Thirty years of FFI research: a recap on epidemiology, pathology and possibilities of treatment and future recommendations

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Abstract

Fatal familial insomnia (FFI) is a rare autosomal dominant prion disease characterized by sleep disturbances, ataxia, cognitive problems and autonomic dysfunction. The disease occurs in one in a million people per year and is invariably fatal. It affects men and women equally. Disease duration but not age of onset is determined by a polymorphism at codon 129 of the wildtype gene. There is also heterogeneity in the symptoms and course of the disease. Diagnosis of the disease is mainly based on genetic analyses. PET and post-mortem studies reveal hypometabolism, neuronal loss and immunological changes in several brain areas, including the thalamus. Molecular studies reveal that retention of the mutated prion protein (prion) inside cellular components stall cellular trafficking processes that in turn impair neuronal function leading to neuronal loss seen in post-mortem studies. Treatments are focused on alleviating symptoms and to a lesser extent on addressing the underlying mechanisms of disease. It is concluded that polymorphism on the wildtype gene but not age of onset have prognostic value for the progression of the disease. Also, diagnosis should not only rely on genetic analysis but also clinical signs. Furthermore, future studies should focus on behavioural testing in animal models in which pathological molecular mechanisms are addressed. Lastly, promising studies that yet lack any replication should be replicated shortly.

Contents

Introduction

Fatal familial insomnia (FFI) is a prion disease characterized by the core symptoms sleep disturbances, ataxia, tremors and dysautonomia[1–3]. It is associated with a mutation on chromosome 20 in the prion protein (PRNP) gene in codon 178 and a methionine codon at position 129[4]. It is a devastating but rare disease that occurs in one in a million people per year[5]. FFI is invariably fatal and disease duration may be less or more than a year[3,6]. It is hypothesized that a polymorphism at codon 129 of the unmutated PRNP allele determines disease duration, but not age of onset, which are key points in the epidemiology of FFI[7–9].

Furthermore, the clinical heterogeneity of FFI makes it hard to timely diagnose it correctly[2]. Clinical diagnoses may also differ from genetic diagnosis[2]. Usually, FFI is diagnosed with genetic testing[1,10–12]. However, other techniques are also developed to aid in the diagnosis of FFI[13]. Sleep studies are frequently done in FFI and are considered necessary for diagnosis beside genetic confirmations[14]. Magnetic Resonance Imaging (MRI) and positron emission tomography (PET) studies are done to study changes in the brain of FFI patients and may not only help in the diagnosis of FFI[14,15], but also reveal which brain areas are affected in FFI[15,16].

Also, PET studies correlate with neuropathological studies. The latter sort of studies reveals that several brain areas are affected, histologically and immunologically. Studies uncovering the mechanisms of pathology in FFI are upcoming as are treatments[17].

In this review, several aspects of FFI are discussed. Firstly, history, epidemiology and symptomatology of FFI are shortly recapitulated. Secondly, it is assessed which techniques used in the recognition of FFI pathology are really appropriate. Thirdly, recent studies that investigate the mechanisms are discussed as are studies about treatments in FFI. Lastly, the direction in which future research should go is discussed.

Etiology of fatal familial insomnia

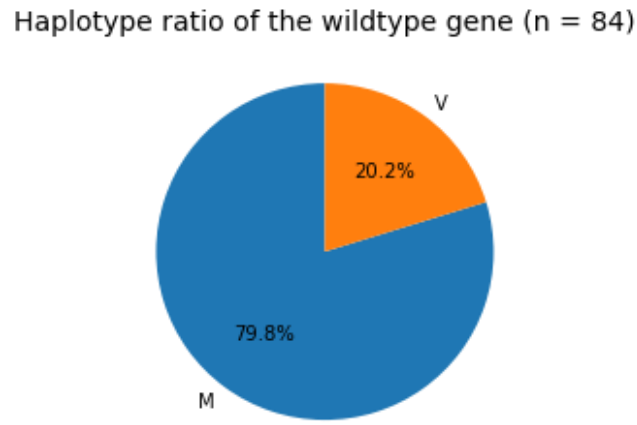
History

Fatal familial insomnia (FFI) is a prion disease that owes its name to the fact that it causes progressive sleeping problems, mostly insomnia or sleeplessness, leading to the death of those who are affected. FFI was first described in 1986 in a 53 year old Italian man. He presented with several symptoms, such as progressive insomnia, high body temperature, speech problems, dreamlike status, tremor and involuntary muscle contractions. Strangely, family members of his died of a similar disease, hence the name fatal 'familial' insomnia as was coined by Lugaresi et al. (1986). As of then, more Italian cases of FFI were reported [18–20]. Later on, investigations on FFI were done in other European countries, the USA and Australia as well, as more cases occurred [8]. Moreover, in 2004, the first case of FFI in China was reported, after which more cases arose in Asia [3,10,11,21–23].

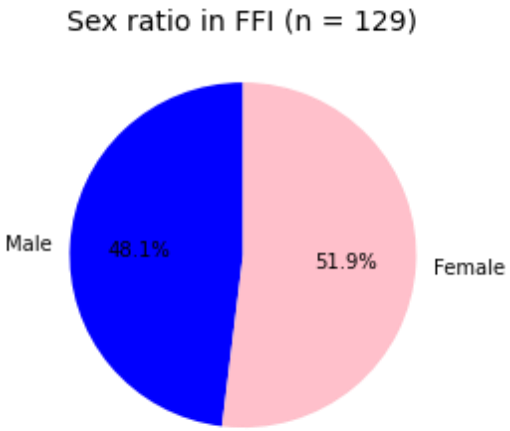
Epidemiology

As implied previously, FFI is a worldwide prion disease nowadays. It is estimated that FFI occurs in one in a million people per year[5]. FFI is mainly associated with a mutation in the prion protein (PRNP) gene that lead to misfolded prion proteins. More specifically, the mutation on this position leads to a conversion of aspartic acid into asparagine on codon position 178 on the PRNP gene[24]. In addition, there is a methionine codon present on position 129 of the gene that carries the D178N mutation[4]. The other (wildtype) gene could either carry a methionine (M) or valine (V) codon at position 129. With this, the distinction between homozygosity and heterozygosity for the methionine codon in FFI is made. **Figure 1** summarizes the epidemiology of FFI [1,8,10–12,22,25–36]. For instance, it was seen that in most of the cases the affected are homozygous for the methionine codon, with a ratio homozygous : heterozygous of approximately 4:1. The male : female ratio in FFI is approximately 1:1. The age of onset and duration of FFI can vary widely. For instance, it was observed that the disease can manifest in the early 20s, but also in the late 50s or early 70s. More specifically, the median age of onset in FFI can be estimated at 48 years. The disease could last from a few months to more than two years[1,3,12,22,30,33], with a median duration of 10.5 months (see figure). Interestingly, some authors state that disease duration is independent from the age of onset[37]. The figure shows that there is indeed no correlation between the age of onset and the duration in FFI. Also, there is debate about whether or not age of onset and disease duration depend on the M/V polymorphism at codon 129[8,9,36,38]. An overarching sample (based on 17 research articles and 1 review) that is used in figure 1 reveals that the age of onset does not depend upon the M/V polymorphism, whereas the duration does. More specifically, if there is a valine codon present at codon position 129 of the PRNP gene that is not mutated, then there is a significantly longer disease duration.

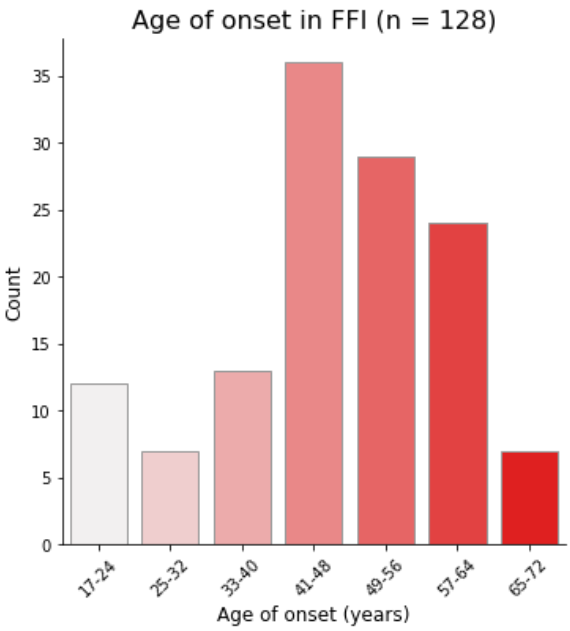
A


Haplotype ratio of the wildtype gene (n = 84)
V
20.2%
79.8%
M

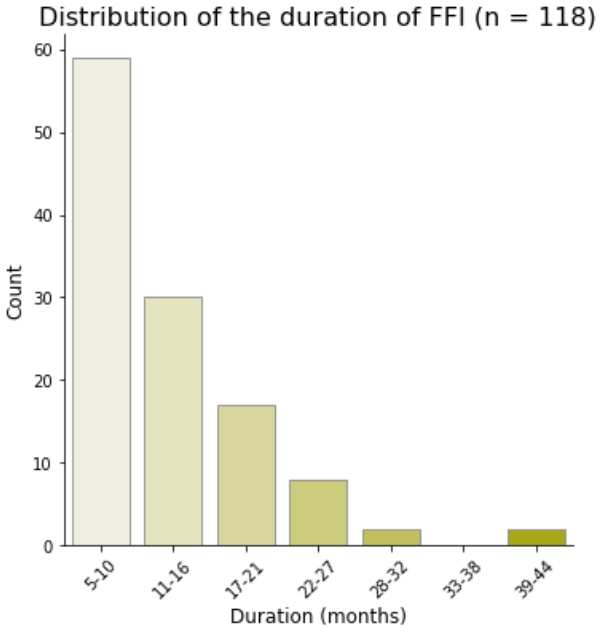
B


Sex ratio in FFI (n = 129)
Male
48.1%
51.9%
Female

C

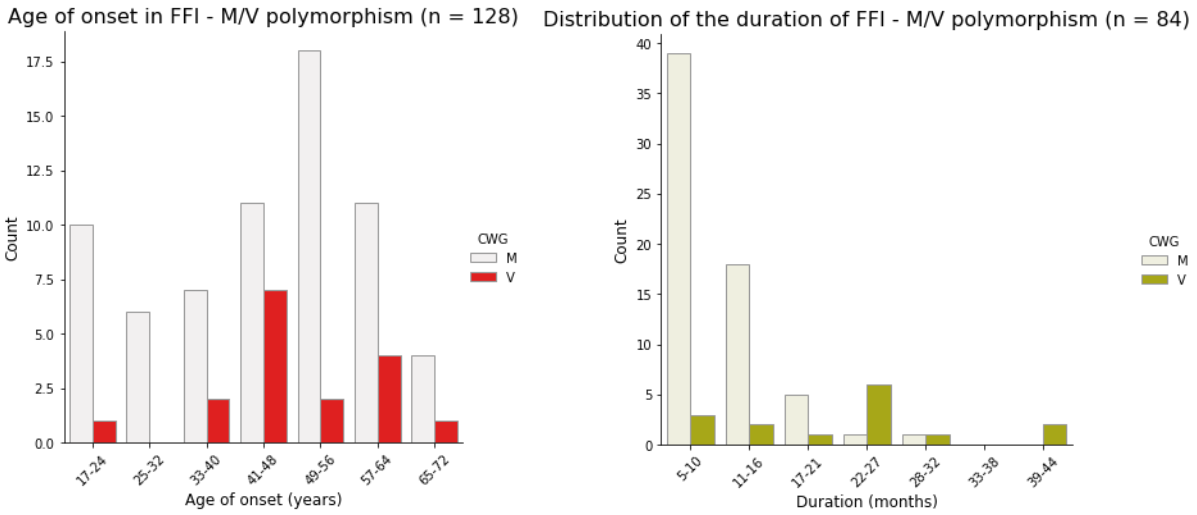

Age of onset in FFI (n = 128)
Count
0
5
10
15
20
25
30
35
17-24
25-32
33-40
41-48
49-56
57-64
65-72
Age of onset (years)

D


Distribution of the duration of FFI (n = 118)
Count
0
10
20
30
40
50
60
5-10
11-16
17-21
22-27
28-32
33-38
39-44
Duration (months)

E

F



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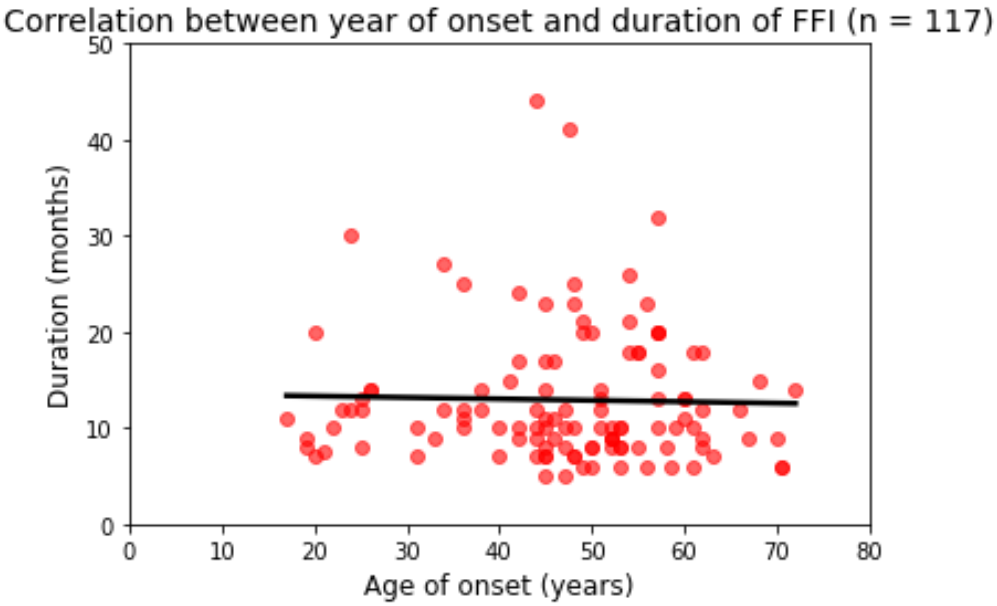


Figure 1. Overview of the epidemiology of fatal familial insomnia (FFI). **A)** the relative prevalence of methionine (M) and valine (V) at position 129 of the wildtype (unmutated) allele in FFI cases. **B)** the relative prevalence of FFI among the two sexes. **C)** the general distribution of the age of onset in years, **D)** duration of the disease in months in FFI, **E)** the age of onset is not affected by the type of codon (M or V) present at position 129 of the unmutated allele (Mann-Whitney test, $U = 498, p > 0.01$) , but **F)** the duration is (Mann-Whitney test, $U = 223.5, p < 0.001$). **G)** the correlation between the year of onset and duration in months is not significant ($r = -0.027, p > 0.01$) Data from multiple studies were summarized into a table and converted to graphs. The sample size between analyses may differ due to missing data. The age in some case representations was also taken as an approximate to the age of onset when it was not explicitly reported. Significance threshold was set at $\alpha = 0.01$.

Symptoms

It can be concluded that FFI has a rapid disease course, with more than half of the cases dying within one year. What happens to the sufferers between the point of onset and death? In other words, what is the course of the disease? In the first half year of the disease, patients present mainly with sleep

problems, such as insomnia[11,29,31,32]. Thereafter, (spatial) memory problems, personality change, hallucinations tremors, involuntary muscle contractions, and disorders of the autonomic nervous system* may develop along with other symptoms[22,28,29]. In much later stages of the disease dementia, swallowing problems, weight loss and breath stoppings during sleep (apnea) develop[11,18,19,29,39]. Pneumonia, an infection of the lungs, was repeatedly reported as the cause of death in these patients[2,11,40].

However, there is also heterogeneity among FFI patients, as is seen from multiple case studies. For instance, insomnia may not always occur first. Sometimes symptoms like social withdrawal and memory impairment may precede insomnia or occur at the same time[2,12]. It was also reported multiple times that insomnia was completely absent or not reported, despite firm genetic diagnoses[31,35,39,40]. In general, when looking at retrospective studies with multiple subjects, there is a profound clinical heterogeneity[1,3,9,26,30]. This might also be the reason why clinical diagnoses differ from genetic diagnoses in FFI[2].

*Including excessive sweating, urinary retention, constipation, hyperthermia, restlessness, tachycardia, irregular breathing, hypertension, impotence[1,3,22,23,26,35].

Diagnosis of FFI

Genetic testing

Genetic testing is nowadays – if not almost – always done in case FFI is suspected, as it is known that this disease is directly related to a mutation on the prion protein gene[3,10–12,22,28,29]. In general, the genetic diagnosis of FFI fulfills if there is a mutation on codon 178 from aspartic acid to asparagine and if the same, mutated gene carries a methionine codon at codon position 129[4]. In short, DNA from blood leukocytes is sequenced after which the result can be read[12,41,42].

Cerebrospinal fluid (CSF) tests

Usually, the cerebrospinal fluid (CSF) of FFI subjects is tested for presence of the 14-3-3 protein. This protein plays a role in several neurodegenerative diseases[43]. The presence of protein 14-3-3 is generally used as a diagnostic criterium for Creutzfeldt-Jakob disease. However, the protein is also implicated in FFI, but less pronounced. In some cases where FFI was diagnosed, the CSF was positive for protein[3,9,29]. In other cases the cerebrospinal fluid was negative, showing heterogeneity in the expression of protein 14-3-3 in the CSF of FFI individuals[3,22,26,28,32,39–41].

Detection of prions

Recently, it has been demonstrated that prions can be detected from the olfactory mucosa (nasal mucus) in patients with FFI, which is called nasal swabbing[13]. This can be done with Protein Misfolding Cyclic Amplification (PMCA) and Real Time Quaking Induced Conversion (RT-QuIC). With Both methods, misfolded prions can be multiplied[13]. With this, Redaelli et al. (2017) were able to detect prions in the samples of nasal mucus of patients with FFI[13]. Interestingly, the nasal mucus was taken from FFI patients between 4 and 10 months after disease onset, which was enough to detect misfolded prions[13]. Normally, the duration between disease onset and diagnosis also ranges between this time

period[26]. This implies that the detection of prions by nasal swabbing is already possible at the time of diagnosis and may thus aid in establishing a diagnosis for FFI.

Sleep studies

Sleep studies, such as electro-encephalography (EEG) and polysomnography (PSG) are frequently done in FFI to measure sleep abnormalities. In brief, characteristics of normal non-REM sleep include the presence of delta waves that occur during a deep sleep, the presence of K-complex waves that occur when one is partially aroused from sleep, the presence of sleep spindles and a high rhythmicity of these waves[44,45]. Non-REM sleep is further divided into three stages that normally occur in healthy people[26] (**Figure 2**). In FFI, however, there are significant alterations of these sleep features and sleep in general. For instance, it was found that there is a decrease in the number of sleep spindles and K complexes and sometimes even an absence of these two, which characterizes a state of insomnia[1,26,40]. Other studies find an excessive amount of theta activity[12,35], which in turn is associated with a state of somnolence that is also observed in FFI[2,44,46]. In addition, aberrant delta-activities also occurred. Where some authors found that delta activity was completely absent[28], others found an excess of delta activity[11,39]. The latter may be associated with hypersomnia that sometimes occurs in later stages of the disease[11,30]. All this shows that non-REM sleep is significantly impaired in patients with FFI. On top of that, REM sleep is also often altered, with either a shortage[22,23,26,32] or excess of it[28,41]. Furthermore, several scientists found a profound reduction in sleep time and sleep efficiency[9,47,48]. It should also be noted that there was absence of periodic sharp wave complexes on the EEGs done in FFI[3,27,30,39]. This is important, as the presence of these complexes often indicate Creutzfeldt-Jakob Disease, which is another related prion disease[49]. Sleep studies might thus aid in the diagnosis of FFI and preclude other prion diseases such as Creutzfeldt-Jakob.

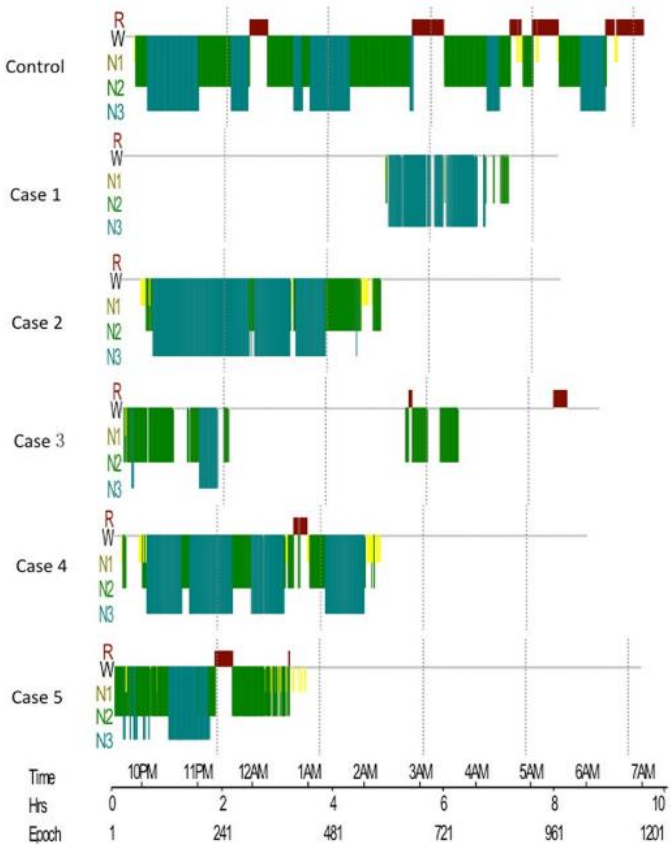


Figure 2. Sleep is severely fragmented in FFI. The figure shows a hypnogram of one control and 5 FFI cases. The 5 FFI patients presented with decreased sleep efficiency and disruption of the normal cyclic sleep organization. The horizontal axis indicates sleep hours. W: wake; R: REM; N1-3: NREM sleep stages. [edited from Wu et al. (2017)[26]]

Brain areas involved in FFI

MRI and CT

In FFI, MRI (Magnetic Resonance Imaging) and CT (Computer Tomography) are regularly used technologies to form pictures of the brain. Notably, results from MRI studies in FFI are inconsistent. For instance, MRI sometimes revealed mild and diffuse cortical atrophy that was not further specified[9,11,32,48,50]. Others report more concrete changes. For instance, in a case study of Sun and colleagues (2017), atrophy of frontal, temporal and parietal lobes was noticed[35]. Hippocampal atrophy was also suggested in another case study[41]. In a more extensive MRI study of Lu et al. (2017) it was found that there were abnormalities in frontoparietal subcortical areas, areas around the lateral ventricles, basal ganglia and the dorsal part of the thalamus[29]. Finally, in a group of 22 FFI patients it was sometimes observed that there was atrophy of the cerebellum and enlarged ventricles[9]. Despite these findings, it was also frequently reported that MRI scans could not reveal any abnormality in FFI patients[10,12,22,26,27,31,34,39,40,47,51].

CT scans have also been done in FFI, but literature on this is scarcer. Here, results are also inconsistent. Where some studies report diffuse cortical atrophy[2,50], other studies report normal CT scans in FFI[12,40].

PET and SPECT

PET (Positron Emission Tomography) and SPECT (Single Photon Emission Computed Tomography) are functional imaging techniques, which use radioactive substances to measure and visualize changes metabolic processes. PET is widely used to map changes in brain metabolism to brain areas and it seems to have a growing importance in FFI studies[9,10,15,16,22,26,28,29,52,53]. Many brain areas have in which reduced metabolism was found have already been discovered. For instance, it was often observed that the thalamus is severely affected in FFI[15,16,23,26,28,51,52]. There is a decreased glucose uptake in this area, indicating hypometabolism[26]. More specifically, one study found a reduction in metabolism in the anterior thalamus, which is in line with histopathological studies as can be seen later on[11,16,37]. Strikingly, not only the thalamus is affected by FFI, but also entire parts of the cortex. These include the frontal, parietal and temporal lobe, while the occipital lobe is hardly or not affected[16,22,29,51,52]. Subareas of these lobes affected include the dorsolateral, medial and orbitofrontal cortex in the frontal lobe[22], together with the lateral, medial and inferior temporal cortex in the temporal lobe[16,22,52] and inferior parietal cortex in the parietal lobe[26]. Other areas affected include the basal ganglia, cerebellum and brainstem[1,29,53], illustrating the diffuse but pervasive pattern of hypometabolism in the brain of FFI patients.

SPECT is a technique similar to PET, but it measures blood perfusion rather than metabolism. As of yet, SPECT is hardly used in FFI research. However, some authors did some remarkable findings. For instance, a reduced blood perfusion was seen in the cerebral cortex, basal ganglia and thalamus[23,26]. SPECT thus detects affected brain areas that are detected with PET as well.

In addition, there are also a couple of longitudinal studies performed on PET in FFI that indicate certain mechanisms of spreading of the hypometabolism. One PET study indicates that the hypometabolism starts in the thalamus 13-21 months before symptomatic onset and then spreads to the basal ganglia and limbic system[15]. Another study shows that once hypometabolism occurs in the cortex, it may occur in the brainstem and cerebellum later on[53]. Lastly, in an association study, it was found that nearly all of the brain areas that had pathologic lesions showed hypometabolism at PET scan. However, not even half of the regions that showed metabolic reduction had lesions. This indicates that metabolic changes are more widespread in the brain and may precede the existence of these lesions[16].

Neuropathological studies

Neuropathological or post-mortem studies are often done after clinical investigations, such as MRI and PET, have been finished, and death of the patients occurs[3,11,29]. These studies open up the possibility to see where and how brain areas are affected in FFI. In the following sentences, it is briefly discussed how some majorly affected brain areas may relate to some symptoms observed in FFI.

A prominent finding was, as observed previously in PET studies, that the thalamus is affected by FFI. This is especially shown by severe neuronal loss in the mediodorsal and anterior nucleus of this brain area[2,11,23,37,40,54]. It is suggested that lesions or infarctions leading to neuronal loss in the mediodorsal thalamus can lead to memory problems and emotional instability[55]. These two symptoms are also seen in cases of FFI, which implies that these symptoms may relate to the neuronal loss in the mediodorsal thalamus[3]. In addition, there is an indication that neuronal loss or lesions in the anterior part of the thalamus may lead to problems related to spatial memory[56]. Furthermore, the entorhinal cortex was also one of the areas of which it was regularly reported to have significant neuronal loss[37,57,58]. The entorhinal cortex plays an important role in spatial memory as well[59–61]. These findings may explain why FFI patients regularly show disorientation and deficits in spatial memory when being admitted to clinical research[1,2,10,22,29]. Another brain area, the inferior olive, which is located in the medulla, repeatedly underwent neuronal loss as well[2,23,34,37,40,57,62]. Lesion studies demonstrate that this brain area plays an important role in movement and that severing of the inferior olive can lead to severe ataxia[63,64]. This might clarify the progressive ataxia that occurs in FFI subjects with a degenerating inferior olive[11,22,31,39]. Other brain areas that underwent neuronal loss to a lesser extent include the brainstem, cerebellum and neocortex[11,34,42]. 'Brain-stem' symptoms seen in FFI, such as dizziness, double vision and dysphagia may be due to neuronal degeneration in the brainstem[65].

In addition to all this, neuropathological studies show that neuronal loss is not the only alteration that takes place in the FFI brain. Gliosis is a reaction on damage to the brain that is manifested by an increase in the number and enlargement of astrocytes and microglia. Gliosis is also a frequently reported phenomenon in FFI and is also seen in the brain areas mentioned previously[2,6,29,34,37,39,40,42,53,54]. Another hallmark of the brain in FFI is the deposition of prions. Different from neuronal loss that seems to be more focal, prions are rather widely distributed across different brain regions[6,51]. It was shown that prions not only emerge in the thalamus, but also in the frontal and temporal lobe, brainstem, hypothalamus, amygdala, hippocampal areas, caudate nucleus and cerebellum[11,29,40,66,67], with a relative sparing of the occipital lobe[24]. In a study by Parchi et al. (1995) it was found that patients with a longer disease duration had a more widespread pattern of prions, indicating that prions spread throughout the brain as the disease progresses[6]. Spongiform degeneration or spongiosis occurs when vacuoles arise inside neurons that are not yet degenerated and seems to be specific for prion diseases[68,69]. Spongiosis in FFI seems to occur as well, but to a lesser

extent than deposition of prions and neuronal loss and may occur in several cortical and subcortical areas[6,34,40,53]. It is also implicated that it occurs in a later stage of the disease[6].

Lastly, in an association study by Cortelli and colleagues (1997) it was demonstrated that by far the most brain areas with lesions also showed hypometabolism on PET scans, as said earlier. Furthermore, brain areas that contained depositions of the misfolded prion protein generally also showed hypometabolism and neuronal loss. On the contrary, nearly none of the regions without misfolded prion protein showed hypometabolism[16]. This indicates that the misfolded prion protein in FFI might be a prerequisite for hypometabolism and neuronal loss seen in FFI. Also, this raises the question of what molecular mechanisms underlie the neurodegeneration that is seemingly caused by these prions.

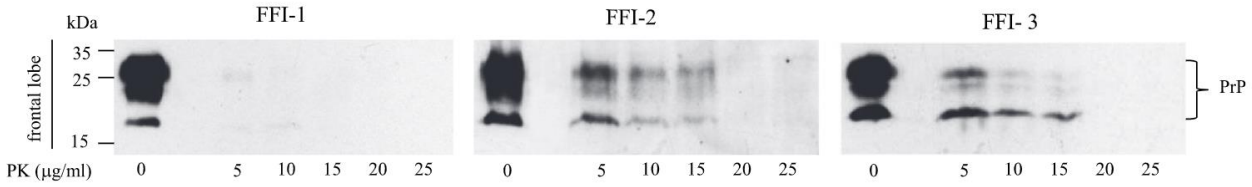
Molecular mechanisms in FFI

Properties of the prion in FFI

The prion protein (PRNP) gene at chromosome 20 encodes for normal prion protein (PrP). Normal PrP plays multiple roles in neuronal cell functioning and normal cognitive functioning[70,71]. The misfolded form of PrP (prion) in FFI arises from a mutation on the prion protein (PRNP) gene in codon 178[72]. The mutation is a substitution the nucleotide guanine into adenine at base position 532 in the PRNP gene and results in the substitution of asparagine (D) for aspartic acid (N)[1,24,34,39]. Hence, the mutation is often abbreviated with D178N in following studies[15,33,40,42]. Together with a methionine codon at position 129 of the PRNP gene, this mutation leads to the production of malfunctioning prions seen in FFI[7].

The prions seen in FFI have certain properties as well that differ from normal PrP. Western blot analysis is a method with which proteins and their sizes can be detected. It was regularly applied for determining the properties of these prions in FFI[6,19,24,51]. In this way, the presence of prions can be indicated in the form of protein fragments with a certain length. Thus, it was found that there distinctive prion fragments with differing length. The length of the prion fragments also depends upon whether it is glycosylated or not[73]. Normally, the size lies between 26 and 29 kDa for glycosylated protein fragments and is around 19 kDa for unglycosylated proteins[6,19,24,51,58,73]. Normal PrP is readily digested by PK[74]. However, it is observed that the abnormal prion is resistant to digestion by protein kinase (PK). More specifically, researchers observed dose-dependent PK-resistances of prions in frontal lobe lysates of three FFI patients (see **Figure 3**)[66].

A



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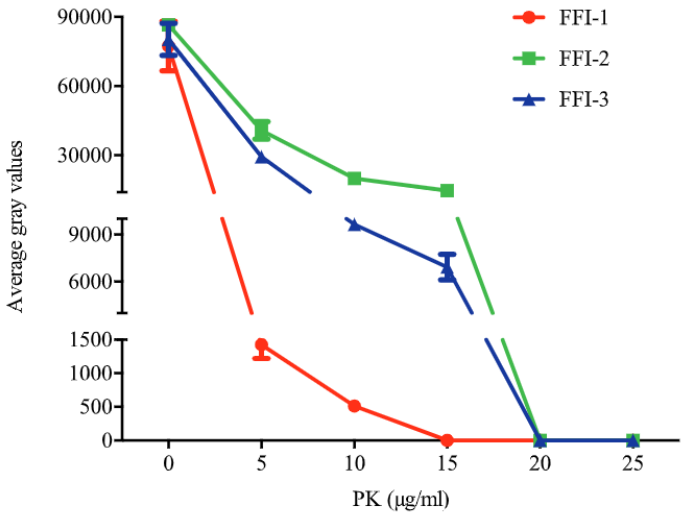

Figure 3. Prions in FFI show a dose-dependent protein-kinase resistance in 3 patients with FFI. A) Western blot of brain homogenate digested by 0, 5, 10, 15, 20 and 25 µg/ml protein-kinase at 37 degrees Celsius for 60 min. Of note is the fading of the grey bands in the Western blot as higher doses of protein-kinase are administered. **B)** quantitative analyses of grey values of the Western blots. Each datapoint represents one triplet of measurements. [edited from Xiao et al. (2020)[66]]

The prions in FFI are not only more resistant to PKs than the normal prion protein. Prions in FFI have infectious properties as well. In a study by Xiao and colleagues, brain homogenates of FFI patients were subjected into RT-QuIC assays together with normal rodent PrP as substrate. In this way, it was observed that FFI prions showed a positive reactivity towards rodent PrP. This means that FFI prions were able to convert normal PrP to misfolded prions and induce the formation of PK-resistant prion fibrils[66]. Bearing this in mind, FFI prions can also induce serious disease in rodents. For example, in a transmission study of Sasaki et al. (2005), it was found that more than half of the mice in two mouse strains developed prion disease-related symptoms (also known as transmissible spongiform encephalopathy). This was observed after the mice were inoculated with tissue homogenate from the frontal cortex of an FFI patient. The mice further developed spongiform degeneration, gliosis and depositions of prions in the cerebral cortex and thalamus, similar to what is seen in FFI[57]. Another recent study showed that mice that were infected with FFI prions had symptoms, reminiscent of FFI, such as ataxia and tremor. Also, these mice showed mild spongiosis, PK-resistant depositions of prions and severe glial activation in several brain areas, including the thalamus[69]. This indicates that the PK-resistant FFI prion causes prion disease in individuals. In the next paragraph, it is studied what mechanisms may underlie the pathologic manifestations seen in FFI.

Possible molecular and cellular mechanisms underlying the pathology of FFI

Animal models of diseases can be used to reveal molecular mechanisms of the concerning disease. There exists an animal model of FFI expressing the core symptoms of FFI (including sleep disturbances, motor dysfunction and spatial memory impairment). Also, these mice express a mutated form of the prion protein that is analogous to the prion seen in FFI with a D177N/128M haplotype instead of D178N/129M[17]. Neurons of FFI prion expressing mice show cellular abnormalities. In particular, the Golgi apparatus, which plays a role in the post-translational processing of proteins, seems affected. Normally, most of the PrP is transported from the Golgi to the plasma membrane. However, in FFI prion expressing mice, the prions are accumulating in the Golgi inside the cell, leading to its enlargement[17,75,76]. Interestingly, several mechanisms have been proposed that show how mutated forms of PrP, including the FFI prion, may lead to neuronal dysfunction and eventually neuronal loss.

As a neuron depolarizes, calcium influx through voltage-gated calcium channels (VGCCs) is triggered. As a consequence, exocytosis of synaptic vesicles with neurotransmitters is induced[77]. One theory is that the anterograde axonal transport of subunits necessary for the assembly of VGCCs is stalled as mutant PrP accumulates inside the cell body of the neuron. It was observed that mutant PrP physically interacts with these subunits. As a consequence of that, retention of mutant PrP intracellular accumulation of these subunits can occur. This could lead to reduced delivery of VGCCs to the presynaptic terminal. In turn, this causes impaired neurotransmission, contributing to neuronal loss[78,79].

Another mechanism is proposed in a study of Ghirardini and colleagues (2020). They showed that mutant PrP interacts with the GluA2-subunit of the AMPA-receptor (AMPAR) as well and impairs the transport of this subunit to the synaptic membrane. As a consequence, there is a greater number of AMPA-receptors expressed at the synaptic membrane that lack the GluA2-subunit. Also, it is known that this subunit makes AMPARs impermeable to calcium[76]. The authors demonstrated that lack of this subunit at the synaptic membrane resulted in increased permeability to calcium after activation of AMPARs with glutamate or AMPA. furthermore, it was shown that this leads to increased neuronal cell death[76].

Lastly, it is known that Src family kinases (SFKs) mediate secretory trafficking in the Golgi complex and that PrPs are involved in this trafficking by coupling with these SFKs[80]. To clarify, it was recently shown that pharmacological activation of SFK in fibroblasts of FFI patients reduced the number of fibroblasts that showed retention of cell-specific molecules (**Figure**. This indicates that activation of SFKs facilitates cellular transport that is otherwise hampered by mutant PrPs (FFI prions)[79]. In summary, the above mechanisms show how FFI may interrupt normal neuronal function that leads to hypometabolism and neuronal loss already seen in PET and neuropathological studies. Possible ways or treatments to ameliorate neuronal functioning, such as with SFKs, are on their way.

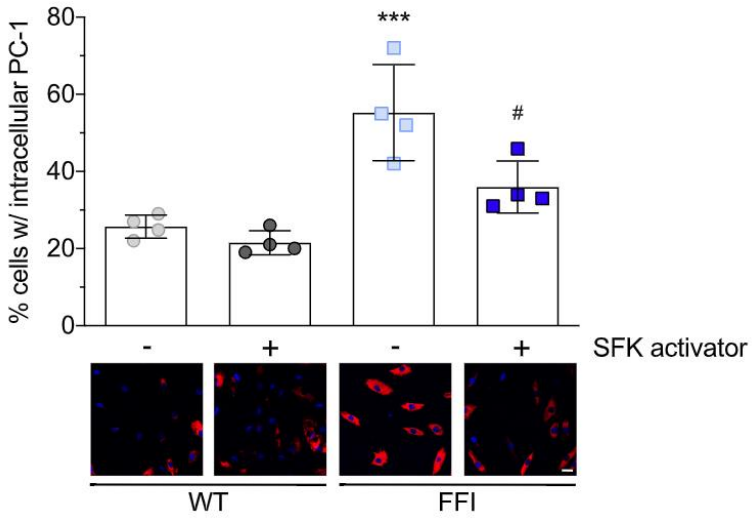

Figure 4. Src activation partially rescues the trafficking impairment of procollagen-I (PC-1) in FFI fibroblasts. Cells were treated with either vehicle or src family (SFK) activator. The bar graph indicates the percentages of cells with intracellular accumulation of PC-I. '#' indicates that FFI with SFK activator is significantly different from FFI without SFK activator. [Edited from Restelli et al. (2021)[79]]

Treatment of FFI

Neuropsychiatric treatment

In FFI, treatments are often done to solely alleviate the symptoms that occur. One major symptom of FFI on which treatments are regularly attempted is the insomnia. However, this symptom is often treatment-resistant, despite transient periods of improvement[10,81]. It was even observed that one of the reported cases had a history of insomnia that was resistant to treatment with benzodiazepines, neuroleptics and antidepressants[2]. In one study, several medicaments used for psychosis and anxiety were used in an attempt to alleviate the symptoms occurring with FFI, but without success[41].

However, in a study by Froböse et al. (2012), the effect of agomelatine on sleep was investigated on a case of FFI with no history of neuropsychiatric illness. The result was an improved sleep efficiency, more deep, slow wave sleep and fewer intermittent awakenings during sleep periods. Furthermore, the previously discussed K-complexes as a component of sleep were preserved, but not sleep spindles. This indicates that sleep was improved in the patient, with better daytime functioning as result[32].

Neuro-immunological treatments

Yet few but notable studies have been done with regard to the effect of immunotherapy on the manifestation of FFI. It is thought that the immune system plays an important role in the aggravation of prion diseases in general[82]. Therefore, steps are being made by scientists to assess if immunotherapy could help in the treatment of FFI[28,74,81]. Here, immunotherapy is a general name for a treatment in which the immune system is suppressed. For example, it was reported that treatment of an FFI patient with corticosteroids – that suppress the immune system – led to a small improvement in the quality of sleep, cognitive function and tremor. (It is, however, unclear whether death in this patient could have been prevented, as the patient was tapered off treatment before death)[81]. In another study, steroids, immunoglobulin and doxycycline were administered, but without effect[28]. Of the antibiotic doxycycline it is known that it inhibits prion infectivity by decreasing protein-kinase resistance of the misfolded prions[83,84]. However, its effect in treating at early stages of prion diseases remains speculative[85,86]. Currently, there is one ten year on-going trial on treating FFI with doxycycline[87]. In the meantime, the effect of doxycycline is tested in animal models of FFI.

For example, in a very recent study of Lavigna et al. (2021) the FFI mouse model showing the core symptoms of FFI was used to test doxycycline (doxy). At a behavioural level, it was found that doxy reduced the memory impairment seen in transgenic FFI mice in the novel object recognition test after 13 weeks of treatment (see figure). On top of that, doxy restored circadian motor activity in mice by rescuing levels of activity during the night. However, doxy failed to prevent the progression of motor dysfunction in transgenic FFI mice. Doxy had neither an effect on the clinical course and survival of these mice (see **Figure 5**). Furthermore, it turned out that doxy had effect on the protease resistance and amount of the insoluble misfolded prion protein. From this, the authors conclude that some, but not all core symptoms in transgenic FFI mice and that it is insecure whether doxy is sufficient to prevent the manifestation and death of FFI in humans[74].

A

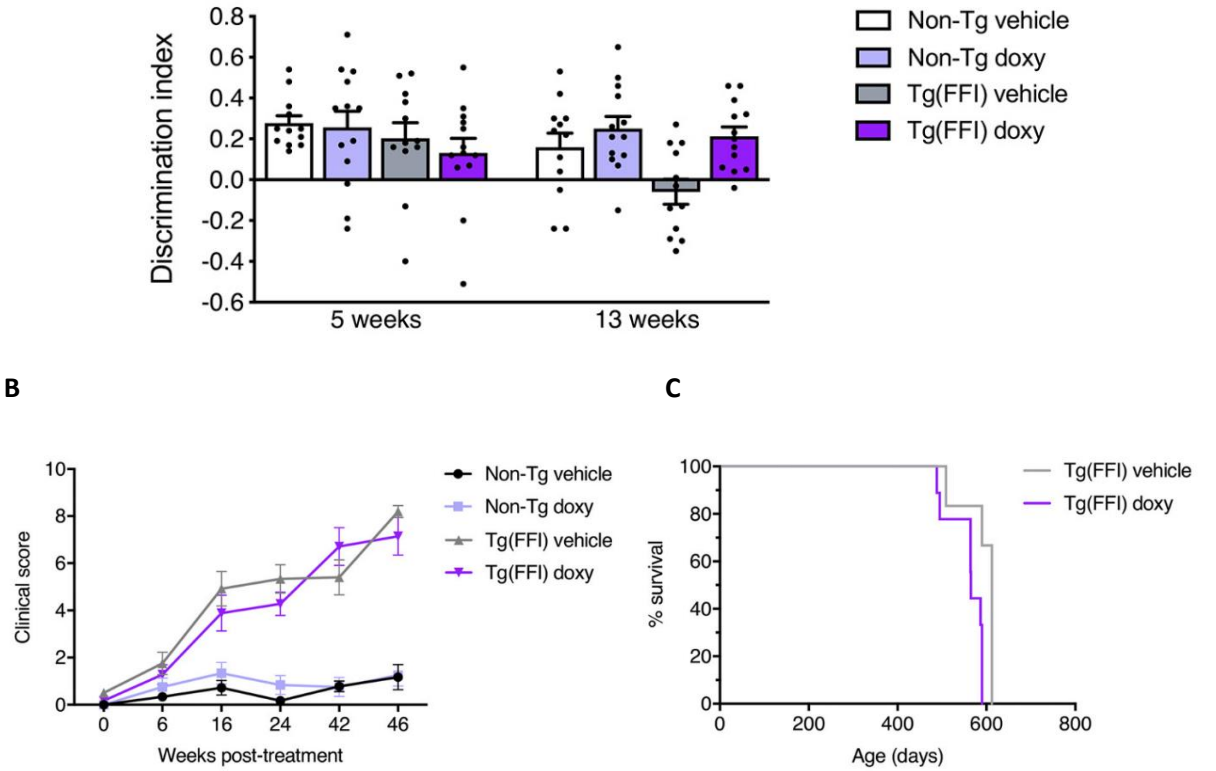

Figure 5. Doxycycline (doxy) rescues some symptoms in FFI (a.o. spatial memory) but does not affect the course of the disease and survival. A) Doxy improves spatial memory after 13 weeks of administration. A higher discrimination index indicates better spatial memory improvement as was detected by the novel object recognition test. **B)** doxy does not alter the clinical course and **C)** survival of transgenic FFI mice. [Edited from Lavigna et al. (2021)[74]]

Mechanical interventions

Nocturnal noninvasive ventilation (NIV) is a technique with which respiratory problems can be alleviated during the night. In one study by Casas-Mendez and colleagues (2011), it was assessed whether NIV could reduce the respiratory problems seen in a woman diagnosed with FFI. The respiratory problems manifested by shallow breathing, breath stoppings (apnea), low oxygen and high carbon dioxide levels and could not be explained by a respiratory infection. The authors observed that NIV induced an improvement in oxygen and carbon dioxide levels, the level of consciousness and subjective perception of falling asleep. However, the state of the patient worsened and led to death two months after the use of NIV. Still, cause of death due to respiratory failure may have been prevented. This implies that NIV could be helpful in the management of FFI cases and that NIV should also be used in other FFI cases at early stages of the disease[88].

Discussion

Here, about thirty years of FFI research was reviewed. From its epidemiology, it can be concluded that it does not discriminate between sexes and that it is a highly heterogenous disease, not only in the manifestation of its symptoms, but also in its course. It was revealed that there is no significant correlation between age of onset and disease duration, which implies that age of onset has no prognostic value for the course of the disease. The polymorphism at codon 129 of the PRNP gene did

not influence the age of onset of FFI but had a significant effect on the duration of FFI. This is also consistent with what was reported in earlier studies in which a smaller sample size was used[7–9]. Still, it should be mentioned that the sample used here consists for the biggest part of a sample collected in a study and review of Harder and colleagues (2004). However, in their case no significant association was found between the polymorphism at codon position 129 of the wildtype gene and duration of FFI. Interestingly, in this review, a significant association was found. This may have to do with the fact that many Asian cases were included here. Also, there were yet no reports about Asian cases of FFI at the time Harder and colleagues (2004) reviewed[8], as the first Asian case reported was in the study of Spacey et al. (2004)[11]. On top of that, the sample size of the individuals who were heterozygous for methionine at codon position 129 was approximately twice as big in this review.

The ways in which the diagnosis of FFI can be supported were also studied. However, an important limitation of this part is that it cannot be inferred whether the outcomes seen in FFI are specific for FFI. This is because this review only focused on FFI research. The genetic diagnosis is by definition leading in the diagnosis of FFI, as the diagnosis is based on this genetic testing. Nevertheless, genetic diagnosis does not always match with clinical diagnosis[2]. This is problematic, as there is much clinical heterogeneity among individuals, although they have the same D178N/129M haplotype[3]. In some cases, insomnia was even absent, which does not fit in well with the name of the disease. To solve this, scientists have tried to set up a pathway to a clinical diagnosis for FFI. Herein, the presence of sleep disturbances is necessary, and should be detected with the help of polysomnographies[14]. With this, FFI could obtain an authentic clinical diagnosis that matches with eventual genetic diagnosis. In this way, FFI cases that do not show sleep disturbances can be ruled out and overrepresentation of FFI in prion diseases can be prevented. Furthermore, nasal swabbing could be used in combination with prion amplification techniques to confirm the presence of prions in FFI as the disease progresses. Also, results of CSF analysis with regard to presence of the 14-3-3 protein are not only heterogenous in FFI, but also other prion diseases[89]. Thus, absence does neither indicate FFI nor preclude other prion diseases. Therefore, CSF analysis might be left out from the diagnostic routine in FFI. In summary, diagnosis should not only be based on genetic testing, but also on clinical signs that may be specific to FFI, such as sleep disturbances[14].

Although techniques such as CT, MRI, PET and SPECT can be used to diagnose FFI, the current review limited its focus on which brain areas can be detected that are affected in FFI. After all, to diagnose FFI with these techniques, one needs knowledge about results with these techniques in other diseases in order to say whether an outcome is specific to FFI. However, it is still possible to discuss which brain areas are affected in FFI. First of all, MRI and CT seem to have a remarkably lower sensitivity and consistency than PET and SPECT, because, unlike PET scan, these techniques fail to detect peculiar changes in the thalamus. After all, the changes in the thalamus are also confirmed in neuropathological studies. Furthermore, PET studies are also able to detect activation of microglia in several brain areas, which corresponds with gliosis seen in post-mortem studies[25]. In addition to this, similarity was found between PET and SPECT concerning which brain areas are detected to be affected. This is not surprising, as reduced blood flow in brain areas results in reduced delivery of nutrients such as glucose to these areas. This could explain why PET and SPECT show similar results, given that PET and SPECT detect hypometabolism and hypoperfusion, respectively. Taking this into consideration, future studies that assess progression and pathology of FFI should focus more on the use of PET and SPECT in combination with post-mortem studies and refrain from using MRI and CT.

With regard to the molecular pathology of FFI, several mechanisms were discussed. It should be noted that some of the mechanisms are still hypothetical in FFI, although they have been established in mouse models that mimic other prion diseases. Therefore, the studies that demonstrated a

pathological mechanism with regard to other prions than the prions in FFI [e.g. Senatore and colleagues (2012)] should be replicated in transgenic FFI mouse models. Also, despite the observation that SFKs improve cellular trafficking, its effect still needs to be validated at the level of behaviour in transgenic FFI mice. Thus, in this case research should focus on replicating above studies, after which scientists should focus on behavioural testing of possible helpful molecular agents. In line with this, case studies that investigated the effect of a particular treatment on FFI or the progression of it (e.g. Froböse et al. [2012] and Casas-Mendez et al. [2011]) should be replicated in group trials to assess whether these interventions are helpful in FFI in general.

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Appendices

Data for figure 1

Year of onset	Duration (months)	Sex	CWG*
45	14	male	V
60	13	male	M
49	6	male	M
45	8	female	M
22	10	male	M
17	11	male	M
49	20	female	M
44	7	male	M
55	18	female	M
57	10	female	M
62	12	male	M
60	11	female	M
19	8	female	M
36	10	female	M

55	18	male	M
37	**	male	
57	13	male	M
57	20	female	
45	10	male	M
26	14	female	M
53	10	male	M
24	30	female	M
19	9	female	M
25	8	male	M
55	8	male	M
47	10	female	M
50	8	female	M
31	7	male	M
31	10	male	M
48	10	male	M
26	14	female	M
36	12	male	M
47	12	female	
21	7.5	male	M
53	6	male	M
70.5	6	female	M
44	44	female	V
70.5	6	female	M
58.5	6	male	M
47	8	male	M
47.5	41	female	V
34	27	male	M
44	10	female	M
52	9	male	M
52	9	male	M
53	8	male	M
53	10	male	M
62	8	male	M
51	10	male	M
56	23	male	V
48	25	female	V
48	23	female	V
45	23	male	V
36	25	female	V
54	26	male	V
57	32	female	V
60	13	female	
20	7	male	V
54	21	female	M
44	12	male	

42	17	male	
50	20	male	
46	9	female	
63	7	female	
58	8	female	M
62	18	female	
20	20	female	M
25	13	male	M
36	11	male	M
50	8	male	
45	11	male	
52	10	female	M
45		female	M
40		female	
50	6	female	
38	12	male	M
66	12	female	V
42	24	female	
23	12	male	M
40	7	female	
34	12	female	
42	10	male	
24	12	male	M
59	10	male	V
51	12	female	M
57	16	female	M
44	9	male	M
67	9	male	M
53	8	male	
70	9	female	M
48		female	V
59		female	M
48	7	female	
20		female	
45	7	female	
54	18	male	
45	7	male	
61	18	male	
49	21	female	
45	17	male	
52	8	male	
57	20	male	
51	14	female	M
42		female	
58		female	
56	6	male	M

47	5	female	
40	10	female	M
48	7	male	M
61	10	female	M
38	14	female	M
33	9	male	V
57	20	female	V
	20	female	
68	15	female	
41	15	male	M
51	13	male	
52	9	female	
62	9	male	
72	14	female	
61	6	female	
45	5	female	
46	17	female	
41		female	
25	12	female	
46	11	male	
42	9	female	
63		male	V
23		female	M

*CWG: codon 129 of the wildtype (unmutated) gene

**empty cells indicate missing data

Code for figure 1

```
import os

import numpy as np

import pandas as pd

from matplotlib import pyplot as plt

import scipy.stats as stats

from scipy.stats import pearsonr

import seaborn as sns

from scipy.stats import pearsonr

from scipy.stats import mannwhitneyu
```

In [ ]:

print("Current working directory:\n\n", os.getcwd()) *# Look at what directory we're in*

In [ ]:

os.chdir("C:\\Users\\jensd\\OneDrive\\Documenten\\Helemaal Jens\\Jens Master BMS jaar 1 en 2\\BMS jaar 2\\MScThesis")

In [ ]:

FFI = pd.read_csv("FFI_onset_duration.csv", sep = ";")

In [ ]:

FFI

In [ ]:

plt.pie([sum(FFI["CWG"] == "M"), sum(FFI["CWG"] == "V")],

    labels = ["M", "V"], startangle = 90, autopct='%1.1f%%')

plt.title("Haplotype ratio of the wildtype gene" + " (n = " + str(sum(FFI["CWG"].notna())) + ")", fontsize = 14)

plt.show()

In [ ]:

```
plt.pie([sum(FFI["Sex"] == "male"), sum(FFI["Sex"] == "female")],
      labels = ["Male", "Female"], startangle = 90, colors = ["blue", "pink"], autopct='%1.1f%%')
plt.title("Sex ratio in FFI" + " (n = " + str(sum(FFI["Sex"].notna())) + ")", fontsize = 14)
plt.show()
```

In [ ]:

```
FFI["CWG"] = FFI["CWG"].astype("category")
```

In [ ]:

```
print(pd.cut(FFI["Year of onset"],
     7, retbins = True, labels = ["17-24", "25-32", "33-40", "41-48", "49-56", "57-64", "65-72"]))


FFI["YOO_cat"] = pd.cut(FFI["Year of onset"],
     7, retbins = True, labels = ["17-24", "25-32", "33-40", "41-48", "49-56", "57-64", "65-72"])[0]
```

In [ ]:

```
sns.catplot(x = "YOO_cat", kind = "count",
          palette="light:r", edgecolor = ".6",
          data = FFI)
plt.xlabel("Age of onset (years)", fontsize = 12)
plt.ylabel("Count", fontsize = 12)
plt.title("Age of onset in FFI" + " (n = " + str(sum(FFI["Year of onset"].notna())) + ")", fontsize = 16)
```

```
plt.xticks(rotation = 45)

plt.show()
```

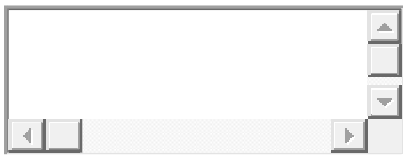
In [ ]:

```
median_age_of_onset = FFI["Year of onset"].median()

print(median_age_of_onset)
```

In [ ]:

```
sns.catplot(x = "YOO_cat", kind = "count", hue = "CWG",
            palette="light:r", edgecolor = ".6",
            data = FFI)
plt.xlabel("Age of onset (years)", fontsize = 12)

plt.ylabel("Count", fontsize = 12)

plt.title("Age of onset in FFI - M/V polymorphism" + " (n = " + str(sum(FFI["Year of onset"].notna())) +
")", fontsize = 16)

plt.xticks(rotation = 45)

plt.show()
```

In [ ]:



```
difference_MV_age_of_onset = mannwhitneyu(FFI[FFI['CWG'] == "M"]["Year of onset"],
            FFI[FFI['CWG'] == "V"]["Year of onset"],
            alternative = "two-sided")
print(difference_MV_age_of_onset)
```

In [ ]:

```
print(pd.cut(FFI["Duration"],
      7, retbins = True, labels = ["5-10", "11-16", "17-21", "22-27", "28-32", "33-38", "39-44"]))


FFI["Duration_cat"] = pd.cut(FFI["Duration"],
      7, retbins = True, labels = ["5-10", "11-16", "17-21", "22-27", "28-32", "33-38", "39-44"])[0]
```

In [ ]:

```
sns.catplot(x = "Duration_cat", kind = "count",
            palette="light:y", edgecolor = ".6",
            data = FFI)
plt.xlabel("Duration (months)", fontsize = 12)
plt.ylabel("Count", fontsize = 12)
plt.title("Distribution of the duration of FFI" + " (n = " + str(sum(FFI["Duration"].notna())) + ")", fontsize = 16)
plt.xticks(rotation = 45)
plt.show()
```

In [ ]:

```
median_duration = FFI["Duration"].median()
print(median_duration)
```

In [ ]:

```
percentage_less_than_year = sum(FFI[FFI["Duration"].notna()]["Duration"] < 12)/sum(FFI["Duration"].notna()) * 100

percentage_less_than_year
```

In [ ]:

```
sns.catplot(x = "Duration_cat", kind = "count", hue = 'CWG',
            palette="light:y", edgecolor = ".6",
            data = FFI)
plt.xlabel("Duration (months)", fontsize = 12)
plt.ylabel("Count", fontsize = 12)
plt.title("Distribution of the duration of FFI - M/V polymorphism" + " (n = " + str(sum(FFI["CWG"].notna())) + ")", fontsize = 16)
plt.xticks(rotation = 45)
plt.show()
```

In [ ]:

```
difference_MV_duration = mannwhitneyu(FFI[FFI['CWG'] == "M"]["Duration"],
                                      FFI[FFI['CWG'] == "V"]["Duration"],
                                      alternative = "two-sided")
print(difference_MV_duration)
```

In [ ]:

```
sns.regplot(data = FFI, x = FFI["Year of onset"], y = FFI["Duration"], ci = None,
            line_kws={'lw': 2.5, 'color' : 'black'},
            scatter_kws={'color' : 'red', 'alpha': 0.6})
```

```
plt.xlabel("Age of onset (years)", fontsize = 12)
plt.ylabel("Duration (months)", fontsize = 12)
plt.title("Correlation between year of onset and duration of FFI"
      +" (n = " + str(sum(FFI['Year of onset'].notna() & FFI['Duration'].notna())) + ")", fontsize = 14)
plt.xlim([0,80])
plt.ylim([0,50])
plt.show()
```

In [ ]:

```
correlation_age_of_onset_duration = pearsonr(FFI["Year of onset"].notna(), FFI["Duration"].notna())
print(correlation_age_of_onset_duration)
```

In [ ]:

```
plt.pie([sum(FFI["Sex"] == "male"), sum(FFI["Sex"] == "female")],
      labels = ["Male", "Female"], startangle = 90, colors = ["blue", "pink"], autopct='%1.1f%%')
plt.title("Sex ratio in FFI" + " (n = " + str(sum(FFI["Sex"].notna())) + ")", fontsize = 14)
plt.show()
```

In [ ]: