Table of Contents

I. List of Figures V

II. List of Tables VII

III. List of Abbreviations VIII

IV. Abstract XI

Chapter 1 - General introduction 1

1.1 The mammalian retina 2
   1.1.1 The outer nuclear layer and photoreceptor structure 3
   1.1.2 Subsequent layers and signal transmission 3

1.2 Inherited retinal degeneration 4
   1.2.1 Non-syndromic inherited retinal degeneration 5
   1.2.2 Syndromic inherited retinal degeneration 6

1.3 Animal models for retinal degeneration 7

1.4 Ccdc66 in retinal degeneration 8
   1.4.1 Ccdc66 gene and products in the mouse 9
       1.4.1.1 Ccdc66 RNA 9
       1.4.1.2 CCDC66 protein 9
       1.4.1.3 Localization 10
   1.4.2 The Ccdc66-deficient mouse – a retinal degeneration model 11

1.5 CCDC66 mutations in humans 12

1.6 Objectives 13

Chapter 2 - Functional investigation of Ccdc66 gene products in the mouse retina 16

2.1 Ccdc66 RNA and reporter gene expression 16
   2.1.1 Introduction 16
   2.1.2 Materials and methods 17
       2.1.2.1 Animals 17
       2.1.2.2 Tissue preparation 17
       2.1.2.3 Preparation of cryosections 17
       2.1.2.4 In situ hybridization 18
       2.1.2.5 X-gal staining (enzyme histochemistry) 20
   2.1.3 Results 21
       2.1.3.1 Ccdc66 RNA expression in the postnatal Ccdc66+/+ and Ccdc66−/− mouse retina 21
2.1.3.2 *Ccdc66* reporter gene expression in the postnatal *Ccdc66*−/− mouse retina and comparison to *Ccdc66* RNA expression (2.1.3.1) 22

2.1.3.3 *Ccdc66* reporter gene expression during postnatal retinal development of the *Ccdc66*−/− mouse retina 24

2.1.4 Discussion 28

2.1.4.1 Methodological aspects of *Ccdc66* RNA, reporter gene expression and protein CCDC66 in the mouse retina 28

2.1.4.2 Retinal processes in parallel with temporal and spatial *Ccdc66* reporter gene expression changes – functional association of *Ccdc66* expression 32

2.2 Interaction partners of protein CCDC66 in the mouse retina 34

2.2.1 Introduction 34

2.2.2 Materials and methods

2.2.2.1 Yeast strains 35

2.2.2.2 Generation of CCDC66 bait constructs 35

2.2.2.3 Chemical transformation of yeast cells 37

2.2.2.4 Auto activity test of baits and prey 38

2.2.2.5 Yeast two-hybrid screen 39

2.2.2.6 Confirmation of positive interaction 40

2.2.3 Results 41

2.2.3.1 Auto activity tests of the baits 41

2.2.3.2 Interaction partner candidates 42

2.2.3.3 Verification of the candidates open reading frame (ORF) 44

2.2.3.4 Auto activity test of the prey and validation of their positive interaction with CCDC66 constructs 46

2.2.4 Discussion 48

2.2.4.1 Mpdz and Eps8 – prime candidates to interact with CCDC66 49

2.2.4.2 ZnHit3, C21orf2, Ppdpf and Med4 – false positive interaction partners? 52

Chapter 3 - Functional investigation of *Ccdc66* gene products in the mouse brain 55

3.1 *Ccdc66* RNA and reporter gene expression in the mouse brain 55

3.1.1 Introduction 55

3.1.2 Materials and methods

3.1.2.1 Animals, tissues and preparation of cryosections 55

3.1.2.2 *In situ* hybridization 56

3.1.2.3 X-gal staining 56

3.1.3 Results 56

3.1.3.1 *Ccdc66* RNA expression in the postnatal *Ccdc66*+/+ and *Ccdc66*−/− mouse brain 56
3.1.3.2  *Ccde66* reporter gene expression in the postnatal *Ccde66*–/– mouse brain and comparison to *Ccde66* RNA expression 62

3.1.3.3  *Ccde66* reporter gene expression in the postnatal developing mouse brain 68

3.1.4  Discussion 78

3.1.4.1  *Ccde66* RNA, *Ccde66* reporter gene expression and protein CCDC66 in the mouse brain; on the role of potential short *Ccde66* transcripts and CCDC66 isoforms 78

3.1.4.2  *Ccde66* reporter gene expression in the mouse brain and parallelizing developmental processes – functional association of *Ccde66* expression 80

3.2  Degeneration in the *Ccde66*– mouse brain 85

3.2.1  Introduction 85

3.2.2  Material and methods 85

3.2.3  Results 86

3.2.3.1  Olfactory bulb 86

3.2.3.2  Brain ventricular ependyma 87

3.2.3.3  Hippocampus 87

3.2.4  Discussion 91

Chapter 4 - Compartment localization of protein CCDC66 in mouse retina and brain 93

4.1  Introduction 93

4.2  Materials and methods 93

4.2.1  Animals and tissues 93

4.2.2  Cell compartment fractionation and Western blot 94

4.2.3  Double immunofluorescence on neurospheres derived mixed neural cell culture 95

4.3  Results 97

4.3.1  Cell compartment fractionation 97

4.3.2  Co-localization of CCDC66 and neural marker proteins by double immune detection in a mixed neural cell culture 99

4.4  Discussion 101

Chapter 5 – Extra-retinal and extra-cerebral *Ccde66* reporter gene expression sites 104

5.1  Introduction 104

5.2  Materials and methods 104

5.2.1  Animals, tissues and preparation of cryosections 104

5.2.2  X-gal staining 104

5.3  Results 105
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.4 Discussion</td>
<td>110</td>
</tr>
<tr>
<td>Chapter 6 - General discussion</td>
<td>111</td>
</tr>
<tr>
<td>6.1 Summary of presented results</td>
<td>111</td>
</tr>
<tr>
<td>6.2 Concluding discussion and open questions</td>
<td>112</td>
</tr>
<tr>
<td>6.3 Conclusions</td>
<td>115</td>
</tr>
<tr>
<td>7. References</td>
<td>116</td>
</tr>
<tr>
<td>8. Appendices</td>
<td>137</td>
</tr>
<tr>
<td>8.1 Buffers and solutions</td>
<td>137</td>
</tr>
<tr>
<td>8.1.1 Chapter 2.1</td>
<td>137</td>
</tr>
<tr>
<td>8.1.2 Chapter 2.2</td>
<td>139</td>
</tr>
<tr>
<td>8.1.3 Chapter 3.2</td>
<td>140</td>
</tr>
<tr>
<td>8.1.4 Chapter 4</td>
<td>141</td>
</tr>
<tr>
<td>8.2 Curriculum vitae</td>
<td>143</td>
</tr>
<tr>
<td>8.3 List of publications</td>
<td>144</td>
</tr>
<tr>
<td>8.4 Acknowledgements</td>
<td>145</td>
</tr>
</tbody>
</table>