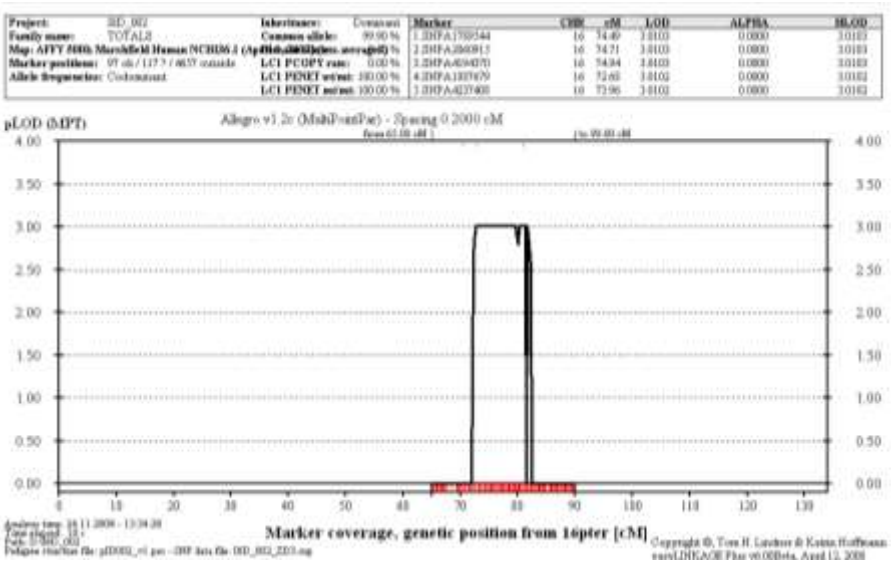
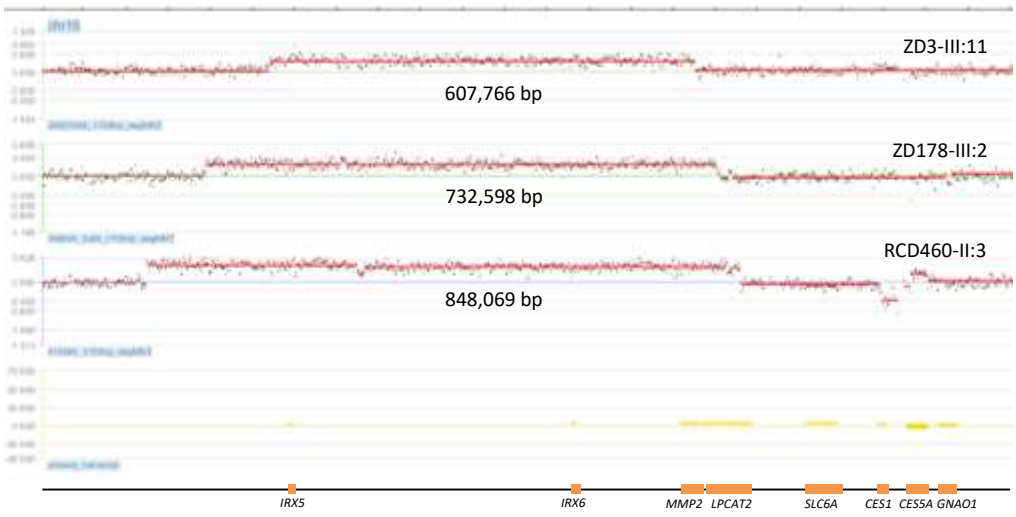
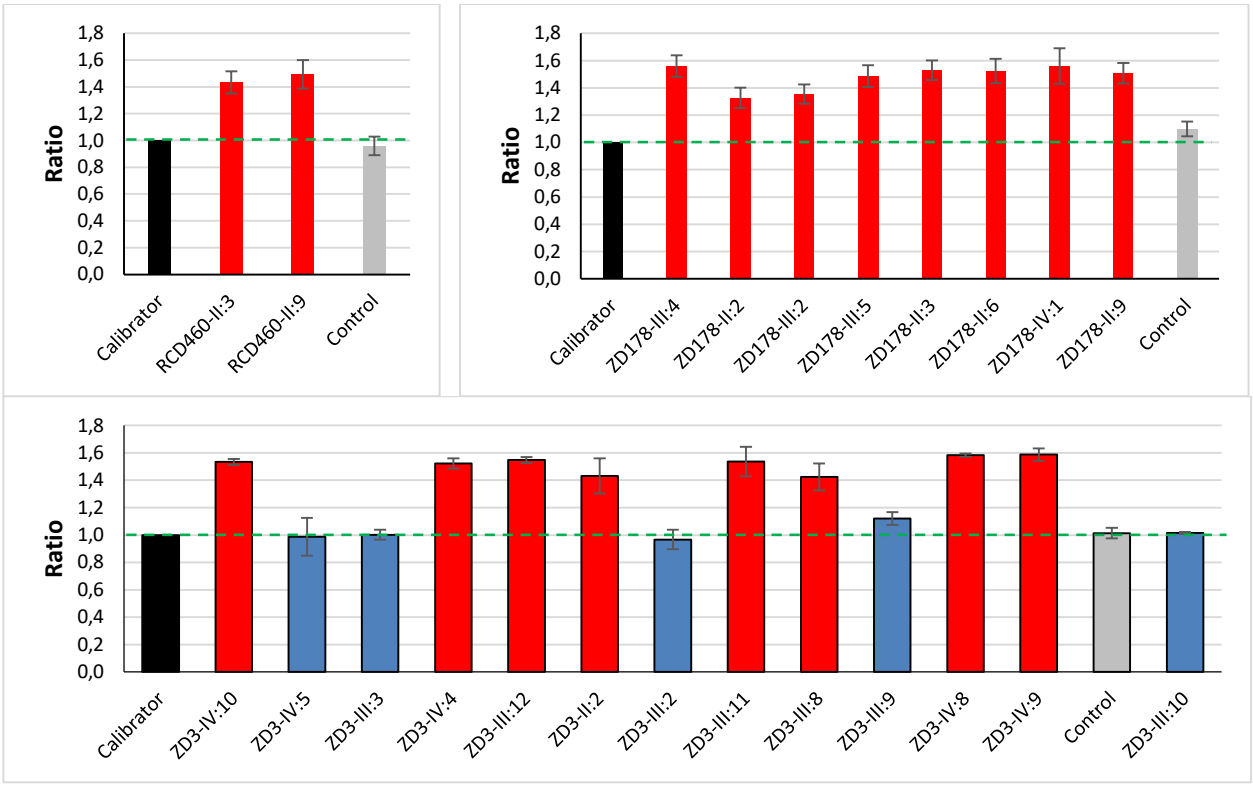
Supplementary Material and Methods



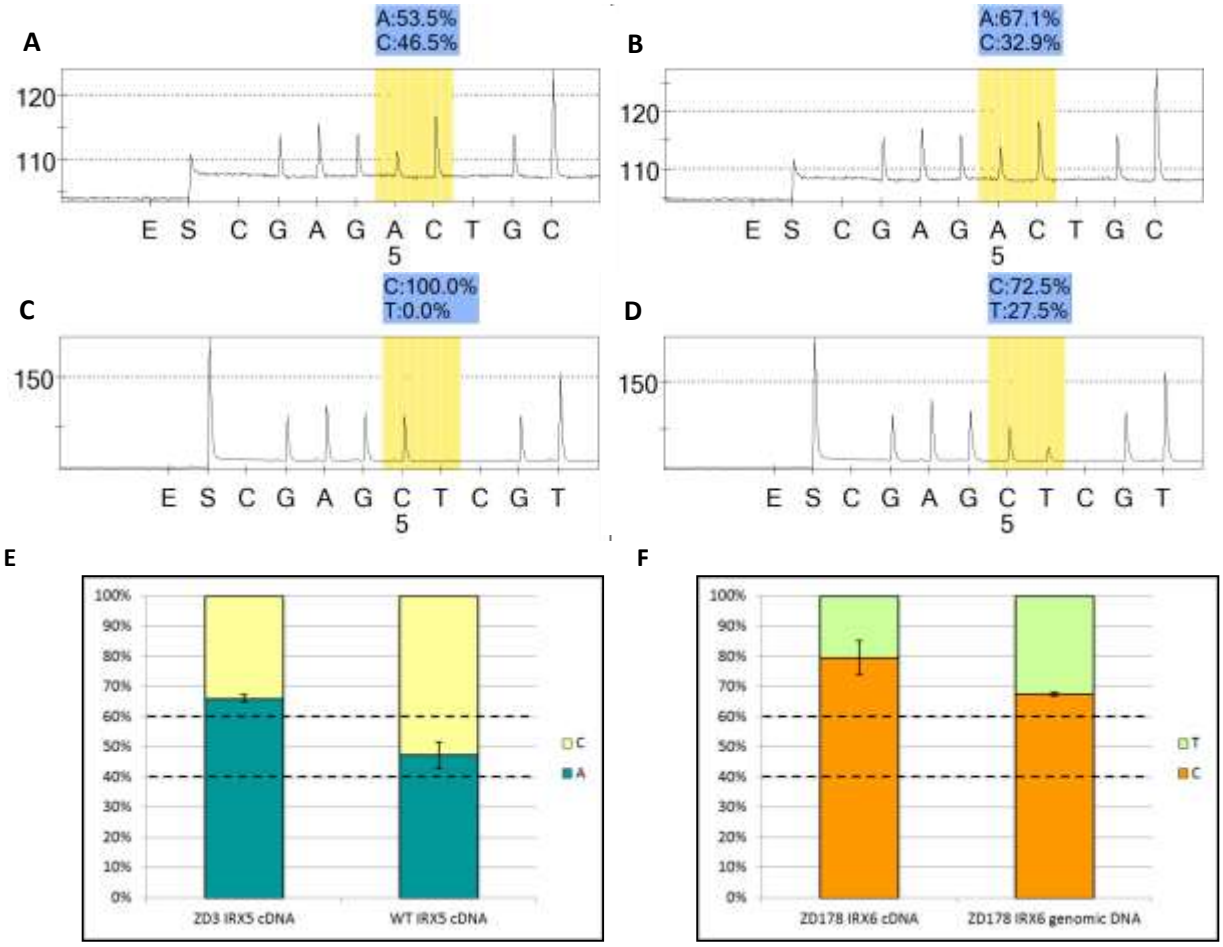
Supplementary Figure 1: **Parametric multipoint LOD score analysis applying ALLEGRO implemented in the easyLINKAGE package under a dominant model was applied (1-3).** A maximal and significant LOD score of 3.0103 was reached on chromosome 16. Recalculation for chromosome 16 only confirmed a disease-critical region of 9.77cM.



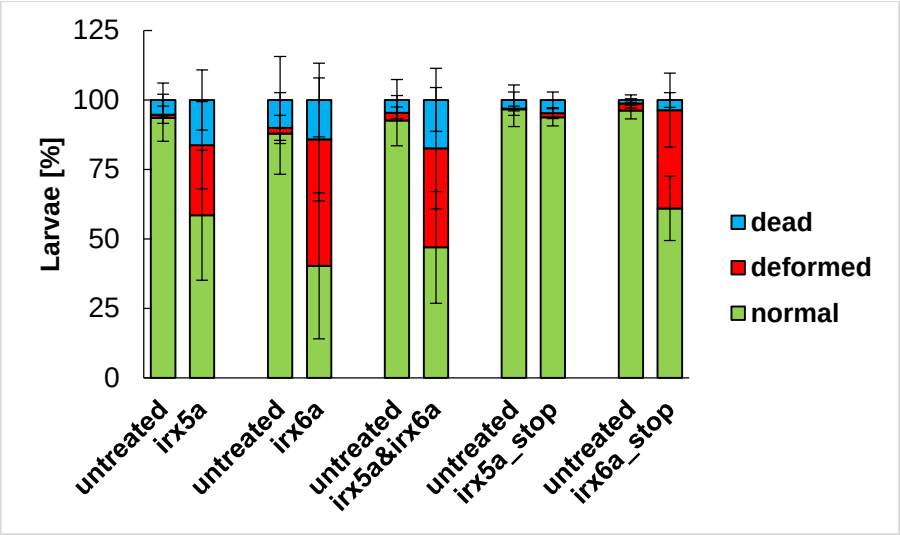
Supplementary Figure 2: **Graphical image of the SignalMap analysis for the ArrayCGH (NimbleGen Human CGH 385K chromosome 16 tiling array) for individuals ZD3-III:11, ZD178-III:2 and RCD460-II:3 affected by autosomal dominant cone dystrophy.** The genomic sizes of the duplications on chromosome 16q12.2 are indicated for each familial duplication as determined by breakpoint mapping. The genes located in or near the duplicated region are shown at the bottom.



Supplementary Figure 3: *IRX6* specific qPCR for segregation analysis in families RCD460, ZD178 and ZD3. The tested individual identifiers correspond to the pedigrees in Figure 1. The calibrator is set to 1.0. Values >1.0 are indicative for a duplication, values <1.0 for a deletion (green dashed line). The graphs show that all affected family members (red) have an increased ratio between reference gene *SDC4* and target gene *IRX6*, while all unaffected family members (blue) show a value of ~1.0. Calibrator (black) and Control (grey) samples from normal probands.



Supplementary Figure 4: Expression analysis of *IRX5* and *IRX6* transcripts out of patient fibroblasts indicate increased expression of *IRX5* and *IRX6* transcripts due to the duplication at the genomic level. (A-D) Pyrogrammes to assess allelic quantification out of patient versus normal proband fibroblast cDNA. (A) cDNA derived from a fibroblast cell line of a normal healthy proband heterozygous for SNP rs13336114 in *IRX5*. Both alleles A/C are expressed at about equal amounts. (B) cDNA generated from fibroblasts of individual ZD3-IV:8. The relationship between the normal C and the mutant (duplicated) *IRX5* A allele at rs13336114 was 70:30 – compatible with a duplicated state and a doubled expression of the *IRX5* A allele compared to the healthy control. (C) cDNA of a normal proband fibroblast sample homozygous C/C for SNP rs74412242 in *IRX6*. (D) cDNA from fibroblasts of individual ZD178-III:2. The relationship between the normal wildtype-associated T and mutant (duplicated) C allele was 70:30, and is in line with a doubled expression for the C allele. E: Enzyme mix, S: Substrate mix. (E,F) Quantitative comparison of *IRX5* and *IRX6* on cDNA and genomic DNA level. Per definition an allelic imbalance is existent if the relationship between two alleles is not 50%:50% – changes above and below 60:40% are considered significant, which corresponds to a difference factor of ≥1.5. The results are displayed as percentage of the C allele compared to the duplicated A allele of SNP rs13336114 in *IRX5*, and duplicated C versus T of SNP rs74412242 in *IRX6*, respectively. Mean value and standard deviation are outcome of at least two independent PCR experiments. (E) Pyrosequencing quantification for SNP rs13336114 in *IRX5* for ZD3-IV:8 and a normal proband showing an imbalanced (duplicated) and a normal ratio, respectively. (F) Pyrosequencing quantification for SNP rs74412242 in *IRX6* in sample ZD178-III:2 both in cDNA and genomic DNA. Consequently, also the quantification of the genomic sample resulted in an imbalanced (duplicated) ratio.



Supplementary Figure 5: Toxicity evaluation of *irx5a* and *irx6a* cRNA microinjections. Zebrafish oocytes were injected with 50pg *irx5a* and/or *irx6a, irx5a*$_{stop}$ or *irx6a*$_{stop}$ cRNA in the one-cell stage. At least three clutches from at least two independent experimental days were evaluated and a portion of eggs from each clutch was kept as untreated control. The fraction of dead, deformed or morphological normal larvae that developed were analyzed up to 5 dpf. For the different conditions studied, at last 47% of the treated larvae developed morphologically normal. Data are shown as mean ± SD.

Supplementary Table 1: Detailed ophthalmological findings in patients with a heterozygous duplication at the *IRXB* cluster.

(see separate Excel sheet)

Supplementary Table 2: Query of Ultraconserved Genomic Regulatory Blocks (UGRBs) that reside on chromosome 16 in the human genome (hg19 assembly). The UGRB related to the *IRXB* cluster is highlighted in grey. The genes affected by the duplications identified in our adCD families in this study and retrieved from UCNEbase (https://ccg.epfl.ch/UCNEbase/) are highlighted in yellow(4).

Cluster name	Cluster ID	Position	#UCNEs	Associated genes
TMEM114_cluster	212	chr16:7241448-8292920	8	*RBFOX1*; *TMEM114*
ZNF423_cluster	200	chr16:49623295-49736168	4	*ZNF423*
IRXB_cluster	201	chr16:50493248-55425289	60	*AKTIP*; *BRD7*; *CHD9*; ***CRNDE***; *CYLD*; *FTO*; ***IRX3***; ***IRX5***; ***IRX6***; *LOC100505619*; *LOC388276*; *LOC643714*; *LOC643802*; ***MMP2***; *NKD1*; *NOD2*; *RBL2*; *RPGRIP1L*; *SALL1*; *SNX20*; *TOX3*
ZFHX3_cluster	207	chr16:72407110-73979963	31	*HTA*; *LOC100506172*; *PMFBP1*; *PSMD7*; *ZFHX3*
WWOX_cluster	208	chr16:78210437-79382369	20	*MAF*; *WWOX*

Supplementary Table 3: Breakpoint mapping primers

	Primer	Primer sequence 5‘-3‘
Family ZD178	LPCAT2 Intron 6 for ZD178-BP-f	TTCTGTTCCAGTTGGGTACATTGTT
	IRX5 upstream for ZD178-BP-r	GGGTTTATCTCATTATCTGGGTTGC
Family RCD460	LPCAT2 Intron 11 for RCD460-BP-f	TGCATCAAAGGACACTAACAACAGA
	IRX5 upstream for RCD460-BP-r	ATATGGATTTCTGGCTTTCCCTCAA
Family ZD3 / ZD346	ZD346-BP-MMP2-f	CACCCTGCCCTCATGTAGTT
	ZD346-BP-IRX5up-r	GTCAGAGCCAGGAGATCAGG

Supplementary Table 4: Stimulus protocol optokinetic response measurement

	Stimulus time (sec)	Angular velocity (°/s)	Bars/ 360°	Contrast
Initiation condition	5	0	20	0.99
	5	7.5	20	0.99
	5	-7.5	20	0.99
Contrast sensitivity	8	7.5	20	1
	8	-7.5	20	1
	8	7.5	20	0.7
	8	-7.5	20	0.7
	8	7.5	20	0.4
	8	-7.5	20	0.4
	8	7.5	20	0.2
	8	-7.5	20	0.2
	8	7.5	20	0.1
	8	-7.5	20	0.1
	8	7.5	20	0.05
	8	-7.5	20	0.05
Control condition	3	7.5	20	0.99
	3	-7.5	20	0.99
Temporal frequency	8	5	20	1
	8	-5	20	1
	8	10	20	1
	8	-10	20	1
	8	15	20	1
	8	-15	20	1
	8	20	20	1
	8	-20	20	1
	8	25	20	1
	8	-25	20	1
	8	30	20	1
	8	-30	20	1
Control condition	3	7.5	20	0.99
	3	-7.5	20	0.99
Spatial frequency	8	7.5	7	0.7
	8	-7.5	7	0.7
	8	7.5	14	0.7
	8	-7.5	14	0.7
	8	7.5	21	0.7
	8	-7.5	21	0.7
	8	7.5	28	0.7
	8	-7.5	28	0.7
	8	7.5	42	0.7
	8	-7.5	42	0.7
	8	7.5	56	0.7
	8	-7.5	56	0.7

Supplementary Table 5: *P*-values for OKR analyses using different contrast stimuli. Two-way ANOVA with Bonferroni correction was performed to test significant differences between groups

	Contrast	*irx5a*	*irx6a*	*irx5a & irx6a*	$irx5a_{stop}$ & $irx6a_{stop}$
untreated	100%	0.75	<0.001	<0.001	0.858
	70%	1.00	<0.001	<0.001	1.000
	40%	1.00	0.028	<0.001	1.000
	20%	1.00	1.000	<0.001	1.000
	10%	1.00	1.000	0.002	1.000
	0.05%	1.00	1.000	1.000	1.000
irx5a	100%	-	<0.001	<0.001	1.000
	70%	-	0.008	<0.001	1.000
	40%	-	1.000	<0.001	1.000
	20%	-	1.000	<0.001	1.000
	10%	-	1.000	0.225	1.000
	0.05%	-	1.000	1.000	1.000
irx6a	100%	-	-	0.004	<0.001
	70%	-	-	<0.001	0.005
	40%	-	-	<0.001	0.380
	20%	-	-	<0.001	1.000
	10%	-	-	0.202	1.000
	0.05%	-	-	1.000	1.000
irx5a & irx6a	100%	-	-	-	<0.001
	70%	-	-	-	<0.001
	40%	-	-	-	<0.001
	20%	-	-	-	<0.001
	10%	-	-	-	0.692
	0.05%	-	-	-	1.000

Supplementary Table 6: *P*-values for OKR analyses using different spatial frequencies. Two-way ANOVA with Bonferroni correction was performed to test significant differences between groups.

	Spatial frequency	***irx5a***	***irx6a***	***irx5a & irx6a***	***irx5a$_{stop}$ & irx6a$_{stop}$***
untreated	0.02 cycles/°	0.087	0.002	<0.001	1000
	0.04 cycles/°	1.000	<0.001	<0.001	1.000
	0.06 cycles/°	1.000	<0.001	<0.001	0.823
	0.08 cycles/°	1.000	<0.001	<0.001	0.177
	0.12 cycles/°	1.000	0.444	1.000	1.000
	0.16 cycles/°	1.000	1.000	0.100	1.000
irx5a	0.02 cycles/°	-	1.000	<0.001	1.000
	0.04 cycles/°	-	<0.001	<0.001	1.000
	0.06 cycles/°	-	<0.001	<0.001	1.000
	0.08 cycles/°	-	0.001	<0.001	1.000
	0.12 cycles/°	-	1.000	1.000	1.000
	0.16 cycles/°	-	1.000	0.861	1.000
irx6a	0.02 cycles/°	-	-	0.001	0.772
	0.04 cycles/°	-	-	<0.001	<0.001
	0.06 cycles/°	-	-	<0.001	0.010
	0.08 cycles/°	-	-	0.532	0.069
	0.12 cycles/°	-	-	1.000	1.000
	0.16 cycles/°	-	-	0.161	1.000
irx5a & irx6a	0.02 cycles/°	-	-	-	<0.001
	0.04 cycles/°	-	-	-	<0.001
	0.06 cycles/°	-	-	-	<0.001
	0.08 cycles/°	-	-	-	<0.001
	0.12 cycles/°	-	-	-	1000
	0.16 cycles/°	-	-	-	0.013

Supplementary Table 7: *P*-values for OKR analyses using different temporal frequencies. Two-way ANOVA with Bonferroni correction was performed to test significant differences between groups.

	Temporal frequency	*irx5a*	*irx6a*	*irx5a & irx6a*	*irx5a$_{stop}$ & irx6a$_{stop}$*
untreated	0.3 cycles/sec	1.000	0.393	0.166	1000
	0.6 cycles/sec	1.000	<0.001	<0.001	1.000
	0.8 cycles/sec	1.000	<0.001	<0.001	0.600
	1.1 cycles/sec	1.000	<0.001	<0.001	0.016
	1.4 cycles/sec	1.000	<0.001	<0.001	0.040
	1.7 cycles/sec	1.000	<0.001	<0.001	0.016
irx5a	0.3 cycles/sec	-	0.662	0.343	1.000
	0.6 cycles/sec	-	<0.001	<0.001	0.923
	0.8 cycles/sec	-	<0.001	<0.001	0.431
	1.1 cycles/sec	-	<0.001	<0.001	0.080
	1.4 cycles/sec	-	<0.001	<0.001	0.057
	1.7 cycles/sec	-	<0.001	<0.001	0.086
irx6a	0.3 cycles/sec	-	-	1.000	1000
	0.6 cycles/sec	-	-	0.028	0.171
	0.8 cycles/sec	-	-	0.007	0.011
	1.1 cycles/sec	-	-	0.026	0.002
	1.4 cycles/sec	-	-	<0.001	0.430
	1.7 cycles/sec	-	-	0.207	0.251
irx5a & irx6a	0.3 cycles/sec	-	-	-	1000
	0.6 cycles/sec	-	-	-	<0.001
	0.8 cycles/sec	-	-	-	<0.001
	1.1 cycles/sec	-	-	-	<0.001
	1.4 cycles/sec	-	-	-	<0.001
	1.7 cycles/sec	-	-	-	<0.001

Reference Supplementary Material:

1. Gudbjartsson, D.F., Jonasson, K., Frigge, M.L. and KongA. (2000) Allegro, a new computer program for multipoint linkage analysis. *Nat. Genet*., **25**, 12-13.
2. Gudbjartsson, D.F., Thorvaldsson, T., Kong, A., Gunnarsson, G. and Ingolfsdottir A. (2005) Allegro version 2. *Nat. Genet*., **37**, 1015-1016.
3. Lindner TH and Hoffmann K. (2005) easyLINKAGE: a PERL script for easy and automated two-/multi-point linkage analyses. *Bioinformatics*., **21**, 405-407.
4. Dimitrieva, S. and Bucher, P. (2013) UCNEbase--a database of ultraconserved non-coding elements and genomic regulatory blocks. *Nucleic Acids Res*., **41** (Database issue), D101-109.