tremor severity; this was also similar to our observations.

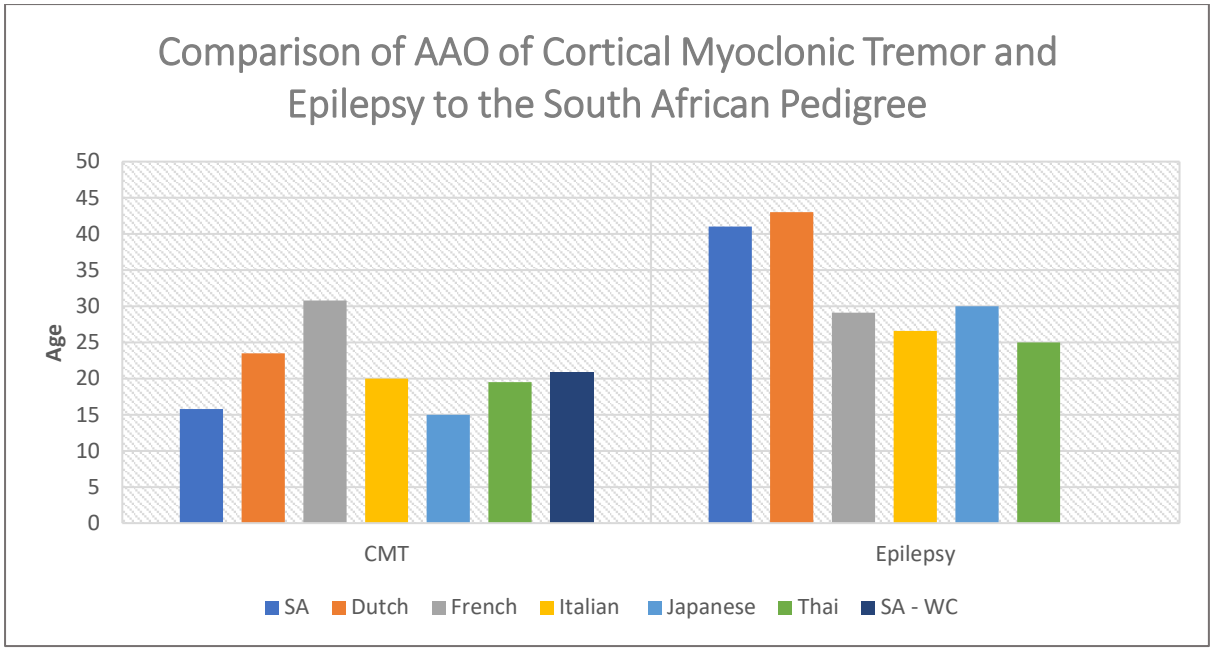

Figure 8: Comparison of Age at onset of Cortical Myoclonic Tremor and Epilepsy in individuals from the South African pedigree to published data from pedigrees from different geographical regions.

Abbreviations: SA – South African FCTME family; SA-WC – South African family from Wetsren Cape with CMT and progressive mycolonic epilepsy[25]

Lower limb myoclonus in later life was prominently seen in one of our patients after 30 years. This feature was not present in the Italian and Japanese families, but did occur in the Dutch and French pedigree[63]. In our patients, there was no evidence of additional neurodegenerative disorders developing over the follow-up period, which corresponds to the findings of the Italian pedigree. This is an important finding since other causes for cortical myoclonic tremor and epilepsy are associated with progressive ataxia, cognitive deterioration and refractory epilepsy[67]. The South African pedigree with myoclonus and epilepsy described by Carr et al[25] also featured prominent progression with ataxia, cognitive decline and refractory epilepsy more distinctive of the progressive myoclonic epilepsies. In our family, epilepsy was mild and easily controlled in most cases and did not lead to progressive debilitation later in life.

Anticipation evident in clinical and neurophysiological parameters were shown in Japanese pedigrees[64,83]. No features of anticipation could be demonstrated in our family although there was a trend toward earlier onset of cortical myoclonic tremor in following generations. Age at onset for epilepsy was affected by the use of phenobarbitone and primidone to manage cortical myoclonic tremor and severity of cortical tremor is unreliable to judge retrospectively. The age of onset, natural progression with the development of seizures and response to treatment were similar in the different generations examined. The size of the cortical SEP response did not increase in later generations. It was evident that members of the same generation were affected differently. Some patients in the third generation were severely affected with cortical myoclonic tremor and epilepsy while others in the same generation and age group were clearly less affected with milder cortical myoclonic tremor and no epilepsy.

Conclusion

We describe the initial presentation and 30 year follow-up of a five generation South African family with autosomal dominantly inherited cortical myoclonic tremor and epilepsy, fitting the descriptions of FCMTE reported by Ikeda[45], van Rootselaar[10] and Striano[67].

Some clinical features in our family differ from those of previously described European and Japanese pedigrees but these do not involve the major features of the condition. Phenotypical similarities are shared mostly with the Dutch and French pedigrees although age of epilepsy onset was older in our pedigree but possibly explained by the use of anti-epileptic drugs to treat cortical myoclonic tremor. Further analyses of candidate genes in this family are planned. The unique long term follow up of this pedigree supports the findings of Copolla[47] that the condition does not cause additional progressive

neurological deterioration and quality of life is mostly influenced by worsening of the cortical myoclonic tremor with age and not by progressive epilepsy, cognitive decline or reduced life expectancy.

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Familial cortical myoclonic tremor and epilepsy: Description of a new South African pedigree with 30 year follow up

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ABSTRACT

Aim: The aims of this study were to report the index case of a South African family with cortical myoclonic tremor and epilepsy, to describe the pedigree with the clinical findings and results of additional investigations, and to report the unique follow-up evaluation of affected and unaffected family members after 30 years.
Methods: The index case led to evaluation of the clinical files of patients from 1978/1979 and clinical assessment and investigation of patients from this cohort as well as newly identified family members. Patients were examined clinically; cortical myoclonic tremor severity was scored by using the Fahn-Tolosa- Marin-Tremor Rating Scale and the Myoclonus Rating Scale. Cortical origin of myoclonus was proven. Statistical analyses were done to assess the impact of cortical myoclonic tremor on quality of life.
Conclusion: Clinical data was available for 23 patients. Increase in cortical myoclonic tremor and age showed a statistically significant correlation with worsening of the sub-score for Quality of Life (FTMTRS) and myoclonus rating scale.
After 30 years eleven of fourteen patients could be followed up. Progression of cortical myoclonic tremor severity was noted but epilepsy control was adequate with all patients reporting less than two seizures per year. No clinical features of neurodegeneration were found.
Discussion: We describe the initial presentation and 30 year follow-up of a four generation South African family with FCMTE. The unique long term follow up of this pedigree supports previous findings that the condition does not cause additional progressive neurological deterioration and quality of life is mostly influenced by worsening of the cortical myoclonic tremor with age.

Familial Cortical Myoclonic Tremor with Epilepsy (FCMTE) (ADCME/BAFME/FAME [1]) with autosomal dominant inheritance, has been reported in more than sixty families since its first description in 1990 [2]. Four genetic loci have now been found and these can possibly be divided into geographical patterns – FCMTE1 (Japanese [3] [4] and one Chinese family [5]), FCMTE2 (Italian [6]), FCMTE3 (French [7] and Chinese family [8]), and FCMTE4 (Thai [9]), as well as unclassified Chinese [10] and Dutch families [11]. The condition is characterized by fine distal myoclonic jerks with cortical origin - termed Cortical Myoclonic Tremor, found in most affected patients, and epilepsy that usually occurs later in the course of the disease. FCMTE appears to be mildly progressive and features of neuro-degeneration, i.e. progressive ataxia, spasticity or cognitive decline are usually absent in the affected patients [12] [13]. This study describes the first South African family with FCMTE that was initially identified in 1977/8 and followed up in 2011–2013.

1. Aim

The aims of this study are to report the index case with cortical myoclonic tremor and epilepsy, to fully describe the pedigree with the clinical findings and results of additional investigations, and to report the follow-up evaluation of the patients after 30 years. The findings will be compared to other described pedigrees and used to comment on the natural history of this disorder.

2. Patients and methods

The index case (IV-3) was seen in clinical practice in 2011 and

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investigated for cortical myoclonus. It was then discovered that this patient was part of a large, four generation pedigree with cortical myoclonic tremor and epilepsy that had first come under the attention of a neurologist (KvdM) in 1977/8 at a university hospital in Bloemfontein, South Africa. At the time the identified affected family members were considered novel with essential tremor-like distal movements, myoclonus and generalized epilepsy. Affected and unaffected family members were examined and their clinical and neurophysiological findings were well documented. The clinical notes of patients belonging to this pedigree were retrieved.

After approval of the medical ethics committee of the University of Pretoria, affected and unaffected family members were contacted in order to compile the pedigree (Table 1) and delineate the clinical characteristics and course of the condition. Signed informed consent was obtained in all patients. From those patients who had been seen in 1977/8, the previous clinical information, including history of tremor and epilepsy, severity of tremor, treatment and electro-encephalography recordings, was retrieved from patient files. The following parameters were recorded on patients available for follow up and newly identified patients: full neurological examination, clinical assessment of tremulous hand movements, analysis of seizure frequency and control on anti-epileptic medication, as well as eventual patient outcome (Table 1). Follow up neurophysiological recordings were performed: a standard electroencephalogram (EEG) using the international 20/10 method; tremor recording with surface electromyography (EMG) recordings of the first dorsal interosseous, extensor indices and deltoid muscles with off-line analysis to assess duration, frequency and rhythm of muscle contraction; somatosensory evoked potentials to investigate the presence of a giant potential (g-SEP); and long loop reflex analysis (LLR/C-reflex) [14].

The Fahn-Tolosa-Marin Tremor Rating Scale (FTMTRS) [15] was completed at last follow up with all the patients who could be contacted from the previous cohort as well as all newly identified patients to assess the severity of tremulous hand movements and the subset on activities of daily living (ADL) to assess the impact of cortical myoclonic tremor on ADL. The impact of myoclonus on ADL was assessed by a retrospective myoclonus rating – the Myoclonus Rating Scale (MRS) - scale that was used before in a similar study [16]. The epilepsy severity was classified according to the number of seizures per year and number of anti-epileptic drugs used to secure seizure control. The data sets were compared with an ANOVA regressional analysis model to assess the correlation of cortical myoclonic tremor with activities of daily living and the myoclonus rating scale and the correlation of age with cortical myoclonic tremor and the myoclonus rating scale.

3. Results

3.1. Index case

The index case of this South African family with cortical myoclonic tremor and epilepsy was a 52 year old male of European descent (case IV:3) (video 1). The tremulous hand movements started at the age of 16 years and were characterized by jerky distal movements resembling postural tremor and distal myoclonus. These jerky movements were more pronounced with sleep deprivation and emotional stress; later, the patient noticed that alcohol reduced the intensity but caused a rebound effect the following day with exacerbation of the tremulous hand movements. Treatment with propranolol was ineffective, but valproic acid, started at the age of 30, reduced the movements. When the patient was 18 years old, an EEG had shown paroxysms of generalized spike and wave activity, with normal background activity and normal photic stimulation (Fig. 1). At that stage, the patient had never had a convulsion. During the course of the next three decades, progression consisted of more frequent proximal myoclonic jerks and more severe tremulous movements in his fingers. Epilepsy was diagnosed when the patient had his first seizure at 30 years age. Another EEG was performed at age 53, which showed similar features as before (Fig. 1). C-reflexes with latencies of 45 ms and g-SEP with P25-N33 amplitudes of 9,4uV were recorded, consistent with cortical myoclonus.

Supplementary video related to this article can be found at http://dx.doi.org/10.1016/j.parkreldis.2017.02.016.

3.2. Clinical description of the pedigree

The South-African FCMTE pedigree currently spans five generations. Of the 23 family members of whom information was available, 19 had cortical myoclonic tremor and 8 of them had epilepsy. Fourteen patients (including the index patient) were seen by one of the investigators (KvdM) in 1977/8. Eleven of the original patients and six additional patients were seen in 2011-13 (RvC). The median age of cortical myoclonic tremor onset was 16 years (average 15,8; range 11–20); the cortical myoclonic tremor appeared to be progressive, causing mild disability and generally responding with moderate reduction in the cortical myoclonic

Table 1
Summary of clinical and neurophysiological characteristics of known affected family members.

Pedigree	Sex, Age	Age of Onset		AED's used	Neurophysiology		
		Cortical Tremor	Epilepsy		EEG	g-SEP	LLR
III:1	M,#	17	37	PHB, MYS	G	ND	ND
III:2	M,#	20	32	#	ND	ND	ND
III:3	M,#	*	*	MYS	ND	ND	ND
III:5	F, 79	16	42	PHB	G	ND	ND
III:9	F, 92	15	40	PHY; CLN	ND	ND	ND
III:12	M,82	16	50	PHY	TLE	ND	ND
IV:3	M,52	16	30	VPA; MYS	G	+	+
IV:5	M,44	15	No seizures	NONE	NOR	ND	ND
IV:6	M,40	17	No seizures	MYS	NOR	+	NEG
IV:12	M,52	14	45	VPA, LTG, LEV	G	+	+
IV:34	F,59	18	No seizures	NONE	TLE	NEG	NEG
IV:36	F, 52	15	No seizures	NONE	ND	ND	ND
V:4	F, 25	12	No seizures	MYS	NOR	+	+
V:5	F, 24	15	No seizures	MYS	NOR	+	+
V:6	F,13	13	No seizures	NONE	NOR	NEG	+

ND: not done; G: generalized epileptiform discharges; TLE: temporal lobe epileptiform discharges; +: positive finding; NEG: negative finding; LLR: long loop reflex; MYS: primidone; PHB: phenobarbitone; CLN: clonzepam; LTG: lamotrigine; LEV: levetiracetam; VPA: valproate; #: detail not known; *: only approximate age known.

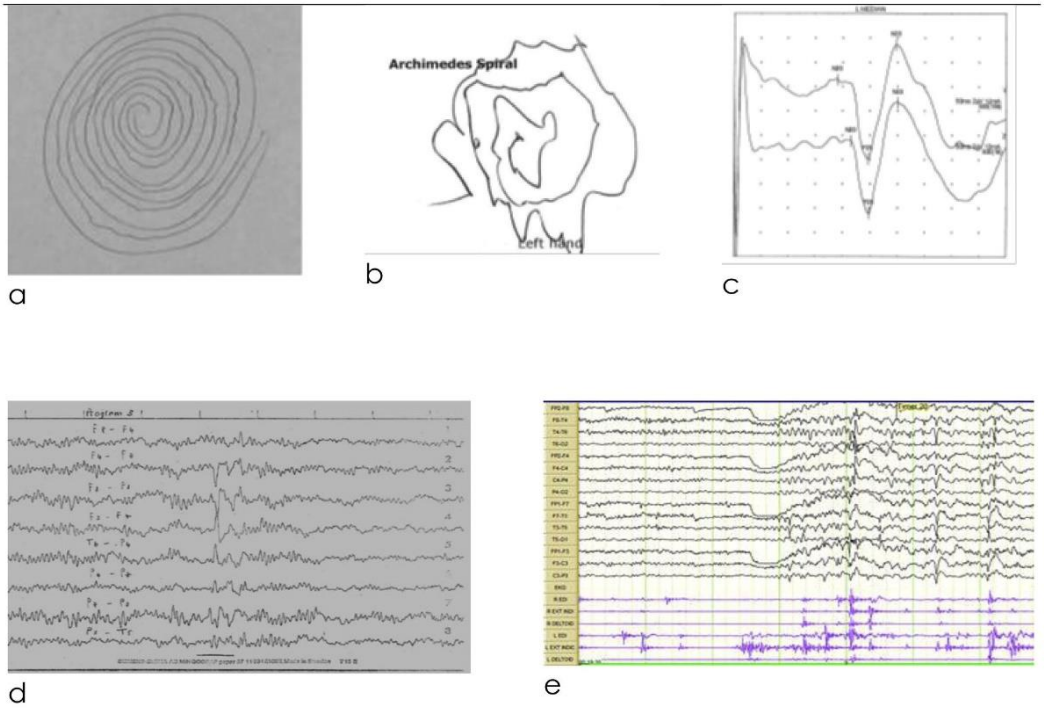

Fig. 1. Spiral drawing of patient IV:12 in 1978 (a) and 2012 (b); Somato-sensory evoked potential done in 2012 with giant-SEP over contralateral scalp (c). EEG done in 1978 showing generalized spike and wave discharges (d) and repeated in 2012 with similar findings (e). Polymyography findings shown on (e) with generalized myoclonic jerk.

tremor to alcohol and anticonvulsants (valproic acid (average dosage 1000 mg daily), primidone (250 mg bd) and levetiracetam (750 mg bd)).

The Fahn-Tolosa-Marin tremor rating scale showed a median value of 24 (average 24,6, range 11–43). The myoclonus rating revealed a mean rating of 2,5 (average 2,4, range 1–4) on activities of daily living. Increased age showed a clear correlation to worsening of cortical myoclonic tremor and subsequent impact on ADL. Increase in the FTMTRS showed significant correlation with impairment of ADL as reflected in the ADL sub-score of the FTMTRS and the MRS. In addition, increased age correlated with worsening of cortical myoclonic tremor and increased impact on ADL as reflected in the ADL sub-score and the MRS. We compared the marginal effect of cortical myoclonic tremor and increase in age on the MRS and activities of daily living sub-score of the FTMTRS individually and combined. We were able to show that the activities of daily living sub-score and MRS were significantly affected by the severity of the cortical myoclonic tremor (p-value 0.001), age (p-value 0.036) and cortical myoclonic tremor and age combined (p-value 0.005)(Supplementary data Fig. 1).

The median age of seizure onset was 42 years (average 41; range 30–50). Seizures were generalized with tonic-clonic convulsions without aura or prodrome in all cases. The frequency of seizures was generally less than two per year. All patients with cortical myoclonic tremor who were above 35 years had epilepsy, except one patient (IV:34) who had never had a clinically apparent seizure, and had not received anti-epileptic medication for cortical myoclonic tremor but showed epileptiform abnormalities on EEG.

Other neurologic symptoms and signs included myoclonic leg movements, causing mild gait dysfunction in one case, and facial myoclonic movements in two patients. Progressive neurological deficit (ataxia, dementia, spasticity) was not noted.

The cortical origin of the distal tremulous finger movements (myoclonic tremor/distal myoclonus) was confirmed by increased amplitudes of the cortical g-SEP and LLR in 7 patients (Table 1). Simultaneous EEG and surface EMG recordings to assess tremor were performed in 7 patients. All 7 patients showed irregular, short duration distal jerks.

3.3. Clinical findings at follow up after 30 years

Of the fourteen patients with complete clinical notes available from 1977/8, eleven patients were followed up personally by the investigator in 2011–2013 while three had already passed away (aged 54 years, liver disease complications; aged 54 lung cancer; and 50 years, gunshot accident). Treatment with propranolol was ineffective and primidone or phenobarbitone as standard second line treatment for essential tremor at the time was used, while phenytoin or phenobarbitone had been used to treat the convulsions.

Of these eleven patients, five were from the 3rd generation of the pedigree and five from the 4th generation. Three patients of the 3rd generation had shown cortical myoclonic tremor and epilepsy at the first visit, persisting at follow-up after more than 30 years.

Cortical myoclonic tremor severity appeared to be more in older patients without development of ataxia or cognitive impairment. One patient, however, complained of significant impairment of mobility due to lower limb myoclonus. Impairment of mobility was noted in one other patient (93 year old female with cortical myoclonic tremor and epilepsy), but this was ascribed to complications of age and not to ataxia or myoclonus. Two patients of this group subsequently passed away from natural causes at the ages of 76 and 79 years respectively (Table 2).

Three patients who were followed up from the fourth generation of the pedigree initially had presented with tremulous fingers movements. The cortical myoclonic tremor was still present in all of them at follow-up, and in addition, two patients had developed seizures. No other neurological features had developed over a period of more than 30 years. The last subject of the fourth generation had initially been seen as part of the family, but had no symptoms in 1978. At follow-up in 2011, he had now developed cortical myoclonic tremor, but no seizures (Table 2).

EEG's were recorded in 10 subjects in 1977/8 – seven patients were clinically affected and three were not affected. These three subjects showed normal EEG's. Of the seven affected patients, five had abnormal EEG's-four with generalized spike and wave paroxysms and one with a left temporal lobe epileptiform focus. Of the five patients with abnormal EEG's three were affected with cortical myoclonic tremor and epilepsy and two only with cortical myoclonic tremor. These two patients both eventually developed epilepsy. Four EEG's were performed in newly diagnosed patients with cortical myoclonic tremor and epilepsy in 2011, showing a left temporal epileptiform focus in one patient and three normal recordings. All four of these patients were affected with cortical myoclonic tremor only.

Of the 1977/78 group, EEG's were repeated in three patients in 2011. Two patients were initially clinically only affected by cortical myoclonic tremor but had had abnormal EEG's. With follow up their EEG's still showed generalized paroxysmal spike and wave activity and clinically they were both now affected with cortical myoclonic tremor and epilepsy. The third subject, also mentioned above, had not been clinically affected and had had a normal EEG initially. With follow up he had now developed cortical myoclonic tremor but the EEG remained normal.

3.4. Epilepsy frequency and control

Eight patients were affected with epilepsy. Of these, useful information on epilepsy control was available in seven. Judging epilepsy severity according to number of seizures per year and anti-epileptic drugs (AED) used, six were rated as having mild epilepsy (one or less seizures/year) and one as moderate (2–5 seizures per year). The patient with moderate epilepsy had more than five seizures per year but was only on a single AED at follow up, possibly indicating sub-optimal seizure control. Two patients developed epilepsy in the period between the first and the second follow up.

4. Discussion

Our patients' clinical and electrophysiological findings are compatible with a diagnosis of FCMTE with symptoms and signs as described in several publications related to this condition [13]. The disorder is autosomal dominantly inherited, the cortical myoclonic tremor appears early in the course of the disease while generalized epilepsy has a later onset. On EEG's, epileptiform dysfunction is seen but no other features of neurodegenerative conditions are apparent; anti-epileptic medication is effective to control the seizures in most subjects.

The cortical myoclonic tremor is the main feature of FCTME and present in all affected family members described. Initially starting as fine tremulous movements in the fingers, this cortical myoclonic tremor progressed over the follow-up period to become the most disabling component of this disorder. The cortical myoclonic tremor onset in our family with FCTME was in the second decade of life in all individuals, with the mean age of onset of 15,8 years. This was earlier than in the Dutch (mean 23.5) [11], French (mean 30.8) [17] and Italian (older than 20) [6] [18] series but possibly in keeping with the Japanese (range 15–45). Lower limb myoclonus with effect on gait stability was also reported in the French family [7] but not in the Japanese or other European pedigrees [13].

The patients in our study developed epilepsy at an average age of 41 years; this compared well with the mean age of epilepsy onset in the Dutch pedigree (43 – range 13–44) [11] but was older than in the French (mean 29.1) [17], Italian (Mean 26.6) [6]and Japanese families (older than 30) [1].All our patients had generalized tonic-clonic seizures and epileptiform dysfunctions on EEG. All patients developing epilepsy were on treatment with phenobarbitone or primidone at the onset of seizures which might have influenced the age at onset and frequency of seizures. The seizures were generally controlled on standard anti-epileptic medication.

Our patients with FCTME have been followed up for a period of more than 30 years, enabling us to document the progression of

Table 2
Comparison of clinical findings and neurophysiological tests in the eight patients that were available for follow up in 2009–2011.

Pedigree number	Patients from the 1977–1979 cohort							Patients from the 2010–2013 follow up						
	Clinical picture			Neurophysiology			Treatment	Clinical picture			Neurophysiology			Treatment
	Age	Tremor	Epilepsy	EEG	g-SEP	c-reflex		Age	Tremor	Epilepsy	EEG	g-SEP	c-reflex	
III:4	49	–	–	N	ND	ND	None	82	–	–	ND	ND	ND	None
III:5	46	+	+	G	ND	ND	PHB	79	+	+	ND	ND	ND	PHB
III:6		–	–	ND	ND	ND	None	79	–	–	ND	ND	ND	None
III:9	60	+	+	ND	ND	ND	PRI	93	+	+	ND	ND	ND	PRI,CLN
III:12	49	+	+	ND	ND	ND	PHB	79	+	+	TLE	ND	ND	PHY
IV:3	18	+	–	G	ND	ND	PRI	53	+	+	G	+	+	VPA, PRI
IV:5	15	+	–	ND	ND	ND	None	44	+	–	ND	ND	ND	
IV:6	11	–	–	ND	ND	ND	None	40	+	–	N	+	+	PRI
IV:9	18	–	–	N	ND	ND	None	51	–	–	ND	ND	ND	None
IV:11	13	–	–	N	ND	ND	None	52	–	–	ND	ND	ND	None
IV:12	17	+	–	G	ND	ND	PRI	49	+	+	G	+	+	LTG, LEV, VPA

"+" indicates feature being present and "-" feature is absent.
ND = not done; G = generalized epileptiform findings; N = normal; PHB = phenobarbitone; PRI = primidone; CLN clonazepam; PHY = phenytoin; VPA = valproate; LTG = lamotrigine; LEV = levetiracetam.

this disorder in detail. To our knowledge, there is only one other report of long-term follow-up in a group of patients with FCTME [16]. This is a study from Italy that investigated the long term outcome of cortical myoclonic tremor and epilepsy in 14 patients from three families affected with FCMTE and localized to chromosome 2p11.1-q12.2. In their study, the mean disease duration was 27.5 years (mean follow up 14.0 years) and cortical myoclonic tremor was minor in all patients at onset but progressed to significant effect on activities of daily living. This was also noted in our group of patients. As also found in our patients, disease duration was the only risk factor identified as a predictor of cortical myoclonic tremor severity in the Italian pedigree. Seizures were rare and seizure frequency was not associated with cortical myoclonic tremor severity; this was also similar to our observations.

Lower limb myoclonus in later life was prominently seen in one of our patients after 30 years. This feature was not present in the Italian and Japanese families, but did occur in the Dutch and French pedigree [17]. In our patients, there was no evidence of additional neurodegenerative disorders developing over the follow-up period, which corresponds to the findings of the Italian pedigree. This is an important finding since other causes for cortical myoclonic tremor and epilepsy are associated with progressive ataxia, cognitive deterioration and refractory epilepsy [19]. The South African pedigree with myoclonus and epilepsy described by Carr et al. [20] also featured prominent progression with ataxia, cognitive decline and refractory epilepsy more distinctive of the progressive myoclonic epilepsies. In our family, epilepsy was mild and easily controlled in the majority of cases and did not lead to progressive debilitation later in life.

Anticipation evident in clinical and neurophysiological parameters were shown in Japanese pedigrees [21] [22]. No features of anticipation could be demonstrated in our family. The age of onset, natural progression with the development of seizures and response to treatment were similar in the different generations examined. The size of the cortical SEP response did not increase in later generations. It was evident that members of the same generation were affected differently. Some patients in the third generation were severely affected with cortical myoclonic tremor and epilepsy while others in the same generation and age group were clearly less affected with milder cortical myoclonic tremor and no epilepsy.

5. Conclusion

We describe the initial presentation and 30 year follow-up of a five generation South African family with autosomal dominantly inherited cortical myoclonic tremor and epilepsy, fitting the descriptions of FCMTE reported by Ikeda [2], van Rootselaar [13] and Striano [19].

Some clinical features in our family differ from those of previously described European and Japanese pedigrees but these do not involve the major features of the condition. Phenotypical similarities are shared mostly with the Dutch and French pedigrees although age of epilepsy onset was older in our pedigree but possibly explained by the use of anti-epileptic drugs to treat cortical myoclonic tremor. Further analyses of candidate genes in this family are planned. The unique long term follow up of this pedigree supports the findings of Copolla [16] that the condition does not cause additional progressive neurological deterioration and quality of life is mostly influenced by worsening of the cortical myoclonic tremor with age and not by progressive epilepsy, cognitive decline or reduced life expectancy.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.parkreldis.2017.02.016.

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Intronic ATTTC repeat expansions in *STARD7* in Familial adult myoclonic epilepsy linked to chromosome 2

Review of the literature, description of a pentanucleotide repeat expansion in a South African family with FCTME and in an international consortium of patients with FCTME2

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1. *Locus heterogeneity*
2. *Discovery of pentanucleotide repeat expansions*
3. *Intronic ATTTC repeat expansions in STARD7 in Familial Adult Myoclonic Epilepsy linked to chromosome 2 in a South African family with FCMTE*
4. ***Nature Commun. 10, 4920 (2019).***

Locus Heterogeneity: finding a TTTTA/TTTCA pentanucleotide repeat mutation in pedigrees with Familial Myoclonic Tremor with Epilepsy from Japan and around the world

Familial Cortical Myoclonic tremor with Epilepsy (FCMTE) or Familial Adult Myoclonic Epilepsy (FAME) has been described in various families around the world with remarkable clinical and genetic heterogeneity. Clinical heterogeneity is limited to differences in age at onset of cortical myoclonic tremor and epilepsy as well as severity of epilepsy; genetic heterogeneity follows geographic patterns with four loci described in distinct geographical locations (table 6).

OMIM description and phenotype number	OMIM gene number	Geographic area	Linkage analysis
FCMTE1 601068	618073	Japan, China	8q22.1-8q24.13
FCMTE2 607876	616712	Italy, Australia and New Zealand	2p11.2-q11.2
FCMTE3 613608	613297	France, The Netherlands	5p15.31-p15.1
FCMTE4 615127	613373	Thailand	3q26.32-q28

Table 6: Summary of linkage analysis locations of known pedigrees with FCMTE/FAME

Despite various studies, pathological mutations in these described loci remained elusive. The first publication to describe a possible pathological mutation came from the Dutch group. Missense variant mutations were described in affected individuals of the Dutch FAME3 family, although one affected family member did not carry the mutation and at the time was considered a phenocopy.

Failure to find pathological mutations in the exons of the loci described led to examination of the non-coding areas of the FAME1 locus in Japanese patients and the discovery of a specific pentanucleotide repeat mutation in the *SAMD12* gene[50]. Repeating the same methodology in other pedigrees with FCMTE produced similar results with repeat expansion mutations found in the non-coding areas flanking exons in these specific locations already described with linkage analysis. These findings proved locus heterogeneity: similar repeat expansion mutations on different chromosomal positions related to distinct unrelated genes, producing a similar clinical syndrome. In none of the mutations the associated gene product could be shown to be pathological and further studies to find the pathogenesis of these mutations are needed.

Discovery of a pentanucleotide repeat expansion in *SAMD12* in 48 Japanese families.

In affected Japanese families with FAME 1 linkage analysis identified a single peak with a cumulative LOD score of 3.1 at 8q22.1-8q24.13 encompassing 30Mb. This area was later refined to a 4.9Mb area (fig 9)[80–83]. Haplotype analysis identified a core haplotype in six families. This region delimited by D8S03791 to rs4876833 contains only exon 4 and the portions flanking introns of *SAMD12* (sterile alpha motif domain-containing 12)[50,94]. Whole genome sequencing analysis of exon 4 was negative for non-synonymous variations. With WGS analysis a repeat TTTTA sequence was noted in intron 4 that was absent in the reference genome; repeat lengths were inconsistent with Mendelian inheritance and raised the possibility that the disease related alleles were not amplified by PCR. By inspecting paired reads with either of the reads designed with the unique sequence of intron 4 of *SAMD12* an extra TTTCA repeat sequence was found. This led to development of a repeat-primed PCR to identify TTTTA and TTCA repeat expansions in the intron of *SAMD12*. Southern

blot analysis confirmed the presence of the allele expansions. The repeat-primed PCR was then used to analyse the intron 4 of *SAMD12* in 51 families with 82 affected individuals. The pathological repeats could be confirmed in 49 families and in less than 5% of the control subjects. The repeat sequence was confirmed and refined by single-molecule real-time sequencing and nanopore sequencing.

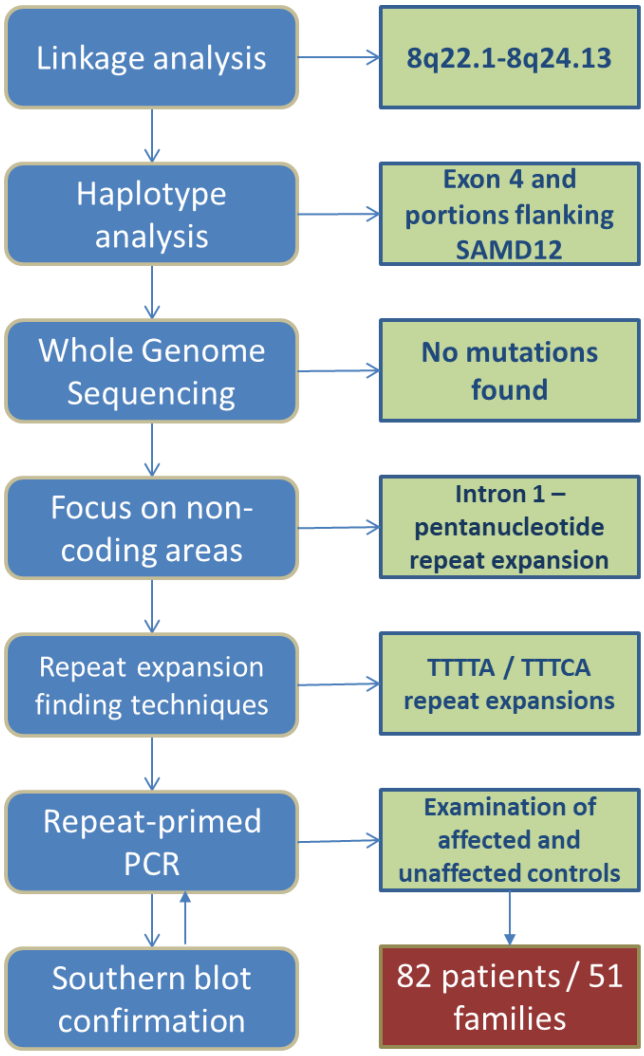


*Figure 9: **Scheme showing the process to identify the causative pentanucleotide repeat expansion in the intron of SAMD12.***

However, repeat expansions were not found in two families in this location, which led to the hypothesis that similar repeat expansions might be present in

other genes involved in the pathogenesis. A search for repeat motifs using data from Whole Genome Sequencing analysis and Southern blot analysis showed that these two families had abnormal expansions of TTTCA and TTTTA repeats in *TNRC6A* and *RAPGEF2* (table 6).

Expanding the discovery to other loci – Locus Heterogeneity

Using similar molecular techniques, repeat expansions were found in Chinese (FAME1)[65,95], Thai (FAME4)[51] and then in European families with loci on chr2 (FAME2)[36] and 5 (FAME3)[36] as summarised in table 6.

When analysing the results from the Dutch FAME3 family, the affected individual who did not harbour the *CTNND2* mutation proved also to have the pentanucleotide insertion mutation, thus providing further evidence for the pathological nature of the pentanucleotide expansion mutation and therefore the benign nature of the *CTNND2* mutation[96].

OMIM	FAME1		FAME2		FAME3	FAME4	FAME6	FAME7
Country	Japanese	Chinese	European	South African	Dutch/French/ German	Thai	Japanese	Japanese
Chromo-some position	8	8	2	2	5	3	16p21.1	4q32.1

Intronic mutation gene	*SAMD12*	*SAMD12*	*STARD7*	*STARD7*	*MARCH6*	*YEATS2*	*TNRC6A*	*RAPGEF2*
Publication	Ishiura, 2018	Cen, 2019 Zeng, J 2019	Colbert 2019	Colbert 2019	Florian, 2019	Yeetong, 2019	Ishiura, 2018	Ishiura, 2018
Number of families	48	19	22	1	4	1	1	1
Affected cases	82	160	158	15	40	13	5	1
Antici-pation	Yes	Yes	Yes	Yes	Yes	No	Unk	Unk

Table 7: Summary of published pentanucleotide repeat mutations in FCMTE/FAME pedigrees

TTTTA repeat expansions were found in small numbers of unaffected family members and unaffected controls in all the gene locations; however, TTTCA repeat insertions were only found in affected individuals. This possibly indicated that although TTTTA repeat expansions are necessary in the pathogenesis but also to facilitate the insertion of the TTTCA expansion; the essential presence of the TTTCA repeat insertion is pathological[36]. This phenomenon was already described in SCA37[97], another pentanucleotide repeat disorder.

Anticipation

Ishiura showed an inverse correlation with age of onset of cortical tremor and epilepsy to the length of the repeat mutation[50]. Clinical anticipation was already demonstrated in Japanese families with FAME before this mutation was known[64,83] but this confirmed the molecular pathogenesis of the clinical anticipation. Cen also confirmed anticipation in repeat length mutation

inversely corresponding to clinical onset in at least two later generations of a Chinese family with FAME1[95].

Anticipation could also be demonstrated in the Australian/New Zealand family with FAME2[36]c and the Dutch/French FAME3 families[96], and correlated with the molecular finding of TTTCA insertion repeat length. In the Australian/New Zealand family the mean age at onset in each generation decreased: from 30 years in generation III, to 17 in generation IV and 12 in generation V. Although no inverse correlation between expansion length and onset of cortical myoclonic tremor could be shown in the FAME3 families, there was a significant inverse correlation of the repeat expansion length and onset of seizures. This is an interesting and unique finding in all of the published data[96].

In the Thai (FAME4) family, anticipation was not shown. In one generation identical twins with identical repeat lengths had different age at onset of cortical tremor. Various explanations could be considered for the absence of anticipation in this family - possibly small numbers and too few generations studied[51].

Pathological mechanism of pentanucleotide repeat expansions not related to gene function

Repeat expansions of non-coding regions can cause disease in different ways: 1) RNA foci consisting of expanded RNA aggregates that sequester RNA binding proteins or other essential cellular factors that can lead to spliceopathy driving cortical hyperexcitability, 2) non-cononical transcription of RNA forming short peptides that can be neurotoxic and 3) pathological gain or loss of function of the associated gene product.

In none of the families with pentanucleotide repeat expansions, pathological production or gain or loss of function of the associated gene or gene product could be proven In spino-cerebellar ataxia-37 (SCA37), another pentanucleotide repeat expansion disorder with a similar insertion expansion as found in FCMTE-genes, expression of the transfected $(ATTTC)_{58}$ insertion, but not $(ATTTT)_n$, leads to abnormal nuclear RNA accumulation. Zebrafish embryos injected with RNA of the $(AUUUC)_{58}$ insertion, but not $(AUUUU)_n$, showed lethal developmental malformations[97]. Evidence for RNA toxicity has not yet been shown in FCMTE patients. At this time accumulation of abnormal AUUUC repeat containing RNA was observed in the brain tissue of some individuals with FCMTE1 only[50]. Similar studies were not done in patients with STARD7 mutations as access to similar tissue was not available[36]. The role of RNA toxicity is suspected but not proven in these patients with non-coding DNA mutations[98].

The function of *SAMD12* is unknown; its levels seem to increase with age initially but remain stable from adolescence onward. Expression in the central nervous system is abundant in the frontal lobes and cerebellum. For an analysis of the levels of *SAMD12* transcripts, a reverse-transcription-PCR analysis was performed on autopsied brains of patients with heterozygous TTTCA repeat expansions and did not show a significant change in the level of *SAMD12* transcript. However, Western blot analysis did show a slight decrease in *SAMD12* levels[50].

No abnormalities in *STARD7* expression could be shown with different techniques in FAME2 affected individuals: analysis of four fibroblast cell lines from one family showed no difference in *STARD7* mRNA or protein expression compared to controls and RNA-sequencing data from patient-derived

fibroblasts from 2 other families showed no significant difference in gene expression of *STARD7* between affected and unaffected individuals[36].

MARCH7 levels in peripheral tissue (skin, lymphocytes) were normal when compared to unaffected individuals; neuronal tissue was not examined though. Similarly, in *YEATS2* no abnormality of the gene product or function could be shown[96].

Expression of these genes in the brain is important and the distribution in the cortex (frontal lobes) and cerebellum of specific importance in the pathogenesis. Focus on the role of the cerebellum was already shown with imaging studies in Italian[72] and Dutch[73] families and post-mortem histology findings in Dutch patients[61]. This is in contrast with the neurophysiological evidence in all patients localising the origin of myoclonus and epilepsy to the cortex[6]. In SCA37 specific expression of the mutated *DUB1* gene were shown in the cerebellum of affected patients in contrast to the more wide-spread expression of mutated genes in patients with FCMTE. This could possibly explain the absence of epilepsy in patients with SCA37.

FAME1 patients with homozygous mutations exhibited more severe disease with severe disabling myoclonus affecting gait, cognitive decline and brain atrophy. In a homozygous FAME1 patient mild and diffuse loss of Purkinje cells and halo-like amorphous materials around the cytoplasm of several Purkinje cells were evident on post-mortem histology. On the other hand, in the cerebellum of patients with heterozygous mutations this feature was inconspicuous. No other findings were made in other brain regions in either the homo- or heterozygous patients who came to post-mortem in the Japanese study[50].

The discovery of pathogenic intronic repeat expansions in *STARD7* on chromosome 2 in families with FCMTE2/FAME2[36]

With the use of bioinformatic analysis of short-read whole genome sequencing ATTTT and ATTTC repeat expansions were identified in the FCTME2 linkage interval on chromosome 2; by screening for a repeat ATTTC expansion in the first intron of *STARD7* by repeat-primed PCR it could be shown to segregate with FCTME2 in 158 affected individuals from 22 families. Long-read sequencing was used to show that these mutations are somatically unstable and pathological. In available patient cell lines, it could be shown that these mutations have no effect on protein or mRNA expression levels of *STARD7*, suggesting the repeat sequence alone is pathogenic.

Molecular process of discovery:

1. Repeat expansion search on the FCMTE interval on Chr2:

- Initially the whole genome sequencing data from two individuals from a large Australian and New Zealand family[52], one from an Italian[68] and two from a French-Spanish[87] family with FCMTE2 were analysed with two repeat expansion detection methods: ExpanionsHunter[99] and exSTRa[100] to look for combined ATTTT and ATTTC repeat expansions within the FAME2 interval on chromosome 2.
- This revealed an expansion of an ATTTT repeat and insertion of an ATTTC repeat in the context of the reverse strand of chr2 within the first intron of *STARD7*.
- *STARD7* (StAR-related lipid transfer domain-containing 7) is a member of the START (StAR-related lipid transfer) domain-contain family of lipid transfer

proteins with functions including intra-mitochondrial lipid transfer of phosphatidylcholine[101,102].

- The endogenous ATTTT repeat was also found to be variable in length in the control samples but not expanded in the same extend as in individuals with FCMTE.
- The ATTTC repeat was not present in any WGS data from 69 control samples.

2. Segregation of *STARD7* ATTTC expansions by repeat-primed PCR:

- A repeat-primed PCR (RP-PCR) assay was developed to rapidly identify the expansion in 137 affected individuals from 16 independently reported families world-wide.
- An additional 72 individuals from 6 families with FCMTE2 were tested and shown to be positive for the repeat mutation.
- The ATTTC repeat expansion did not amplify in any of the 28 control DNA samples extracted from unrelated individuals.
- In all 158 individuals who tested positive for the ATTTC expansion, priming from ATTTT repeats was only successful from the telomeric end of the endogenous repeat and priming from ATTTC repeats was only positive from the centromeric end of the endogenous repeat. This suggested that the structure of the pathologic repeat in the context of the forward strand on chr2 was (AAATG)n[N](AAAAT)n, where (n) represents the unknown number of each repeat sequence.

3. Long-read sequencing:

    - The total number of repeats could not be determined with the rp-PCR and was investigated with long-read sequencing using single molecule real-time (SMRT) read and Oxford Nanopore reads.
    - This showed somatic variation in repeat sizes of the TTTTA repeat sequence in single individuals. This natural variability of the TTTTA repeat sequence meant it was not feasible to use for mutation screening; the

ATTTC repeat-primer however was 100% sensitive in all families with suggestive linkage to chr2.

Intronic ATTTC repeat expansions in *STARD7* in affected South African individuals with FCMTE

A South African family with FCMTE spanning five generations and with follow up data available for 30 years was discussed in chapter 6[35]. Linkage analysis in this family had failed to demonstrate a pathogenic mutation. With the discovery of the pentanucleotide repeat expansion in the Japanese family, DNA of the South African family was again analysed as part of the FAME2 consortium. The FAME2 consortium is an international collaboration coordinated by Mark Corbett in Adelaide, Australia, that was organised to search for the mutation and pathogenesis of FAME2 (FCMTE2) in patients from Europe, Australia and South Africa[36]. DNA of five affected individuals from the South African family with FCMTE was subsequently analysed by RP-PCR (fig 10). In all these individuals the repeat expansion ATTTT with insertion of ATTTC were found. Analysis of the clinical data and expansion length revealed features to suggest anticipation.

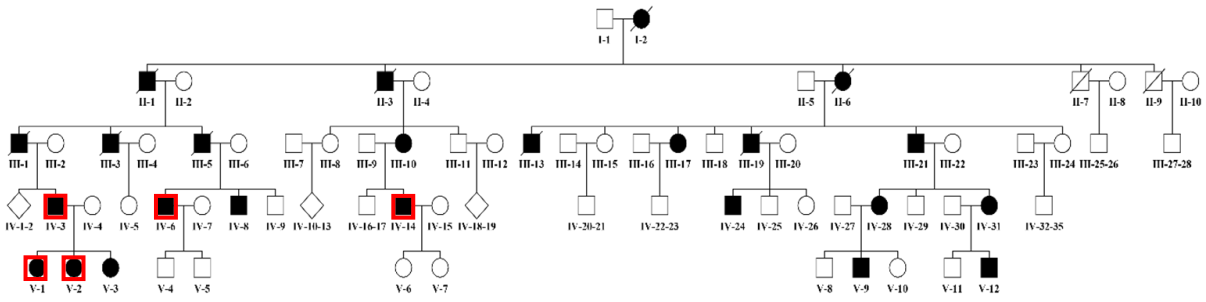


Figure 10: Pedigree of the South African family with FCMTE. DNA form the individuals marked with a red box were analysed.

Patients

Clinical data of tested affected individuals

The clinical and follow up data of the affected individuals in this South African family with CMTE were discussed in chapter 6 and the accompanying article[35]. The five individuals from whom DNA was extracted and analysed is summarised in table 8.

Pedigree number	Gender	Age at examination	Age at onset cortical tremor	Age at onset epilepsy	Neurophysiology		
					EEG	g-SEP	LLR
IV:3	Male	52	16	30	G	+	+
IV:6	Male	40	17	No seizures	N	-	-
IV:14	Male	52	14	45	G	+	+
V:1	Female	25	12	No Seizures	N	+	+
V:2	Female	24	15	No Seizures	N	+	+

Table 8: Clinical and neurophysiology data on the five South African patients of who DNA was analysed.

Methods

1. DNA was analysed as discussed in the previous section of this chapter using rp-PCR directed to find the repeat TTTTA and TTTCA repeat expansions in *STARD7*.
2. Clinical anticipation was examined by calculating the average age at onset of cortical myoclonic tremor and epilepsy in the last three generations (III, IV and V) of the South African family and was compared to repeat length of the ATTTC expansion.

Conclusions

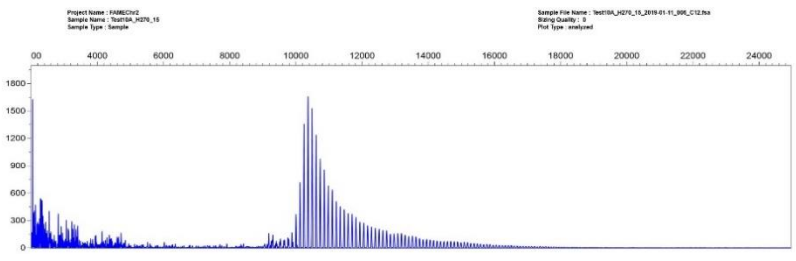
Clinical data

All five patients were affected with cortical myoclonic tremor and 2 with epilepsy. VI-6 did not have any seizures by the time of examination despite being older than the median age for seizure onset. This might be explained by the treatment for the cortical myoclonic tremor with primidone.

In four of the five patients' neurophysiologic investigations confirmed cortical origin of myoclonus with positive long-loop reflexes and giant somato sensory potentials. In patient IV-6 these tests were negative and the results were again attributed to taking primidone at the time of the examination.

ATTTT and ATTTC repeat expansions

DNA analysis proved the presence of the ATTTC repeat expansion insertion in all five individuals examined, confirming that the pathological mutation in the South African family with FCMTE is a pentanucleotide repeat expansion insertion of ATTTC in *STARD7*.

18	15	A	IV-3	

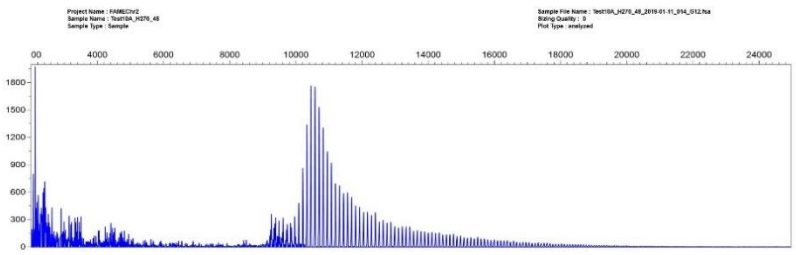
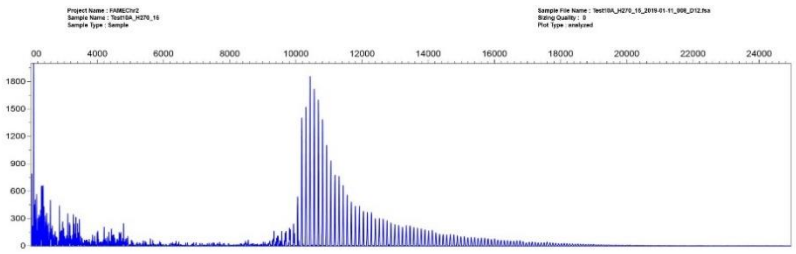
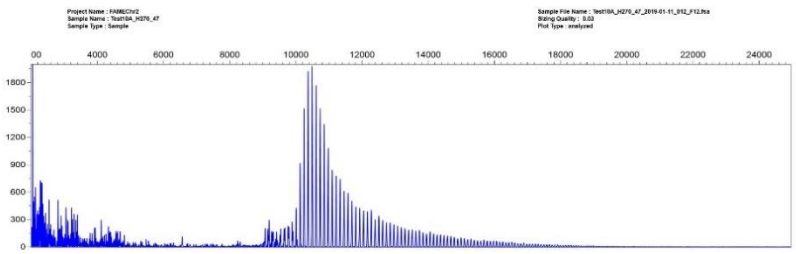
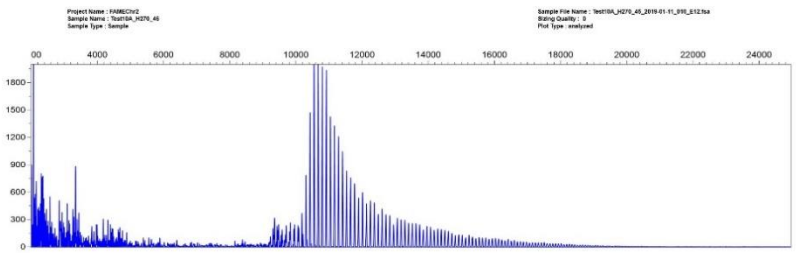
18	48	A	IV-6	
18	16	A	IV-14	
18	47	A	V-1	
18	46	A	V-2	

Table 9: Repeat-Primed PCR of the five affected South African individuals with FCMTE2/FAME2.

Anticipation

1. The average age of cortical myoclonic tremor onset in the three generations with comparable data showed a small decrease in the average age at onset from one generation to the next which was previously regarded as not significant: generation III: 16.8 years, generation IV: 15.8 years and generation V: 13.3 years.

2. The average age at onset of epilepsy was 40.2 years in generation III and 37.5 years in generation IV. This was calculated with 5 affected members with epilepsy in generation III and two in generation IV. The use of primidone and phenobarbitone to suppress cortical myoclonic tremor is thought to be a major influence in the age at onset of epilepsy, making this data less reliable.
3. In the Thai family (FAME4) it was speculated that the small number of affected individuals in each generation influenced the demonstration of lack of anticipation. The number of individuals that were available for analysis for the age at onset of cortical myoclonic tremor was small: 5 individuals in generation III, 6 in generation IV and 3 in generation V. This might have influenced the small decrease in age at onset between different generations.
4. Anticipation is further supported by the increase in the length of the ATTTC repeat expansion found in each of the two generations that was examined the study, as shown in table 9[36].
5. This data supports the already known conclusion from the examined Australian/New Zealand family that FAME2 exhibits clinical anticipation that is caused by increased length of the pathological repeat expansion of ATTTC on *STARD7*.

Conclusion

DNA primed-repeat PCR confirmed the pathogenic repeat mutation in the South African family with FCMTE to be a pentanucleotide repeat expansion insertion of ATTTC in the intron of *STARD7* on chromosome 2. This family shares the mutation with families from Southern Europe, Australia and New Zealand although the presence of a founder effect was not proven. Clinical anticipation regarding the age at onset of cortical tremor could be demonstrated and was corroborated by the repeat expansion length. Analysis of *STARD7* could not show that abnormal expression of the gene product or

function was the mechanism of neurotoxicity and further analysis of the pathophysiology of FAME2/FCMTE2 is required.

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Intronic ATTTC repeat expansions in *STARD7* in familial adult myoclonic epilepsy linked to chromosome 2

Mark A. Corbett et al.#

Familial Adult Myoclonic Epilepsy (FAME) is characterised by cortical myoclonic tremor usually from the second decade of life and overt myoclonic or generalised tonic-clonic seizures. Four independent loci have been implicated in FAME on chromosomes (chr) 2, 3, 5 and 8. Using whole genome sequencing and repeat primed PCR, we provide evidence that chr2-linked FAME (FAME2) is caused by an expansion of an ATTTC pentamer within the first intron of *STARD7*. The ATTTC expansions segregate in 158/158 individuals typically affected by FAME from 22 pedigrees including 16 previously reported families recruited worldwide. RNA sequencing from patient derived fibroblasts shows no accumulation of the AUUUU or AUUUC repeat sequences and *STARD7* gene expression is not affected. These data, in combination with other genes bearing similar mutations that have been implicated in FAME, suggest ATTTC expansions may cause this disorder, irrespective of the genomic locus involved.

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FAME (also referred to as Familial Cortical Myoclonic Tremor and Epilepsy or Benign Adult onset Familial Myoclonic Epilepsy [OMIM phenotypic series: PS601068]) is characterised by cortical myoclonic tremor and overt myoclonic and later generalised tonic-clonic seizures (GTCS)[1]. Onset of symptoms occurs in the second to third decade with variable expressivity within and between families; anticipation has been noted in some families[1]. The frequency of GTCS varies from 15 to 100% in 22 different families reported here (Table 1)[2]. Seizures are typically controlled with anti-epileptic drugs for generalised epilepsies, although rarely individuals have drug resistant epilepsy. FAME has been mapped to four distinct chromosomal loci. Most families link to chromosomes 8q24[3] or 2p11.2-q11.2[4], with an additional two families mapping to chromosome 5p15.31-p15[5] and one to chromosome 3q26.32-q28[6]. There is one report of autosomal recessive FAME caused by mutation in *CNTN2* where the phenotype was disputed[7,8]. Candidate genes and variants that fall within these common linkage intervals have been suggested for chr2 (*ADRA2B*) and chr5 (*CTNND2*); however, none of these genes have been shown to be allelic in all FAME families with linkage to the same interval[1]. We previously showed using identity-by-descent mapping that there are at least four distinct founder loci linked to FAME2 (OMIM:607876) on chr2[9].

The genetic cause of FAME has long remained elusive. The cause of FAME1, which is linked to chr8 (OMIM:601068), has recently been shown to be a complex repeat expansion of pentameric TTTTA and inserted TTTCA repeats into the fourth intron of the *SAMD12* gene[10,11]. In the same study, *TNRC6A* (chr16) and *RAPGEF2* (chr4) were implicated as FAME genes within single families, respectively, found via direct detection of the same repeated TTTTA and TTTCA sequences[11].

Here, we use bioinformatic analysis of short-read whole-genome sequencing to identify ATTTT and ATTTC repeat expansions in the FAME2 linkage interval. We screen for an intronic ATTTC expansion in the first intron of STARD7 by repeat-primed PCR and show it segregates with FAME2 in 158 affected individuals from 22 families. We use long-read sequencing to suggest the ATTTT and ATTTC expansions may be somatically unstable. We analyse clinical data and show evidence of anticipation over multiple generations of a large FAME2 family. Finally, we demonstrate that the presence of the ATTTC repeat has no effect on protein or mRNA expression levels of STARD7 in available patient cell lines. These data suggest the repeat sequence alone is pathogenic, independent of an effect on the coding sequence of the encompassing gene.

Results

Discovery of a repeat expansion in STARD7. We analysed Illumina HiSeq X-10 whole-genome sequencing data initially from two individuals from a large Australian-New Zealand FAME family, one from an Italian family and three from a French-Spanish family (Table 1 and Supplementary Table 1; Families 1, 3 and 19, respectively)[2,12,13] with two repeat expansion detection methods, ExpansionHunter and exSTRa[14,15], to look for similar combined ATTTT and ATTTC repeat expansions on both the forward and reverse chromosome strands within the FAME2 interval. This revealed an expansion of an ATTTT repeat and insertion of an ATTTC repeat in the context of the reverse strand of chr2 within the first intron of *STARD7* (StAR-related lipid transfer domain-containing 7) in all FAME samples tested (Fig. 1a, Supplementary Fig. 1). The endogenous ATTTT repeat in intron 1 of *STARD7* was also found to be variable in length in the normal population but not expanded to the same extent as repeats found in individuals with FAME. The ATTTC repeat was not present in any whole-genome sequencing data from 69 control

Table 1 Clinical summaries of 22 investigated FAME families

Family	Nationality	Total affected	Mean onset [range]	Myoclonus/CT	TCS	Focal Sz	References
1	Australian/New Zealand of European ancestry	55	18.6 y [4–59,60 y]	55/55 (100%)	8/55 (15%)	2/55 (4%)	2
2	Italian	2	15–25 y	2/2 (100%)	2/2 (100%)	0/2 (0%)	
3	Italian	4	2–18y	4/4 (100%)	4/4 (100%)	2/4 (50%)	12
4	Italian	11	22.3 y [12–49,50 y]	11/11 (100%)	11/11 (100%)	3/11 (27%)	4,17
5	Italian	25	26.6 y [5–39,40 y]	25/25 (100%)	10/25 (40%)	0/25 (0%)	38
6	Italian	12 (3 studied)	12 y [8–17,18 y]	11/12 (91.6%)	6/12 (50%)	1/12 (8.3%)	9,39
7	Italian	4	22.75 y [10–35,36 y]	4/4 (100%)	3/4 (75%)	0/4 (0%)	
8	Italian	10 (6 studied)	18.5 y [17–19,20 y]	6/6 (100%)	4/6 (66.6%)	0/6 (0%)	40
9	Italian	13 (11 studied)	17 y [12–21,22 y]	11/11 (100%)	9/11 (81.1%)	0/14 (0%)	41
10	Italian	16 (14 studied)	15.8 y [13–19,20 y]	14/14 (100%)	10/14 (71%)	0/14 (0%)	41
11	Italian	10 (5 studied)	15.5 y [13–17,18 y]	5/5 (100%)	4/5 (80%)	0/5 (0%)	42
12	Italian	21 (17 studied)	39.2 y [24–55,56 y]	17/17 (100%)	13/17 (76.4%)	0/17(0%)	43
13	Italian	3	17.7 y [12–22,23 y]	3/3 (100%)	1/3 (33%)	0/3 (0%)	42,44
14	Italian	3	16.3 y [15–18 y]	3/3 (100%)	3/3 (100%)	0/3 (0%)	44,45
15	Italian	4	30.3 y [18–48,49 y]	4/4 (100%)	3/4 (75%)	2/4 (50%)	17
16	Iraqi of Sephardic Jewish ancestry	15 (10 studied)	21 y [12–31,32 y]	10/10 (100%)	4/10 (40%)	2/10 (20%)	
17	Israeli of Sephardic Jewish ancestry	2	21 y [21 y]	2/2 (100%)	2/2 (100%)	0/2 (0%)	
18	South African of European ancestry	24 (15 studied)	15.8 y [11–19,20 y]	15/15 (100%)	7/15 (47%)	1/15 (7%)	46
19	French/ Spanish	13	41 y [30–59,60 y]	13/13 (100%)	8/13 (62%)	0/13 (0%)	13,47
20	French	7 (2 studied)	20 y ($n = 1$) Childhood ($n = 1$)	2/2 (100%)	1/2 (50%)	0/2 (0%)	9
21	Syrian	1	20 y	1/1 (100%)	1/1 (100%)	0/1 (0%)	
22	Italian	11 (10 studied)	25.1 y [14–39,40 y]	9/10 (90%)[a]	4/10 (40%)	1/10 (10%)	

CT cortical tremor, *Focal Sz* focal seizures, *TCS* tonic-clonic seizures, *y* years, *n* number of individuals
[a]One family member last evaluated at 9 years of age

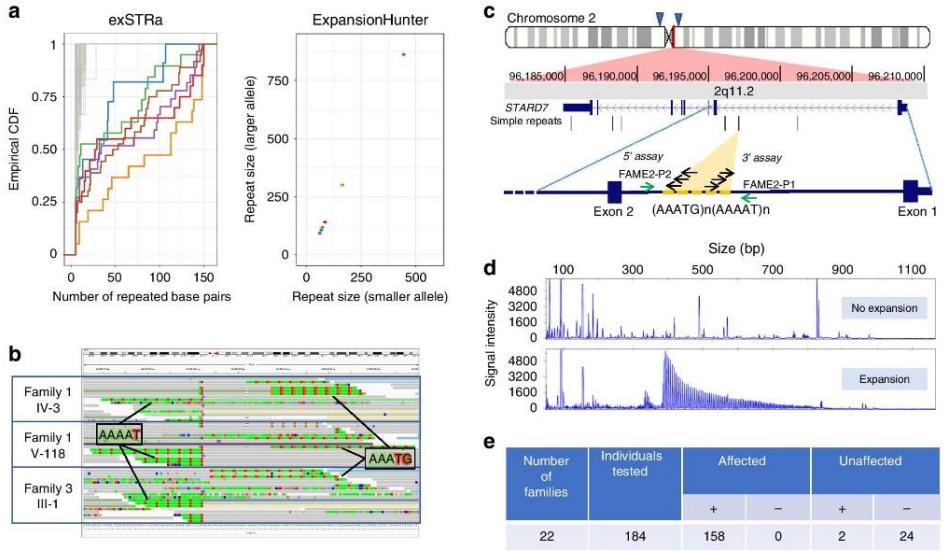

Fig. 1 Identification of an expanded pentameric ATTTC repeat causing FAME2. **a** Estimated sizes of the AAATG repeats in two affected individuals from Family 1 (red, orange), one from Family 3 (brown) and three affected individuals from Family 19 (blue, green, purple), compared to 69 individuals without FAME using TruSeq Nano (grey) or KAPA Hyper (tan) library preparation. Left panel shows empirical cumulative distribution functions from exSTRa panel while the right panel shows the estimated repeat size by Expansion Hunter (the sum of both alleles suggests repeat sizes of 0.75–2.3 kb). Data underlying this part of the figure are available in Source Data. **b** WGS data from two individuals in Family 1 and one from Family 3 show reads suggesting expansion of AAAAT and insertion of AAATG repeats in the chr2 linkage interval. **c** Upper section shows the location of the repeat in the context of chr2. The approximate location of the FAME2 minimal linkage interval is shown above the ideogram with two blue arrow heads. The *STARD7* gene is on the reverse chromosome strand and the endogenous AAAAT repeat is found in the first intron of the gene. Schema in the lower section shows the primers used in the RP-PCR to detect the ATTTT "3′ assay" and ATTTC "5′ assay" expanded repeats, respectively. **d** Example results of the RP-PCR 5′ assay obtained in an individual negative for the ATTTC insert (top panel) and in an individual affected by FAME, positive for the ATTTC repeat insertion (bottom panel). Full screening results are provided in Supplementary Data 1. **e** Summary of 184 individuals from 22 families tested with the RP-PCR assay. Individuals under category (+) tested positive for the ATTTC repeat and individuals under category (−) tested negative for the repeat

samples (Supplementary Fig. 1), nor is it reported in the Simple Repeats track in the UCSC genome browser (build hg38)[16].

Segregation of STARD7 ATTTC expansions by repeat-primed PCR. We developed a repeat-primed PCR (RP-PCR) assay to rapidly identify the expansion in 137/137 affected individuals from 16 independently reported FAME2 families worldwide (Fig. 1c, d, Table 1, Supplementary Table 1, Supplementary Data 1, Supplementary Fig. 2; Families 1, 3–6, 8–10, 12–16, 19, 20 and 22). Of the 24 individuals tested in these families that did not have a FAME diagnosis, two were positive for the ATTTC expansion; both were from younger generations and likely pre-symptomatic. We tested an additional 72 individuals (52 unrelated and 20 cases from six families with multiple affected individuals) with clinical similarity to FAME. Of these, 20/20 familial and 1/52 singleton cases were positive for an ATTTC expansion in *STARD7* (Table 1, Supplementary Fig. 2; Families 2, 7, 11, 17, 18 and 21 [singleton case]). The 52 unrelated subjects comprised 13 subjects with generalised epilepsy and tremor and 39 with myoclonic epilepsy with onset over the age of 19 years; 8/52 cases had a family history of epilepsy. Finally, within the families we tested, there were 13 individuals where the diagnosis of FAME was uncertain, usually due to a history of tremor with no other diagnostic features. Of these, 8/13 carried the ATTTC expansion. Two of the individuals with uncertain diagnosis that tested negative, were a mother and daughter pair from Family 1 (Supplementary Fig. 2a [red box] III-13 and IV-65) and subsequent analyses with microsatellite markers showed that these individuals did not have the same haplotype as affected carriers of the ATTTC expansion (Supplementary Fig. 3). The ATTTC repeat expansion did not amplify in any of 28 control DNA samples extracted from unaffected individuals unrelated to FAME.

In all 158 individuals that tested positive for the ATTTC expansion, we observed that priming from ATTTT repeats was only successful from the telomeric end of the endogenous repeat and priming from ATTTC repeats was only possible from the centromeric end of the endogenous repeat. This suggested the structure of the pathogenic repeat in the context of the forward strand of chr2 was (AAATG)n[N](AAAAT)n, where (n) represents the unknown number of each repeat sequence.

Long-read sequencing reveals the repeat structure. The total numbers of repeats could not be determined by the RP-PCR assay, therefore we investigated some of these with long-read sequencing (Fig. 2). In one individual from the Australian-New Zealand family (Family 1: IV-98) a single molecule real-time (SMRT) read and a single Oxford Nanopore read were found that spanned the repeat. The SMRT read generated to 99% base accuracy by circular consensus calling was comprised of four subreads and contained 274 AAATG and 387 AAAAT repeats, without interruption from other sequences. The Oxford Nanopore read contained 345 AAATG and 390 AAAAT repeats with some interruptions, suggesting somatic variation of repeat sizes

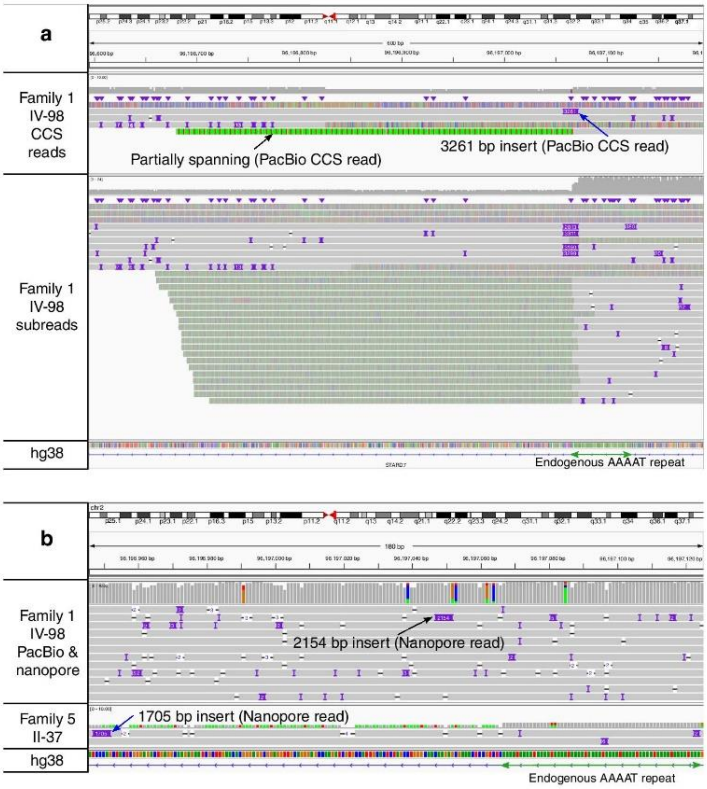

Fig. 2 Long-read sequencing identifies the structure of the AAATG/AAAAT repeat expansion in intron one of *STARD7*. **a** Upper panel shows CCS reads from one member of Family 1 (IV-98) mapped to GRCh38. A read with a 3261 bp insert (blue arrow) which contains both AAATG and AAAAT sequences and flanking sequences that map to either side of the endogenous AAAAT repeat is present. Lower panel shows the component subreads mapped to the same region. **b** Top panel shows combined PacBio and nanopore reads mapped to hg38, following correction with Canu v1.7, with base pair mismatches in the reads masked for clarity. For Family 1 IV-98 (upper panel), a 2154 bp insert is shown (black arrow) on IGV; however, the read sequence contains a 3672 bp combined AAATG/AAAAT repeat insertion. Lower panel shows a nanopore read in one individual from Family 5 (II-37) with a 1705 bp insert on IGV (blue arrow), however the read contains a 4645 bp combined AAATG/AAAAT repeat insertion. Complete sequences for all reads that span the repeat expansion are included in Supplementary Data

may occur within the one individual. In a second individual (Family 5; III-37), a single Oxford Nanopore read spanned the expanded repeats with 588 AAATG and 340 AAAAT repeats; 4645 bp in total length. The natural variability in the length of the endogenous ATTTT repeat sequence meant that is was not feasible to use that sequence for mutation screening; however, the ATTTC repeat primer was diagnostic for FAME with a sensitivity of 100% in all families with linkage or suggestive linkage to chr2. This included two families with the previously identified *ADRA2B*; c.675_686delTGGTGGGGCTTTinsGTTTGGCAG; p.H225_L229delinsQ225_F_G_R228 variant strongly suggesting that allele is not causative (Table 1; Family 4 & 15)[17].

Evidence of anticipation in a large FAME2 family. In view of the discovery that FAME2 and FAME1 are caused by similar dynamic mutations of ATTTC repeats, and the demonstration of clinical anticipation in FAME1[11], we searched for evidence of anticipation in our pedigrees. We examined the median onset age of any relevant symptom, where available, for each generation in the Australian/New Zealand family (Family 1). We found evidence of anticipation; generation III had a median onset of 30 years (range 14–60 y, $n = 6$), in generation IV median onset was 17 years (8–50 y, $n = 30$) and the median onset in generation V was 12 years (4–19 y, $n = 16$). The remaining families were either too small or onset data were unavailable for anticipation to be robustly assessed.

STARD7 transcript and protein abundance are not altered. Reverse transcriptase, quantitative PCR using primer pairs spanning the repeat containing intron between exons one and two and a second pair spanning between exons three and four showed no significant differences in STARD7 transcript expression in patient-derived fibroblast cell lines (Fig. 3a). Protein abundance was also unaltered, confirmed by western blotting using an antibody to STARD7 protein that was previously validated using STARD7-knockout cell lines (Fig. 3b)[18]. RNA-Seq data from six patient-derived fibroblasts (four from Family 1 and two from Family 5) showed there was no significant difference in gene expression of STARD7 between affected and unaffected individuals along the entire length of the gene (Supplementary Fig. 4; $p = 0.838$; False Discovery Rate = 1). Reads containing ATTTC repeats were not present in the RNA-Seq data despite robust expression of

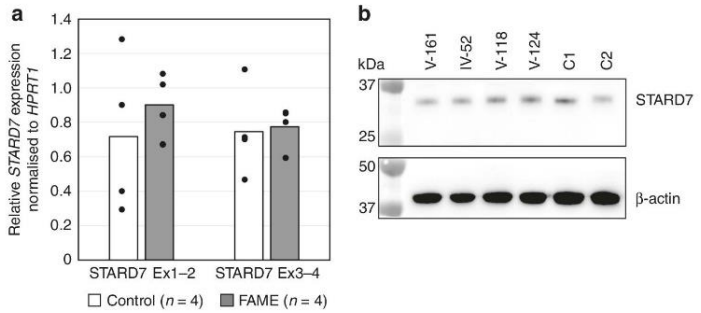

Fig. 3 Expression of STARD7 is unaltered in patient-derived skin fibroblasts. **a** Graph shows average STARD7 expression by relative standard curve quantitative PCR (qPCR) normalised to HPRT1 expression in fibroblast cell lines from four control donors (white bars) and four affected male individuals from Family 1 (IV-52, V-118, V-124 and V-161; black bars). Individual data points overlay the each bar. Tests for significance were performed using Student's two-tailed *t*-test assuming unequal variances ($p = 0.50$ Exon 1-2; $p = 0.85$ Exon 3-4). **b** Western blot of STARD7 protein compared to β-actin on the same blot of fibroblasts from the same four individuals from Family 1 as assayed by qPCR and two male control donors (C1 and C2). Data underlying this figure are available in Source Data

STARD7. This is consistent with the observations from lymphoblastoid cell lines (LCLs) derived from individuals with FAME1, where no reads with repeats were found[11].

Discussion

The pathogenic ATTTC insertion and expansion was always accompanied by the endogenous ATTTT pentanucleotide repeat in all cases of FAME2 that we describe here, replicating the findings in the cases of FAME with expansions in *SAMD12*, *TNRC6A*, *RAPGEF2*[10,11,19] and the report of a similar expansion in *MARCH6* causing chr5-linked FAME[20]. The same observation also holds for spinocerebellar ataxia 37 (SCA37, OMIM: 615945), which is caused by the same repeat expansion in the first intron of *DAB1*[21]. For SCA37, it has been hypothesised that the thymidine to cytosine transition occurs after expansion of the endogenous ATTTT repeat to ~200 copies followed by further expansion of the mutant ATTTC sequence[22]. The ATTTT/ATTTC strand of the repeat is aligned with the direction of gene expression in all genes reported thus far, regardless of their chromosomal orientation. The mechanism of disease pathogenesis has been suggested to be RNA toxicity[21]. In zebrafish embryos, direct injection of RNA containing 58 copies of the AUUUC repeat was lethal or caused developmental defects in 81%, while the effect of injecting RNA containing 139 AUUUU repeats was not significantly different from controls[21]. Accumulation of AUUUC repeat containing RNA was observed in the brain of some individuals with FAME1, but we did not have access to similar biopsy tissue from individuals with FAME2[11]. While we found no significant change in expression of STARD7 in patient-derived cell lines, it is possible that expression of this gene is regulated differently in the non-proliferating cells of the brain. Profiling expression of all known genes implicated with pathogenic ATTTC dynamic mutations using gene expression data from the GTEX portal https://www.gtexportal.org[23] shows that *DAB1* has high expression specifically in cerebellum while the five genes implicated in FAME thus far are more broadly expressed throughout the brain (Fig. 4). This difference in expression may partly explain the absence of epilepsy in individuals with SCA37.

STARD7 is a member of the START (StAR-related lipid transfer) domain-containing family of lipid transfer proteins with functions including intra-mitochondrial lipid transfer of phosphatidylcholine[24]. Previously, increased levels of choline have been detected by proton magnetic resonance spectroscopy (^{1}H-MRS) in the cerebellum of 11 individuals from three Italian families all shown here to have the ATTTC dynamic mutation[25] (Table 1). This observation may be peculiar to FAME2 families since the *SAMD12*, *RAPGEF2*, *TNRC6A* and *MARCH6* genes do not have overlapping molecular functions.

In conclusion, we have identified the molecular basis of FAME2 is an inserted expanded ATTTC repeat in the first intron of the *STARD7* gene, in 22 pedigrees with 266 affected individuals. The insertion segregates with disease status in 100% of individuals tested from families with linkage or suggestive linkage to chromosome 2 providing substantial genetic evidence that this mutation is causal in this syndrome. The FAME2 locus is the most frequently observed linked region for Caucasian individuals affected by this disorder whereas chromosome 8 thus far is limited to Asian individuals, therefore molecular genetic testing should take this into consideration if choosing to screen by RP-PCR. Identification of the gene and causative mutation for FAME2 opens the opportunity to explore the origins of the ATTTT/ATTTC expansion through a detailed comparison of the haplotypes and repeat structures of these individuals as has been done for SCA37[22]. There may be many additional undiagnosed individuals with a spectrum of FAME-related symptoms whose genetic causes may be due to ATTTC insertion and expansion at one of the FAME loci. This is especially likely in families that have multiple individuals with tremor and a low frequency of GTCS. As no preventive or curative treatments are currently available for FAME, these findings may have important therapeutic implications, including RNA-targeting treatments, such as antisense oligonucleotides or RNA-targeting Cas9 (RCas9)[26].

Methods

Ethics. This study was approved by the Human Research Ethics Committees of the University of Melbourne and the University of Adelaide. Written, informed consent was obtained from all participants in the study.

Whole-genome sequencing. Adelaide: Human genomic DNA extracting from peripheral blood lymphocytes was prepared from two individuals in Family 1 (IV-3 and V-118) for sequencing using the TruSeq Nano DNA Library Preparation Kit (Illumina). Mapping of 150 bp, paired-end sequence reads to the UCSC hg19 build of the genome and calling of single nucleotide variants from whole-genome sequencing (WGS) data generated using an Illumina HiSeqX10 platform (Kinghorn Centre for Clinical Genomics, Sydney, Australia), was performed as previously described with the minor modification of using the Genome Analysis Toolkit (GATK) version 3.8 software[27,28]. Filtering of both coding and non-coding variants within the chr2 linkage interval shared between both individuals under a dominant model and absent from the gnomAD variant database[29] at a frequency >0.001 was performed using the *bcftools isec* command from htslib v1.9. Single nucleotide variants and indels were annotated with ANNOVAR[30]. Reads

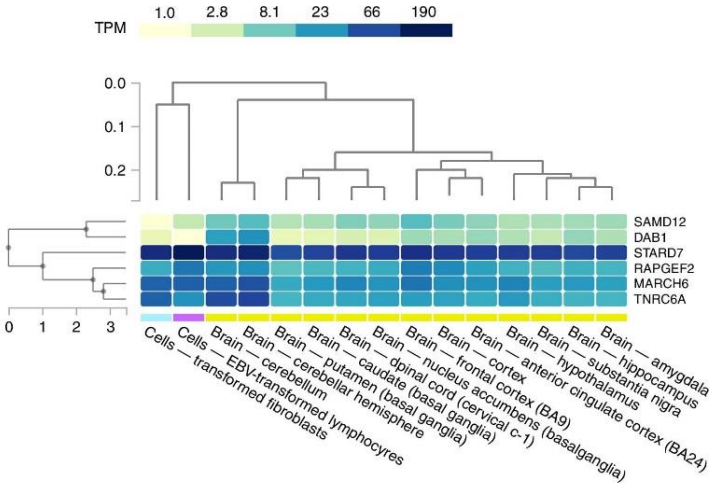

Fig. 4 Expression patterns of ATTTC repeat genes in brain, skin fibroblast and lymphoblastoid cell lines. The heatmap shows relative gene expression expressed as transcripts per kilobase per million mapped reads (TPM) based on the colour scale as shown. Data and image downloaded from the GTEx Portal https://www.gtexportal.org

containing the expanded repeat were visualised using the Integrative Genomics Viewer (IGV) v2.4.5 with soft-clipped reads unmasked[31].

Rome: WGS library was prepared from the genomic DNA of the individual (PM195; Family 4) by using TruSeq DNA PCR-Free KIT (Illumina, San Diego, CA, USA) and sequenced 150 bp paired-end reads on an Illumina HiSeq producing 470,174,247 fragments, corresponding to about 39X coverage after mapping and removal of duplicated reads. Reads were quality filtered and aligned to the reference human genome sequence (GRCh38/hg38) with BWA-MEM v.0.7.15[32]. Resulting BAM files underwent local realignment around insertion-deletion sites, duplicate marking and recalibration steps with GATK v3.8[28]. Variant calling was performed with HaplotypeCaller v3.8 with standard parameters, and output VCF files were recalibrated with VariantRecalibrator from GATK v3.8. Genomic variant annotation was carried out with VarSeq v1.4.7 (Golden Helix, Inc., Bozeman, MT, www.goldenhelix.com) and only variants with a minimum read depth of 5X were included in the downstream analysis. Thereafter, only variants in the pericentromeric region of interest of chr2 (chr2: 91,800,000–106,700,000) were considered.

Prioritisation of variants of potential interest was carried out through three distinct analyses. For the first analysis, all variants reported to be pathogenic or potentially pathogenic in the clinical databases of ClinVar, HGMD Professional v2017.2 and/or Centogene CentoMD v4.1 were retained. For the second analysis, we focused on variants in exonic regions without a reported clinical annotation. We excluded variants with a population frequency above 1% in the databases of 1000 Genomes Project, National Heart, Lung and Blood Institute (NHLBI, https://www.nhlbi.nih.gov/) Exome Sequencing Project (ESP, http://evs.gs.washington.edu/), ExAC (Exome Aggregation Consortium, http://exac.broadinstitute.org/) and gnomAD (The Genome Aggregation Database, https://gnomad.broadinstitute.org/), along with variants recorded in the Personal Genomics internal database. We retained all the non-synonymous variants predicted to alter the protein structure or function by at least three of the following in silico prediction tools: Mutation Taster, SIFT, Polyphen-2, MutationAssessor and FATHMM. For the third analysis, we prioritised the variants outside exonic regions by considering rare variants (frequency below 1% in frequency population databases, including the Personal Genomics internal database) and with a predicted significant effect on the protein structure or function by at least three of the in silico prediction tools. Variants were then prioritised by considering their presence in regulatory regions as reported in the ENCODE database (https://www.encodeproject.org/). The manual inspection of the BAM files, by using Integrative Genomics Viewer (IGV), allowed us to evaluate the coverage of the variants and the quality of the aligned reads.

The identification of putative genomic expansions, structural variants or copy number variations was carried out by using Lumpy v0.2.13[33] and Manta v1.2.2[34] software. The ExpansionHunter tool v2.5.3[14] was adopted to estimate the size of potential repetitions of short unit sequences.

Long-read sequencing. DNA was extracted for all long-read sequencing protocols using the QIAsymphony system from skin fibroblasts (passage 6) cultured in Dulbecco's modified Eagle's Medium (DMEM; Life Technologies) with 10% fetal calf serum. Pacific Biosciences (PacBio) single molecule real-time (SMRT) sequencing data were obtained in two batches: In the first batch, two Australian FAME2 carriers (Family 1: IV-44 and IV-98) were sequenced with two flow cells per sample. Resulting bam files were converted to fastq using the SMRT Link software v5.1.0 *bam2fastq* program. Resulting fastq files were either mapped directly to the human genome hg38 build using NGM-LR[35] with structural variants called by Sniffles[35] or used as input for de novo assembly with Canu v1.7. In the second batch, a single sample (Family 1: IV-98) was sequenced. DNA fragment sizes were determined with the Femto Pulse capillary electrophoresis system (Agilent Technologies, Santa Clara, CA). DNA fragments of size greater than 6 kb were selected with BluePippin (Sage Science, Beverly, MA) pulsed field gel electrophoresis system. Sequencing was carried out for 20 h per SMRT cell on the Sequel system with Binding Kit 3.0 (PacBio, 101–500–400) and Sequencing Kit 3.0 (PacBio 101–427–800). Circular consensus calling was performed using CCS 3.2.1 software. Reads were mapped to the GRCh38 build of the human genome using *pbmm2* with "-c 0 -L 0.01" for CCS reads and "-c 0 -L 0.1" for subreads.

Oxford nanopore data were obtained for DNA samples extracted from fibroblasts from two individuals from Family 1, as described above, and two from Family 5 (II-37 and IV-29 Fig. S2e). For each of the four participant samples, 3 µg of DNA was prepared for Oxford Nanopore 1D genomic sequencing by ligation using the SQK-LSK108 kit and was run on a FLO-MIN106 flow cell for 48 h. Basecalling was performed on MinKNOW 18.01.6 with MinKNOW Core 1.11.5 and Albacore v2.1. Data were either mapped with NGM-LR or assembled with Canu v1.7 as described below, using suggested settings for nanopore sequencing reads.

De novo whole-genome assembly of one individual with input of both PacBio and nanopore sequencing from one individual from Family 1 was carried out using the Canu v1.7 assembler with default starting parameters for a genome size of 3.6 Gbp. Recalibrated reads from Canu were mapped to the hg38 build of the human genome using NGM-LR as described above.

Repeat expansion analysis. WGS was performed for two affected individuals from Family 1 on the Illumina HiSeq X10 platform, one individual from Family 3 as described above, and three affected individuals from Family 19 on the Illumina HiSeq platform. A cohort of 69 individuals without FAME were used for comparison, with 150 bp paired-end sequencing performed on the Illumina HiSeq X platform (Kinghorn Centre for Clinical Genomics, Sydney, Australia). Library preparation for 53 of the samples used the Illumina TruSeq Nano DNA HT Library Preparation Kit; the other 16 samples used KAPA Hyper Prep Kit PCR-free library preparation.

Reads were aligned to the hg19 reference genome with BWA-MEM v0.7.17-r1188[32], then duplicate marking, local realignment and recalibration were performed with GATK v4.0.3.0[28]. Repeat expansion analysis targeting two FAME2 loci, the ATTTT repeat and predicted ATTTC insertion in *STARD7*, was performed using ExpansionHunter v2.5.5[14] and exSTRa v0.88.3 with Bio-STR-exSTRa v1.0.1[15]. Custom files defining the FAME2-AAAAT and FAME2-AAATG repeat loci were created for ExpansionHunter (below) and exSTRa (Supplementary Table 2).

```
{
"OffTargetRegions": [
"chr3:151086374151086421",
"chr4:2171641221716717",
"chr6:4867170448671855",
"chr8:119379052119379154",
"chr9:113463975113464205"],
"RepeatId": "FAME2-AAAAT",
"RepeatUnit": "AAAAT",
"TargetRegion": "chr2:9686280596862859"
}
{
"OffTargetRegions": [
"chr3:151086374151086548",
"chr4:2171641221716717",
"chr6:4867170448671855",
"chr8:119379052119379154",
"chr9:113463975113464205"],
"RepeatId": "FAME2-AAATG",
"RepeatUnit": "AAATG",
"TargetRegion": "chr2:9686282596862826"
}
```


Supplementary Figure 1 shows the repeat sizes predicted by ExpansionHunter and empirical cumulative distribution function of repeated bases from exSTRa for the two FAME2 loci. Significance testing was performed using the exSTRa tsum_test function with 100,000 permutations in case-control mode comparing each affected individual with FAME to the 69 unaffected individuals without FAME. All FAME2 carriers were significant outliers for the FAME2-AAATG locus ($p < 0.0001$ for all individuals) while only four samples were significant outliers ($p < 0.05$) for the FAME2-AAAAT locus.

RNA-Seq. Total RNA was extracted from patient-derived primary skin fibroblasts of four Australian/New Zealand FAME, two Italian FAME and four age-matched controls using QIAGEN RNeasy kits, as per the manufacturer's protocol. Library preparation and RNA-Seq were performed as a service by the UCLA Neuroscience Genomics Core Facility. The TruSeq v2 kit (Illumina) was used to generate unstranded libraries with 150-bp mean fragment sizes and 50-bp paired-end sequencing performed using the HiSeq2500 (Illumina). Sequence data were mapped to the GRCh38 build of the human genome using *HISAT2 v2.1.0*[36]. Read counts were generated with *StringTie v1.3.3*[36]. Differential expression between FAME and control samples was determined using the exact test from the *edgeR v3.26.5* package in *R v3.6.0*[37]. Differentially expressed genes were filtered to false discovery rate (FDR) < 0.05 and log base 2-fold change (LFC) >= 1 or <= −1.

Quantitative PCR. RNA was extracted from four patient-derived primary skin fibroblast cell lines from Family 1 and four control fibroblast cell lines from adult donors not affected by FAME as described above under *RNA-Seq*. cDNA were generated from 1 µg of total RNA using the iScript reverse transcription kit (Bio-Rad, Gladesville, NSW, Australia; cat# 1708891), according to the manufacturer's protocol.

Quantification of differentially expressed transcripts was performed with the relative standard curve method using SYBR green fluorescence intensity for detection. Products were amplified in 1 × iTaq Universal SYBR Green supermix (Bio-Rad; cat# 1725121) with primers at 1µM final concentration. Each sample and standard was amplified with three technical replicates on an Applied Biosystems StepOnePlus. Expression values were determined relative to a dilution curve of a cDNA standard made from pooled control fibroblast cDNA. Specificity of products was determined by melt curve analysis at the conclusion of each run. Expression values of each gene were normalised to *HPRT1* expression values from the same sample.

Western blotting. Fibroblasts were cultured as described in Supplementary methods then lysed with lysis buffer (150 mM NaCl, 1% Triton X-100, 1 mM EDTA, 0.25% Sodium deoxycholate, 50 mM Tris. Added protease inhibitor, 50 mM NaF and 0.1 mM Na_3VO_4). Extracts were separated by 4–12% polyacrylamide gel and transferred to nitrocellulose membrane by electroblotting. STARD7 was detected with rabbit polyclonal anti-human/mouse/rat STARD7 (Proteintech cat# 15689–1-AP) at 1:500 dilution followed by anti-rabbit IgG conjugated to horseradish peroxidase (HRP) at 1:2000 (Dako cat# P0448). Enhanced chemiluminescent detection (Bio-Rad cat# 1705061) was visualised with the chemidoc detection system (Bio-Rad). Full blots are available in the Source Data file.

PCR amplification and sequencing of repeats (Rome). Pentanucleotide repeats were analysed in duplicate by long-range PCR with Expand Long Template PCR System (Roche) according to the manufacturer's recommendation. Some 200 ng genomic DNA were amplified with primers STARD7F and STARD7R (300 nM), dNTP (350 µM) buffer 1 (1×) Enzyme 0.5 U (×50 µl reaction). After 2 min of initial denaturation at 94 °C, DNA samples underwent 10 cycles of amplification (denaturation 94 °C for 10 s, annealing 56 °C for 30 s, elongation 68 °C 3 min) followed by an additional 20 cycles (94 °C for 15 s, annealing 56 °C for 30 s, elongation 68 °C 45 s + 20 s each cycle elongation for each successive cycle). PCR products were separated by electrophoresis on 1% agarose gel. DNA was extracted from the agarose gel slice and the number of repeat units was determined by Sanger sequencing (Eurofins Genomics Sequencing Service).

Repeat-primed PCR. Primers for both Adelaide and Rome are shown in Supplementary Table 3.

Adelaide: Reaction mixes included 100 ng genomic DNA, 0.5 µM FAM-labelled locus specific (RP-PCR-FAME2-P1 or P2) and RP-PCR-P3 primers, and 0.05 µM repeat specific primer (one of RP-PCR-FAME2–4.5 to 4.8) with Expand Long Template polymerase (Roche, cat# 25524324) or Taq polymerase (Roche, cat# 18697220). The initial RP-PCR step was at 95 °C for 5 min followed by 10 cycles (95 °C for 30 s, 48 °C + 1.0 °C each cycle for 45 s and 65 °C + 1.0 °C each cycle for 5 min) continuing to 30 cycles (95 °C for 30 s, 58 °C for 1 min and 72 °C for 5 min) and ending with 72 °C for 7 min. Fragment analysis was performed on the RP-PCR products with an ABI3730 DNA analyser.

Rome: The pentanucleotide repeat sequence in *STARD7* gene was amplified by ATTTT and ATTTC RP-PCR with the following primers: STARD7R* 5′FAM-labelled (locus specific primer), RP-PCR-STARD7-P3 (generic primer) and RP-PCR-STARD7-P4 primers specific for the short pentanucleotide repeat (ATTTT) and for the possible expanded (ATTTC) repeat or possible (ATTTC) repeat interruption. PCR was performed with 100 ng DNA, 1.5 mM $MgCl_2$, 200 µM dNTP, 0.4 µM locus specific primer, 0.4 µM generic primer, 0.2 µM repeat primer, 2.5 U Polymed Taq in 25 µl volume. The initial PCR step was at 94 °C for 15 min followed by 35 cycles (94 °C for 45 s, 60 °C for 30 s and 72 °C for 2 min) and 72 °C elongation for 30 min. Capillary electrophoresis was performed on ABI310 GEN ANALYZER (Applied Biosystems).

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

Source data for Figs. 1a, 3a, 3b, Supplementary Figs. 1a, b and 4b are provided in the Source Data files of this manuscript. RNA-Seq data are available from the NCBI BioProject PRJNA563467. Whole-genome sequencing data are available from the corresponding author on request, subject to human research ethics approval and patient consent.

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Competing interests

A.W. and S. Chakraborty. are employees and shareholders of Pacific Biosciences. There are no other competing interests to declare.

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A South African family with myoclonus-dystonia syndrome with a novel mutation in the *SGCE* gene responding to deep brain stimulation

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Introduction

Myoclonus-dystonia syndrome (MDS) is a rare movement disorder characterized by the co-occurrence of childhood-onset dystonia and myoclonus usually affecting the upper body and cervical area. The myoclonus is frequently the predominant feature and often alcohol responsive. The condition is often associated with psychiatric disturbances including obsessive compulsive disorder (OCD), anxiety and possibly depression.

MDS is inherited in an autosomal dominant pattern with several known genetic mutations. The most common mutation described is in the epsilon sarcoglycan gene on chromosome 7q21 (*SGCE* or DYT-11)[103]. Additional methylation of the maternal allele carrying the *SGCE* gene results in maternal imprinting, with subsequent expression of only the paternal allele in the offspring. This reduces the penetrance of maternally inherited mutations, and most patients therefore inherit the disorder from their fathers[104]. Another locus (DYT-15) for MDS was identified in a single large pedigree from Canada and subsequently in two other families with an unknown mutation; one family were shown with a *KCTB17* gene mutation on chromosome 22q12.3 (DYT-*KCTB17* / DYT-26); and some patients with co-existing myoclonus and dystonia may also have mutations in the DYT-DRD, DYT-*THOR1A*, *RELN* and *TUBB2B* locations[43,105]. However, in some cases, none of the common mutations have been found and the search for new mutations causing a similar clinical picture is ongoing.

Onset of MDS is usually in the first or second decade with focal hand dystonia, often writer's cramp, and subsequent myoclonus and dystonia of the upper body and cervical area. Myoclonus and dystonia can occur in the same or in different body regions. Clinical patterns might indicate mutation status and

patients with predominant axial (truncal) myoclonus and dystonia and myoclonus and dystonia in the same body region, more often seem to be *SGCE* negative[106].

Treatment of MDS is generally disappointing with little response to various drugs used in dystonia and myoclonus. Botulinum toxin may have some efficacy in focal dystonia, a recent publication showed mild improvement with zonisamide[107] and response to tetrabenazine and carbamazepine has been reported in case reports. Deep brain stimulation (DBS) of the internal segment of the Globus Pallidum (GPi) and in some cases also the Ventral intermediate nucleus of the thalamus (Vim) have shown efficacy in single case studies and case series [108].

In South Africa, no patients with MDS have been reported. Here, we describe findings of a clinical and genetic study of a South African Afrikaner family with autosomal dominantly inherited severe axial myoclonus, limb-, axial- and cervical dystonia, associated OCD and anxiety.

Methods and patients

The index patient and thereafter the family members were assessed clinically and with video recordings (Supplementary video data to the published article[37]: video 1 and 2[37]; https://www.prd-journal.com/article/S1353-8020(19)30426-2/fulltext) with the Unified Myoclonus Rating Scale (UMRS), the Hospital Anxiety and Depression Scale (HADS) and the Yale OCD scale. Dystonia rating was done retrospectively using video data and a blinded investigator (JC) with the Unified Dystonia Rating Scale (UDRS). Assessments were done before surgery as well as at six months and at four years after DBS surgery. Radiological and electrophysiological investigations were performed in all patients.

The study participants signed informed consent to participate in a study examining the clinical outcome of DBS in dystonia that was approved by the Clinical Ethics Committee of the University of Pretoria (36/2015). Consent to be videotaped and for the submission and publication of video was obtained in writing.

DBS surgery was performed targeting the posteroventral segment of the GPi using intraoperative microelectrode monitoring and macroelectrode stimulation in a procedure described before[34] (Activa RC with model 3389 leads; both Medtronic Inc., Minneapolis, Minn, USA).

Clinical assessment of the index patient

The index patient, a 40-year-old female (Figure 11A, individual II-2; Table 10), presented with severe axial myoclonus of the upper body which was refractory to treatment. Limb myoclonus was less prominent, but the patient also had cervical dystonia and writer's cramp. The writer's cramp had developed at the age of 12 and was followed by torticollis when she was 16 years old. Progressive myoclonus developed in the 2nd decade of life. Action induced myoclonus was prominent with upper body myoclonus triggered by walking. Drinking, eating and dressing were difficult due to action limb myoclonus. The patient also showed features of OCD, anxiety and depression; she had a score of 15 on HADS.

Clinical assessment of family members

The index patient's sister, a 43-year-old female (Figure 11A, individual II-1; Table 10), had axial myoclonus from the age of 13; focal left leg dystonia preceded this with onset at around 6 years old. She had severe action induced axial myoclonus with dystonia of the trunk causing impairment of mobility. She also

complained of anxiety and had debilitating OCD which rendered her house bound. DBS was implanted bilateral in the GPi using the same technique as in the index patient.

Special investigations done as routine clinical practice did not indicate cortical origin myoclonus with normal magnetic resonance imaging of the brain, and electroencephalogram; and somato-sensory evoked potentials without evidence of a giant-SEP.

Patient II-I has two sons aged 20 and 17 (Figure 11A). The younger son (patient III-2) had been diagnosed with writer's cramp at the age of 8. He has not developed myoclonus or dystonia in other regions. The older son (patient III-1) is treated for attention deficit disorder with co-morbid impulsivity and depressive mood. He has mild postural tremor but no features of dystonia or myoclonus.

The father of the two affected sisters (Figure 11A, individual I-1) had postural action induced arm myoclonus which was not disabling; and he also had a history of depressive episodes.

All affected family members reported improvement of myoclonus with alcohol use and deterioration thereof under emotional strain.

Genetic analysis

Individual II-1, the sister of the index patient, had undergone genetic testing at the Afdeling Genoomanalyse, Academisch Medisch Centrum in Amsterdam, Netherlands. Subsequently, whole blood samples were collected from five

available family members and genomic DNA was isolated using the Nucleospin Blood (XL) DNA extraction kit (Macherey-Nagel) at the Human Molecular Genetics Laboratory at Stellenbosch University in South Africa. Polymerase chain reaction (PCR) primers were designed flanking exon 6 of the *SGCE* gene (Forward: 5'-TGTAGTCAAGAAATGGAGCCTGT-3'; and Reverse: 5'-CAAACGTTAACTCCAGCCACA-3') and the PCR product was screened using Sanger Sequencing at the Central Analytical Facilities, Stellenbosch University. Allele frequencies were determined in 246 ethnically matched controls by means of high-resolution melt (HRM) analysis using the RotorGene 6000 analyzer (Corbett Life Science). The controls had been recruited from the Western Province Blood Transfusion Service clinics in Cape Town, South Africa. Samples with altered heat denaturation profiles were Sanger sequenced to characterize the sequence variants.

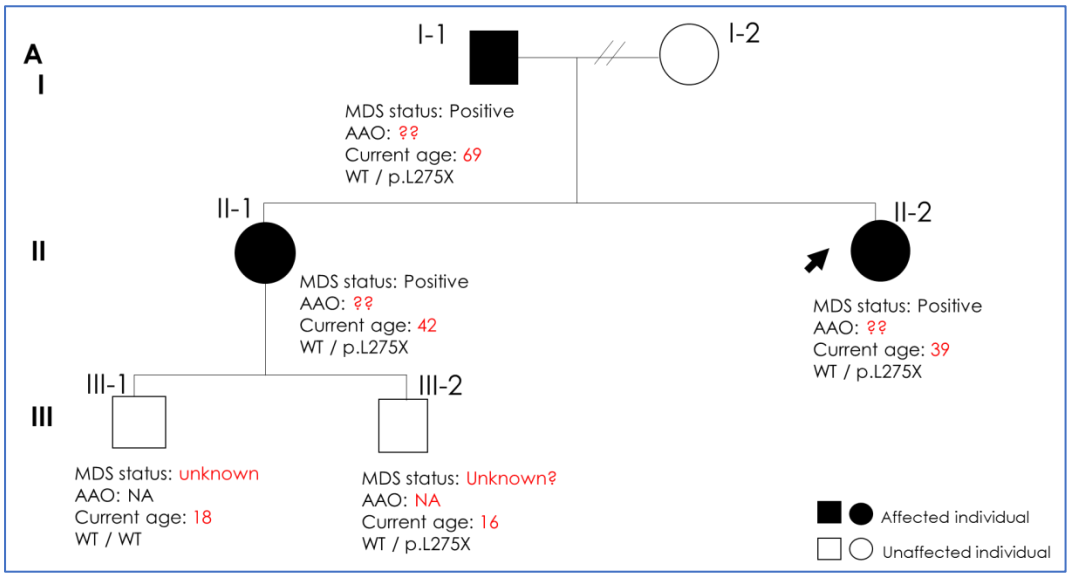
Results and discussion

Clinical assessment of this family showed mildly heterogeneous clinical features and progression with mild postural limb myoclonus in one family member, severe axial and cervical myoclonus with associated dystonia, severe anxiety and OCD in two female siblings in the same generation and focal task-specific dystonia (writer's cramp) in one male member of the third generation. The inheritance pattern is autosomal dominant with possible mother-to-son transmission evident. The clinical syndrome started in childhood in all affected individuals with focal limb dystonia: leg in one patient and writers' cramp in two.

Genetic screening of the family revealed a new variant in the *SGCE* gene, a heterozygous c.824T>A nucleotide substitution in exon 6 that leads to a premature stop codon in the protein at amino acid position 275 (p.L275X). All three affected individuals as well as an individual with unknown disease status

(possible MDS) (individual III-2) are heterozygous carriers of p.L275X. The Sanger sequencing results are shown in Figure 11B.

This variant is absent from the ExAC database (http://exac.broadinstitute.org/) and, to our knowledge, has not previously been reported. The mutation p.L275X could not be identified in 246 ethnically matched controls. Additional support for the potential pathogenicity of this variant is provided by *in silico* prediction software Mutation-Taster (http://www.mutationtaster.org) that predicted the variant to be "disease-causing". Furthermore, the leucine residue at position 275 is evolutionarily conserved and the mutation was predicted to have a very high impact with a Combined Annotation Dependent Depletion (CADD) score of 43 (Variant Effect Predictor, VEP; http://www.ensembl.org/Homo_sapiens/Tools/VEP). Although this mutation is likely to be pathogenic since it is predicted to lead to a truncated protein, future studies including gene and/or protein expression studies are necessary to characterize its functional effect.



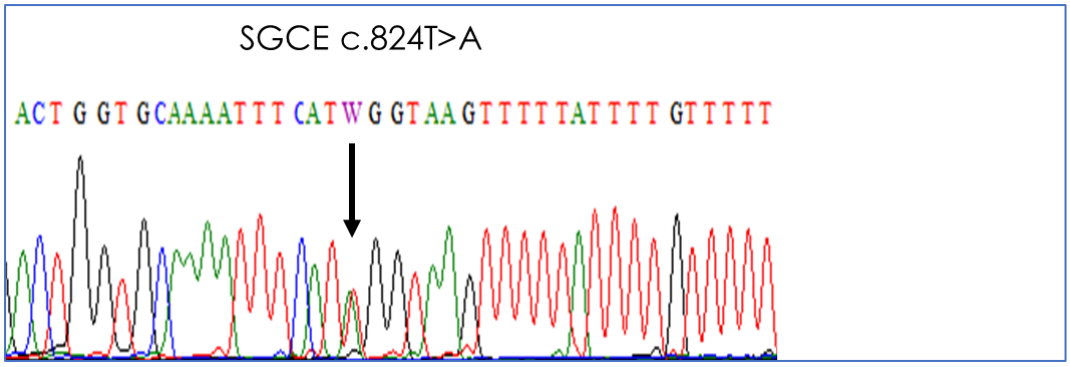


Figure 11: ***A.*** *Pedigree of the South African family with myoclonus-dystonia syndrome. The variant is present in three affected individuals (I-1, II-1 and II-2) and one possibly affected individual (III-2). The black arrow indicates the index patient. Squares indicate male individuals while circles are indicative of females.* ***B.*** *Sequencing chromatogram indicating the p.L275X mutation.*

Abbreviations: AAO, age at onset; L, Leucine; MDS, myoclonus-dystonia syndrome; NA, not applicable; WT, Wild-type; X, Stop

The outcome of DBS treatment is summarized in Table 10 and shown in video 1 and 2 (Supplementary data[37]). In patient II-2 the UMRS score improved by 52% at six weeks post-surgery, 56% after three months, and 58% after six months, still sustained at last follow up five years after surgery; the HADS was stable in the depression section but showed mild deterioration in the anxiety section. The pre- and postsurgical videos of patient II-1 (Supplementary data, video 2) show similar improvement of the movement disorder. Patient II:1 also exhibited sustained improvement in anxiety and OCD (Table 10).

Both patients showed significant improvement in dystonia at four years follow up; patient II-1 had 47% and patient II-2 66% improvement as summarized in Table 10. Both our patients remained on stable stimulation settings for the entire follow-up period (more than 4 years after surgery) using moderate-high pulse width (120 - 180us) and standard frequency (130Hz) stimulation (Table 10). No consistent or compared DBS stimulation data are reported in the literature and reported parameters vary in pulse width and frequencies chosen similar to the data available from dystonia studies.

Patient	Sex	Age at onset	Age at surgery	UMRS			UDRS			HADS			y-BOCS			DBS Setting	
				B	M	Y	B	M	Y	B	M	Y	B	M	Y	L	R
II-1	F	6	37	ND	28	ND	43	ND	14.5	34	8	8	36	3	5	3.2V	3.0V
																180us	180us
II-2	F	12	33	69	33	ND	29.5	ND	15.5	15	13	13	ND	ND	ND	3.5V	3.5V
																120us	120us

Table 10: Summary of DBS outcome measures in patients II-1 and II-2 at 6 months and 4 years follow up.

Abbreviations: UMRS – United Myoclonus Rating Scale; UDRS – United Dystonia Rating Scale; HADS – Hospital Anxiety and Depression Scale; Y-BOCS – Yale Brown Obsessive Compulsive Disorder Scale; V – volt; us – micro-seconds. DBS settings at last follow up in constant voltage mode, frequency in both patients set at 130Hz.

DBS has been shown to be effective in 22 publications including case reports and short case series involving 49 patients with MDS as reviewed by Rughani[109] and more recently by Smith[108]. Both reviews conclude that thalamic DBS is as effective as pallidal DBS in managing myoclonus, but that pallidal stimulation seems more effective in managing dystonia. Two more recent case series reports also showed similar outcomes targeting the pallidum[110] and the thalamus[111]. Nine patients showed sustained improvement in dystonia and myoclonus when targeting the pallidum with a mean of 8.7 year follow up, and in five cases targeting the thalamus with an average follow up of 50 months was equally effective. The number of cases reported with long-term outcomes is small and additional case reports remain valuable to contribute to the robustness of the data. Similar to what has been done for Tourette syndrome, an international database might be an excellent way to improve the quality and strength of the data and support DBS as a first line treatment modality in patients with MDS.

The first reported and most common mutation causing MDS was found in the sarcoglycan epsilon (*SGCE*) gene on chromosome 7[103]. Since then more than 100 different mutations were found with around 50% of patients harboring a *SGCE*-mutation[105]. The initial designation for this mutation was DYT-11 that was subsequently changed to DYT-*SGCE* with the acceptance of the new nomenclature[112]. Due to maternal imprinting reduced penetrance was evident with several unaffected individuals in large families with mutations[103,113,114]. Imprinting with silencing of the maternal allele by methylation of CpG dinucleotides within the promotor region of the gene cause reduced maternal expression. Sporadic cases were also shown to be from paternal expression of imprinted genes in affected individuals with no family history of MDS[104]. In 5% of cases incomplete imprinting was demonstrated with less severe symptoms in affected cases of maternal transmission[113]. Maternal uniparental disomy of chromosome 7 lead to Silver-Russel syndrome (short stature, cognitive impairment) and silencing of the *SGCE* gene but only single cases in the literature with both Silver-Russel syndrome and MDS are reported. Truncal involvement and myoclonus and dystonia in the same region seem to predict a negative *SGCE* status but in both patients II-1 and II-2 truncal involvement and myoclonus and dystonia in the cervical region was evident[106]. Patient III-2 developed writers' cramp with at last follow up no progression or myoclonus. This might be an epiphenomenon or indicate possible maternal inheritance with milder involvement. Further follow up and possible genetic analysis will be beneficial[115].

The clinical pattern of truncal dystonia and myoclonus in the same body region; and possible expression of maternal transmission emphasize the uniqueness of this mutation in the *SGCE* gene.

Patients with MDS can have a variety of psychiatric symptoms. Anxiety, depression and OCD are mostly reported. The response of psychiatric

symptoms in the reported cases seems variable. Contarino reports deterioration of affective symptoms in the Dutch cohort [116]. In the French case series [110] patients did not report significant anxiety or OCD before or after surgery but enhanced social adjustment was reported post-surgery, reflecting improved psycho-social skills. In our two cases affective mood symptoms remained mostly unchanged and OCD features improved in both cases. This improvement was sustained at follow up after four years. Multiple factors, including social adjustment and support, genetic mutations and specific psychiatric diagnosis might play an important prognostic role in the outcome of psychiatric symptoms after surgery.

Conclusion

We report a South African family with a novel mutation in *SGCE*, possible loss of maternal imprinting and successful long-term improvement of motor and obsessive-compulsive symptoms with DBS of the dorsolateral GPi. This report contributes to the existing but sparse data on genetic mutations and DBS treatment outcomes in MDS. It can be argued that earlier DBS surgery is indicated in patients with MDS to avoid unnecessary and ineffective pharmacological treatment and disability. The formation of an international database for DBS in MDS is suggested to facilitate accruement of clinical outcome data for regulatory use.

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Correspondence

A South African family with myoclonus-dystonia syndrome with a novel mutation in the SGCE gene responding to deep brain stimulation

ARTICLE INFO

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Myoclonus-dystonia syndrome (MDS), a childhood onset movement disorder, is characterised by axial and predominantly upper-body myoclonus with dystonia, and is often associated with disabling psychiatric symptoms [1]. Mutations in the epsilon-sarcoclygan gene (*SGCE, DYT-SGCE, DYT11*) is mostly described as the cause with additional methylation of the maternal carrying allele causing maternal imprinting; and subsequent expression of only the paternal allele [2]. Pharmacological treatment may be disappointing, although zonisamide may be efficacious (Level 1 evidence) and other drugs (valproate, levodopa, sodium oxybate) may be of benefit. Deep Brain Stimulation (DBS) of the Globus Pallidus interna and Ventral intermediate nucleus thalamus (Vim) is an alternative therapy with evidence in case studies and case-series showing efficacy and safety [3]. There are no published reports on South African patients with MDS. We identified a South African Afrikaner family with MDS and report on the findings of clinical and genetic studies performed on selected family members.

The index patient, a 40-year-old female (Fig. 1 (supplementary data), individual II-2), presented with severe treatment refractory axial myoclonus of the upper body, cranio-cervical dystonia and writer's cramp. The writer's cramp developed at the age of 12 and was followed by torticollis at 16 years old. Progressive myoclonus developed in the second decade of life. Action induced upper body myoclonus was prominent, triggered by walking, with impairment of activities of daily living. She had prominent anxiety, depressive mood and obsessive-compulsive disorder (OCD). The index patient's sister, a 43-year-old female (Fig. 1(supplementary data), individual II-1), had axial myoclonus from the age of 13; preceded by focal left leg dystonia and debilitating OCD. Patient II-I has two sons aged 20 and 17 (Fig. 1 (supplementary data)). The younger son (III-2) had been diagnosed with writer's cramp at the age of 8; without development of myoclonus or dystonia by 16 years old. At this stage it is uncertain whether he is affected. The older son (III-1) is treated for attention deficit disorder with co-morbid impulsivity and depressive mood. He has mild postural tremor. The father of the two affected sisters (Fig. 1 (supplementary data), individual I-1) had postural action induced arm myoclonus which was not disabling; and a history of depressive episodes.

Genetic screening of the family revealed a new variant in *SGCE*, a heterozygous c.824T > A substitution in exon 6 that leads to a premature stop codon in the protein at amino acid position 275 (p.L275X). All three affected individuals (I-1, II-1, II-2) as well as individual (III-2) are heterozygous carriers of p.L275X. This variant is absent from the ExAC database (http://exac.broadinstitute.org/) and, to our knowledge, has not previously been reported. The frequency of p.L275X was screened in 246 ethnically-matched controls and was not found. Additional support for the pathogenicity of this variant is provided by *in silico* prediction software Mutation-Taster (http://www.mutationtaster.org) that predicted the variant to be "disease-causing". Furthermore, the leucine residue at position 275 is evolutionarily-conserved and the mutation was predicted to have a very high impact with a Combined Annotation Dependent Depletion (CADD) score of 43 (Variant Effect Predictor, VEP; http://www.ensembl.org/Homo_sapiens/Tools/VEP).

DBS surgery was done in patient II-1 and II-2 targeting the posteroventral segment of the GPi. Videos of the patients showing them before, and four years after surgery are shown in the supplementary video segments 1 and 2. These were assessed by a blinded rater (JC). Significant improvement was observed at initial follow up six weeks after surgery and was sustained at four year follow up. Outcomes were gratifying with improvements in dystonia (Unified Dystonia Rating Scale) of 47% (II-1) and 66% (II-2), and improvement of myoclonus (Unified Myoclonus Rating Scale) of 58% (II-2) four years after surgery (Table 1). Regarding psychiatric symptoms, there was a significant improvement in OCD and moderate improvement of anxiety and depression in both individuals (Table 1). Both patients remained on stable stimulation settings for the entire follow-up period stimulating the two deepest contacts with moderate-high pulse width (120 - 180 μs) and standard frequency (130Hz). No surgical or stimulation-induced side-effects were reported.

Supplementary video related to this article can be found at https://doi.org/10.1016/j.parkreldis.2019.10.001.

Pre- and post DBS videos of individual II-1 (video 2) and II-2 (video 1). In both videos segment 1 shows myoclonus and dystonia before DBS surgery and segment 2 at last follow up, 4 years after surgery. In both videos head and cervical myoclonus is prominent (oscillatory myoclonus) and is completely suppressed after DBS.

Here, we report on an African Afrikaner family with a novel mutation in the *SGCE* gene causing MDS with long-term improvement of motor and obsessive-compulsive symptoms with DBS of the posteroventral GPi. These findings are in keeping with previous reports with

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Table 1
Summary of clinical outcomes before and after GPi Deep Brain Stimulation surgery.

Patient	Sex	Age at onset	Age at surgery	UMRS			UDRS			HADS			Y-BOCS			DBS Settings at last follow up[a]	
				B	M	Y	B	M	Y	B	M	Y	B	M	Y	Left GPi	Right GPI
II-2 [b]	F	12	33	69	33	ND	29.5	ND	15.5	15	13	13	ND			3.5V/120 μs	3.5V/120 μs
II-1	F	6	37	ND	28	ND	43	ND	14.5	34	8	8	36	3	5	3.2V/180 μs	3.0V/180 μs

Abbreviations: F: female; B: Score before surgery; M: Score six months after surgery; Y: Score at last follow up after surgery at 4 years; UMRS: United Myoclonus Rating Scale; UDRS: United Dystonia Rating Scale; HADS: Hospital Anxiety and Depression Scale; Y-BOCS: Yale Obsessive Compulsive Scale; ND: Not done.

[a] Both sides were programmed in monopolar with most ventral contact as the anode and case as the cathode. (0-/C+ and 8-/C+). Frequency in both cases set at 130Hz.

[b] Index case.

long-term follow up of GPi DBS in MDS [4]. Although previous reports indicated worsening of some psychiatric features [5], an improvement in social adjustment was reported, reflecting improved psycho-social skills, at longer than 5 years in a French cohort [4]. Multiple factors, including social support, genetic mutations and specific psychiatric diagnosis might play an important prognostic role in the outcome of psychiatric symptoms after surgery. We hypothesise that certain pre-surgical psychiatric co-morbidity, like OCD, might respond well to DBS and warrant further study. The outcome of DBS treatment in these patients support surgery as an early treatment option, contributes to the sparse data available on genetic causes and treatment options in MDS and argues for the establishment of an international register of pooled genetic and outcome data on MDS.

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Documentation of authors' roles

RvC and CS conceptualized the study. RvC was responsible for accumulation of data and write up of the manuscript, SB and AN were responsible for the genetic analysis and assisted with write up of the manuscript, JC performed blinded clinical assessments and provided advice on the manuscript and CS supervised the data accumulation and interpretation and was involved in the write up of the manuscript. All authors critically reviewed and approved the final version of the manuscript.

Ethics statement

The study was approved by the Clinical Ethics Committee University of Pretoria, number 36/2015. All patients signed written informed consent to participate in the study. Additional written consent was obtained from individual II-1 and II-2 for the online publication and dissemination of the video material; and is on file.

The authors confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this work is consistent with those guidelines.

Declaration of competing interest

RvC received honoraria for teaching from Medtronic and CiplaMedpro.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parkreldis.2019.10.001.

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Supplement 1

Paroxysmal Nonkinesigenic Dyskinesia: sporadic cases of a genetic condition with complex mixed phenomenology including myoclonus

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Successful Treatment of Disabling Paroxysmal Nonkinesigenic Dyskinesia with Deep Brain Stimulation of the Globus Pallidus Internus

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Paroxysmal Nonkinesigenic dyskinesia (PNKD) is a rare, often familial disorder that causes recurrent episodes of uni- or bilateral involuntary movements, which may include dystonia, myoclonus, chorea, ballism or a combination of these. The movements are precipitated by alcohol, caffeine, fatigue or emotional triggers, and most patients develop symptoms by early adolescence.

In this report we describe two patients with severe unilateral, sporadic, PNKD with a complex phenomenology characterised by dystonia combined with chorea and myoclonic-like jerks. The two patients were treated successfully with DBS of the internal segment of the Globus pallidus (GPi) with six months and six year follow up. These two cases were included in the overall report of familial myoclonic disorders to illustrate the complexity of the clinical phenomenology of movement disorders, including myoclonus, the importance of case series in rare disorders, and to emphasize that many genetic conditions do not necessarily readily show a positive family history in disorders with varying penetrance.

PNKD is classified as part of the paroxysmal dyskinesias and is rare, occurring at an estimated incidence of 1 in 1 million people[117] - significantly less frequent than the paroxysmal kinesigenic dyskinesias. As previously mentioned, the abnormal movements can be triggered by emotional stress, caffeine or alcohol consumption[118], but spontaneous episodes may also occur. The condition is often genetic with an autosomal dominant pattern of inheritance, but sporadic and idiopathic as well as secondary causes are well-described[119]. Dyskinetic episodes generally last several minutes and can occur several times a week. Genetic studies in familial forms of PNKD have shown at least two mutations in the myofibrillogenesis regulator 1 (*MR1*) gene where alanine is replaced with valine at either position 7 or 9[120]. These mutations alter the structure of the PNKD protein, probably causing a functional defect, but the

precise mechanism in the pathogenesis of familial PKND remains uncertain. Mutations in the *MR-1* gene have not yet been found in sporadic cases[121] and some families with no *MR-1* mutations have also been seen[122]. This is in contrast to findings in patients with paroxysmal kinesigenic dyskinesias (PKD), where the same mutation is often also detected in sporadic cases.

Treatment of PNKD is usually difficult and most patients do not respond well to any pharmacological treatment regimen. Familial cases have been shown to respond to clonazepam and other benzodiazepines[117] and some case studies of successful treatment with levetiracetam were reported[123]. Deep Brain Stimulation (DBS) of the internal segment of the Globus Pallidus is now an accepted treatment modality in primary dystonia, myoclonic dystonia and some specific forms of secondary dystonia[124,125]. Three case studies of successful treatment of PNKD with DBS were found in the literature: two publications describe stimulation of the internal part of the Globus pallidus[126,127] and in one, DBS of the thalamus was performed[128] (table 11).

Publication year	First author	Number of cases	Family history	*MR-1* genetics	Associated condition	DBS target	Outcome	Follow up durations
2001	Loher	1	No	Not done	Peripheral trauma	Vim-Th	Positive	4 years
2006	Yamada	1	No	Not done	Peripheral trauma	GPi	Positive	9 months
2010	Kaufman	1	No	Not done	Complex neurological disorder	GPi	Positive	18 months
2014	Van Coller	2	No	Not done	Peripheral trauma / Ehlers Danlos syndrome	GPi	Positive	6 months

Table 11: Summary of published cases of DBS in PNKD.

Abbreviations: Vim-Th - ventro-intermediate nucleus of thalamus; GPi - internal segment globus pallidum

Patients and Methods

Patient 1 was a 34-year-old male with a 12 year history of intermittent dystonic posturing of the right arm and leg (video - supplementary data in published article[34]; https://www.karger.com/Article/Abstract/365226; video stills in fig 12). Patient 1 had no reported family history.

Initially, the patient noticed abnormal posturing with writing and writer's cramp was considered as possible cause. In addition, an ulnar nerve entrapment was diagnosed and surgically treated. After this procedure, the patient noticed the development of abnormal and involuntary spasms of the right arm and eventually also of the right leg. The episodes usually lasted less than 10 minutes, but some episodes lasting hours and one-episode lasting days were also documented. These episodes occurred several times a week, but sometimes weeks would pass without an episode. Episodes could be triggered by emotional stress but not by exercise, caffeine intake or movement. Most episodes were spontaneous. After the onset of the dystonic movements, treatment for a depressive mood disorder was started with escitalopram. During attacks, dystonic posturing was found affecting the right leg, arm and face. Gait was severely involved and the arm was not functional during an episode. Examination between episodes was normal. Treatment with several drugs, including clonazepam, valproate, carbamazepine, biperiden and tetrabenazine, was ineffective. Investigations including electro-encephalogram and MRI of the brain were normal.

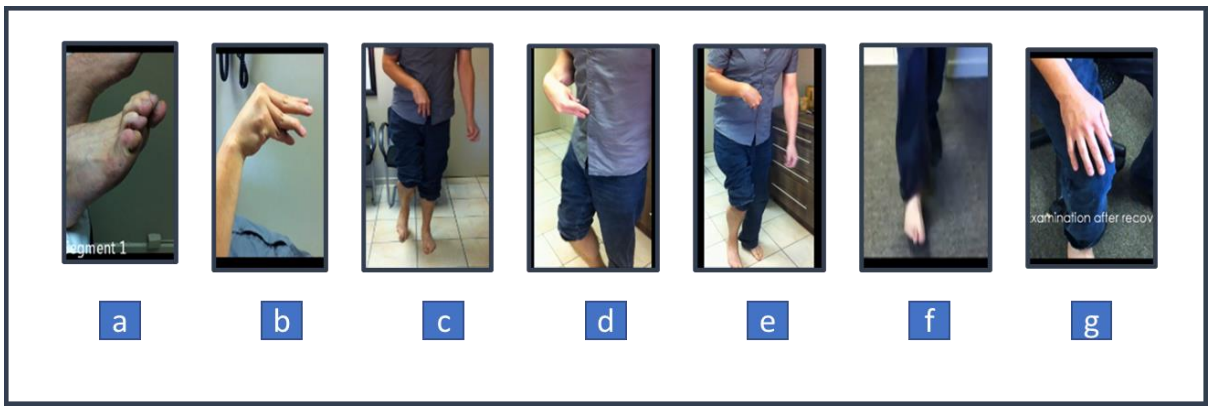

Figure 12: Stills from video (patient 1) showing acute onset of dystonia in the right foot (a), subsequent involvement of the hand (b) and then the right hemi-body (c,d,e) with spontaneous recovery (f,g).

Patient 2 was a 24-year-old male with a 5-year history of episodic painful spasms of the left side of the face, neck and arm. The episodes occurred three to four times a week and generally lasted for several minutes. Two episodes lasted longer than 12 hours and were only terminated with intravenous midazolam. Episodes were mostly spontaneous but could be triggered by emotional stress and caffeine intake. Exercise did not induce episodes. The movements were mostly sudden, dystonic with lateral posturing of the neck (laterocollis) and anterior displacement of the left shoulder and a combination of chorea, jerks and dystonic movements of the left arm.

In adolescence, the patient had been diagnosed with Ehlers-Danlos syndrome and had had bilateral arthrodesis of his shoulder joints to prevent repeated dislocations. He was treated for a depressive mood disorder with venlafaxine for the previous four years, but there was no history of neuroleptic drug use. As a teenager, he experimented with recreational drugs (cannabis and cocaine). He did not have a family history of neurological or neuromuscular disorders.

The episodic movements were mostly dystonic with laterocollis and anterior displacement of the left shoulder and dystonic tremor of the left arm. Gait was not affected during episodes. Electro-encephalogram and MRI of the brain were normal. Standard investigations to exclude other secondary causes for dystonia were negative. Treatment with botulinum toxin type A, biperiden, baclofen, valproate, carbamazepine, clonazepam and tetrabenazine was not effective in managing the frequency of the episodes. The intensity of the episodes was mildly reduced with clonazepam.

Deep Brain Stimulation surgery of the Globus Pallidus internus was performed in a one stage procedure in both patients. Micro-electrode recordings and standard macro-electrode stimulation were used to assist with optimal lead placement (3389, Medtronic)[129–131]. Post-operative CT/MRI merge confirmed placement of the leads in the postero-ventral GPi. Implantation of the Implantable Pulse Generator (IPG) (Activa PC, Medtronic) was then performed as part of the same procedure.

Non-stereotactic imaging was performed for both patients under general anaesthesia. Stereotactic planning software was used (Framelink version 5.2.4, Medtronic Inc, Minneapolis, MN, USA). Neuronavigation compatible sequences were acquired which were contiguous. T1 weighted 3D isotropic voxel (1 mm) sequences were acquired before and after double dose Gadolinium contrast. T2 sequences (2 mm) were acquired; coronal sequences were centred on the mid-commissural point (MCP). T2 axial sequences were inverted to enhance the visualization of the lentiform nucleus and the posterior limb of the internal capsule.

Direct targeting was used to identify the posterior limb of the internal capsule, internal medullary lamina (separating internal and external pallidum) and

optic tract[131] . At the intercommissural plane level the long axis of the GPi was drawn and this was the most medial margin of the GPi. This long axis of the GPi was parallel to the optic track, 4-6 mm inferior to the intercommissural plane. The long axis was divided into four quadrants. The centre of the third quadrant from anterior was chosen as the target. This was along a line drawn perpendicular to the long axis of the GPi and at least 2 mm away from the posterior limb of the internal capsule. Additionally, it was verified that the target was medial to the internal medullary lamina. This produced the lateral and anterior-posterior functional coordinates (with respect to MCP). The vertical functional coordinate was calculated by scrolling inferiorly in 1 mm steps from the intercommissural plane to the slice on which the optic tract was first visualized. Once the optic tract was identified, a vertical coordinate superior to this slice and just above the ambient cistern was selected and. a safe trajectory was planned. The functional plan was fused with the stereotactic post-contrast CT to transform functional coordinates to stereotactic coordinates. The Luminant universal localiser (Integralife Sciences Corporation, Burlington, Massachusetts, USA) was used in both patients.

Two channel simultaneous recording was performed in the central and anterior trajectory of the Ben's gun in the "+" orientation (Stardrive, FHC Inc, Bowdoin, Maine, USA). CRW-Precision stereotactic frame (Integralife Sciences Corporation, Burlington, Massachusetts, USA) with a phantom was used to perform the procedures. MER and MES (Leadpoint 5+3, Medtronic Inc, Minneapolis, USA) were used to physiologically confirm the anatomically determined target. It was ascertained by verifying on the stereotactic planning workstation that the medial and posterior trajectory would be very close to the posterior limb of the internal capsule and the lateral trajectory would be in the Globus pallidus externa or the internal medullary lamina. In the + configuration of the Ben's gun (Microdrive) each of the trajectories are offset by 2 mm centre to centre. MER was performed from 10 mm above the calculated target in steps of 0.5 mm. The pattern of MER was classified as silent (depicting laminae,

fluid filled spaces/peri-vascular spaces) or active (depicting GPe or GPi). In both patients the differences between GPe and GPi were not apparent, though this could be inferred with respect to reviewing the complete trace retrospectively. In all instances, the target was taken as the inferior-most limit and no MER/MES was performed beyond this limit. MES was performed at 120 µ seconds and 450 µ seconds within safe charge density limits up to supramaximal thresholds. Amplitudes of up to 5 mA were used at 120 µ seconds and up to 1.5 mA were used at 450 µseconds. Patients were asked to report phosphenes at target stimulation.

Patient 1

MER was performed with anterior and central micro-electrodes and recordings were obtained 4mm up to target on the right and 2mm up to target on the left.

MES was done with the test electrode (model 22670 FHC Inc, Bowdoin, Maine, USA) in position using the central electrode on both sides and stimulating with 1-5mA, 120 µ seconds and 130Hz on both sides. Stimulation was done at three levels above target with 2 mm intervals. Capsular side effects were recorded at supra-maximal stimulation, mostly consisting of pulling of the hand and foot at stimulation above 4.5mA. No side effects were observed at 1.5 mA on 450 µ seconds. No visual side effects were noted at target.

The micro/macro-stimulation electrodes were removed and permanent leads (3389 lead, Medtronic Inc, Minneapolis, USA) implanted in the central trajectory as directed by imaging, MER and MAS under fluoroscopy guidance in the lateral orientation using crosshairs.

Patient 2

The central and anterior electrodes were again used and positive activity recorded 2.5 mm above target on both sides. Macro-electrode stimulation was done in the same way as in patient 1. Capsular side effects (pulling of the thumb) were found with stimulation above 2.5 mA on the right and above 4.0 mA on the left (pulling of the hand) at 120 μ seconds. No side effects were observed at 1.5 mA on 450 μ seconds. No visual side effects indicating stimulation of the optic tract were found.

The permanent lead (3389 lead, Medtronic Inc, Minneapolis, USA) was implanted using the central MER guide under fluoroscopy guidance in the lateral orientation using crosshairs.

Post-operative imaging

An immediate post-operative audit was conducted on both patients using the Framelink planning software. A CT scan was acquired with the base ring of the stereotactic frame without the localiser. This was fused with the pre-operative plan and a detailed audit of the trajectory followed by the permanent lead in probes eye view was done. The depth at which the leads were implanted was evaluated in the trajectory view. Thereafter the blended exams were studied (80% of T2 weighted axial inverted MRI and 20% of CT). Windowing in the post-operative CT scan ensured that the artefact of the lead was reduced to a realistic geometry (actual lead diameter 1.27 mm; artefact about 1.5 mm). No pneumocephalus was observed in either patient. The lead location was studied with respect to the planned target depth and chosen trajectory and found to be satisfactory in both patients.

Programming of the IPG was undertaken the day after surgery in both patients. As both patients predominantly had symptoms unilaterally, only the

contralateral GPi was programmed. Standard programming procedures were used to identify optimal electrode selection and settings. Stimulation settings are summarized in Table 12.

At six-month follow-up, patient 1 reported complete resolution of symptoms with no further episodes of dystonia. All his oral medications had been discontinued. No stimulation related side effects were reported or found on examination and he was fully functional and able to return to work. Patient 2 had a weeklong episode of repeated short duration dystonic episodes of the left arm that settled with addition of a second electrode and then in month six also developed dystonic episodes involving the right arm that settled with additional stimulation of the left GPi: deepest electrode at minimal stimulation settings.

Follow up after six years showed continued benefit from stimulation in both patients. Patient 1 developed short-lived dystonic episodes related to emotional stress that occurred less than once per month. In year four he had 2 admissions to hospital with long lasting spasms needing intravenous treatment. This settled after adjustment of the DBS to dual monopolar stimulation. He remains off all pharmaceutical treatment. Patient 2 had an average of 12 dystonic days per month before surgery with emergency room admissions every week. After surgery he had no emergency room admissions and less than 2 dystonia days per month. Patient 2 developed intracranial sepsis four years after the original surgery and six months after pulse generator replacement, necessitating the removal of the entire system for three months. During this period, he had daily dystonic episodes and frequent admissions to hospital for intravenous treatment. After replacement of the system and programming of bilateral interleaving settings to avoid capsular spread of current he has not had any further hospital admissions.

Discussion

PNKD is a movement disorder characterized by episodic, sudden, unprovoked episodes of abnormal dystonic or choreiform movements causing varying degrees of disability. Treatment is generally difficult and most patients have to adjust their lives according to the disability.

Genetic mutations in *MR-1* have been described in several families with PNKD with high clinical penetrance of 98%. Patients with the mutation seem to have a different clinical and treatment course[118]. Patients with *MR-1* mutations have a young age at onset, median 4.0 +/- 4.6 years compared to non-mutation carriers 12.3 +/- 10.8 years; with frequent dystonic episodes more than once per week and they are more likely to develop attacks when exposed to emotional stress or fatigue, alcohol and caffeine. Due to logistic limitations, we were unable to perform genetic analysis in our patients. Patient 2 has a clinical diagnosis of Ehlers-Danlos syndrome (EDS), classified as type IV. Interestingly, Type IV or vascular EDS is often associated with mutations in *COL3A1* situated on chromosome 2q32.2 (OMIM 120180; www.omim.org) in close proximity to *MR-1* which is situated on chromosome 2q35 (OMIM 609023).

The use of DBS in dystonia has made a significant difference in the treatment and disability of patients with other forms of dystonia. Although most large studies showed efficacy in patients with primary dystonia, some evidence for the use of DBS in dystonia-plus syndromes are emerging. In myoclonus-dystonia, DBS has been shown to be effective and safe in treating the movement disorder[132] but not in improving the psychiatric co-morbidity[116].

The use of deep brain stimulation in PNKD has been reported in three patients since 2001. Loher[128] described chronic thalamic stimulation in a patient who was severely affected with PNKD. Unilateral stimulation of the contralateral thalamic ventral intermediate (Vim) nucleus improved the movement disorder, with continued success over a period of at least four years. Dystonic episodes recurred when the device was accidentally switched off. Long term follow up was reported after 9 years and showed mild loss of stimulation effect. The effect was regained when the target was changed to the GPi[133]. Two other case reports described DBS of the Globus pallidus internus. Yamada[126] presented a case of unilateral post traumatic PNKD with complete suppression of abnormal movements after implantation of GPi DBS, and Kaufmann[127] reported a difficult case with a generalized movement disorder with superimposed bilateral PNKD which, although with atypical elements, showed a significant reduction in the frequency and intensity of the episodic dystonic episodes (table 12).

Conclusion

We present two patients with PNKD in whom the episodic severe involuntary movements resulted in significant disability and loss of employment. All treatment options had failed. DBS was then performed with targeting of the GPi using standard procedures as for dystonia. Both patients responded extremely well with low stimulation parameters and no stimulation side effects. One patient had complete resolution of symptoms and although the second patient initially had some further episodes, these also settled with adjustment of stimulation. Both patients were functional and able to return to work.

Patients with dystonia who are treated with DBS may take several weeks to show improvement, but in patients with levodopa induced dyskinesia and phasic dystonia, GPi DBS causes rapid reduction in abnormal movements[134].

Our patients showed immediate benefit post DBS and although the follow up time might still be short in the original publication, current follow up after 6 years showed sustained improvement with suppression of involuntary movements in these two cases, reflecting long-term improvement brought about by pallidal stimulation. The remarkable feature of reversibility in DBS allows for blinded assessments: patients can participate in a blinded study in both roles as active and placebo participant when switching the stimulation off. Unfortunately, neither patient was willing to participate in a blinded evaluation since the response to DBS had been so impressive.

Although PNKD is often a familial condition, sporadic cases occur, raising the question of reduced penetrance and asymptomatic individuals in this condition, as has been described previously[118,121]. Thus, it remains possible that our patients are part of families with a gene defect that unfortunately has not been identified.

In rare medical conditions it may be difficult to find enough patients to perform controlled trials for the assessment of the efficacy of a treatment modality. In these conditions, case reports and small case series assume greater importance. We propose that GPi DBS is a possible treatment option in patients with disabling PNKD not responding to standard medical treatment. Further evaluation with larger numbers of patients and blinded evaluation will be necessary to support this statement. In addition, standardized criteria for the diagnosis of PNKD need to be agreed upon[119] to avoid overlap with other movement disorders.

Patient	Follow up	Side programmed	Electrode configuration	Amplitude	Pulse width	Frequency
Patient 1	3 months	Left GPi	0 -, C+	2.0V	120us	130Hz

	6 months	Left GPi	0-, C+	2.0V	120us	130Hz
	6 years	Left GPi	0-1-; C+	3.6V	90us	120Hz
Patient 2	3 months	Right GPi	8-, C+	1.5V	120us	130Hz
	6 months	Right GPi	8-, C+	2.5V	120us	125Hz
			9-, C+	2.0V	120us	
		Left GPi	0-/C+	1.5V	120us	125Hz
	6 years	Right GPi	8-, C+	2.3V	120us	120Hz
			9-; C+	1.5V	120Us	
		Left GPi	0-; C+	2.5V	120us	120Hz
			1-:C+	2.0V	120us	

Table 12: ***Stimulation parameters of two patients with PNKD at first programming and at 12 week follow up.***

	Patient 1: left GPi	Patient 1: right GPi	Patient 2: left GPi	Patient 2: right GPi
Lateral	-21.3	+18.3	-25.7	+17.5
AP	-6.2	-0.4	-3.4	-0.7
Vertical	-23.2	-22.0	-13.7	-12.2

Table 13: ***Stereotactic coordinates for DBS surgery of the GPi in two patients with PNKD.***

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Case Report

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Successful Treatment of Disabling Paroxysmal Nonkinesigenic Dyskinesia with Deep Brain Stimulation of the Globus Pallidus Internus

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Key Words
Paroxysmal nonkinesigenic dyskinesia · Paroxysmal dyskinesia · Deep brain stimulation · Globus pallidus internus

Abstract
Paroxysmal nonkinesigenic dyskinesia (PNKD) causes episodes of treatment-resistant involuntary movements. Previous case reports showed effective treatment of PNKD with deep brain stimulation (DBS). We report 2 patients in whom DBS was highly successful when other treatment modalities had failed. ***Methods:*** Two patients aged 34 and 24 years with a longstanding history of PNKD were treated with globus pallidus internus (GPi) DBS. Motor effects were monitored and followed up postoperatively and again at 6 months after surgery. ***Results:*** Both patients responded very well to GPi DBS with complete suppression of dyskinesia after surgery in 1 patient and in the second after an additional adjustment of stimulation. ***Conclusion:*** GPi DBS might be an effective alternative treatment modality for PNKD.

Paroxysmal nonkinesigenic dyskinesia (PNKD) causes episodes of uni- or bilateral involuntary movements, which may include dystonia, chorea, ballism or a combination of these [1]. The disorder is classified as part of the paroxysmal dyskinesias and is rare, occurring at an estimated incidence of 1 in 1 million people [2] – significantly less frequent than the paroxysmal kinesigenic dyskinesias. The abnormal movements can be triggered by emotional stress, caffeine or alcohol consumption [3] or can occur spontaneously. Although mostly sporadic and idiopathic, familial and secondary causes have been described [4]. Dyskinetic episodes generally last several minutes and can occur several times a week. Genetic studies in familial forms of PNKD have shown at least two mutations in the myofibrillogenesis regulator 1 (MR-1) gene where alanine is replaced with valine at either position 7 or 9 [5]. These mutations alter the structure of the PNKD protein, probably causing a functional defect, but the precise mechanism in the pathogenesis of familial PNKD remains uncertain. Mutations in the MR-1 gene have not yet been found in sporadic cases [6] and some families with no MR-1 mutations have also been seen [7].

Treatment of PNKD is usually difficult and most patients do not respond well to any pharmacological treatment regimen. Familial cases have been shown to respond to clonazepam and other benzodiazepines [2] and some case studies of successful treatment with levetiracetam were reported [8]. Deep brain stimulation (DBS) of the internal segment of the globus pallidus is now an accepted treatment modality in primary dystonia, myoclonic

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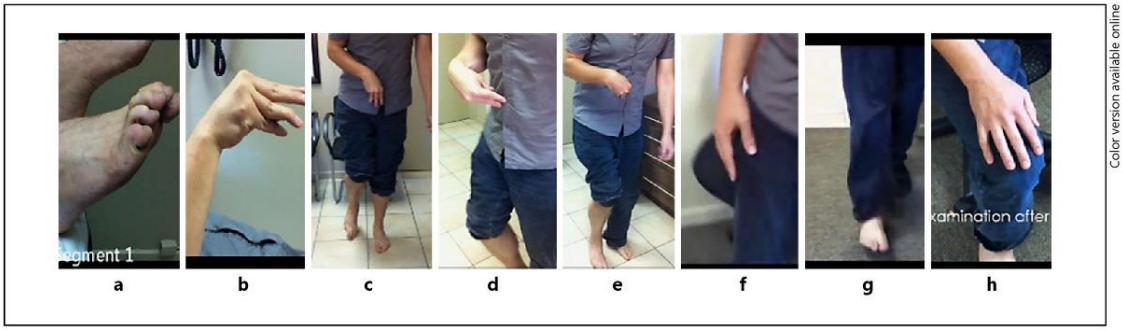

Color version available online

Fig. 1. Stills from video indicating onset of paroxysmal hemidystonia in the right foot (**a**), involvement of the hand (**b**) and the whole right side of the body (**c–e**). Note the normal hand (**f**, **h**) and foot (**g**) position after spontaneous recovery.

dystonia and some specific forms of secondary dystonia [9, 10]. Three case studies of successful treatment of PNKD with DBS were found in the literature: 2 publications describe stimulation of the internal part of the globus pallidus [11, 12] and in 1, DBS of the thalamus was performed [13].

In this report, we describe 2 patients with unilateral PNKD who were treated successfully with DBS of the internal segment of the globus pallidus (globus pallidus internus; GPi) with 6 months of follow-up.

Patients and Methods

Patient 1 was a 34-year-old male with a 12-year history of intermittent dystonic posturing of the right arm and leg (fig. 1). Initially, he noticed abnormal posturing with writing and writer's cramp was considered as possible cause. In addition, an ulnar nerve entrapment was diagnosed and surgically treated. After this procedure, the patient noticed the development of abnormal and involuntary spasms of the right arm and eventually also of the right leg. The episodes usually lasted less than 10 min, but some episodes lasting hours and 1 episode lasting days were also documented. These episodes occurred several times a week, but sometimes weeks would pass without an episode. Episodes could be triggered by emotional stress but not by exercise, caffeine intake or movement. Most episodes were spontaneous. After the onset of the dystonic movements, treatment for a depressive mood disorder was started with escitalopram. During attacks, dystonic posturing was found affecting the right leg, arm and face (online suppl. video 1; for all online suppl. material, see www.karger.com/doi/10.1159/000365226). Gait was severely involved and the arm was not functional during an episode (online suppl. video 2). Examination between episodes was normal (online suppl. video 3). Treatment with several drugs, including clonazepam, valproate, carbamazepine, biperiden and tetrabenazine, was ineffective. Investigations including electroencephalogram and magnetic resonance imaging (MRI) of the brain were normal.

Patient 2 was a 24-year-old male with a 5-year history of episodic painful spasms of the left side of the face, neck and arm. The episodes occurred 3–4 times a week and generally lasted for several minutes. Two episodes lasted longer than 12 h and were only terminated with intravenous midazolam. Episodes were mostly spontaneous but could be triggered by emotional stress and caffeine intake. Exercise did not induce episodes. The movements were mostly sudden, dystonic with laterocollis posturing of the neck and anterior displacement of his left shoulder and a combination of chorea and dystonic movements of the left arm.

In adolescence, the patient had been diagnosed with Ehlers-Danlos syndrome (type VII-D) and had had bilateral arthrodesis of his shoulder joints to prevent repeated dislocations. He had been treated for a depressive mood disorder with venlafaxine for the last 4 years, but there was no history of neuroleptic drug use. As a teenager, he experimented with recreational drugs (cannabis and cocaine). The episodic movements were mostly dystonic with laterocollis and anterior displacement of the left shoulder and dystonic tremor of the left arm. Gait was not affected during episodes. Electroencephalogram and MRI of the brain were normal. Standard investigations to exclude other secondary causes for dystonia were negative. Treatment with botulinum toxin type A, biperiden, baclofen, valproate, carbamazepine, clonazepam and tetrabenazine was not effective in managing the frequency of the episodes. The intensity of the episodes was mildly reduced with clonazepam.

DBS surgery of the GPi was performed in a one-stage procedure in both patients. Microelectrode recordings and standard macroelectrode stimulation were used to assist with optimal lead placement (model 3389, Medtronic Inc., Minneapolis, Minn., USA) [14, 15]. Postoperative computed tomography (CT)/MRI merge confirmed placement of the leads in the posteroventral GPi. Implantation of the implantable pulse generator (Activa PC, Medtronic Inc.) was then performed as part of the same procedure.

Nonstereotactic imaging was performed for both patients under general anesthesia. Stereotactic planning software was used (Framelink version 5.2.4, Medtronic Inc.) [16]. Neuronavigation-compatible sequences were acquired which were contiguous. T1-

weighted 3D isotropic voxel (1 mm) sequences were acquired with and without double-dose contrast. T2 sequences (2 mm) were acquired; coronal sequences were centered on the mid-commissural point. T2 axial sequences were inverted to enhance the visualization of the lentiform nucleus and the posterior limb of the internal capsule.

Direct targeting was used to identify the posterior limb of the internal capsule, internal medullary lamina (separating internal and external pallidum) and optic tract [17]. At the intercommissural plane level, the long axis of the GPi was drawn and this was the most medial margin of the GPi. This long axis of the GPi was parallel to the optic track, 4–6 mm inferior to the intercommissural plane. The long axis was divided into 4 quadrants. The center of the third quadrant from anterior was chosen as the target. This was along a line drawn perpendicular to the long axis of the GPi and at least 2 mm away from the posterior limb of the internal capsule. Additionally, it was verified that the target was medial to the internal medullary lamina. This gave us the lateral and anterior-posterior functional coordinates (with respect to the mid-commissural point). The vertical functional coordinate was calculated by scrolling inferiorly in 1-mm steps from the intercommissural plane to the slice on which the optic track was first visualized. Once the optic track was identified, we selected a vertical coordinate superior to this slice and just above the ambient cistern (table 2). A safe trajectory was planned. The functional plan was fused with the stereotactic post-contrast CT to transform functional coordinates to stereotactic coordinates. The Luminant universal localizer (Integra LifeSciences Corporation, Burlington, Mass., USA) was used in both patients.

Two-channel simultaneous recording was performed in the central and anterior trajectory of the Ben's gun in the '+' orientation (STar Drive, FHC Inc., Bowdoin, Me., USA). Cosman-Roberts-Wells (CRW) Precision stereotactic frame (Integra LifeSciences Corporation) with a phantom was used to perform the procedures. Microelectrode recording (MER) and macroelectrode stimulation (MES; Leadpoint 5 + 3, Medtronic Inc.) were used to physiologically confirm the anatomically determined target. It was ascertained by verifying on the stereotactic planning workstation that the medial and posterior trajectory would be very close to the posterior limb of the internal capsule and the lateral trajectory would be in the globus pallidus externus (GPe) or the internal medullary lamina. In the '+' configuration of the Ben's gun (Microdrive), each of the trajectories are offset by 2 mm center to center. MER was performed from 10 mm above the calculated target in steps of 0.5 mm. The pattern of MER was classified as silent (depicting laminae, fluid-filled spaces/perivascular spaces) or active (depicting GPe or GPi). In both patients, the differences between GPe and GPi were not apparent, though this could be inferred with respect to reviewing the complete trace retrospectively. In all instances, the target was taken as the inferior-most limit and no MER/MES was performed beyond this limit. MES was performed at 120 and 450 μs within safe charge density limits up to supramaximal thresholds. Amplitudes of up to 5 mA were used at 120 μs and up to 1.5 mA at 450 μs. Patients were asked to report phosphenes at target stimulation.

Patient 1

MER was performed with anterior and central microelectrodes and recordings were obtained 4 mm up to target on the right and 2 mm up to target on the left.

MES was done with the test electrode (model 22670, FHC Inc.) in position using the central electrode on both sides and stimulating with 1–5 mA, 120 μs and 130 Hz on both sides. Stimulation was done at 3 levels above target with 2-mm intervals. Capsular side effects were recorded at supramaximal stimulation, mostly consisting of pulling of the hand and foot at stimulation above 4.5 mA. No side effects were observed at 1.5 mA on 450 μs. No visual side effects were noted at target.

The micro-/macrostimulation electrodes were removed and permanent leads (3389 lead, Medtronic Inc.) implanted in the central trajectory as directed by imaging. MER and MES were performed under fluoroscopy guidance in the lateral orientation using crosshairs.

Patient 2

The central and anterior electrodes were again used and positive activity recorded 2.5 mm above target on both sides. Macroelectrode stimulation was done in the same way as in patient 1. Capsular side effects (pulling of the thumb) were found with stimulation above 2.5 mA on the right and above 4.0 mA on the left (pulling of the hand) at 120 μs. No side effects were observed at 1.5 mA on 450 μs. No visual side effects indicating stimulation of the optic tract were found.

The permanent lead (3389 lead, Medtronic Inc.) was implanted using the central MER guide under fluoroscopy guidance in the lateral orientation using crosshairs.

Postoperative Imaging

An immediate postoperative audit was conducted on both patients using the Framelink planning software. A CT scan was acquired with the base ring of the stereotactic frame without the localizer. This was fused with the preoperative plan and a detailed audit of the trajectory followed by the permanent lead in probe's eye view was done. The depth at which the leads were implanted was evaluated in the trajectory view. Thereafter, the blended exams were studied (80% of T2-weighted axial inverted MRI and 20% of CT). Windowing in the postoperative CT scan ensured that the artefact of the lead was reduced to a realistic geometry (actual lead diameter 1.27 mm; artefact about 1.5 mm). No pneumocephalus was observed in either patient. The lead location was studied with respect to the planned target depth and chosen trajectory and found to be satisfactory in both patients.

Programming of the implantable pulse generator was undertaken the day after surgery in both patients. As both patients predominantly had symptoms unilaterally, only the contralateral GPi was programmed. Standard programming procedures were used to identify optimal electrode selection and settings. Settings are summarized in table 1.

At 6-month follow-up, patient 1 reported complete resolution of symptoms with no further episodes of dystonia. All his oral medications had been discontinued. No stimulation-related side effects were reported or found on examination and he was fully functional and able to return to work. Patient 2 had a week-long episode of repeated short-duration dystonic episodes of the left arm that settled with addition of a second electrode and then, in month 6, also developed dystonic episodes involving the right arm that settled with additional stimulation of the left GPi (refer to table 1 for programming settings).

Table 1. Stimulation parameters at first programming and 12-week follow-up

Patient	Follow-up, months	Side programmed	Electrode configuration (Activa PC, Medtronic)	Volt	Pulse width, μs	Frequency, Hz
Patient 1	3	left GPi	monopolar 0–, case+	2.0	120	130
	6	left GPi	monopolar 0–, case+	2.0	120	130
Patient 2	3	right GPi	monopolar 8–, case+	1.5	120	130
	6	right GPi	interleaving monopolar 8–, case+	2.5	120	125
		right GPi	interleaving monopolar 9–, case+	1.5	120	125
		left GPi	monopolar 0–/C+	1.5	120	125

Discussion

PNKD is a movement disorder characterized by episodic, sudden, unprovoked episodes of abnormal dystonic or choreiform movements causing varying degrees of disability. Treatment is generally difficult and most patients have to adjust their lives according to the disability. Genetic mutations in MR-1 have been described and patients with the mutation seem to have a different clinical and treatment course [3]. Due to logistic limitations, we did not do genetic analysis in our patients.

The use of DBS in dystonia has made a significant difference in the treatment and disability of patients with other forms of dystonia. Although most large studies showed efficacy in patients with primary dystonia, some evidence for dystonia-plus syndromes are emerging. In myoclonus dystonia, DBS has been shown to be effective and safe in treating the movement disorder [18] but not in improving the psychiatric comorbidity [19].

The use of DBS in PNKD has been reported in 3 patients since 2001. Loher et al. [13] described chronic thalamic stimulation in a patient who was severely affected with PNKD. Unilateral stimulation of the contralateral thalamic ventral intermediate nucleus improved the movement disorder, with continued success over a period of at least 4 years. Dystonic episodes recurred when the device was accidentally switched off. Long-term follow-up was reported after 9 years and showed mild loss of stimulation effect. The effect was regained when the target was changed to the GPi [20]. Two other case reports described DBS of the GPi [11, 12]. Yamada et al. [11] presented a case of unilateral posttraumatic PNKD with complete suppression of abnormal movements after implantation of GPi DBS, and Kaufman et al. [12] reported a difficult case with a generalized movement disorder with superimposed bilateral PNKD which, although with atypical elements, showed a significant reduction in the frequency and intensity of the episodic dystonic episodes.

Table 2. Stereotactic coordinates

	Patient 1		Patient 2	
	left GPi	right GPi	left GPi	right GPi
Lateral	–21.3	+18.3	–25.7	+17.5
AP	–6.2	–0.4	–3.4	–0.7
Vertical	–23.2	–22.0	–13.7	–12.2

AP = Anterior-Posterior.

We present 2 patients with PNKD in whom the dystonic episodes resulted in significant disability and loss of employment. All treatment options had failed. DBS was then performed with targeting of the GPi using standard procedures as for dystonia. Both patients responded extremely well with low stimulation parameters and no stimulation side effects. One patient had complete resolution of symptoms and although the second patient had some further dystonia, this also settled with adjustment of stimulation. Both patients were functional and able to return to work.

Patients with dystonia who are treated with DBS may take several weeks to show improvement, but in patients with levodopa-induced dyskinesia and phasic dystonia, GPi DBS causes rapid reduction in abnormal movements [21]. Our patients showed immediate benefit after DBS and although the follow-up time might still be short in these 2 cases, the effect of microlesioning and placebo should have worn off, thus reflecting actual improvement brought about by stimulation. The remarkable feature of reversibility in DBS allows for blinded assessments: patients can participate in a blinded study in both roles as active and placebo participant. Unfortunately, neither

patient was willing to participate in a blinded evaluation since the response to DBS had been so impressive.

In rare medical conditions, it may be difficult to find enough patients to perform controlled trials for the assessment of the efficacy of a treatment modality. In these conditions, case reports and small case series assume greater importance. We propose that GPi DBS is a possible treatment option in patients with disabling PNKD not responding to standard medical treatment. Further evaluation with larger numbers of patients and blinded evaluation will be necessary to support this statement. In addition, standardized criteria for the diagnosis of PNKD need to be agreed upon [22] to avoid overlap with other movement disorders.

Disclosure Statement

R.v.C. received a travelling grant in the last year from Medtronic Inc.

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Supplement 2

Statistical analysis comparing age, severity of tremor and myoclonus to activities of daily living in patients with Familial Cortical Myoclonic Tremor with Epilepsy from South Africa

SUMMARY OUTPUT: **Activity of daily living vs. Tremor**

Regression Statistics	
Multiple R	83.07%
R Square	69.00%
Adjusted R Square	62.80%
Standard Error	4.067
Observations	7

ANOVA

	Df	*SS*	*MS*	*F*	*Significance F*
Regression	1	184.14	######	11.13	0.02
Residual	5	82.72	16.54		
Total	6	266.86			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-10.850	6.186	-1.754	0.140	-26.752	5.053
ln(Tremor)	10.021	3.004	3.336	0.021	2.300	17.743

SUMMARY OUTPUT: **Myoclonus rating scale vs. Tremor**

Regression Statistics	
Multiple R	96.10%
R Square	92.36%
90.83%	
Standard Error	0.247
Observations	7

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	3.69	3.69	60.44	0.00
Residual	5	0.31	0.06		

Total	6	4.00				
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-0.832	0.376	-2.212	0.078	-1.798	0.135
ln(Tremor)	1.419	0.183	7.774	0.001	0.950	1.889

SUMMARY OUTPUT: **Myoclonus rating scale vs. Age**

Regression Statistics	
Multiple R	78.62%
R Square	61.82%
Adjusted R Square	54.18%
Standard Error	0.553
Observations	7

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	2.47	2.47	8.10	0.04
Residual	5	1.53	0.31		
Total	6	4.00			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	0.655	0.517	1.266	0.261	-0.674	1.983
Age	0.036	0.013	2.845	0.036	0.004	0.069

Conclusion and Future directions

Myoclonus is a complex disorder of rapid repetitive muscle jerks that can occur in proximal or distal appendicular or axial muscles. It can be of cortical, sub-cortical or spinal cord origin; part of progressive and severely disabling epilepsy syndromes, basal ganglia conditions, benign and physiological or even functional (psychogenic)[1].

Cortical myoclonic tremor (CMT) is a unique form of cortical myoclonus that appears rhythmical and is often confused with Essential tremor. Delayed diagnosis of CMT lead to mismanagement and defers the opportunity to prevent seizures and pharmacological complications. Although CMT can occur in a number of rare conditions it is phenotypically associated with Familial Cortical Myoclonic Tremor with Epilepsy (FCMTE) (Familial Adult Myoclonic Epilepsy(FAME)/Benign Adult Familial Myoclonic Epilepsy (BAFME)), a condition that bridges the fields of movement disorders and epilepsy[48]. FCMTE is a true 21st century condition that was first described in the 1990's in Japan and eventually classified as a single clinical entity in 2005[10]. The large five generation South African family that is described here was already identified in 1977 with postural tremor-like movements, myoclonus and epilepsy with an autosomal dominant inheritance pattern[35]. The long-term follow up data of this unique family confirms the nature of the phenotype – benign course over 30 years with increasing myoclonus and cortical myoclonic tremor and with rare generalised tonic-clonic seizures and no cognitive or motor disability.

The discovery of pentanucleotide repeat expansions in the introns of different genes in families with a similar phenotype from different geographical regions is unique. Pentanucleotide ATTTC/ATTCA expansion insertion mutations were

found in this South African family in the intronic region of the *STARD7* gene with no abnormality detected in the gene product, implicating RNA toxicity as a possible pathophysiological cause[36].

Finding novel pathological mutations in patients from different geographical areas, in known genes, is an important tool to expand diagnostic testing and improving phenotype-genotype correlation. The finding of a novel mutation: heterozygous c.824T>A substitution in exon 6 of the epsilon-sarcoclygan (*SGCE*) gene in a South African family with myoclonus dystonia syndrome (MDS) (*DYT-SGCE; DYT-11; OMIM 604149*) is therefor an important contribution to the genotype-phenotype profile of DYT-*SGCE*[37]. The phenotype of MDS in this family compares well with described families with adolescent onset focal dystonia and development of severe and disabling axial and appendicular myoclonus with accompanied obsessive-compulsive disorder and anxiety. In this family co-occurrence of marked truncal features and dystonia and myoclonus in the same body regions were remarkable[135].

MDS is known from small patient series studies and case reports to respond well to GPi and thalamic DBS[136]. Small studies and single case reports with detailed clinical information and outcome data make up an important part of the knowledge about diagnosis and treatment of many rare neurological disorders[108]. This is demonstrated by the long-term data in this study that shows exceptional motor and non-motor outcomes after GPi DBS in two family members of this family and paths the way for early treatment in other affected family members and patients with DYT-*SGCE* in South Africa.

The study of n=1 has led to important discoveries in neurology in the past: using amantadine in Parkinson's disease, thalamotomy for tremor and possibly even the original description of Wilson's disease[44]. In very rare disorders case reports

and case series are best available evidence and treatment decisions are often based on these. In Paroxysmal Non-Kinesigenic Dyskinesia (PNKD) this is demonstrated well with the acceptance of DBS as an early treatment modality in this rare and very disabling condition[137]. The two South African patients with a sporadic form of this disorder, described here, showed the typical phenotype of disabling episodic dystonia, myoclonus and chorea often triggered by caffeine and emotional stress and lasting for hours and sometimes days[34]. Both patients were treated with pallidal DBS with sustained suppression of dystonic episodes and no adverse events after six years' follow up. The PNKD or *MR-1* (*PNKD-MR-1; OMIM 118800*) mutation is the only common genetic mutation described in patients with familial PNKD, although in many patients with the typical phenotype this mutation is absent[121]. No data is yet available whether PNKD patients with the *MR-1* mutation have an improved outcome with DBS when compared to non-mutation carriers.

The internal segment of the globus pallidum is the most common target in MDS, and was also the target in the described South African cases[110]. There are however case reports and series targeting the thalamus with significant myoclonus improvement but less robust dystonia control[109,111]. DBS of the posterior sub-thalamic area was shown to be effective in reducing myoclonus and improvement of ataxia in South African patients with North Sea Progressive Myoclonic Epilepsy[40]. The subthalamic nucleus and substantia nigra was also successful in myoclonus improvement in two case studies[138]. DBS of the lateral thalamic nucleus – ventral intermediate nucleus (Vim) – is the preferred target in tremor disorders with long-term evidence of effective tremor control in different conditions causing tremor[139]. The most promising target for DBS in epilepsy is the anterior thalamus that might also be an effective target for DBS in myoclonus[140]. DBS is hypothesised as a possible treatment option for patients with treatment refractory myoclonus in PME and possibly FCMTE. Further study to explore this treatment option and optimal brain target is necessary.

The complexity of the pathophysiology in FCMTE with neurophysiological evidence of cortical origin and radiological and pathological evidence of cerebellar involvement also need further investigation[74]. Sensing-DBS is a new development in DBS treatment that offers the clinician the opportunity to record local field potentials in the area where the DBS lead is situated. It offers the future possibility of automatic response from the DBS device to specific neurophysiological brain activity[141]. This technology offers a unique opportunity to investigate local field potentials in the thalamus and other brain targets in real-time and possibly shed light on the pathophysiology of the network involved in FCMTE and myoclonic disorders[142].

Precision diagnosis in rare and complex neurological conditions is challenging but vital to avoid unnecessary diagnostic tests and inappropriate treatment. The development of accessible diagnostic genetic studies with Next Generation Sequencing (NGS) techniques have made this an achievable goal[14]. The downside to this development is the conflict created in phenotype-genotype matching and judging the pathogenicity of newly found mutations. Accurate clinical analysis of phenotype and genotype, with the inclusion of patients from different geographical areas and ethnic backgrounds have therefore become imperative.

Next Generation Sequencing offer a unique opportunity to perform genetic testing of the whole genome or large parts of the genome in a shorter time and reduced cost than previous linkage and other sequencing techniques[1,14,77,143]. Still, investment in new technology is costly and a low priority in countries facing large scale medical and socio-economical challenges[16]. International collaboration and local continental networks

should be better utilised to investigate rare conditions. Narrowing the differential diagnosis of rare conditions like CMT, PME and dystonia will ultimately lead to time and cost saving, not only, in an era of precision and personalised medicine.

Movement disorders and complex epilepsy syndromes often involve central nervous system networks as opposed to single pathological areas of the brain[144]. Study of the phenotype and genotype of these conditions in patients from different ethnical and geographical regions form an essential element in the understanding of these rare conditions. The phenotype-genotype and treatment descriptions of these South African families with rare and unique movement disorders therefor form an important part of global knowledge and contribute to further cost-effective treatment in South Africa and internationally; with improved understanding of the pathological brain networks involved in these complex disorders. Further study of these conditions is necessary in the South African, and rest of the developing world, context to advance focussed and effective individualised patient care.

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Faculty of Health Sciences

Institution: The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.

- FWA 00002567, Approved dd 22 May 2002 and Expires 03/20/2022.
- IORG #: IORG0001762 OMB No. 0990-0279 Approved for use through February 28, 2022 and Expires: 03/04/2023.

22 January 2021

Approval Certificate
Amendment

Ethics Reference No.: 152/2011
Title: A clinical, neurophysiological and genetic study of South African familial combined myoclonic syndromes.

Dear Dr R van Coller

The **Amendment** as supported by documents received between 2021-01-06 and 2021-01-20 for your research, was approved by the Faculty of Health Sciences Research Ethics Committee on 2021-01-20 as resolved by its quorate meeting.

Please note the following about your ethics approval:

- Please remember to use your protocol number (152/2011) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

Dr R Sommers
MBChB MMed (Int) MPharmMed PhD
Deputy Chairperson of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health).

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