

## *A Pharmacogenetic protocol for Fluoxetine response during a period of rapid brain development and weight gain in animal model.*

### Um protocolo de Farmacogenética para resposta à Fluoxetina durante um período de rápido crescimento cerebral e ganho de peso em modelo animal.

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#### ABSTRACT

We developed a pharmacogenetics protocol based on the treatment of Wistar rats with Fluoxetine, a selective serotonin re-uptake inhibitors during the neonatal period. The first step of this protocol is a pilot study to evaluate the feasibility of finding statistically significant differences in the response to Fluoxetine, treated during the suckling period. Two groups of animals were identified as “good” and “bad” responders based on their extreme pattern of weight change. We searched for Single nucleotide Polymorphisms reported during the re-sequencing of the serotonin transporter gene of rat in various databases A Blast of both sequences allowed the identification of 10 sites of putative variations and latter we found Rat homologues for human genes involved with weight gain.

**Key words:** pharmacogenetics, animal models, Fluoxetin, weigh gain, brain development.

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## RESUMO

Foi desenvolvido um protocolo farmacogenético para Fluoxetina baseado no tratamento de ratos Wistar durante o período neonatal. A primeira etapa deste estudo piloto se caracteriza pelo estudo de viabilidade de identificar diferença estatisticamente significativa em relação à resposta ao antidepressivo, durante o período da amamentação. Dois grupos de animais foram identificados como “bons” e “maus” respondedores” baseados nos seus padrões de ganho de peso. Através de um estudo de Bioinformática identificamos variações de DNA na seqüência do gene do transportador da serotonina do rato em vários bancos de dados. Um Blast de ambas seqüências permitiu identificar 10 variações e posteriormente identificamos genes homólogos em Ratos que possam estar envolvidos neste fenômeno.

**Palavras chave:** Farmacogenética, modelos animais, Fluoxetina, ganho de peso, desenvolvimento cerebral.

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Ansorge et al. (2004) reported transgenic mice lacking one or two copies of the Serotonin Transporter Gene (5-HTT) exposed to neonatal treatment with Fluoxetine. These animals presented depression-like and anxiety related behaviors, a similar result when compared with the normal (non transgenic) mice also receiving Fluoxetine.

Numerous studies confirmed that the neonatal use of selective serotonin re-uptake inhibitors (SSRIs), such as fluoxetine, sertraline and citalopram, are known to reduce weight gain during the nursing period in rats and decrease depressive-like behavior in the adult animal (Barreto-Medeiros et al, 2002; Manhaes-de-Castro et al, 2001; Mendes da Silva, 2002; Leite et al, 2005).

The rapid brain development of *Rattus norvegicus* occurs during pregnancy and nursing periods. There is little data about the influence of antidepressants on brain development and the genetic factors that interfere with drug response during this period. (Geula et al, 1995; Barreto-Medeiros et al, 2002). These drugs inhibit the action of the serotonin transporter (5-HTT), allowing serotonin (5-HT) to act longer in the synaptic gap. Serotonin seems to play a role in regulating the development of mammalian brain through its action on self-producing serotonergic neurons in target tissues, innervated by them (Whitaker-Azmitia et al., 1991; Shemer et al., 1991; Lauder et al., 1993).

Studies with other groups of drugs suggest that there is variability in drug response influenced by genetic variations in Wistar rats. Vöikar et al.(1999) analyzed the individual differences of sensitization for apomorphine-induced behaviors and found that the behavioral response was different between the “weak” and “strong” responders. ntlly, Germeyer et al.(2002) identified a dopamine D2 receptor polymorphism associated with apomorphine-induced stereotypes.

Other neurotransmitters panels, such as the serotonergic, might be influenced by common genetic polymorphisms in the rat genome, affecting drug responses. However, none have been reported so far.

We developed a pharmacogenetics protocol based on the treatment of Wistar rats with Fluoxetine, a selective serotonin re-uptake inhibitors (SSRIs), during the neonatal period (21 days) (**Figure 1**).

The first step of this protocol is a pilot study to evaluate the feasibility of finding statistically significant differences in the response to fluoxetine in a limited number of Wistar rats, treated during the suckling period.

After the drug treatment, the animals were selected based on their response to daily injections of 10 mg of Fluoxetine per Kilogram of body weight and we tried to identify animals, and the litters, with

the most extreme response to the drug treatment based on this criteria.

The standard response to this kind of experiment is a progressive and maintained reduction of weight gain, when compared to the control animals, only injected with Saline solution.

*Vivarium* conditions, diet protocols and animals mating system were performed as previously described (Mendes da Silva et al., 2002). With their preserved genealogical identities, the pups were randomized among the dams. This procedure intended to decrease the bias of weight gain due to variability of milking from the females nursing the pups. Litters were reduced to six neonates each.

The groups were composed according to pharmacological treatments: 1) Fluoxetine (n=28, injected with fluoxetine dissolved in vehicle: 10mg/kg, s.c., 1ml/100g). 2) Control (n=18, injected with the vehicle: 0.9% NaCl, s.c., 1ml/100g).

From the 1<sup>st</sup> to 21<sup>st</sup> post-natal days, at 12:00 pm., before injection, body weight of the pups measured using an electronic analytic balance. The animals were analyzed statistically as corresponding to 2 extreme phenotypes: those whose body weight evolution were minimized (“good responders”) and those whose body weight evolution were greater amongst the fluoxetine group (“bad responders”) (See detail in **figure 1**).

After the identification of the rats that have extreme response of weight gain we found animals with a 2 standard deviations related to the average saline sample. Both animals with extreme response were females (See **Figure1**). The heaviest one with 57.7g and the lighter one with 25.2g. This group had an average final weight of 43.13g (SD 7.37) and the control with an average final weight of 51.8g (SD7.47).

Two groups of animals, curiously all female, were identified as “good” and “bad” responders based respectively on their extreme pattern of weight gain or weight lost. Both groups differed significantly

from the controls in a 2.0 standard deviation. The phenomenon seemed to be common to the same litters, suggesting a genetic component.

Previous studies have shown females as more sensitive to drug response variation. Curiously, two recent studies suggest gender dependent differences in drug response in Wistar rats and in corticotropin-releasing factor receptor-2-deficient mice (Bales., 2003; Solberg., 2003).

Additionally, we searched for SNPs reported during the re-sequencing of the 5-HTT gene in rat in various genetic databases. The variations reported so far in the 5-HTT gene come from the deposit of two cDNA sequences available at the EMBL accession no. M79450 and Y11024. A pairwise Blast of both sequences allowed identification of 10 sites of putative variations (**Figure 2**).

Different strains of the common *Rattus norvegicus*, such as Wistar, Wistar-Kyoto (WKY) and Sprague-Dawley, have been used to study complex traits, in an attempt to simulate behavior models of depression and anxiety. Some of these traits seem to be genetically inherited and sex dependent (Solbeg et al. 2003).

Curiously, there are also strain differences in the behavioral effects induced by antidepressant such as Fluoxetine (Lopez-Rubalcava et al 1999). However, there is few data about the intra-strain variability.

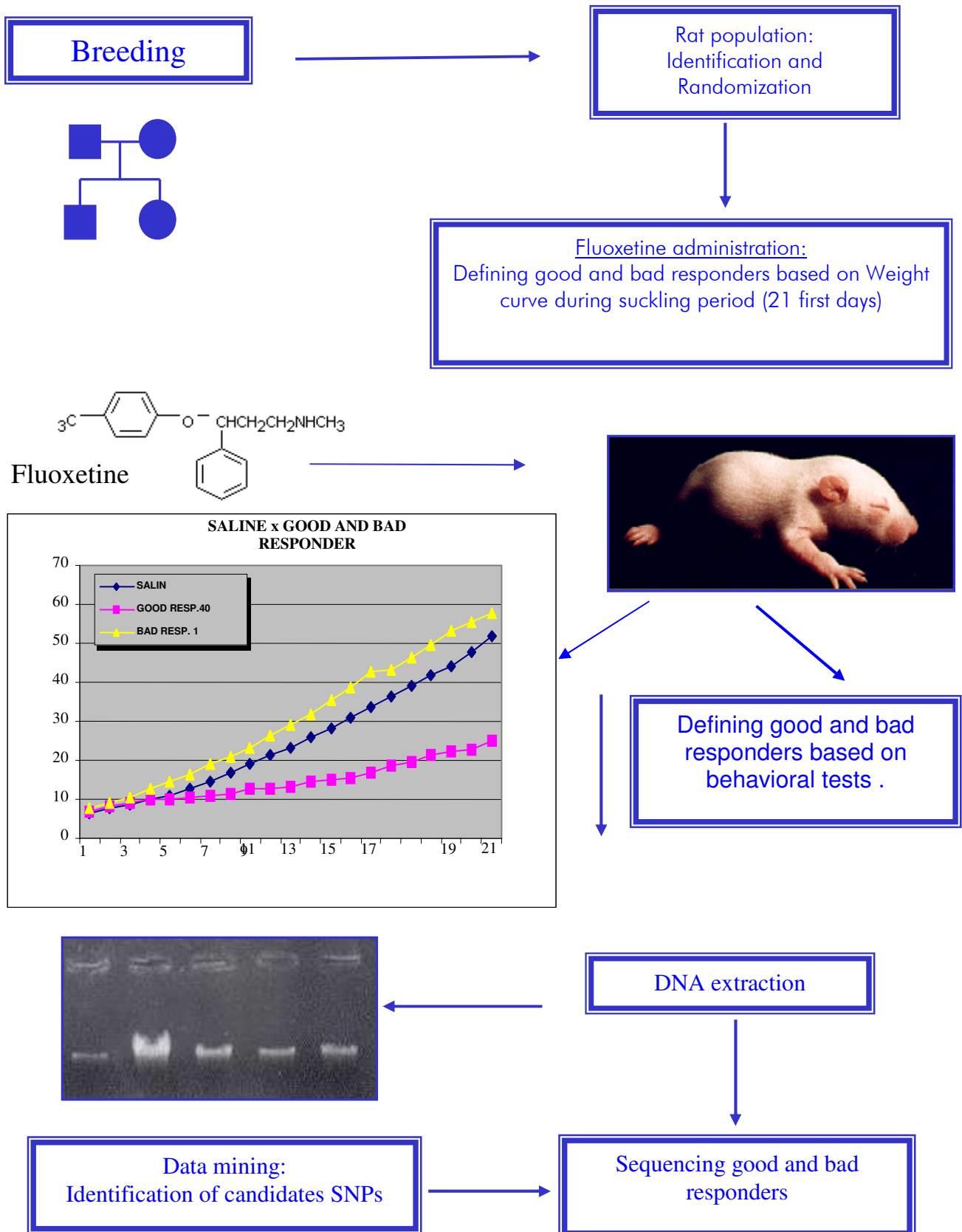
In order to identify candidate genes responsible for this finding we searched various data bases trying to identify human genes already involved with weight gain based on the work of Irizarry et al (2001) whom studied genes involved with weight gain.

Around 70 genes were identified in humans. Latter we searched for homologues in Rats to build a panel of candidates to search for Single nucleotide Polymorphisms (SNPs) and eventually identify potential genetics variants relevant for the female response to antidepressants (**Table 1**).

The next step of this work is to compare the sequence of the 5-HTT gene and other genes that might explain these phenotype difference between the animals with extreme response to fluoxetine treatment and in sets of sibships. Also the analysis of the weight gain curve in a bigger sample including the analysis of behavior tests such as exploratory activity and forced swimming test. The final goal is to identify genetic polymorphisms responsible for the variability of drug response among sibships of animals.

The neonatal antidepressant treatment was recently considered a good model for predisposition depression (Willner et al., 2002). However, considering the difficulties comparing animal behavioral response to human depression we suggest that our protocol might be useful as a model for the search of genes involved in Fluoxetine response during period of rapid brain development, rather than a model to human affective disorder.

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**Figure 1.** The steps involved at the proposed pharmacogenetic protocol for Fluoxetine.

M79450 gcaggccccagcctcctctcatcacgtatgcagaggcaatagccaacatgccagcatcc 1260  
Y11024 gcaggccccagcctcctctcatcacgtatgcagaggcaatag**g**caacatgccagcatcc 1491

M79450 acgtctttgccatcatctctctcatgttaatacacgtgggattggacagcacgttc 1320  
Y11024 acgtctttgccatcatctctctcatgttaatacacgtgggattggacagcacgttc 1551

M79450 gcaggcctggaaggtgtgatcacagctgtgctggatgagttccctcacatctgggccaag 1380  
Y11024 gcaggcctggaaggtgtgatcacagctgtgctggatgagttccctcacatctgggccaag 1611

M79450 cgcaggggaatggtcgtgctcatcgtggtcatcacgtgcgtctgggatccctgctcaca 1440  
Y11024 cgcaggggaatggtcgtgctcatcgtggtcatcacgtgcgtctgggatccctgctcaca 1671

M79450 ctgacgtcaggaggggcatacgtggtgactctgctggaggagtatgccacggggccagca 1500  
Y11024 ctgacgtcaggaggggcatacgtggtgactctgctggaggagtatgccacggggccagca 1731

M79450 gtgctaccgtggccctcatcgaggccgtcgcctgtcttggtctatggaatcactcag 1560  
Y11024 gtgctaccgtggccctcatcgaggccgtcgcctgtcttggtctatggaatcactcag 1791

M79450 ttctgcagcgtatgaaggagatgctgggtcagcccgggatggtttggaggatctgc 1620  
Y11024 ttctgcagcgtatgaaggagatgctgggtcag-- cgggatgg-ttggaggatctgc 1848

M79450 tgggtggccatcagccctctgtttctctgttcatcattgcagtttctgatgagccca 1680  
Y11024 tgggtggccatcagccctctgtttctctgttcatcattgcagtttctgatgagccca 1908

M79450 cccagctacggctttccaatacaactatccccactggagtatcgtctgggctactgc 1740  
Y11024 cccagctacggctttccaatacaactatccccactggagtatcgtctgggctactgc 1968

M79450 atagggatgtcgtccgtcatctgcacccctacctatatcattatcggtgatcagcact 1800  
Y11024 atagggatgtcgtccgtcatctgcacccctacctatatcattatcggtgatcagcact 2028

M79450 ccggggacactaaggagcgcattataaaagtatcactcctgaaacacccacagaaatc 1860  
Y11024 ccggggacactaaggagc**g**attataaaagtatcactcctgaaacacccacagaaat- 2087

M79450 ccgtgtggcgacatccgatgaatgctgtgtaacacacccctgggagaggacacctctcc 1920  
Y11024 ccgtgtgg-gacatccgatgaatgctgtgtaacacacccctgggagaggacacctctcc 2146

M79450 cagccacctctcagctctgaaaagccccactggactcctccccttaagccaagcctg 1980  
Y11024 cagccacctctcagctctgaaaag-cccactggactcct---ccttaag -caag- ctg 2201

**Figure 2.** The variations reported are from the deposit of two cDNA sequences available at the EMBL accession no.M79450 and Y11024. Blast of both sequences allowed to identify 610 putative variations, highlighted in red.

**Table 1.** Total combined SNPs from EST data and from public genomic sequencing (HGBASE, SNP Consortium), for the obesity candidate gene

Gene	Title	Location Hommo Sapiens	Location Rat
<i>ACPI</i>	acid phosphatase 1	2p25	6q16
<i>ADA</i>	adenosine deaminase	20q12-q13.11	3q42
<i>ADRA2A</i>	adrenergic, alpha-2A-, receptor	10q25	1 [1618.40(cR3000)]
<i>ADRB2</i>	adrenergic, beta-2-, receptor	5q31-q32	18q12.1
<i>ADRB3</i>	adrenergic, beta-3-, receptor	8p12-p11.2	16q12.3
<i>AGT</i>	Angiotensinogen	1q42-q43	19q12
<i>AK1</i>	adenylate kinase-1	9q34.1	3 [60.70(cR3000)]
<i>APOA4</i>	apolipoprotein A-IV	11q23-q23	8q22
<i>APOB</i>	apolipoprotein B	2p24-p23	6q14
<i>APOD</i>	apolipoprotein D	3q27-qter	11q11
<i>ASIP</i>	agouti signaling protein	20q11.2-q12	3q42
<i>ATP1A1</i>	ATPase, Na/Ktransporting, alpha 1	1p13	2q34
<i>ATP1A2</i>	ATPase, Na/Ktransporting, alpha 2	1q21-q23	13q24q26
<i>ATP1B1</i>	ATPase, Na/Ktransporting, beta 1	1q22-q25	13q23
<i>BF</i>	Factor, properdin	6p21.3	20q12
<i>CBFA2T1</i>	Core-binding factor, alpha unit 2 (MTG8)	8q22	5q13
<i>CCKBR</i>	cholecystokinin B receptor	11p15.4-p15.4	1q33
<i>CD36L1</i>	CD36 antigen (collagen type I receptor, thrombospondinreceptor)-like	12pter-qter	12q15-q16
<i>CPE</i>	Carboxypeptidase E	4q32	16p13
<i>DCP1 (ACE)</i>	Dipeptidyl carboxypeptidase 1 (angiotensin 1 converting enzyme, ACE)	17q23	10q32.1
<i>DRD2</i>	dopamine receptor D2	11q22.2-q22	8q24
<i>ESD</i>	esterase D	13q14.1-q14	15q11
<i>FABP2</i>	fatty acid binding protein 2	4q28-q31	2q42
<i>FGF13</i>	Fibroblast Growth Factor 13	Xq26	Xq36
<i>GCGR</i>	glucagon receptor	17q25	10q32.3
<i>GCK</i>	glucokinase	7p15-p13	14q21
<i>GLO1</i>	glyoxalase	6p21.2-21.1	20p12
<i>GNAS1</i>	guanine nucleotide binding protein, alpha stimulating activity polypeptide 1	20q13.2-q13.3	3q42
<i>GNB3</i>	guanine nucleotide binding protein (G protein), beta polypeptide 3	12p13-p13	4q42
<i>GPC3</i>	Heparan Sulfate Proteoglycan (Glypican) 3	Xq26.1-q26.1	Xq36

Gene	Title	Location Hommo Sapiens	Location Rat
<i>GPC4</i>	Heparan Sulfate Proteoglycan (Glypican) 4	Xq26–q26.1	Xq36
<i>GYP A</i>	glycophorin (includes MN blood group)	4q28–q31	
<i>GYS1</i>	glycogen synthase 1 (muscle)	19q13.3	
<i>HSD3B1</i>	hydroxy-delta-5-steroid dehydrogenase, 3 beta	1p13.1	2q34
<i>HTR2C</i>	5-hydroxytryptamine (serotonin) receptor 2C	Xq24	Xq34-q35.1
<i>IGF1</i>	insulin-like growth factor I	12q22–q23	7q12-q13
<i>IGF2</i>	insulin-like growth factor II	11p15.5	1q41
<i>INS</i>	insulin	11p15.5	1q41
<i>INSR</i>	insulin receptor	19p13.3	12q12
<i>IRS1</i>	insulin receptor substrate 1	2q36–q36	9q34
<i>ISL1</i>	ISL1 transcription factor, LIM/homeodomain (islet-1)	5q22.3	2q15
<i>KEL</i>	Kell blood group	7q33	4q22
<i>LDLR</i>	low-density lipoprotein receptor	19p13.2	8q13
<i>LEP</i>	leptin .	7q31–q32	4q22
<i>LEPR</i>	leptin receptor	1p31	5q33
<i>LIPC</i>	lipase, hepatic	15q2–q22	8q24
<i>LIPE</i>	lipase, hormone sensitive	19q13.1–q13.2	1q21
<i>LPL</i>	lipoprotein lipase	8p22	16p14
<i>MC4R</i>	melanocortin 4 receptors	18q21.3	18q12.1
<i>MYO9A</i>	myosin IXA	15q21–q25	8q24
<i>NPY</i>	neuropeptide Y	7pter–q22	4q24
<i>NPY1R</i>	neuropeptide Y receptor Y1	4q31.3–q32	16p14
<i>ORM1</i>	orosomucoid	9q32	5q24
<i>PCSK1</i>	protein convertase subtilisin/kexin type	15q15–q21	2q11-q12
<i>PGD</i>	phosphogluconate dehydrogenase	1p36.2–36.13	5q36
<i>PGM1</i>	phosphoglucomutase	11p31	5q33
<i>PMM2</i>	phosphomannomutase 2	16p13	10q12
<i>POMC</i>	proopiomelanocortin	2p23	6q14
<i>PON2</i>	paraoxonase 2	7q21.3–q21.3	4q13
<i>PPARG</i>	peroxisome proliferator-activated receptor, gamma	3p25	4q42
<i>SLC2A1</i>	solute carrier family 2 (facilitated glucose transporter), member 1	1p35–p31.3	5q36.1
<i>SLC2A4</i>	solute carrier family 2 (facilitated glucose transporter), member 4	17p13	10q24
<i>SNRPN</i>	small nuclear ribonucleoprotein polypeptide N	15q11–q12	1q22
<i>THRB</i>	thyroid hormone receptor, beta	3p24.1–p22	15p16
<i>TUB</i>	Tubby (mouse) homologue	11p15.5	1q
<i>UCP1</i>	uncoupling protein 1	4q28–q31	19p11-q11
<i>UCP2</i>	uncoupling protein 2	11q13	1q32
<i>UCP3</i>	uncoupling protein 3	11q13	1q32

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