CHAPTER 1

INTRODUCTION

1.1. BACKGROUND

Fragile X mental retardation 1 gene (*FMR1*) is located on the X chromosome and is responsible for producing the fragile X mental retardation protein (FMRP) which is important for synaptic plasticity and spine maturation (1). This gene has a CGG repeat in the 5' UTR with a normal length around 6-54 CGG repeats (2). This CGG repeat is unstable and intergenerational dynamic mutation can cause the repeat to expand beyond the normal size, resulting in more than 200 CGGs (full mutation). In the full mutation range the *FMR1* gene promoter is usually methylated resulting in gene silencing and no FMRP is produced. Individuals without FMRP develop mental retardation, a disease named fragile X syndrome (1, 3). The prevalence of Caucasian individuals with fragile x syndrome is estimated about 1 in 4000 (4). In Indonesia Faradz *et al.* found that 5 of 262 male students (1.9%) with developmental disability in special schools were positive for fragile x syndrome (5).

Individuals with a CGG repeat in the premutation range of 45-200 CGGs show elevated levels of *FMR1* mRNA and slightly reduced levels of FMRP (6). They are at risk to develop another disease which is totally different from fragile X syndrome, a neurological disease called fragile X-associated tremor/ataxia syndrome (FXTAS) (7, 8). This disease affects more than one third of premutation males over 50 years old and increases to 50% in premutation males of 70-90 years

old (9, 10). Patients with FXTAS develop progressive action tremor and gait ataxia. They may also present with executive function deficits, cognitive decline, Parkinsonism associated, autonomic dysfunction and peripheral neuropathy (7, 8, 11). Anxiety, disinhibition, depression, and apathy sometimes appear as psychological problems (12, 13).

FXTAS patients exhibit several neurohistological and neuroanatomical characteristics. They show Purkinje cell loss, Bergmann gliosis and Global brain atrophy (14, 15). On the histological level, intranuclear inclusions are formed in neurons and astrocytes (15). These inclusions contain the expanded premutation CGG RNA as well as proteins (16-18). The presence of ubiquitin in these intranuclear inclusions is considered as the major hallmark of FXTAS neuropathology (14, 19). The increased levels of *FMR1* mRNA, suggest a toxic RNA gain of function as the mechanism underlying the disease. Another fact supporting this mechanism is the presence of the CGG mRNA within the intranuclear inclusions in neurons and astrocytes which leads to cell death (20-22).

To study this disease, a KI (knock-in) mouse model has been set up. In this mouse model, the endogenous CGG repeat is replaced with human CGG repeat in the premutation range (23, 24). These mice imitate the human situation, such as elevated levels of *Fmr1* RNA and intranuclear inclusions in different brain regions. Other similarities with human FXTAS are an increase in number and size of intranuclear inclusions with age, as well as slight learning disturbance and mild decreased of neuromotor task (25). The KI mice also unstably transmit the

CGG repeat to the next generation. But only moderate expansion is observed without methylation, even though the repeat reaches full mutation threshold (26). This KI mouse is a good model to study FXTAS. However inclusions in these mice did not appear in astrocytes, which are likely caused by lack of toxic RNA expression in these cells. Other differences are the absence of neuronal loss, gliosis, and Purkinje cell dropout, while they are commonly seen in human FXTAS. This premutation KI mouse is still not sufficient to develop FXTAS disease as seen in human regarding to neurodegeneration, clinical and behavioral phenotype (24, 26-29).

Hence developing a mouse model which is able to imitate the human situation even better would be necessary for FXTAS study. Since the KI mice do not show inclusions in astrocytes, the question remains whether inclusions in astrocytes are required to produce a severe form of FXTAS as seen in human patients. It is also possible that inclusions only in the astrocytes are sufficient to produce FXTAS. Thus it is important to use a mouse model to study the necessity and sufficiency of different cell types for FXTAS progression. One essential study which requires a decent mouse model is the study to see the possibility to reverse the neuropathology of FXTAS by cessation of toxic RNA expression (28). This study will give information to look for a possible effective therapy, particularly by interfering with the toxic RNA.

Taken together, inducible expression of an expanded premutation CGG repeat together with the use of specific promoters should give a good model which mimics the human FXTAS situation. The tet-regulated inducible system places an

operator sequence which enables to control the onset (tet-on) or termination (tet-off) of expression. Induction relies on the presence of tetracycline (tet) or an analog such as doxycycline (dox). In the tet-on system, induction of tet will activate the gene expression, while in the tet-off system the presence of tet will stop the gene expression (30). In this study, the tet-on system with dox induction was used. This system is able to induce expression of abundant expanded premutation CGG RNA by giving dox and to stop the expression by cessation of dox. It is expected that long administration of dox will produce FXTAS neuropathology. Afterwards it will be proved whether or not the cessation of toxic RNA by stopping the dox could reverse the neuropathology yielding FXTAS outcome improvement.

The specific reverse tetracycline transactivator (rtTA) drivers make it possible to restrict the place of toxic RNA transgene expression. This study uses PrP-rtTA which allows expression in all cell types in the brain except for the Purkinje cells (31); and GFA2-rtTA which restricts the expressions only in Bergmann glia and astrocyte (32). This restriction is a powerful tool to study the sufficiency and necessity of the cells in the development of FXTAS and allow us to know which cell types (neurons, Bergmann glia, astrocytes) contribute to FXTAS neuropathology. Both premutation CGG-bound Tet-on systems and specific rtTA drivers can be placed in a mouse, generating an inducible transgenic mouse for FXTAS.

The transgenic mice have been generated by a research group in Erasmus MC Rotterdam, and will be used for the main projects: reversibility study and

finding the responsible cells which produce FXTAS. Before the mice are ready for those main studies, these mice need to be characterized. Characterization of these mice is mandatory to make sure that these transgenic mice can work properly as expected. While finding the best founder of each line, would be useful to give the best result in the future studies. This particular research is a small part of the main project, aims to characterize the available transgenic mice; hence they would be ready for the big project.

1.2. RESEARCH QUESTIONS

1. Does Tet-on-nCGG-eGFP transgene work *in vivo* in the transgenic mice?
2. Does Tet-on-nCGG-eGFP/GFA2-rtTA transgene work in the bigenic mice?
3. Does Tet-on-nCGG-eGFP/PrP-rtTA transgene work in the bigenic mice?
4. Does the transgene of bigenic mice express specifically in the brain, and not in other tissues outside the brain?
5. From all transgenic mice founders available, which founder is the best to be used for further studies?
6. Is the system able to produce FXTAS primary neuropathological hallmark, the ubiquitin-positive intranuclear inclusions?

1.3. RESEARCH PURPOSES

1.3.1. General purposes

This research aims to characterize the expression of transgenic mice, choose the best founder of available transgenic lines, and observe the ubiquitin inclusions formation of the inducible transgenic mouse model for FXTAS.

1.3.2. Specific purposes

This particular research itself aims to:

1. Characterize the workable of Tet-on-nCGG-eGFP system to express the transgene *in vivo*.
2. Characterize the workable of Tet-on-nCGG-eGFP / GFA2-rtTA transgene in the bigenic mice.
3. Characterize the workable of Tet-on-nCGG-eGFP / PrP-rtTA transgene in the bigenic mice.
4. Characterize the leakage of expression outside the brain of the bigenic mice
5. Choose the best founder of each transgenic line by comparing the expression of GFP and rtTA of the transgenic mice.
6. Observe the formation of FXTAS neuropathology, the ubiquitin-positive intranuclear inclusions, in the bigenic mice.

1.4. RESEARCH BENEFITS

This work provides a powerful animal model to study FXTAS. This animal will be used to gain data and information supporting FXTAS pathophysiology and therapy.

1.5. RESEARCH ORIGINALITY

Up to now there is no study on FXTAS using inducible transgenic mouse model. This study is the first study to generate an inducible transgenic mouse model in which the transgene can be expressed in certain areas of the brain by PrP and Gfa2 driver promoter under control of tetracycline. A closest published paper regarding to this study is a paper by Hashem *et al.* on 2008 (59), which generated a transgenic mouse model for FXTAS using Pcp/L7 driver promoter without an ability to control the transgene expression.