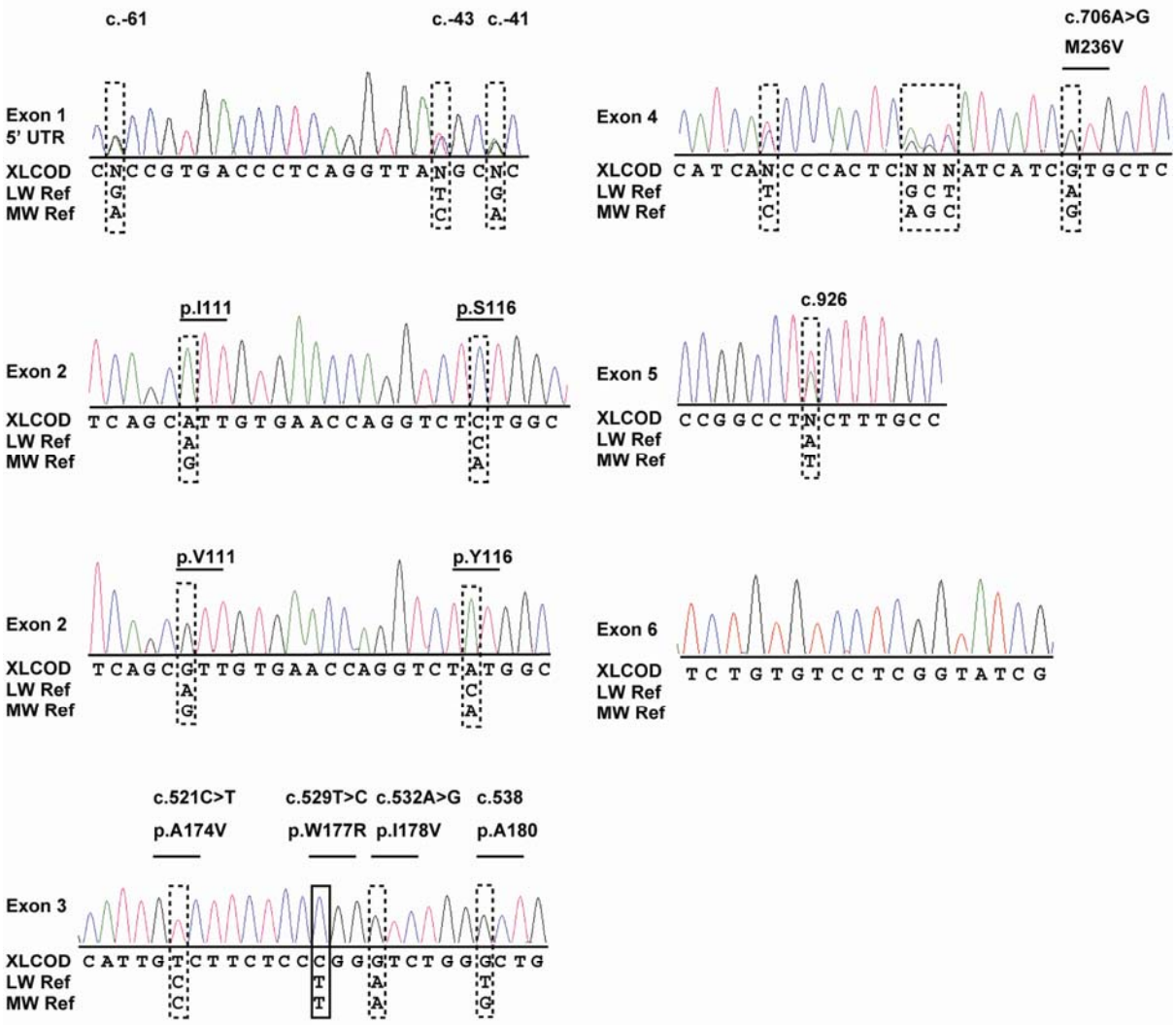
The American Journal of Human Genetics, Volume *87*

Supplemental Data

X-linked Cone Dystrophy XLCOD5 Caused by Mutation of the Red and Green Cone Opsins

Jessica C. Gardner, Tom R. Webb, Naheed Kanuga, Anthony G. Robson, Graham E. Holder, Andrew Stockman, Caterina Ripamonti, Neil D. Ebenezer, Olufunmilola Ogun, Sophie Devery, Genevieve A. Wright, Eamonn R. Maher, Michael E. Cheetham, Anthony T. Moore, Michel Michaelides, and Alison J. Hardcastle

A



B

Genes	Opsin Gene Sequence																		
	Exon 2			Exon 3						Exon 4			Exon 5						
	65	111	116	153	171	174	177	178	180	230	233	236	274	275	277	279	285	298	309
LW Ref	T	I	S	L/M	V/I	A/V	W	I/V	S/A	I/T	A/S	M/V	I	F	Y	V	T	A	Y
MW Ref	I/T	V	Y	M/L	V	A/V	W	I/V	A/S	T	S	V	V	L	F	F	A	P	F
LW XLCOD	T	I	S	M	V	V	**R**	V	A	I	A	V	I	F	Y	V	T	A	Y
MW XLCOD	I	V	Y	M	V	V	**R**	V	A	T	S	V	V	L	F	F	A	P	F

Figure S1. Sequence Analysis of the LW and MW Cone Opsin Array

(A) Partial sequence of all LW and MW cone opsin exons in affected male IV:2. Sequences shown were generated from PCR products obtained using primers which co-amplify both genes, with the exception of exon 2 which was generated using LW or MW specific primers. Boxed sequences highlight sequence differences between the LW and MW genes in the XLCOD5 family compared to reference sequence (shown below). The missense mutation (c529T>C, pW177R) detected in exon 3 is also boxed (solid line). **(B)** Table showing opsin gene sequences for LW, MW and XLCOD. Numbers below the exon number represent the codon number in which either a SNP or a sequence difference between the LW and MW gene sequence is known. In the LW and MW reference gene sequences the first letter designates the more common allele and the second letter designates the less common allele. Note the identical exon 3 haplotype in both genes of the XLCOD family. The exon 3 haplotype block carried by affected individuals contains the mutation (W177R highlighted in bold) in linkage disequilibrium with several SNPs (M153; V171; V174; V178; A180) which essentially define it, spectrally and genetically, as an MW exon variant.

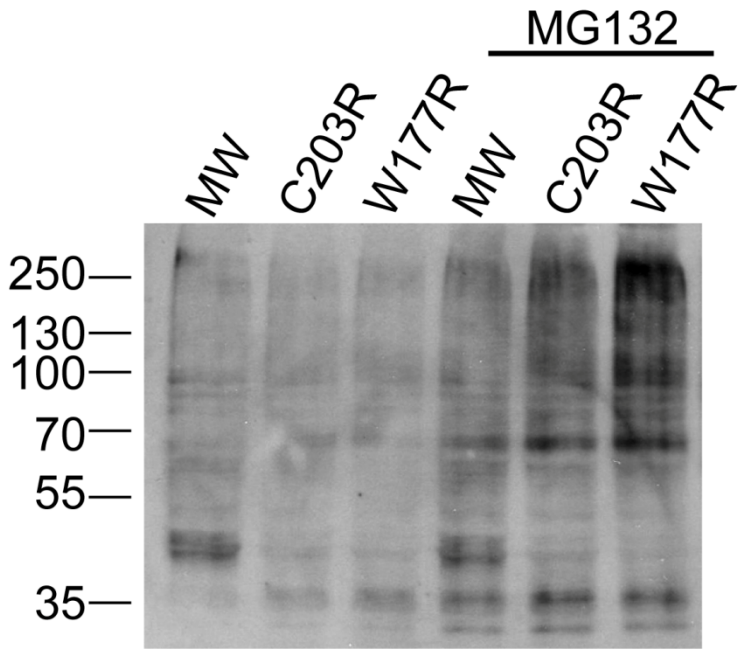


Figure S2. MG132 Treatment Increases the Levels of MW Mutant Proteins

Western blot analysis of SK-N-SH cell lysates transfected with wild-type MW opsin (MW), C203R MW and W177R MW mutant opsins, with and without MG132 treatment, probed with 1D4 opsin antibody**.** MG132 treatment led to an increase in levels of C203R MW mutant and W177R MW mutant proteins.

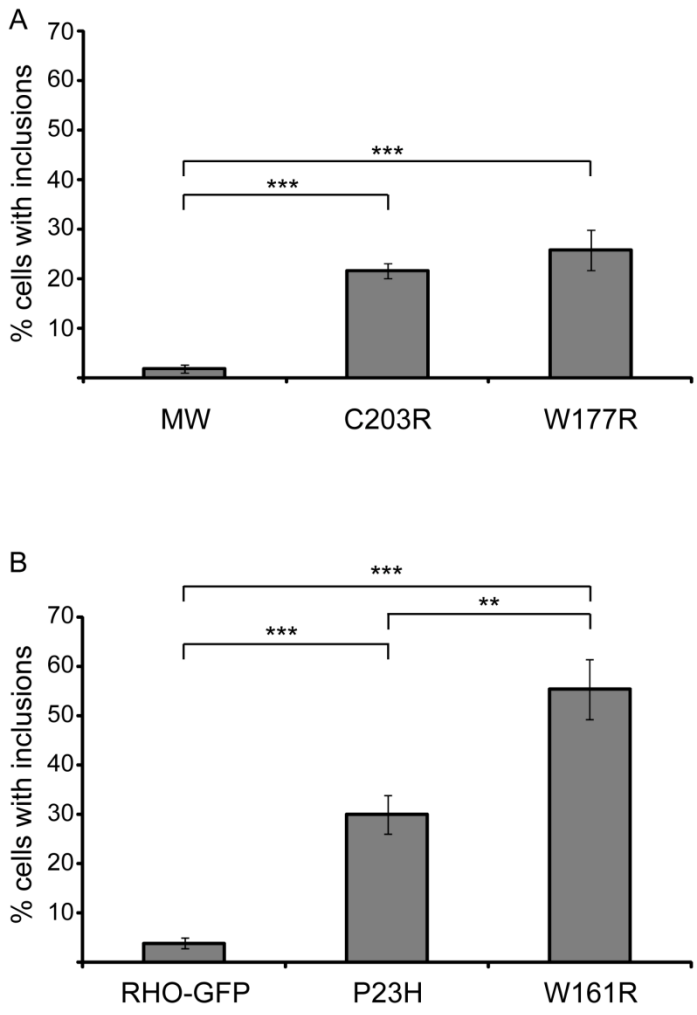


Figure S3. Inclusion Incidence of the Cone Opsin and Rod Opsin Mutations

(A) The incidence of inclusion formation was assessed in SK-N-SH cells transfected with either wild-type MW opsin (MW), C203R MW mutant or W177R MW mutant opsins. Inclusion incidence is significantly higher in cells transfected with C203R MW mutant or W177R MW mutant compared to cells transfected with wild-type MW. **(B)** The incidence of inclusion formation in SK-N-SH cells transfected with GFP-tagged RHO, P23H or W161R. Inclusion incidence is significantly higher in cells transfected with W161R-GFP

compared to cells transfected with P23H-GFP. Data are presented as mean ± 2 SEM. $^{**}P<0.005$, $^{***}P<0.0005$.