Program #: 3367

Whole Exome Sequencing (WES) Identifies a Mutation in ALPK1 Responsible for a Novel, Autosomal Dominant Disorder of Vision Loss, Splenomegaly, and Pancytopenia

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T237M Mutation in ALPK1 is identified as the likely causative mutation in Autosomal Dominant Digre-Williams Syndrome

Phenotype

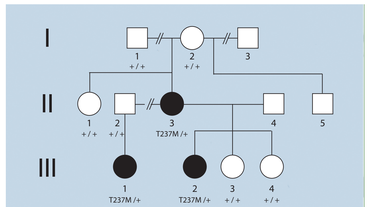


FIG. 1. Family tree: Case 1 is Patient III-2 in the pedigree; Case 2 (the mother) is Patient II-3 in the pedigree; and Case 3 (half-sister) is Patient III-1 in the pedigree.

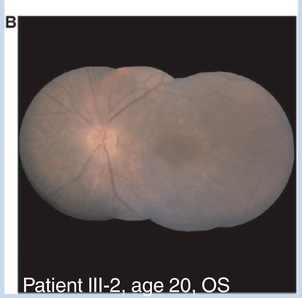


FIG. 2. Representative fundus photos and optic disc photo from this cohort. Shown are fundus photos of Patient III-2. A: Fundus montage photo of the right eye. B: Fundus montage photo of left eye of Patient III-2 at age 20. At the time of the photos, best corrected vision was 20/50 OD, and 20/150 OS. These photographs are representative of the other affected family members. Note: Optic disc edema, flame hemorrhages, and attenuated blood vessels.

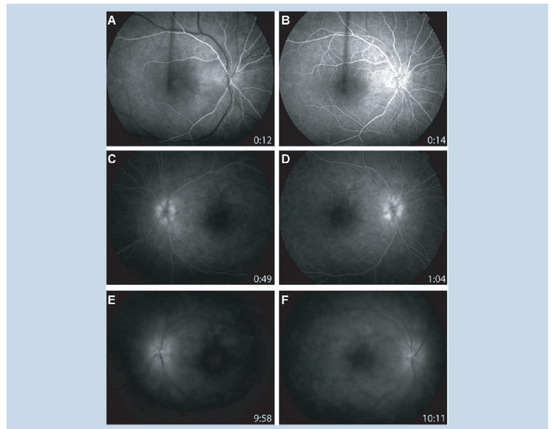


FIG. 6. Fluorescein angiogram from Patient III-2 at age 14. The angiogram shows leakage from the optic nerve, but no vasculitis or cystoid macular edema. The times indicated are in minutes and seconds from injection of fluorescein. Images A, B, D, and F are from the right eye; images C and E are from the left eye.

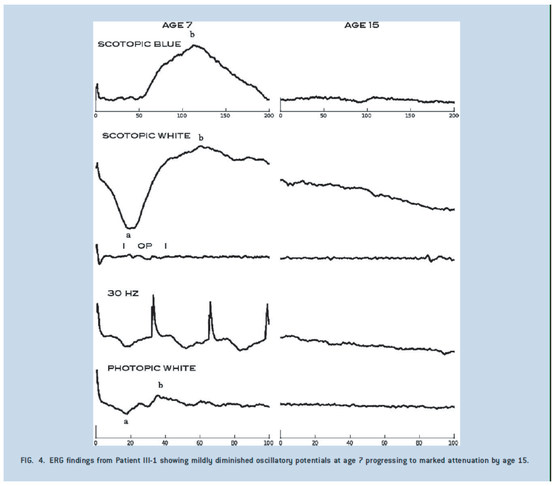
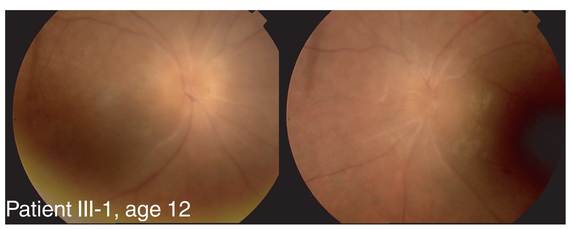
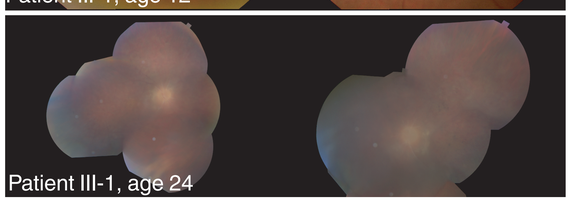


FIG. 4. ERG findings from Patient III-1 showing mildly diminished oscillatory potentials at age 7 progressing to marked attenuation by age 15.



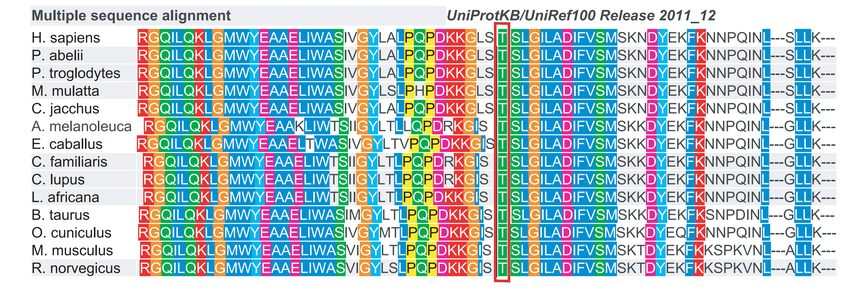


Genotype

Candidate genes identified using VAAST

Gene name	p-value	chromosome	position (hg19)	Allele change	Protein change
PRAMEF11	6.10E-06	chr1	12887174	C->T	R->H
ANKRD20A4	7.32E-06	chr9	69423637	G->A	E->K
MRPL4	8.55E-06	chr19	10367459	C->T	R->W
FAM90A10	9.77E-06	chr8	7629232	G->T	A->S
GOLGA6L10	9.77E-06	chr15	82635194	T->C	E->G
FAM90A20	1.28E-05	chr8	7155458	C->G	A->G
EEF1A1	1.65E-05	chr6	74228474	C->T	R->H
MSI1	1.65E-05	chr12	120800875	C->T	V->M
VDAC2	1.71E-05	chr10	76980685	G->T	A->S
PIM1	2.08E-05	chr6	37138779	A->T	K->M
USP11	2.26E-05	chrX	47104817	G->A	V->M
TAS2R31	2.62E-05	chr12	11183427	T->G	S->R
ABCF1_DUP_06	2.87E-05	chr6	30539278	C->T	P->L
STAU2	3.30E-05	chr8	74529664	T->C	I->V
PRAMEF4	3.48E-05	chr1	12939510	C->T	S->N
FRG1	3.97E-05	chr4	190876196	G->A	A->T
TAS2R19	4.52E-05	chr12	11174390	G->A	L->F
ZNF527	4.64E-05	chr19	37879853	C->T	P->L
NBPF10	4.64E-05	chr1	145368518	C->T	S->L
CTBP2	4.82E-05	chr10	126678163	G->A	A->V
PRSS1	5.43E-05	chr7	142460764	G->A	V->I
ZNF846	6.96E-05	chr19	9869202	T->A	N->I
CLDN25	7.94E-05	chr11	113651170	T->C	F->S
SIGLEC10	9.03E-05	chr19	51917709	G->A	T->M
SIRPA	0.000136	chr20	1895965	C->A	N->K
THNSL1	0.000197	chr10	25314307	G->T	E->*
TBRG1	0.000222	chr11	124495568	T->C	Y->H
PDIA2	0.000263	chr16	334579	C->T	P->L
AKAP8L	0.000302	chr19	15514392	C->T	A->T
CHST15	0.000337	chr10	125780759	G->C	P->A
STARD8	0.000596	chrX	67937592	A->C	E->A
ALPK1	0.000840	chr4	113348736	C->T	T->M
PTPN13	0.001565	chr4	87691019	C->G	S->R
FAM90A13_DUP_020	0.002901	chr8	7575210	G->C	M->I
DMRTA2	0.003176	chr1	50886700	G->C	A->G
DUX4L4	0.003529	chr4	191003471	C->T	S->L
CDKN2A	0.003686	chr9	21971185	C->A	R->L
USP17	0.003960	chr4	9217197	T->C	S->P
FDX1	0.004235	chr11	110300857	G->A	G->R
NBPF16_DUP_01	0.004645	chr1	148754897	A->G	Y->C
MUC17	0.008888	chr7	100678029	G->C	R->T
MLL3	0.009841	chr7	151945225	T->C	E->G

Protein sequence comparison across species for ALPK1 position T237.



1. Yang Zhang. I-TASSER server for protein 3D structure prediction. BMC Bioinformatics, 9:40 (2008).
2. Ambrish Roy, Alper Kucukural, Yang Zhang. I-TASSER: a unified platform for automated protein structure and function prediction. Nature Protocols, vol 5, 725-738 (2010).
3. Ambrish Roy, Jianyi Yang & Yang Zhang. COFACTOR: an accurate comparative algorithm for structure-based protein function annotation. Nucleic Acids Research, vol 40, W471-W477 (2012).
4. Franceschini A, Szklarczyk D, Frankild S, et al., STRING v9.1: protein-protein interaction networks, with increased coverage and integration. Nucleic Acids Res. 2013 Jan;41.

Methods / Results

WES on 4 individuals - 2 affected II-3 III-2, and 2 unaffected II-2 and III-3
Illuimina Truseq Enrichment Kit
Quantitative PCR enrichment
Sequencing with Illumina HiSeq 2000 101 cycle paired end sequencing

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Mapped and aligned with Picard Tools (http://picard.sourceforge.net)
SNPs and indels identified with Genome analysis toolkit (GATK)
Manual inspection and curation was done with Intergrative Genomics Viewer (http://www.broadinstitute.org/igv)

↓

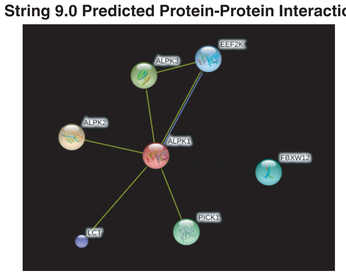
VAAST - compares to HGMD, dbSNP, ESP, and1000 Genomes panels to rule out common variants.

Non-synonymous, synonymous | Exonic

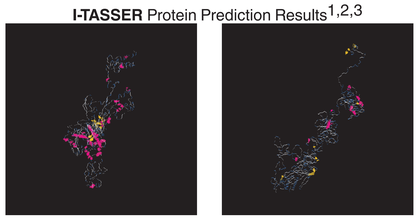
SIFT, PolyPhen 2 GFRP
Alternative Splicing
Splice site prediction

- Swiss model → Functional assays, search for available animal models, qRT-PCR, RNA-Seq
- **In silico bioinformatics:** sequence conservation in species gene expression in retina/spleen gene card (www.genecards.org)
- Secondary Structure Predictions, Solvent Accessibility, Fold Prediction Sequence Information (PredictProtein, PsiPred, I-TASSER)

String 9.0 Predicted Protein-Protein Interactions[4]



I-TASSER Protein Prediction Results[1,2,3]



Conclusions:

WES identifies ALPK1 mutation in Digre-Williams Syndrome
Disease causing mutation is inherited in AD pattern
SNP is chr4:113348736 C→T, located in exon 7 of 16 in *ALPK1*
ALPK1 T237M mutation cosegregates with disease
3 of 3 affected, 0 of 6 unaffected.
T237M mutation is predicted to be damaging - SIFT, PolyPhen2
T237 position is conserved across species

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