TABLE OF CONTENTS

DANKE! 2

ZUSAMMENFASSUNG 7

SUMMARY 9

1 INTRODUCTION 11

1.1 CRISPR/CAS SYSTEM 11

1.1.1 ADAPTIVE IMMUNE SYSTEM IN BACTERIA 11

1.1.2 IMPORTANCE OF PAM SEQUENCE 11

1.1.3 RNA-PROTEIN COMPLEX 12

1.1.4 APPLICATION FOR GENOME EDITING 13

1.1.5 SPECIFICITY 14

1.2 LIMB DEVELOPMENT 16

1.2.1 PATTERNING 17

1.2.2 MUSCLES AND NEURONS 18

1.2.3 CHONDROGENESIS 19

1.3 GENE REGULATION 20

1.3.1 PROMOTER 20

1.3.2 ENHANCER 20

1.3.3 INSULATOR 22

1.3.4 COMPARTMENTS 22

1.3.5 CHROMOSOME CONFORMATION CAPTURE 23

1.3.6 TOPOLOGICALLY ASSOCIATING DOMAINS 23

1.3.7 LOOPING 25

1.4 STRUCTURAL VARIATIONS 26

1.5 AIM 28

2 MATERIALS AND METHODS 29

2.1 MOUSE MODELS 29

2.1.1 CRISPR/CAS9 SYSTEM 29

2.1.2 FRT/FLPE SYSTEM 31

2.1.3 PRONUCLEAR INJECTIONS 32

2.2 EXPRESSION AND PHENOTYPE ANALYSIS 32

2.2.1 LacZ staining 32
2.2.2 Whole Mount In Situ Hybridisation 32
2.2.3 Skeletal staining 33
2.2.4 RNA-sequencing 33
2.3 Human samples 33
2.3.1 Skin biopsy 33
2.3.2 Genomic DNA 33
2.4 4C-sequencing 34
2.4.1 Library preparation 34
2.5 ChIP-seq data 39

3 RESULTS 40

3.1 Structural variations at the *EPHA4* locus lead to different human limb phenotypes 40
3.1.1 Identification of a novel type of preaxial brachydactyly caused by megabase-sized deletions 40
3.1.2 Deletions, inversions, and duplications associated with distinct limb malformations localise within *EPHA4* TAD. 41
3.2 Disease causing genes identification using mouse models 43
3.2.1 Generation of structural variations using CRISPR/Cas9 system 43
3.2.2 Creating a mouse model for Brachydactyly and F-syndrome 43
3.2.3 Expression analysis shows ectopic activation of *Pax3, Wnt6* and *Ihh* 45
3.2.4 Mouse model carrying a deletion develops brachydactyly 48
3.2.5 Pathogenic effect of *Pax3*, *Wnt6*, and *Ihh* when overexpressed in developing limb buds 50
3.3 Chromatin interaction profiles reveal gain of contact between disease causing genes and *Epha4* enhancer cluster 54
3.4 TAD boundaries play a pathomechanistic role in contact establishment between disease causing genes and *Epha4* enhancer 60

4 DISCUSSION 63

4.1 CRISPR/Cas9 system as a tool to model human disease 63
4.2 Recapitulation of human phenotypes 65
4.2.1 Mesodermal expression of *Pax3* and *Wnt6* inhibits chondrogenesis in the limb bud 65

4.3 A CLUSTER OF LIMB ENHANCERS IS RESPONSIBLE FOR ECTOPIC GENE ACTIVATION 67
4.3.1 IS ENHANCER ADOPTION A UNIVERSAL PATHOMECHANISM? 67
4.4 3D CHROMATIN ORGANISATION AND TADS 69
4.4.1 C-TECHNIQUES CAN BE USED IN HUMAN CELLS TO VISUALISE THE EFFECTS OF STRUCTURAL VARIATIONS 69
4.4.2 STRUCTURAL VARIANTS CAN CAUSE INTERACTION BETWEEN PREVIOUSLY ISOLATED TADS, LEADING TO DISEASE-RELEVANCE FOR BOUNDARY ELEMENTS 69
4.4.3 TOPOLOGICAL DOMAIN AS A UNIT FOR GENE REGULATION 72
4.4.4 PRODUCED REARRANGEMENTS ARE USEFUL FOR FUTURE CHROMATIN STRUCTURE STUDIES 73

5 REFERENCES 74

6 ABBREVIATIONS 82

7 SCIENTIFIC PUBLICATIONS 84

8 APPENDIX 85

8.1 EMBRYONIC STEM CELL CULTURE PROTOCOL 85