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Supplemental Data

X-linked Megalocornea Caused by Mutations in *CHRDL1* Identifies an Essential Role for Ventroptin in Anterior Segment Development

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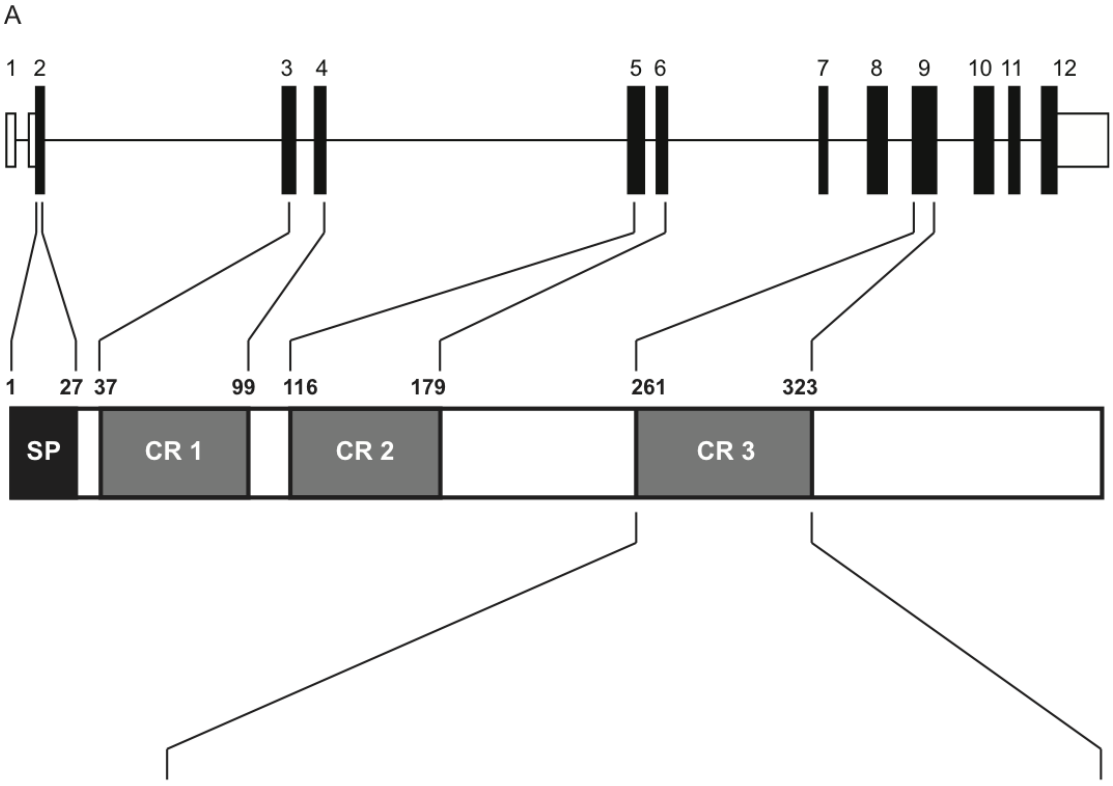

B

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VOPT CR3  CVSN-GKTYS-HGESWHPNLRAFG--IVECVLCTCNV--TKQECKKIHCPNRYPCKYPQKI---DGKCCKVC
VOPT CR1  CMFQ-DKKYR-VGERWHPYLEPYG--LVYCVNCICSE-NGNVLCSRVRCP-NVHCLSPVHI---PHLCCPRC
VOPT CR2  CEYN-GTTYQ-HGELFVAEGLFQNRQPNQCTQCSCSE--GNVYCGLKTCP-KLTCAFPVSV---PDSCCRVC
CHRD CR3  CYFDGDRSWRAAGTRWHPVVPPFG--LIKCAVCTCKGGTGEVHCEKVQCP-RLACAQPVRV--NPTDCCKQC
CHRDL2 CR3 CVHG-GKTYS-HGEVWHPAFRAFG--PLPCILCTCED--GRQDCQRVTCPTEYPCRHPEKV---AGKCCKIC
CRIM1 CR2 CYAN-GLILA-HGDRWRED---------DCTFCQCVN--GERHCVATVCG--QTCTNPVKV---PGECCPVC
NELL1 CR1 CQVS-GLLYR-DQDSWVDGD--------HCRNCTCKS--GAVECRRMSCP-PLNCSPDSLPVHIAGQCCKVC
COL5A2    CTQN-GQMYL-NRDIWKPA---------PCQICVCDN--GAILCDKIECQDVLDCADPVTP---PGECCPVC
KCP CR4   CELD-GEEFA-EGVQWEPDGR-------PCTACVCQD--GVPKCGAVLCP-PAPCQHPTQP---PGACCPSC
THBS1     CYHN-GVQYR-NNEEWTVDS---------CTECHCQN--  VTICKKVSCP-IMPCSNATVP---DGECCPRC
BMPER CR5 CKFG-NKIFQ-DGEMWSSIN---------CTICACVK--GRTECRNKQCIPISSCPQGKIL--NRKGCCPIC
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Figure S1. Conserved Protein Domains and Motifs in Chordin-Like 1 (Ventroptin)

(a) Domain organisation of human Ventroptin (458 aa). SP = signal peptide CR1-3 = three chordin domains. Gene structure is shown above. **(b)** Conservation of cysteine and other residues in the three chordin domains of human Ventroptin (VOPT) and related chordin domains in human proteins Chordin (CHRD), Chordin-like 2 (CHRDL2), Cysteine rich transmembrane BMP regulator 1 (CRIM1), Nel-like 1 (NELL1), Collagen type 5 alpha 2 (COL5A2), Kielin/chordin-like protein (KCP), Thrombospondin 1 (THBS1), BMP binding endothelial regulator (BMPER). The conserved cysteine residue in the third chordin domain mutated in Family 3 (c.782G>T; p.Cys261Phe) is marked by an asterix.

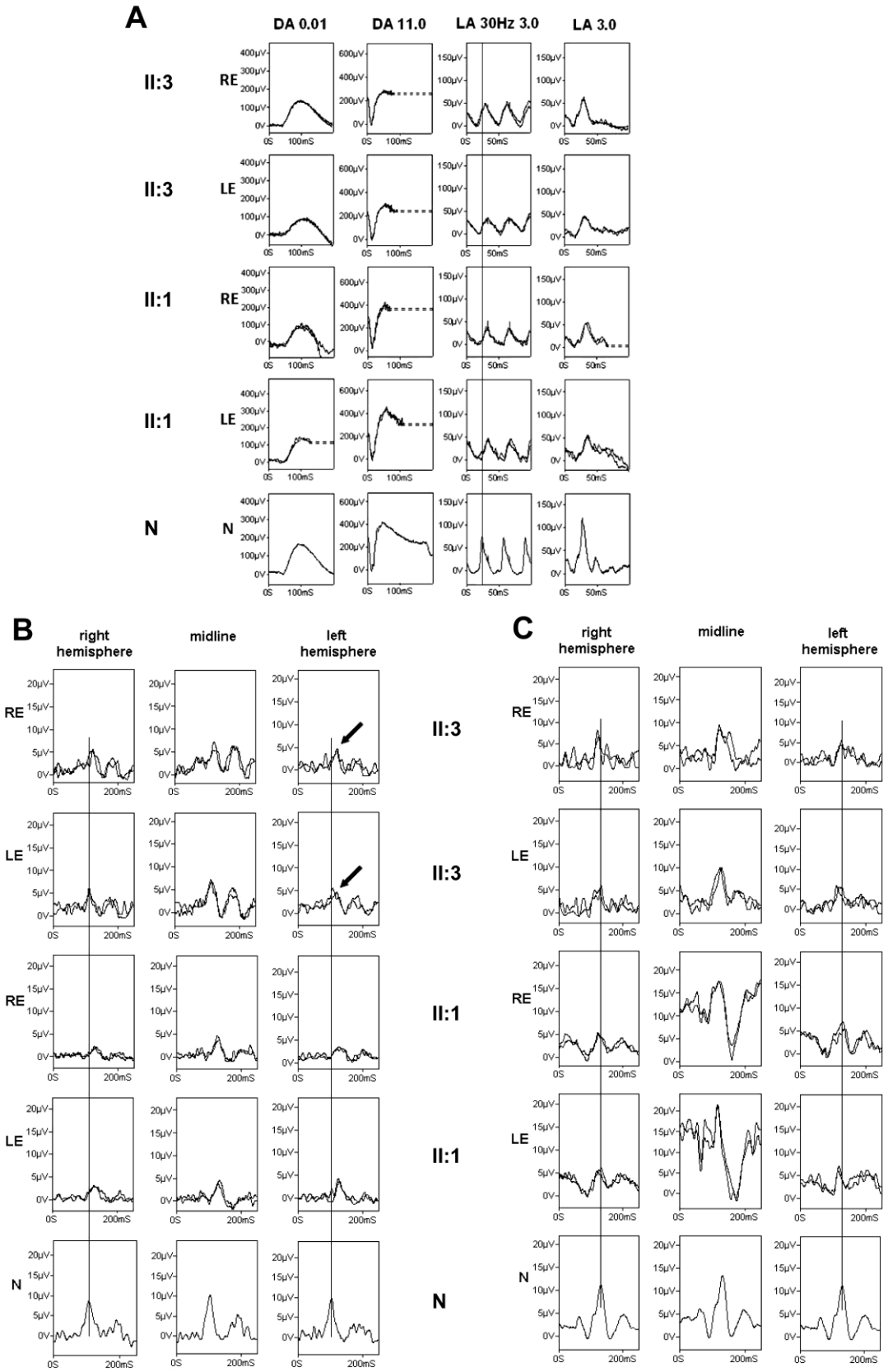

Figure S2. Electrophysiology Showing Mild Generalised Cone System Dysfunction (Subjects II:1 and II:3) and Interhemispheric VEP Asymmetry (Subject II:3)

(a) Full-field ERGs for patients II:3 and II:1. Dark-adapted (DA) ERGs are

shown for flash strengths of 0.01 and 11.0cd.s.m^{-2} and show no definite evidence of rod system involvement; light adapted (LA; background 30cd.m^{-2}) ERGs for flashes of 3.0cd.s.m^{-2} (30 Hz and 2 Hz) are consistent with mild generalised cone system dysfunction in both individuals. Broken lines replace eye movement artifacts. **(b)** Pattern reversal VEPs for patients II:3 and II:1 were recorded to a high contrast black and white reversing checkerboard stimulus (2,2 reversals/second; check size 50 minutes of arc). Arrows indicate increased peak time in the left hemisphere traces for II:3 from both eyes. Delay in pattern reversal VEPs from both the right and left eyes of II:1, and right eye of II:3 compared to normal age-matched control is highlighted (line from normal trace). **(c)** Flash VEPs for II:1 and II:3 were considered normal. RE = right eye, LE = left eye, N = age-matched normal subject for comparison.

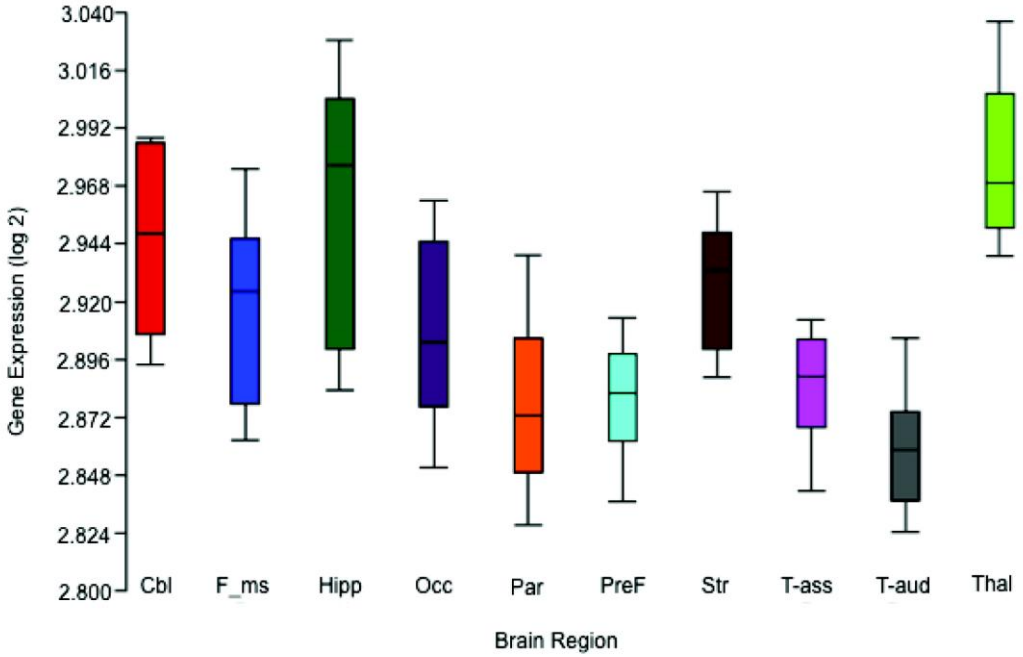

Figure S3. Expression of *BMP4* in Human Fetal Brain Regions
BMP4 is expressed in all brain regions tested. Cbl: Cerebellum; F_ms: Motor somatosensory neocortex; Hipp: Hippocampus; Occ: Occipital visual neocortex; Par: Parietal association neocortex; PreF: Prefrontal cortex (orbital dorsolateral, medial and ventrolateral prefrontal neocortex); Str: Striatum; T-ass: Temporal association neocortex; T-aud: Temporal auditory neocortex; Thal: Mediodorsal thalamus. Box plots represent median and 25–75th percentiles. Upper and lower lines show minimum and maximum values, respectively.

Table S1. Summary of Mutations Identified in *CHRDL1* Causing X-Linked Megalocornea

Family	*CHRDL1* mutation	Exon/Intron	Predicted consequence
1	238,024 bp deletion ChrX:109726503 to ChrX:109964527	Delete exons 6-12	truncated protein product
2	Frameshift c.101delAG	Exon 3	p.Glu34AspfsX14 truncated protein product
3	Missense c.782G>T	Exon 9	p.Cys261Phe non-functional chordin domain
4	Splice site IVS4+2T>G	Intron 4	miss-splicing and truncated protein product
5	Frameshift mutation c.872delG	Exon 9	p.Cys291LeufsX25 truncated protein product
6	Nonsense c.652C>T	Exon 8	p.Arg218X truncated protein product
7	Segmental deletion >270 Kb	Delete entire gene	no protein

Nomenclature based on Ensembl annotation transcript ID ENST00000372042, protein ID ENSP00000361112 and HG19 genome assembly

Table S2. Summary of Association Study for Mean Corneal Diameter and *CHRDL1* SNP Genotype

SNP	Position	Minor Allele	MAF	Functional Category	Cohort	p-value
rs12857107	109813366	G	0.40	Intron	Raine	0.8911
rs5943057	109825861	C	0.40	Intron	Raine	0.8911
rs197023	109855043	G	0.29	Intron	Raine	0.9490
					Chinese	0.6793
rs12689346	109890618	A	0.25	Intron	Raine	0.2176

Table S3. Detail of Electrophysiology for Patients with Mild Generalised Cone System Dysfunction Compared to Controls

	II:3				II:1				Normal (70-79 years, N=50)	
	timing (ms)		amplitude (μV)		timing (ms)		amplitude (μV)		timing (ms) 95th percentile	amplitude (μV) 5th percentile
	RE	LE	RE	LE	RE	LE	RE	LE		
DA 0.01	97	105	145	110	98	100	125	140	111	100
DA 11.0										
a-wave	14	14	240	225	14	13	255	240	14	187
b-wave	55	55	305	310	54	55	380	415	58	296
LA 3.0 30Hz	30	33	45	35	32	32	40	40	30	55
LA 3.0 2Hz										
a-wave	15	16	20	15	15	16	20	25	16	20
b-wave	29	29	60	50	34	34	55	60	32	85

Control data from Neveu MM et al. 2011

Table S4. Summary of Age–Corrected Cognitive Performance for MCG1 Individuals II:1 and II:3 from Family 1

	Case	
	II:1	**II:3**
Age	71	66
Verbal IQ	125	116
Vocabulary (centile)	55 (75th)	49 (63th)
Digit Span	20 (95th)	19 (91st)
Similarities	24 (95th)	22 (84th)
Verbal Learning Trials	55 (75-90th)	48 (50-75th)
Verbal learning delay	12 (75th)	13 (90th)
Verbal Recall Immediate	43 (90th)	42 (75-90th)
Verbal Recall Delayed	36 (75th)	46 (>90th)
Fluency phonemic "s"	19 (>96th)	20 (>96th)
Fluency animals	30 (90th)	24 (90th)
Hayling Test	Average	Average
Cognitive estimates	1 (90th)	0 (95th)

Supplemental Methods, Cognitive Tests

Intellectual Level

The Vocabulary, Digit Span, and Similarities subtests from the WAIS-R were administered and the scores were pro-rated to obtain an estimate of VIQ (Spreen & Strauss, 1998).

Executive Functions

Verbal fluency: The subject had to produce as many words beginning with the letter S (phonemic fluency) and as many animal names (semantic fluency) both in a minute. Performance on fluency tests, particularly for the phonemic category (letter "s"), is a sensitive indicator of frontal lobe functioning (Henry & Crawford, 2004).

The Hayling Test: A response suppression task. The subject has to complete two series of 15 sentences each missing the last word. In the first section a sensible completion is required and in the second a nonsensical completion. The test yields two measures of mental processing speed and an error score for the second series. Performance on this measure has been shown to involve frontal brain regions in healthy individuals (Collette *et al*. 2001) and to be adversely affected by frontal lobe pathology (Burgess *et al.* 1997).

Cognitive estimates: This is a semantic reasoning task that requires the subject to provide a reasonable estimation to ten questions that have no exact answer, based on their available semantic knowledge. The questions are of the format 'How fast do race horses gallop?' Penalties are awarded for inaccurate responses and the higher the score the poorer the reasoning demonstrated. This test has been shown to be sensitive to frontal lobe pathology (Spreen & Strauss, 1998).

Memory

Verbal Recall: This was assessed using the Story Recall subtest from the Adult Memory and Information Processing Battery (AMIPB; Coughlan & Hollows,1986). The subject is read a short story and then has to recall as many details as possible immediately following presentation and again following a delay of 30 minutes. Performance measures used were the immediate recall score and the % retained score (delayed recall/immediate recall x 100).

Verbal Learning: The List Learning test from the AMIPB was employed. The subject is presented with a list of 15 words on five occasions and following each presentation has to recall as many of the words as possible. A second list of words is then presented and following one attempt at recall is required to recall as many words from the first list (delayed recall). The total number of words remembered in the learning phase (verbal learning trails) and in the delayed recall condition (verbal learning delay) were recorded.

Supplementary References

Burgess, P., *et al.* (1997). The Hayling Island and Brixton Test Manual. *Edmunds: Thames Valley Test Co*.

Collette, F.*, et al.* (2001). The functional anatomy of inhibition processes investigated with the Hayling task. Neuroimage ***14***, 258-267

Coughlan, A., and Hollows, S. (1986) The Adult Memory and Information Processing Battery. *Leeds: St James Hospital*.

Henry, J.D., and Crawford, J.R. (2004) A meta-analytic review of verbal fluency performance following focal cortical lesions. Neuropsychology. ***18***, 284-295.
Neveu, M.M., Dangour, A., Allen, E., Robson, A.G., Bird, A.C., Uauy, R., and Holder, G.E. (2011). Electroretinogram measures in a septuagenarian population. Doc. Ophthalmol. ***123***, 75-81.
Spreen, O., and Strauss, E. (1998). A compendium of neuropsychological tests: administration, norms and commentary. *New York: Oxford University Press* (1998).