

Table of Contents

Eidesstattliche Erklärung – Statement under Oath	
Disclaimer	
Dedication	
Acknowledgement	i
Table of Contents	vi
Index of Figures	xviii
Index of Tables	xxii
Acronyms & Abbreviations	xxiv
Summary	1
Zusammenfassung	3
I. Introduction	7
A. Mental retardation	7
A.1. Definition of mental retardation	7
A.2. Costs of mental retardation	8
A.3. Aetiology of mental retardation	8
A.3.1. General considerations	8
A.3.2. X-linked mental retardation	10
A.3.2.1. Biology and DNA sequence of the X chromosome	10
A.3.2.2. X chromosome inactivation	14
A.3.2.2.1. The X chromosome inactivation hypothesis	14
A.3.2.2.2. X chromosome inactivation in X;autosome translocations	15
A.3.2.3. Evidence for X-linked mental retardation from classical genetics	17
A.3.2.4. Clinical variability of X-linked mental retardation	18
A.3.2.5. Heterogeneity of X-linked mental retardation	19
A.4. Molecular mechanisms underlying cognitive (dys)function	23
A.4.1. A note of caution	24
A.4.2. The ‘structural’ and the ‘functioning’ brain	28

A.4.3. Remodelling of the Actin cytoskeleton and dendrite outgrowth in mental retardation	29
A.4.3.1. General overview	29
A.4.3.2. Mental retardation gene products regulate remodelling of the Actin cytoskeleton	32
A.4.4. Chromatin remodelling and regulation of transcription in mental retardation	36
A.4.4.1. General overview	37
A.4.4.2. Mental retardation gene products regulate chromatin remodelling and transcription	40
A.4.5. Synaptic function in mental retardation	44
A.4.5.1. General overview	44
A.4.5.2. X-linked mental retardation gene products regulate synaptic function	45
A.4.6. The Ubiquitin – proteasome pathway in neurodegenerative disease	50
A.4.6.1. General aspects of the Ubiquitin – proteasome pathway	50
A.4.6.2. Overview of the Ubiquitin – proteasome pathway	51
A.4.6.3. The E3 Ubiquitin ligase complexes	52
A.4.6.4. The SCF E3 Ubiquitin ligase complex	53
A.4.6.5. The Ubiquitin – proteasome pathway in human (neurological) disease	56
B. Identification and confirmation of candidate disease genes	57
B.1. Identification methodology for Mendelian disease genes	57
B.1.1. Functional cloning	57
B.1.2. Candidate gene approaches	57
B.1.3. Positional cloning	58
B.1.3.1. Linkage analysis	58
B.1.3.2. Loss of heterozygosity mapping	61
B.1.3.3. Characterisation of disease-associated chromosomal rearrangements	62
B.1.3.3.1. Deletions and insertions	62
B.1.3.3.2. Inversions	62
B.1.3.3.3. Translocations	63
B.2. Confirmation of candidate disease genes	64
B.2.1. Types of pathogenic changes in human disorders	64
B.2.2. Mutation screening	65
B.2.3. Types of mutation screening	68
B.2.3.1. Single-strand conformation polymorphism analysis	68

B.2.3.2. Denaturing high-performance liquid chromatography	68
B.2.3.3. Restriction site polymorphism polymerase chain reaction	69
B.2.3.4. Dideoxynucleotide DNA sequencing	69
B.2.3.5. Southern hybridisation	69
C. Objectives	69
II. Materials & Methods	73
IIA. Materials	73
A. Chemicals and chemical compounds	73
B. Reagents	76
B.1. Biological reagents	76
B.1.1. General	76
B.1.2. RNA/DNA	76
B.1.3. Protein	77
B.1.3.1. General	77
B.1.3.2. Antibodies and immunoglobulins	78
B.1.3.3. Enzymes	81
B.1.3.3.1. General	81
B.1.3.3.2. Restriction endonucleases	82
B.2. Non-biological reagents	82
C. Vectors & Plasmids	84
C.1. Vectors	84
C.2. Plasmids	85
D. Buffers and solutions	86
E. Cell culture	90
E.1. General reagents	90
E.2. Media	90
F. Cells	92
F.1. Mammalian cell lines	92
F.2. Bacterial and yeast strains	92
G. Molecular biology kits	93
H. Materials, plastic- & glassware and disposables	94
I. Laboratory equipment and instruments	97
J. Software, algorithms and databases	100

J.1. Software packages & programs	100
J.2. Algorithms	102
J.3. Databases	104
IIB. Methods	107
A. Experimental procedures	107
A.1. Isolation	107
A.1.1. DNA	107
A.1.1.1. Isolation of DNA from Epstein-Barr virus-transformed lymphocytes	107
A.1.1.2. Isolation of DNA from blood	107
A.1.1.3. Isolation of high-purity plasmid DNA from bacterial cells	108
A.1.1.4. Isolation of ultra pure plasmid DNA from bacterial cells	108
A.1.2. RNA	109
A.1.2.1. Isolation of total RNA from cell lines	109
A.1.2.2. Isolation of total RNA from mouse tissue	109
A.1.2.3. Isolation of poly-A ⁺ RNA from total RNA	109
A.1.3. Protein	109
A.1.3.1. Isolation of total protein from mammalian cells	109
A.1.3.2. Isolation of his-tagged proteins from mammalian cells	110
A.2. Separation	111
A.2.1. Agarose gel electrophoresis	111
A.2.2. Poly-acrylamide gel electrophoresis	111
A.2.2.1. General poly-acrylamide gel electrophoresis	111
A.2.2.2. Single-strand conformation polymorphism analysis	111
A.2.3. High-throughput denaturing high-performance liquid chromatography	112
A.3. Amplification	112
A.3.1. Polymerase chain reaction	112
A.3.2. Reverse transcription polymerase chain reaction	113
A.3.3. Semi-quantitative reverse transcription polymerase chain reaction	113
A.3.4. Rapid amplification of complementary DNA ends	114
A.3.5. Suppression polymerase chain reaction	114
A.3.6. <i>In vitro</i> mutagenesis	115
A.3.7. DNA sequencing	115
A.4. Hybridisation	116
A.4.1. Fluorescent <i>in situ</i> hybridisation	116

A.4.2. Mouse <i>in situ</i> hybridisation	117
A.4.2.1. Mouse <i>in situ</i> hybridisation – $^{33}\alpha[P]UTP$	117
A.4.2.2. Mouse <i>in situ</i> hybridisation – $^{35}\alpha[S]UTP$	120
A.4.3. Southern hybridisation	122
A.4.4. Northern hybridisation	123
A.4.5. Complementary DNA library screening	123
A.4.6. Western hybridisation	123
A.4.7. Serum test on peptide dot blot	124
A.5. Molecular biology	125
A.5.1. DNA restriction	125
A.5.2. DNA Ligation	125
A.5.3. Transformation	125
A.5.4. Immunoprecipitation	126
A.5.5. Chemical cross-linking of proteins	126
A.5.6. Raising an α -hKIAA1202 antibody	127
A.5.7. Automated yeast two-hybrid screening	128
A.6. Cell biological methods	128
A.6.1. Mammalian cell culture	128
A.6.2. Transfection	129
A.6.3. Fluorescence & immunofluorescence	130
A.6.3.1. Proteins tagged with enhanced green fluorescent protein	130
A.6.3.2. V5-tagged and endogenous proteins	130
A.6.4. Cell differentiation	131
A.6.5. Stable transfection of U373 MG cells	132
A.6.6. Microscopy	132
A.6.6.1. Bright field, dark field, phase contrast and epifluorescence microscopy	132
A.6.6.2. Confocal microscopy	132
A.6.6.3. Electron microscopy	133
B. Clinical procedures	133
B.1. 46,X,t(X;8)(p11.2;p22.3) patient characterisation	133
B.2. X-linked mental retardation patient cohort, control panels and <i>hKIAA1202</i> mutation screening	134
C. Computational procedures	134

C.1. Bio-informatics	134
C.1.1. Molecular biological software	134
C.1.2. Prediction algorithms	134
C.1.2.1. Exon and gene prediction algorithms	134
C.1.2.2. General protein sequence analysis	135
C.1.2.3. Transmembrane prediction	135
C.1.2.4. Automated structural homology modelling	135
C.1.3. Sequence alignment and phylogenetic analysis	135
C.1.3.1. Pairwise alignment	135
C.1.3.2. Multiple alignment	135
C.1.4. Databases and nomenclature	136
C.2. Image acquisition, analysis and processing software	136
III. Results	139
A. Patient with mental retardation and a <i>de novo</i> 46,X,t(X;8)(p11.2;p22.3) balanced translocation	139
A.1. Clinical characterisation	139
A.2. Karyotyping	140
A.3. Cytogenetic, molecular and computational analyses	140
A.3.1. X-chromosomal breakpoint cloning	141
A.3.1.1. Fluorescent <i>in situ</i> hybridisation on chromosome X	142
A.3.1.2. Southern hybridisation on chromosome X	144
A.3.1.3. Suppression polymerase chain reaction	145
A.3.2. Chromosome 8 breakpoint cloning	146
A.3.2.1. Fluorescent <i>in situ</i> hybridisation on chromosome 8	146
A.3.2.2. Southern hybridisation on chromosome 8	146
A.3.2.3. Breakpoint-spanning polymerase chain reaction	147
A.4. Computational analysis	148
B. Molecular and computational characterisation of <i>Fbxo25</i>	150
B.1. Nucleic acid studies	150
B.1.1. Genomic organisation of <i>Fbxo25</i>	150
B.1.1.1. Genomic organisation of <i>hFBXO25</i>	150
B.1.1.2. Genomic organisation of <i>mFbxo25</i>	153
B.1.2. Expression analysis of <i>Fbxo25</i>	154

B.1.2.1. Expression analysis of <i>hFBXO25</i>	154
B.1.2.2. Expression analysis of <i>mFbxo25</i>	155
B.1.2.2.1. Northern hybridisation	155
B.1.2.2.2. RNA <i>in situ</i> hybridisation	155
B.2. Computational studies	160
B.2.1. Identification of hFBXO25 homologues	160
B.2.2. Analysis of F-box sequences	160
B.3. Protein studies	164
B.3.1. Cloning of the hFBXO25 open reading frame	164
B.3.2. Subcellular localisation of over-expressed hFBXO25	165
B.3.3. Investigation of hFBXO25 interactions	165
B.3.3.1. Co-immunoprecipitation experiments	165
B.3.3.2. E3 ligase activity assay	167
B.3.3.3. <i>In vitro</i> mutagenesis	167
C. Molecular and computational characterisation of <i>Kiaa1202</i>	170
C.1. Nucleic acid studies	170
C.1.1. Genomic organisation of <i>Kiaa1202</i>	170
C.1.1.1. Genomic organisation of <i>hKIAA1202</i>	170
C.1.1.2. Genomic organisation of <i>mKiaa1202</i>	178
C.1.2. Expression analysis of <i>Kiaa1202</i>	179
C.1.2.1. Expression analysis of <i>hKIAA1202</i>	179
C.1.2.2. Expression analysis of mouse and zebrafish <i>Kiaa1202</i>	180
C.1.3. Mutation screening of <i>hKIAA1202</i>	183
C.1.3.1. Single-nucleotide exchanges	183
C.1.3.2. Genomic rearrangements	185
C.1.3.3. Variable repeats	187
C.1.3.4. Attempt to generate an <i>mKiaa1202</i> knock-out	188
C.2. Computational studies	189
C.2.1. Identification of hKIAA1202 homologues	189
C.2.2. Analysis of protein organisation	191
C.3. Protein studies	195
C.3.1. Cloning of the hKIAA1202 open reading frame	195
C.3.2. Generation and characterisation of an α -hKIAA1202 antibody	199

C.3.2.1. Generation of an α -hKIAA1202 antibody	199
C.3.2.2. Characterisation of the α -hKIAA1202 antibody	200
C.3.3. Subcellular localisation of Kiaa1202	203
C.3.3.1. Subcellular localisation of over-expressed hKIAA1202-V5	203
C.3.3.2. Testing of the α -hKIAA1202 antibody for use in immunofluorescence	206
C.3.3.3. Subcellular localisation of endogenous Kiaa1202	206
C.3.4. Investigation of putative hKIAA1202 interactions	210
C.3.4.1. Generation of a cell line stably expressing hKIAA1202-V5	210
C.3.4.2. Putative interaction between Kiaa1202 and filamentous Actin	211
C.3.4.2.1. Co-localisation studies of over-expressed hKIAA1202-V5 and filamentous Actin	212
C.3.4.2.2. Co-localisation studies of endogenous Kiaa1202 and filamentous Actin	212
C.3.4.2.3. Mitochondrial outer membrane recruitment assay	215
C.3.4.2.4. Co-immunoprecipitation experiments with hKIAA1202 and filamentous Actin	218
C.3.4.3. Putative hKIAA1202 interactions identified by yeast two-hybrid screening	219
C.3.4.3.1. Rationale and overview of the yeast two-hybrid system	220
C.3.4.3.2. Cloning of hKIAA1202 constructs for use in yeast two-hybrid	221
C.3.4.3.3. Auto-activation and homo-oligomerisation	222
C.3.4.3.4. Putative Kiaa1202 – Vimentin interaction	225
IV. Discussion	233
A. Current state of knowledge	233
B. Hypothesis: the t(X;8) translocation probably causes the patient's mental retardation	235
C. It is highly unlikely that the idiopathic seizures of the t(X;8) patient correlate with her mild mental retardation	236
D. <i>hFBXO25</i> encodes an F-box protein and hKIAA1202 associates with the Actin cytoskeleton	241
D.1. Brain-expressed <i>hFBXO25</i> encodes an F-box protein, which is part of the SCF ^{<i>hFBXO25</i>} E3 Ubiquitin ligase	241

D.2. Brain-expressed hKIAA1202 colocalises with filamentous Actin at cellular sites of rapid Actin remodelling and is able to direct its subcellular distribution	244
E. The Ubiquitin – proteasome pathway is important in brain function, but involvement of hFBXO25 in mental retardation is unlikely	247
E.1. The Ubiquitin – proteasome pathway in neurodegenerative disease and mental retardation	247
E.2. The Ubiquitin – proteasome pathway in synaptic function and mental retardation	249
E.3. In pathology, F-box proteins are mainly linked to malignancies	252
E.4. Involvement of hFBXO25 in mental retardation is unlikely	253
F. Several <i>hKIAA1202</i> patient-specific variants, its putative interaction partners, including Actin, and its domain structure suggest a function for it in human cognition	255
F.1. Patient-specific <i>hKIAA1202</i> variants	255
F.2. Several putative hKIAA1202 binding partners link hKIAA1202 to possible mental retardation pathways	263
F.3. hKIAA1202's domain structure and its interaction with filamentous Actin are compatible with a function in cognition	266
G. Conclusion: <i>hKIAA1202</i> is a candidate gene for X-linked mental retardation	270
V. Outlook	275
A. Further confirmation of <i>hKIAA1202</i> as a gene involved in cognition	275
B. Biochemical interactions	276
C. Cell biological & genetic interactions	278
VI. Epilogue – X-linked mental retardation. Is it really on the X?	283
VII. Commentary – Should we place less importance on intelligence?	291
VIII. Appendix A – Intelligence quotient: definition, evaluation and problems as a measure of ‘intelligence’	297
A. Definition and testing of the intelligence quotient	297
B. Problems with testing the intelligence quotient as a measure of general intelligence	297
B.1. Problems of definition	298
B.2. Practical problems intrinsic to testing the intelligence quotient	298
B.3. Principle problems intrinsic to testing the intelligence quotient	298
IX. Appendix B – Molecular mechanism and pattern of X chromosome inactivation	301

A. Molecular mechanism of X chromosome inactivation	301
A.1. Counting of X chromosomes	301
A.2. Onset, establishment and maintenance of X chromosome inactivation	301
B. X chromosome inactivation pattern	302
B.1. Escape from X chromosome inactivation	302
B.2. Reactivation	303
B.3. Skewed X chromosome inactivation	303
B.4. Tissue specificity of the X chromosome inactivation pattern	304
B.5. Variation of the X chromosome inactivation pattern within the population	304
B.6. Variability of the X chromosome inactivation pattern with age	304
X. Appendix C – From the principle of hereditary to the molecular elucidation of inborn errors of metabolism	307
A. Heredity	307
B. Classical genetics	307
B.1. Gregor Mendel and the garden pea	307
B.2. Thomas Morgan and the fruit fly	308
B.3. George Beadle, Edward Tatum and bread mould	310
C. Molecular genetics	310
C.1. The chemistry of life	311
C.2. The molecular nature of heredity	312
C.3. The structural basis of heredity	313
C.4. Human molecular genetics	314
XI. Appendix D – Mutations in human pathology	315
A. Localised mutations	315
A.1. Base substitutions	315
A.1.1. Silent mutations	315
A.1.2. Missense mutations	315
A.1.3. Nonsense mutations	316
A.2. Insertions and deletions	316
A.3. Expansions of triplet repeats	316
A.4. Mutations affecting messenger RNA and transcription	323
A.4.1. Mutations of the poly-adenylation signal	323
A.4.2. Mutations affecting RNA splicing	323

A.4.3. Mutations in the untranslated regions	324
A.4.4. Mutations in regulatory sequences	324
A.5. Epigenetic mutations	325
A.5.1. Imprinting	325
A.5.2. Methylation at the promoter	325
A.6. Mitochondrial mutations	325
B. Global mutations	326
B.1. DNA transposition	326
B.2. Alteration of the chromosomal environment	326
B.3. Unequal cross-over and unequal sister chromatid exchange	326
B.4. Contiguous gene syndrome	327
B.4.1. X-chromosomal	327
B.4.2. Autosomal	327
B.5. Chromosomal aberrations	328
B.6. Numerical chromosomal abnormalities	328
B.6.1. Uniparental disomy	328
B.6.2. Aneuploidy	328
B.6.3. Polyploidy	329
XII. Appendix E – Polymerase chain reaction primers	331
XIII. Appendix F – Polymerase chain reaction cycling conditions	351
XIV. Appendix G – Denaturing high-performance liquid chromatography conditions	353
XV. Appendix H – Gene, mRNA and protein symbols	357
XVI. References	365
A. Textbooks	365
B. Primary literature, reviews & book chapters	365

