Journal club on

"A splice donor mutation in NAA10 results in the dysregulation of the retinoic acid signalling pathway and causes Lenz microphthalmia syndrome."

J Med Genet. 2014 Jan 15. doi: 10.1136/jmedgenet-2013-101660.

Yiyang

2014-1-23

Lenz Microphthalmia Syndrome (LMS)

- First described by Dr. Lenz, 1955.
- Characterized by unilateral or bilateral microphthalmia and/or clinical anophthalmia with malformations of the ears, teeth, fingers, skeleton, and/or genitourinary system; developmental delay and/or mental retardation are also present.
- X-linked pattern, very rare.
- Two disease loci/ (or two diseases?): MCOPS1 (Xq27-q28), MCOPS2(Xp11.4-p21.2): *BCOR* c.254C>T, p.Pro85Leu.

TABLE II. Review of Anomalies Seen in Lenz Microphthalmia Syndrome

	Microcephaly	Ear	Teeth	Palate	Urogenital	Spine	Digital
Lenz, 1955	NA	NA	NA	High arched	Hypospadias	NA	NA
Herrmann and Opitz, 1969	+	Single, anteverted tags,	Agenesis of upper incisors	High arched	Hypospadias, cryptorchidism	Lordosis	Syndactyly, clinodactyly, camptodactyly
Hoefnagel et al., 1963 (propositus)	+	Large, anteverted	—	—	—	—	—
Glanz et al., 1983	+	Simple, anteverted, tags	Widely spaced, peg like	Cleft palate	Hypospadias, cryptorchidism	—	Syndactyly
Baraitser et al., 1982	+	Simple, protruding	Crowded	High arched	—	—	Syndactyly, pseudoclubbing
Traboulsi et al., 1988 (case 1)	+	Low set, rotated	Widely spaced	—	—	—	Hypoplastic thumb, clinodactyly
Traboulsi et al., 1988 (case 2)	–	Cup shaped, tag	—	—	Hypospadias	—	Syndactyly, dup thumb, clinodactyly
Ozkinay et al., 1997	–	Simple, anteverted tags	Widely spaced, hypoplastic	High arched	Hypospadias	—	—
Goldberg and McKusick, 1971 (IV-3)	+	Simple, anteverted	—	—	—	—	—
Goldberg and McKusick, 1971 (III-4)	+	—	Diastema	—	—	Kyphosis	—
Goldberg and McKusick, 1971 (III-18)	+	Simple, anteverted	Widely spaced	—	—	—	—
Antoniades et al., 1993	+	Simple, low set, rotated	Delayed dentition	High arched	—	Lordosis	Syndactyly
Pallota, 1983	+	Low set, anteverted	Agenesis of incisors	High arched	Cryptorchidism	Schisis	Broad thumb, clinodactyly
Present case 1	–	Tag, overfolded helices	—	High arched	Left duplicated renal system	Scoliosis	Fetal pads, clinodactyly
Present case 2	–	—	Crowded	High arched	—	Scoliosis	Fetal pads, syndactyly, clinodactyly
Present case 3	–	Low set, overfolded	—	High arched	—	—	Fetal pads, syndactyly, clinodactyly
Present case 4	–	—	Peg like	High arched	NA	Scoliosis	Fetal pads, syndactyly
Incidence	11/16	15/17	13/17	11/17	8/16	8/17	11/16
Percentage with anomaly	69%	88%	76%	65%	50%	47%	69%

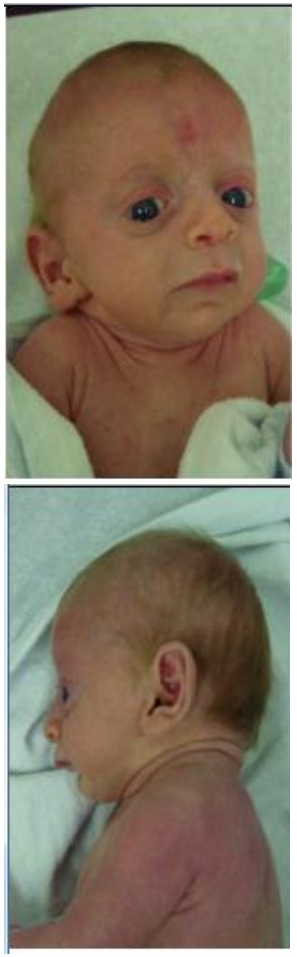
NA, Information not available.

Am J Med Genet. 2001 Jan 1;98(1): 92-100.

VS Ogden Syndrome

Table 1. Features of the Syndrome in Family 1

Category	Features
Growth	postnatal growth failure
Development	global, severe delays
Facial	wrinkled foreheads; prominence of eyes, down-sloping palpebral fissures, thickened lids; large ears; flared nares, hypoplastic alae, short columella; protruding upper lip; microretrognathia
Skeletal	delayed closure of fontanels; broad great toes
Integument	redundancy/laxity of skin, minimal subcutaneous fat, cutaneous capillary malformations, very fine hair and eyebrows
Cardiac[a]	structural anomalies (ventricular septal defect, atrial level defect, pulmonary artery stenoses), arrhythmias (Torsade de points, PVCs, PACs, SVtach, Vtach), death usually associated with cardiogenic shock preceded by arrhythmia.
Genital[a]	inguinal hernia, hypo- or cryptorchidism
Neurologic[a]	hypotonia progressing to hypertonia, cerebral atrophy neurogenic scoliosis



Rope AF, *et al*. Am J Hum Genet. 2011 Jul 15; 89(1): 28-43.

*Phenotype of Carriers

LMS:

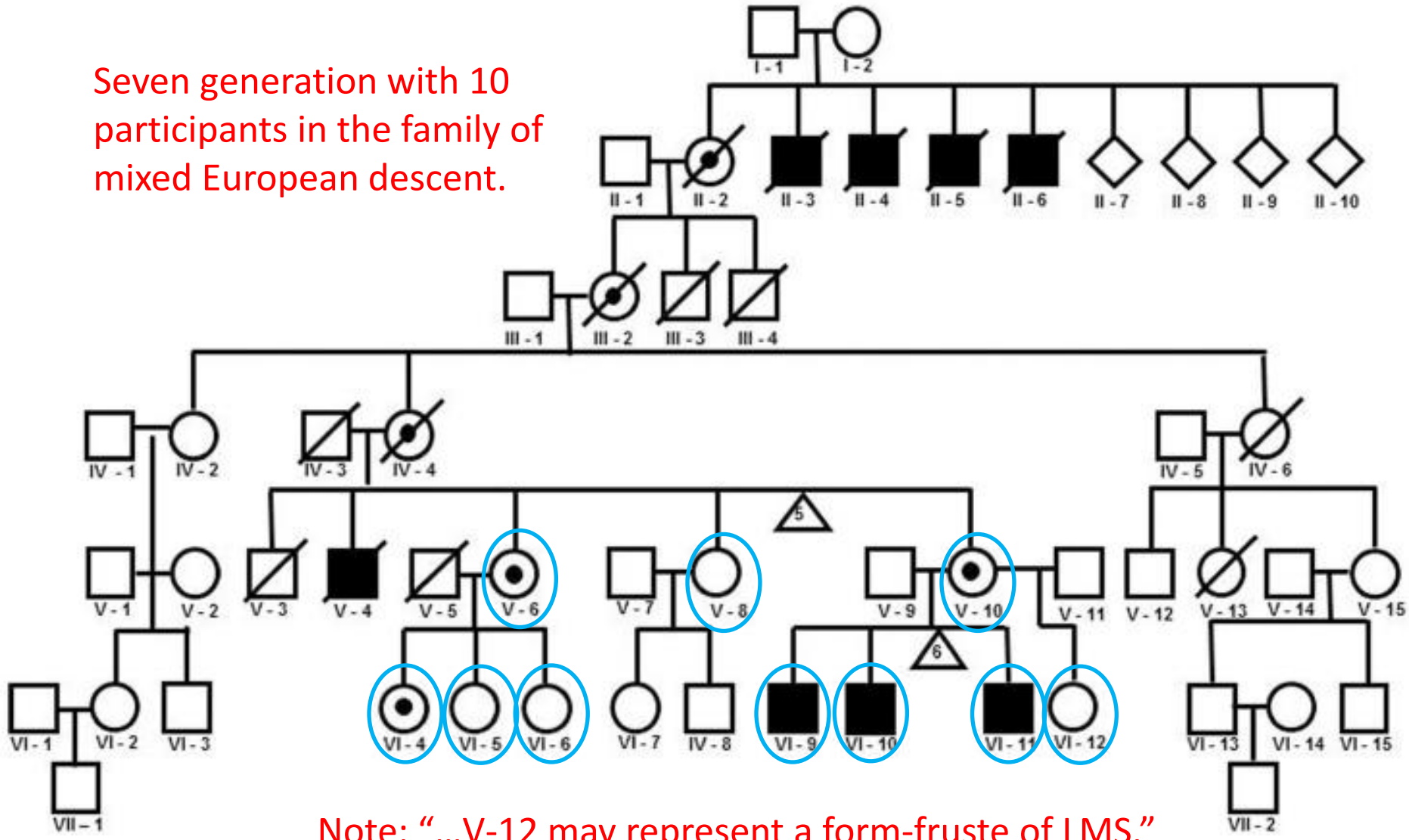
- Three heterozygotes (VI-4, V-6, V-10) had cutaneous syndactyly between the second and third toes and short terminal phalanges.
- Short stature.
- Recurrent spontaneous abortions.
- X chromosome skewing?

Ogden Syndrome:

- X chromosome skewing.

Pedigree

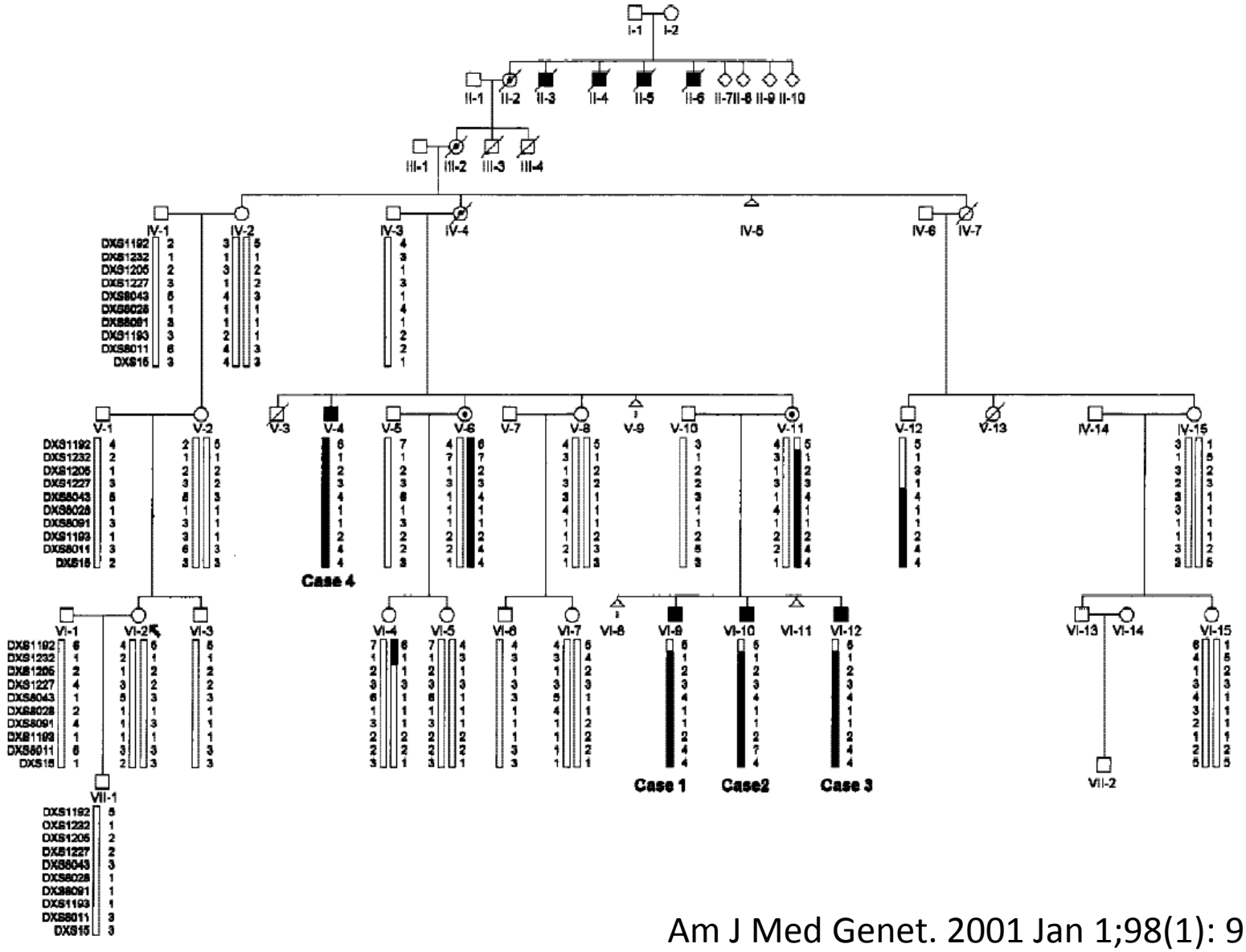
Seven generation with 10 participants in the family of mixed European descent.



Note: "…V-12 may represent a form-fruste of LMS."

-Am J Med Genet. 2001 Jan 1; 98(1): 92-100.

Haplotype Analysis



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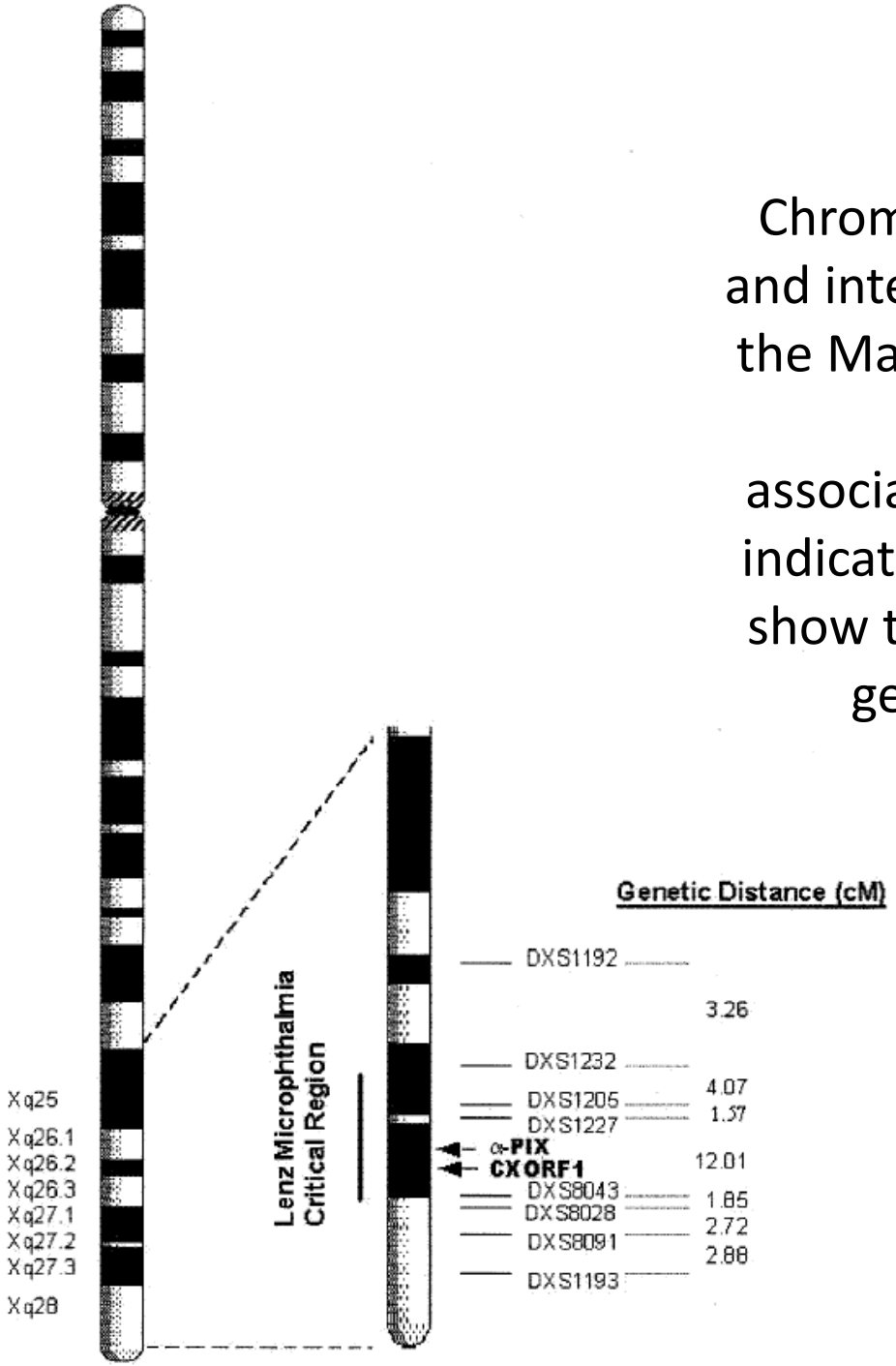


Chromosome X showing marker order and intermarker distances (cM) based on the Marshfield female genetic map. The critical region
associated with Lenz microphthalmia is indicated by a vertical black bar. Arrows show the relative position of candidate genes mapped to the disease.

If V-12 is unaffected, Xq27.1-Xq28, 17.65-cM, DXS1232-DXS8043;
if V-12 is affected, Xq26-qter, 32-cM.

Am J Med Genet. 2001 Jan 1;98(1): 92-100.

Clinical Reports

"Three brothers and a maternal uncle had congenital anophthalmia, delayed motor development, hypotonia, and mental retardation. They also have abnormal ears, high-arched palate, pectus excavatum, finger and toe syndactyly, clinodactyly, fetal pads, scoliosis, and cardiac and renal abnormalities."

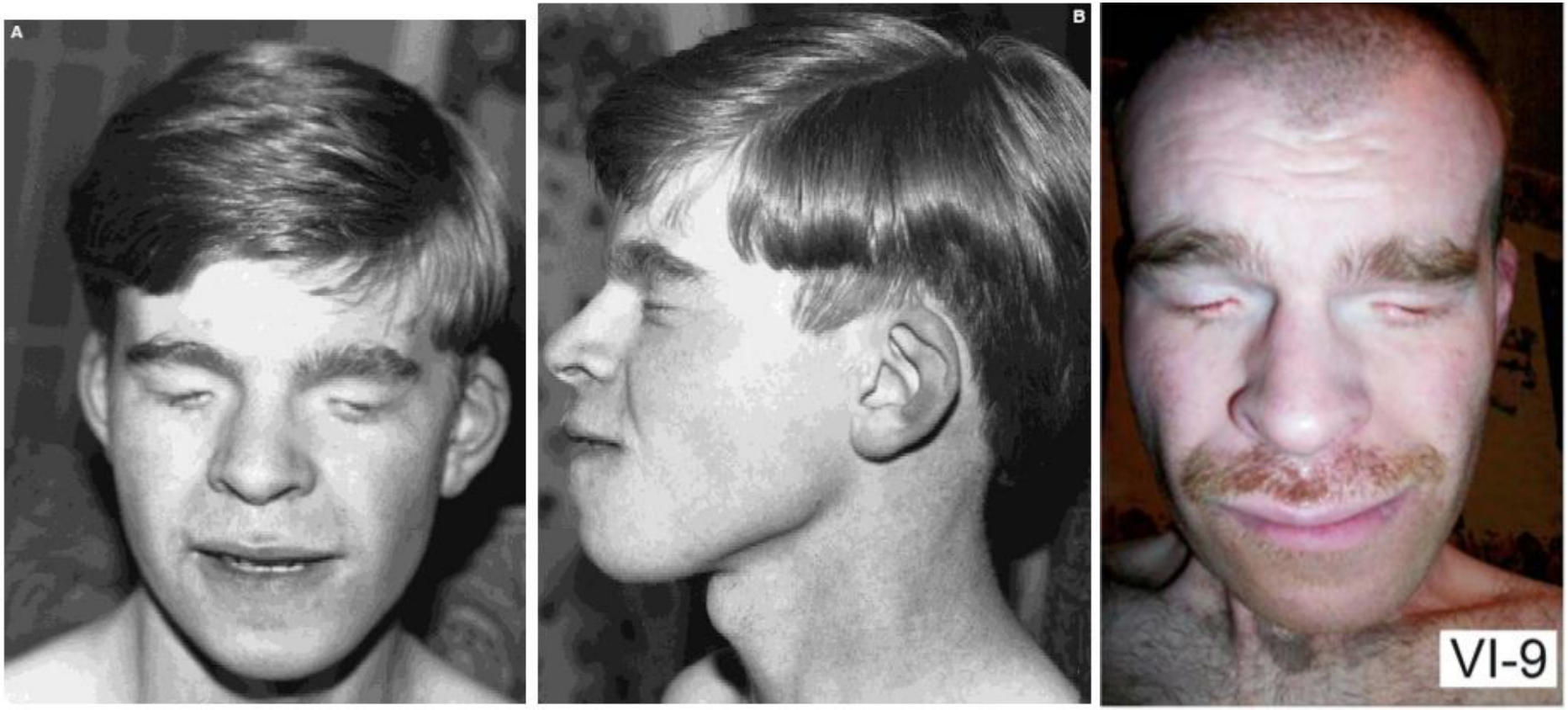
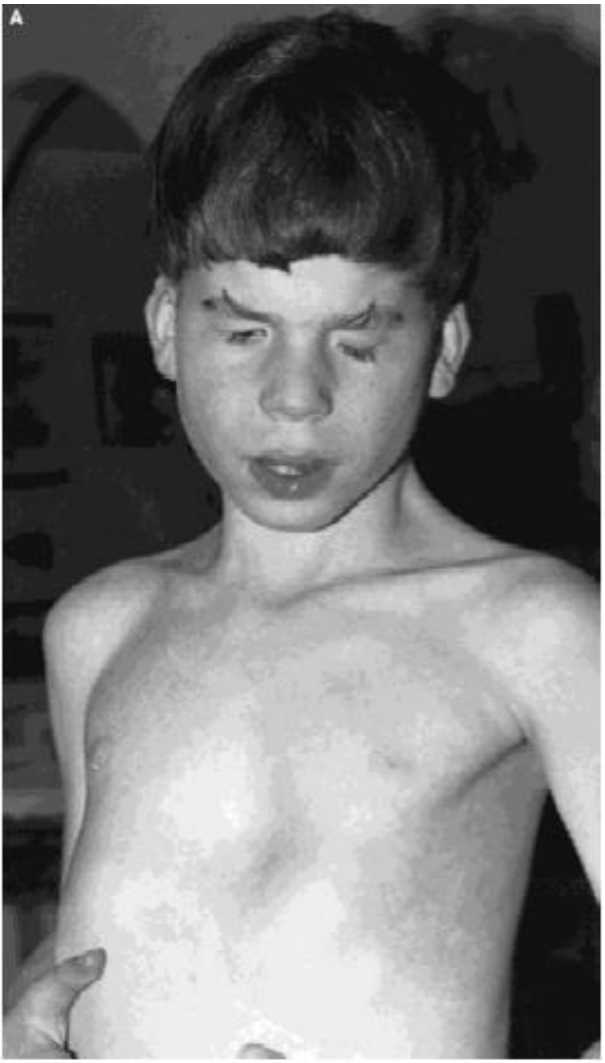
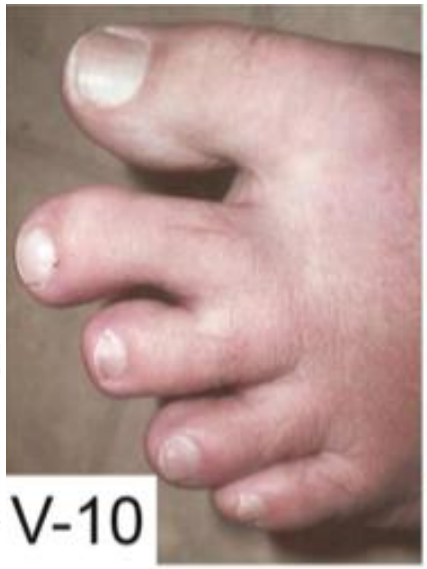


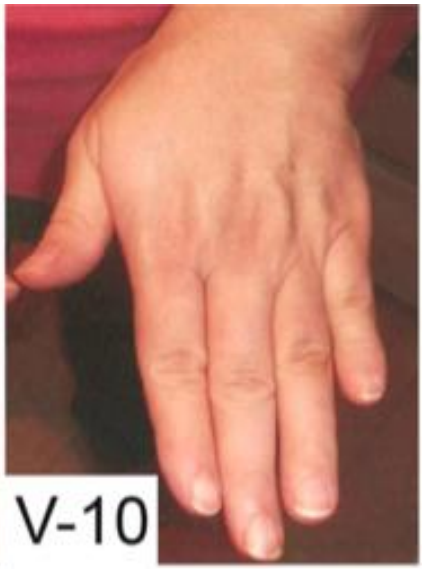
Fig. 1. Patient 1 at age 15 years. Note bilateral anophthalmia and abnormally modeled ears with overfolded helices.

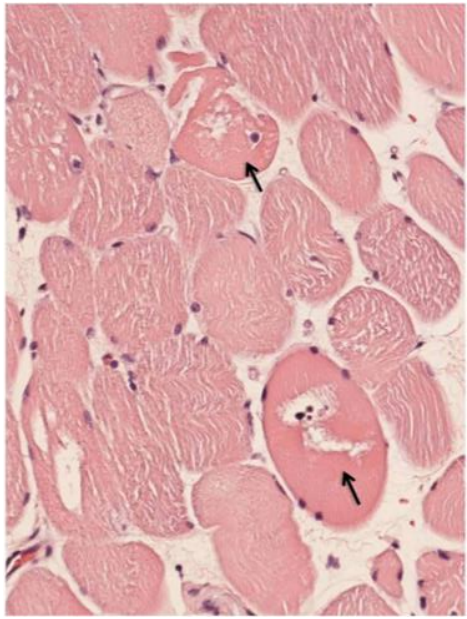
Clinical Reports



V1-11
skeleton
muscle





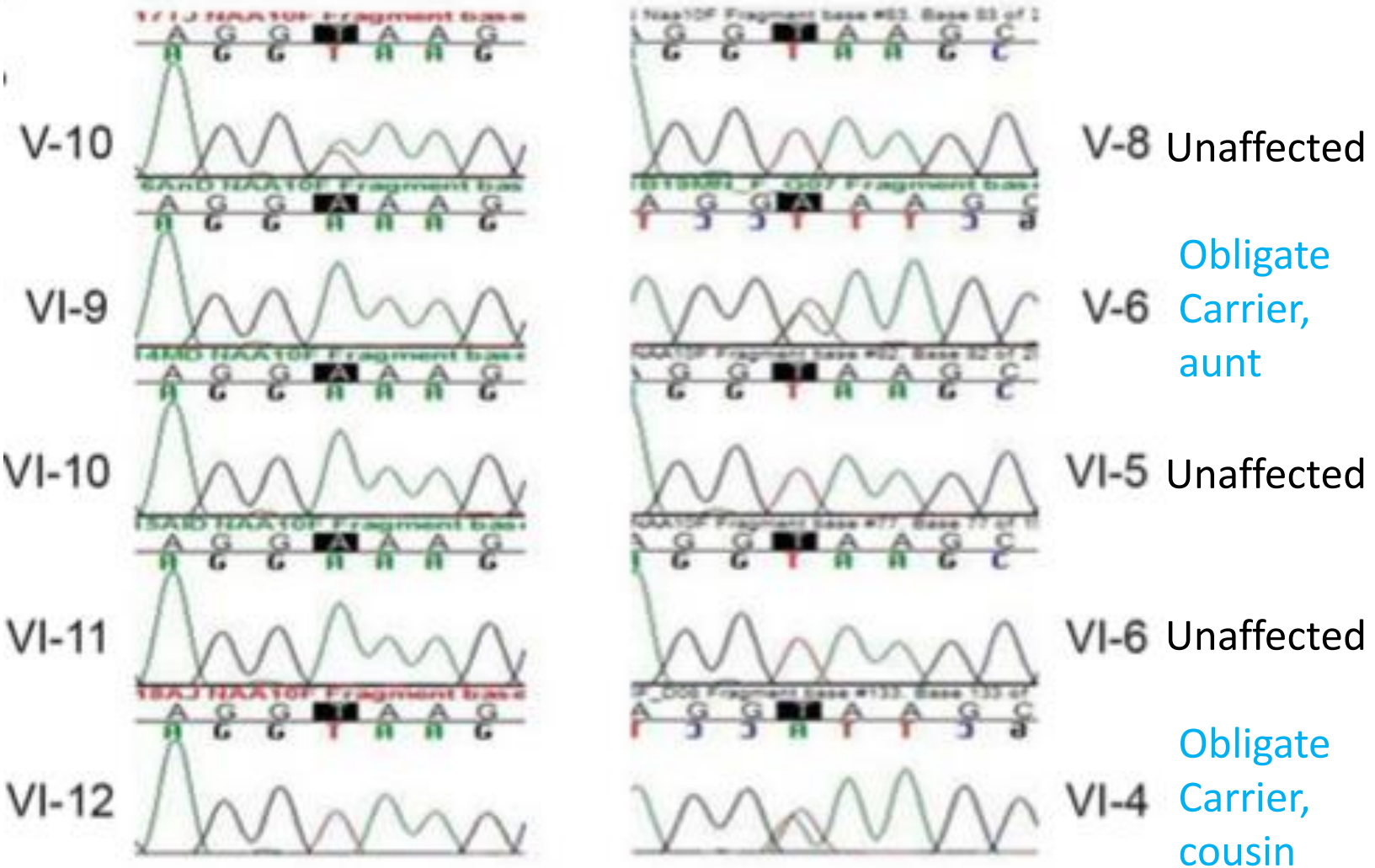


V-10: Obligate carrier

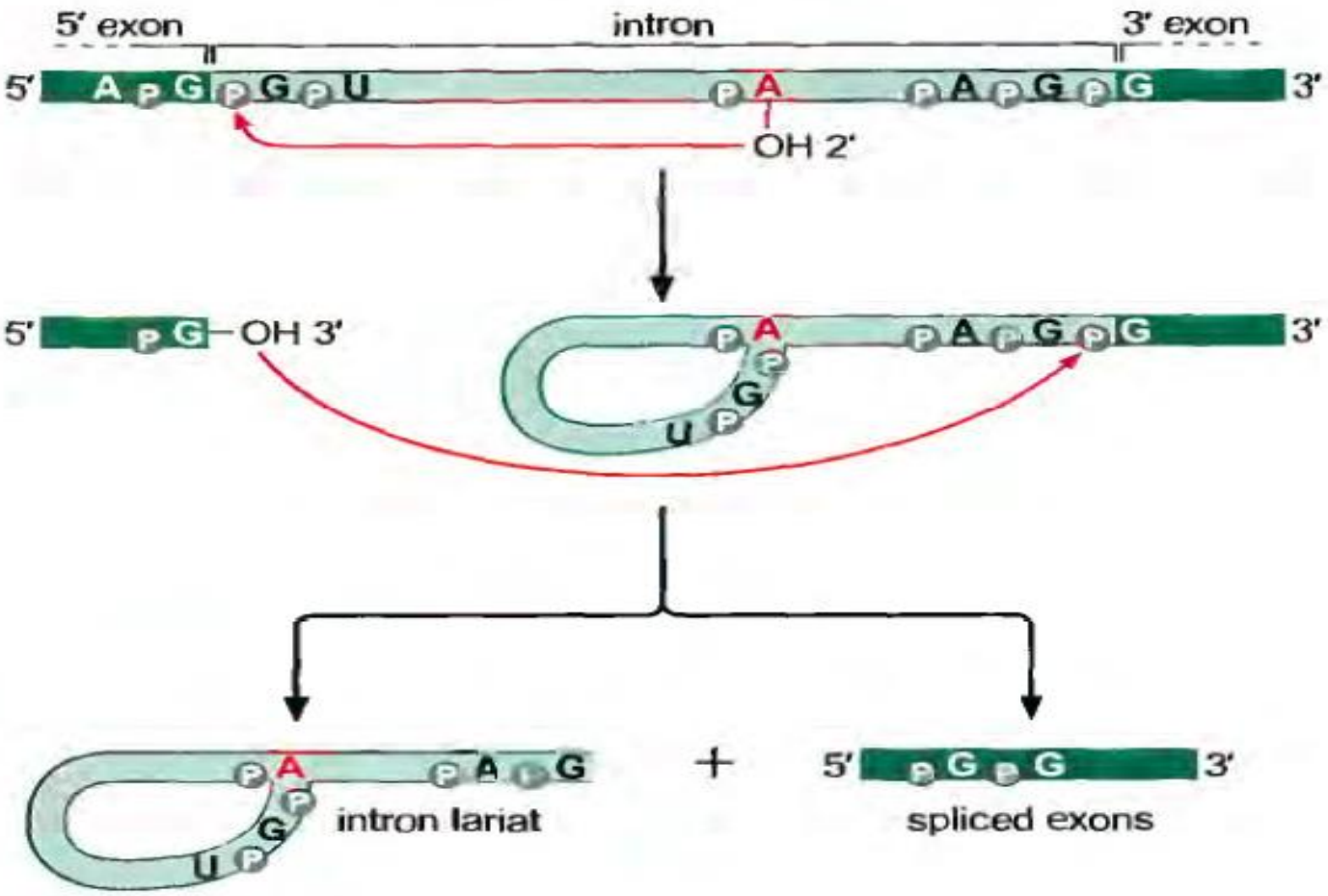
Whole Exome Sequencing

- Whole exome sequencing of three affected individuals (VI-9, VI-10, VI-11). Total DNA was obtained from peripheral blood of the patients and controls using standard protocols.
- HiSeq 2000 (Illumina) platform.
- Extracted all X chromosomal reads.
- ANNOVAR: 32/42 SNP/DIP variants in VI-9, 37/42 SNP/DIP variants in VI-10, and 37/36 SNP/ DIP variants in VI-11.
- Five SNPs and 14 DIPs showed overlap among all three affected male individuals.
- Only one variant fulfilled the criteria to be either in an exon or a splice junction, which was in the *NAA10* gene.
- This variant predicts a mutation at the intron 7 GT splice donor site (c.471+2T→A, ChrX: 153,196,214) of *NAA10*.

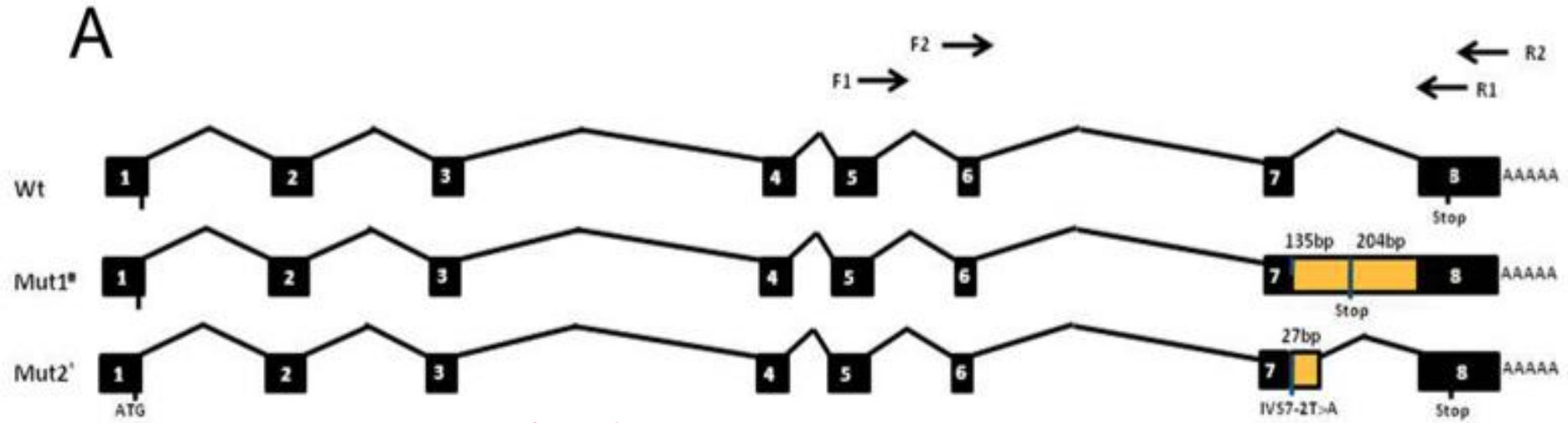
Sanger Validation



Gene Splicing

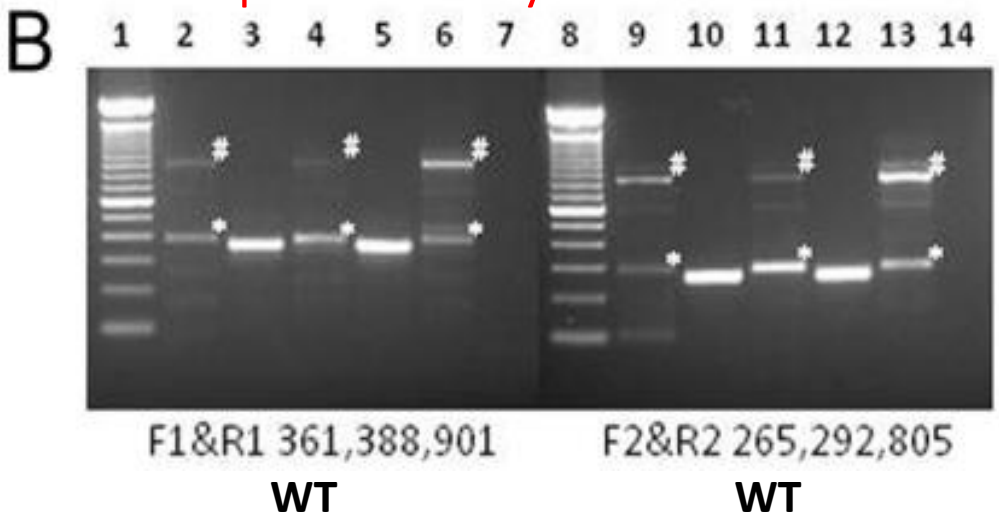


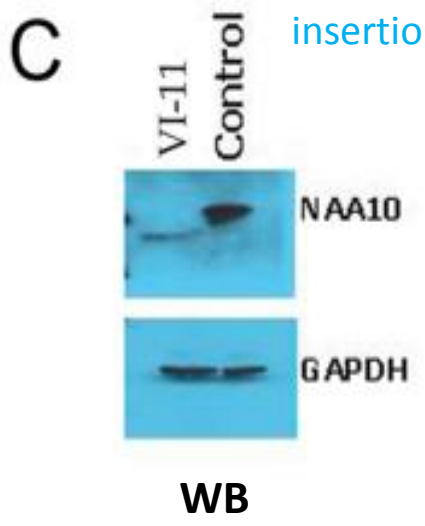
Results, RT-PCR validate the aberrant splicing



No *Naa10* WT band was present in any affected males.

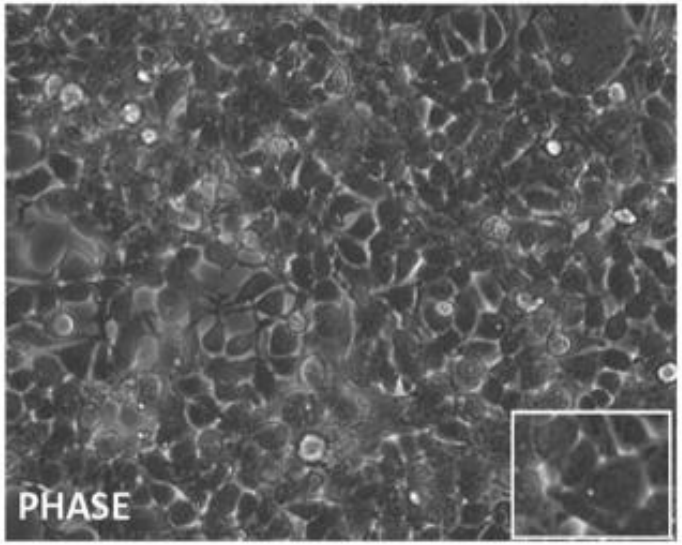
Cryptic splice site at c.471+27, in-frame insertion from intron 7.

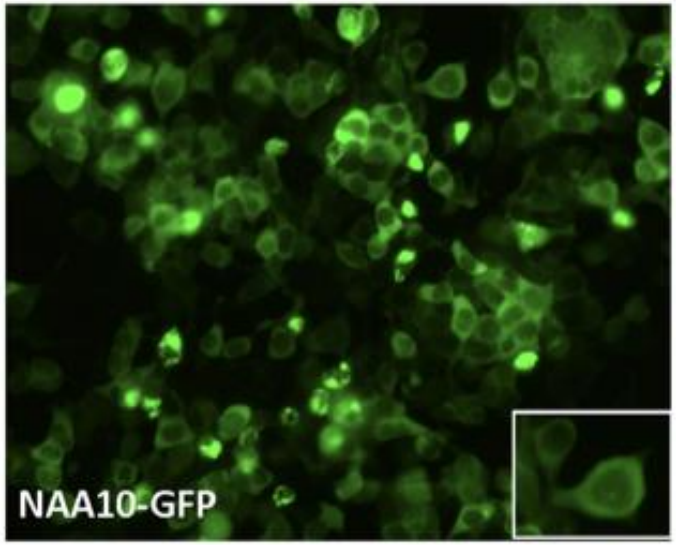


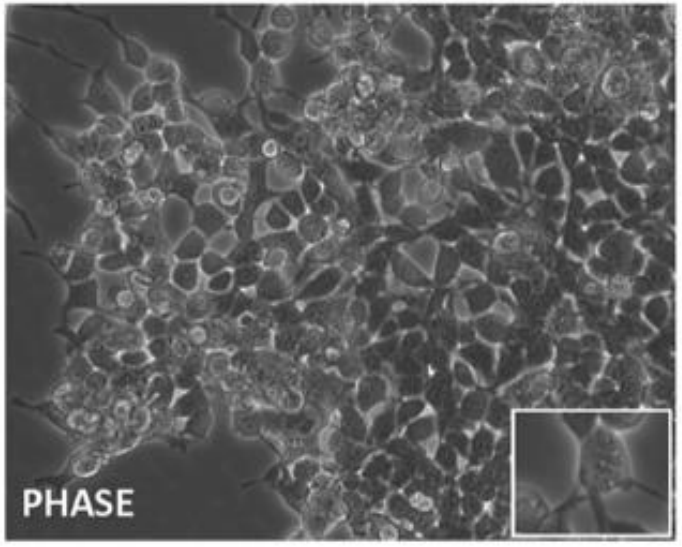


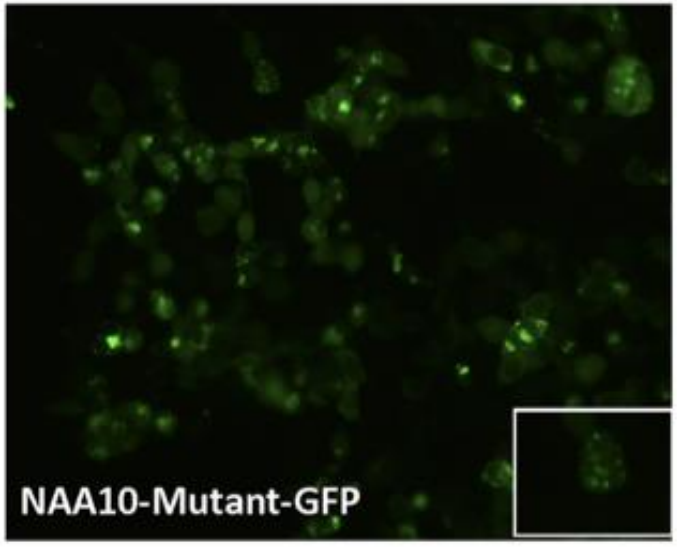
VI-11 is the most severely affected

Results, Naa10 localisation IF assay using 293T cells









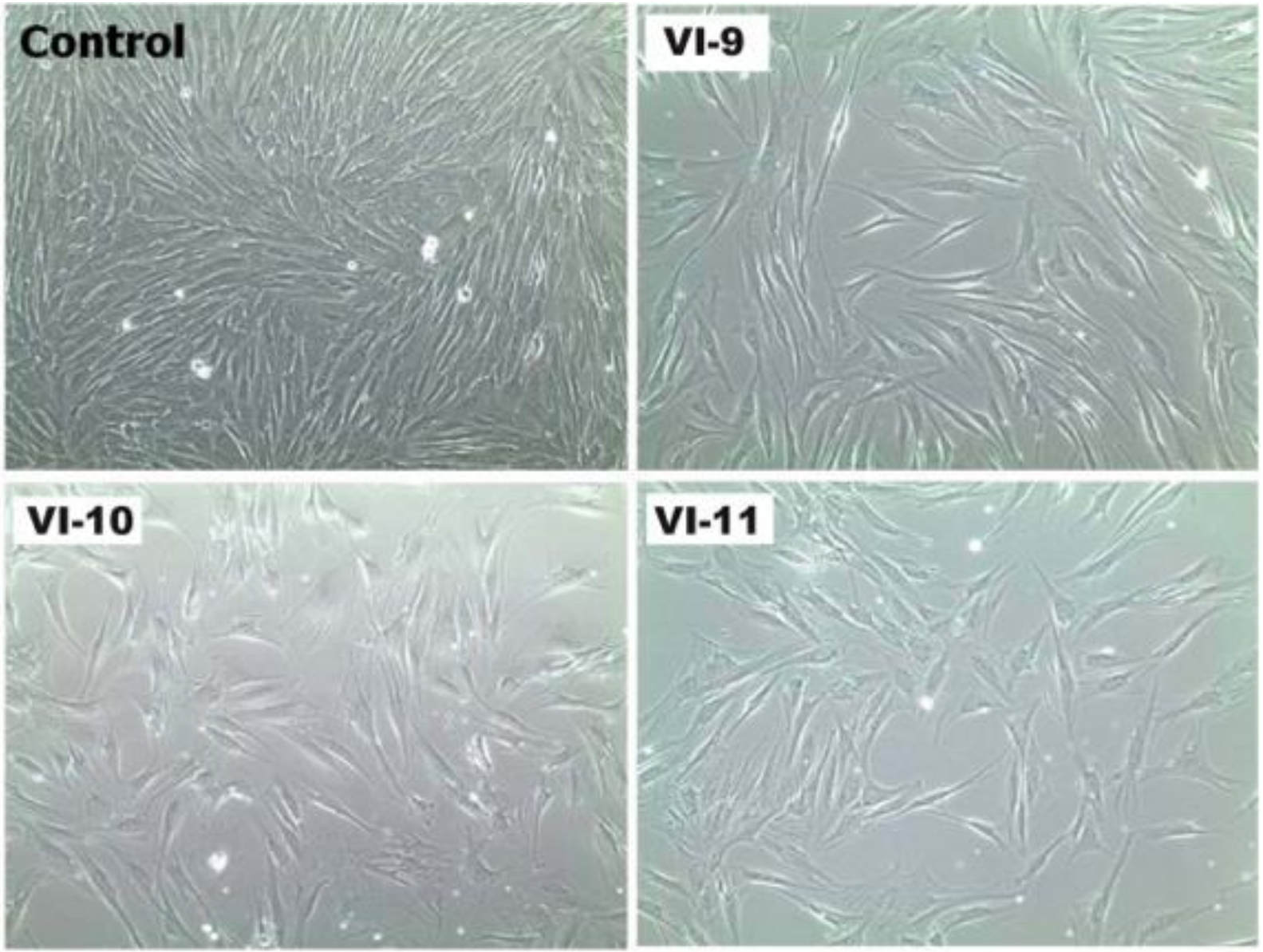
Control Vs VI-11 fibroblasts:

Endogenous Naa10 cell localisation does not change (within the cytoplasm and nucleus).

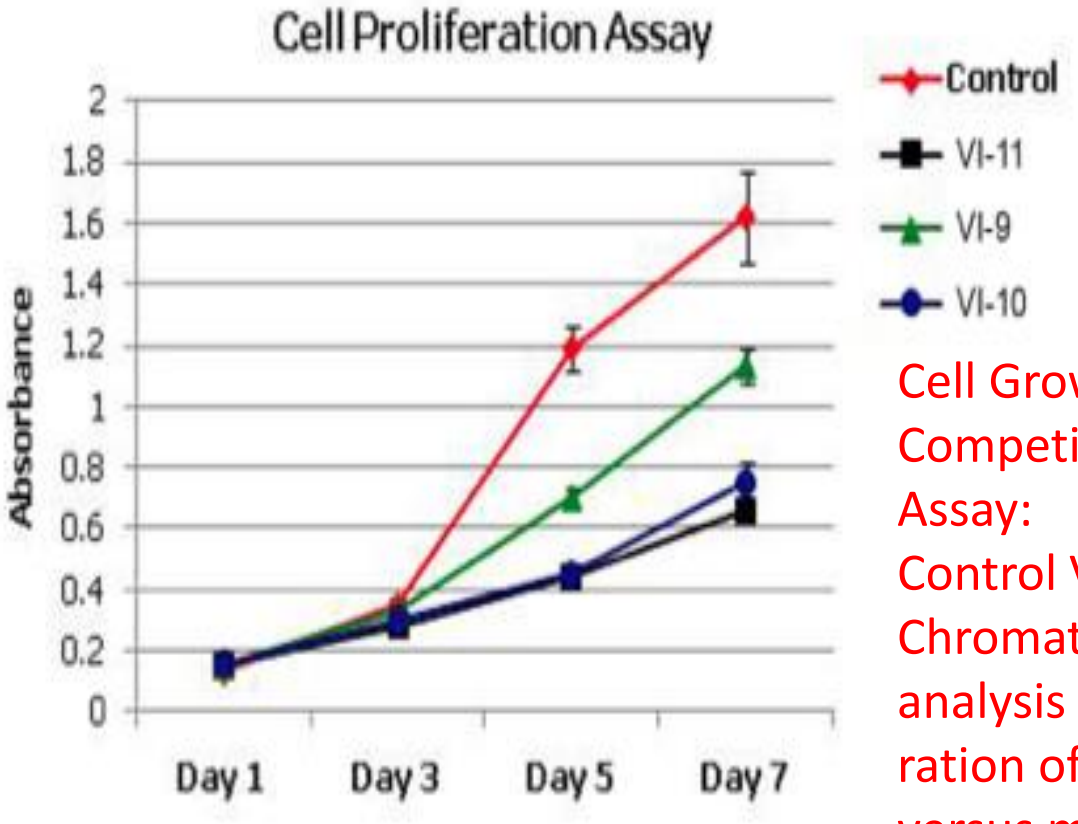
Overexpression:

showed punctate staining, suggesting of protein aggregation within the cytoplasm.

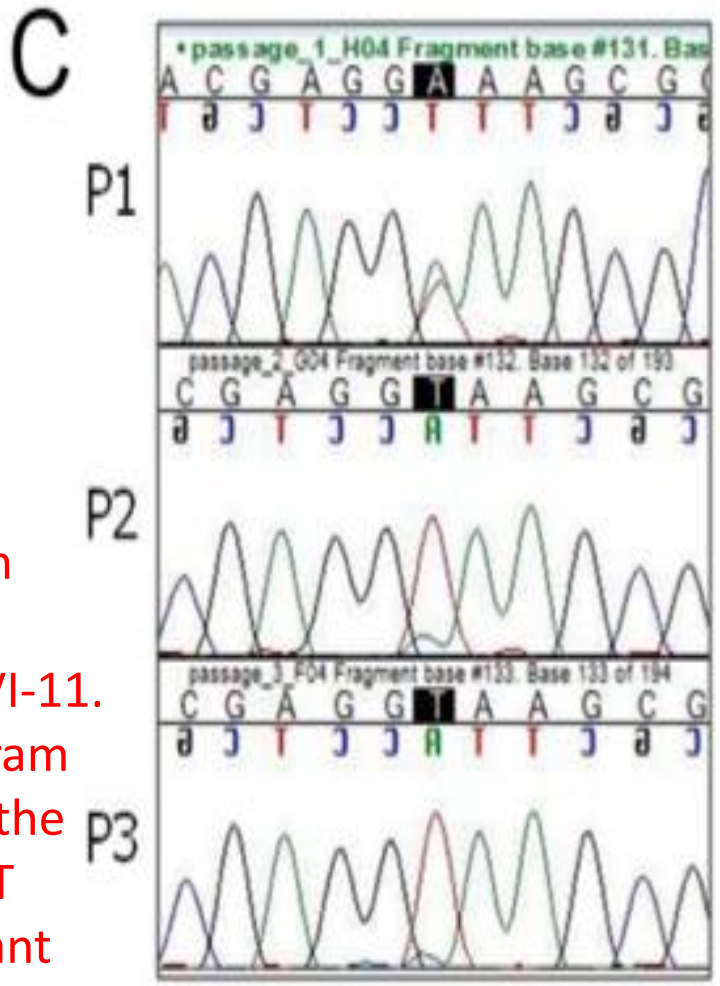
Results, effect on cell proliferation, on Day 5 of culture



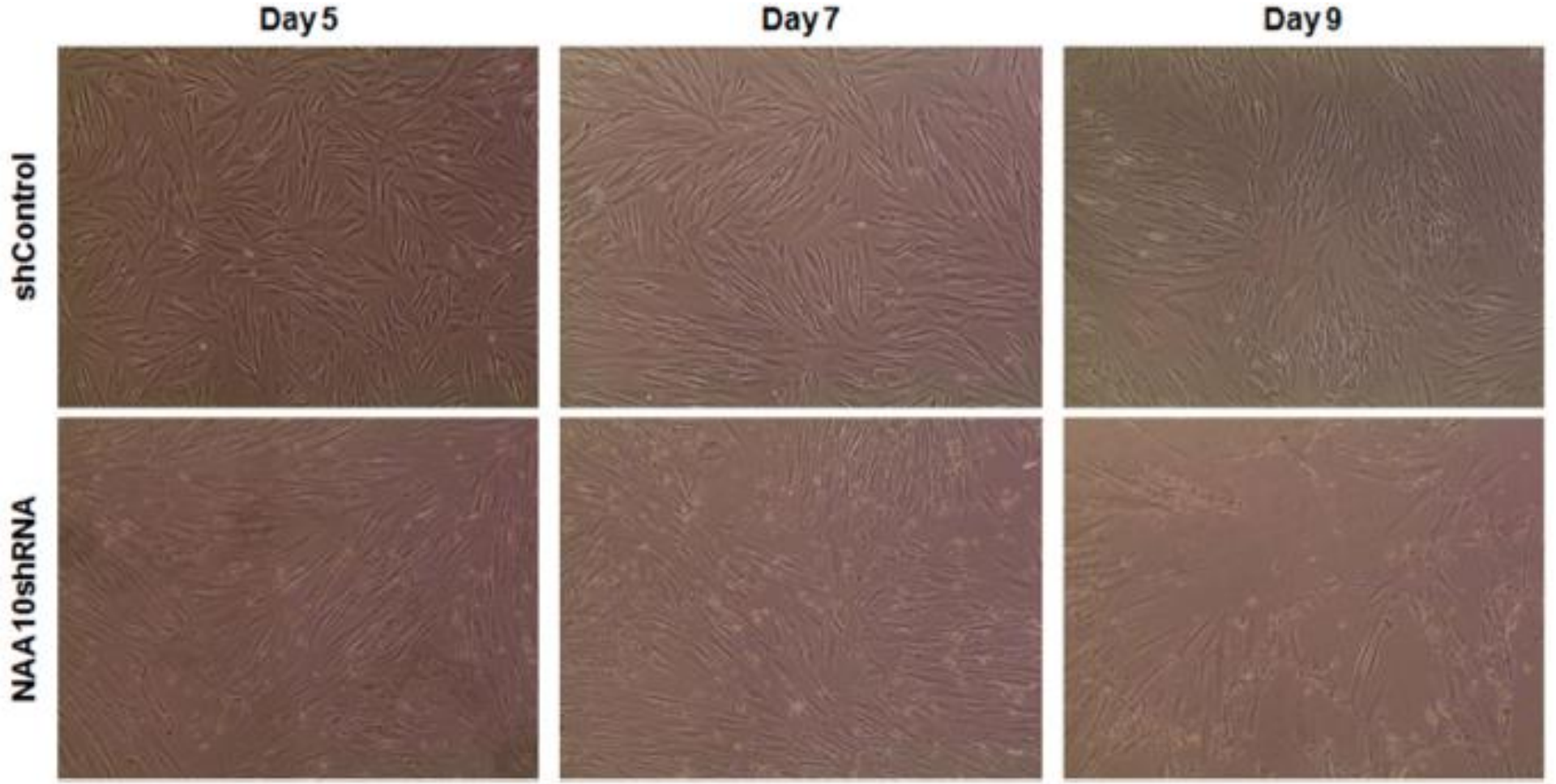
Results, cell growth deficiency



Cell Growth Competition Assay:
Control Vs VI-11.
Chromatogram analysis on the ration of WT versus mutant cells.



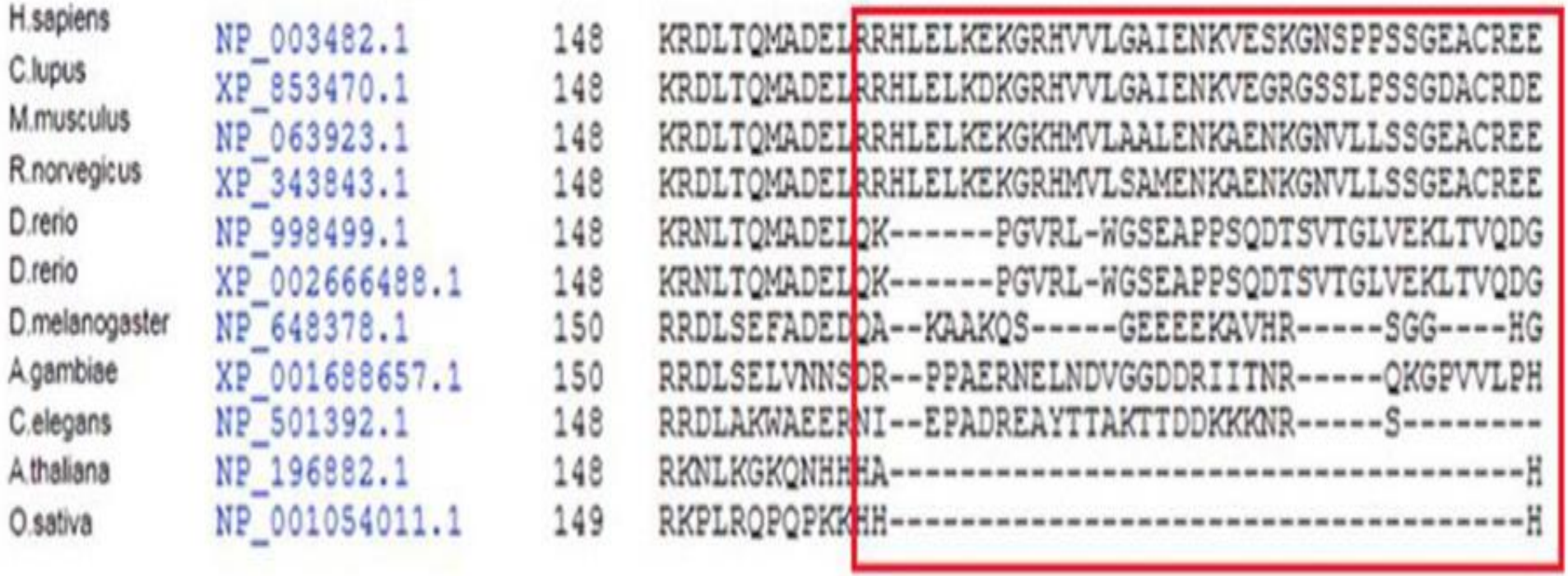
Results, *Naa10* knowdown in NDHF cells using shRNA lentivirus system



PS: four out of five cell lines became apoptotic and proceeded to die within a week of transduction.

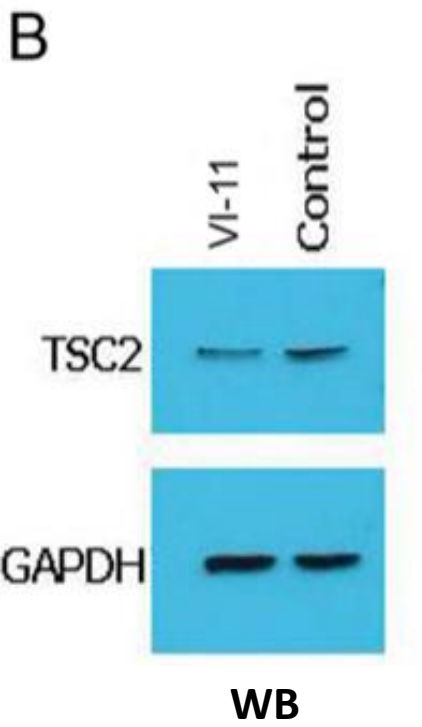
Results, conservation of AA sequences encoded by exon 8 of *Naa10*

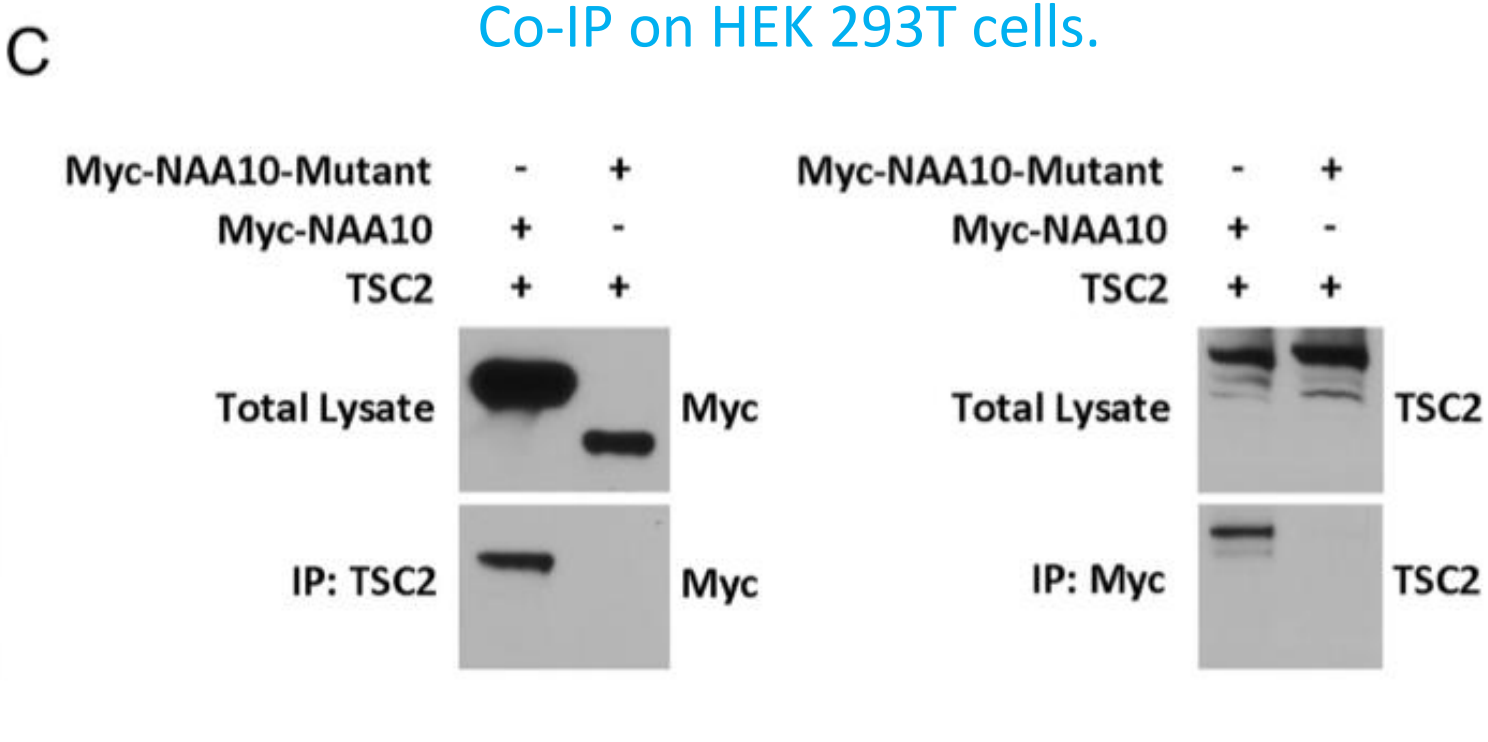
A



H.sapiens	NP_003482.1	148	KRDLTQMADELRRHLELKEKGRHVVLGAIENKVESKGNSPPSSGEACREE
C.lupus	XP_853470.1	148	KRDLTQMADELRRHLELKDKGRHVVLGAIENKVEGRGSSLPSSGDACRDE
M.musculus	NP_063923.1	148	KRDLTQMADELRRHLELKEKGKHMVLAALENKAENKGNVLLSSGEACREE
R.norvegicus	XP_343843.1	148	KRDLTQMADELRRHLELKEKGRHMVLSAMENKAENKGNVLLSSGEACREE
D.rerio	NP_998499.1	148	KRNLTQMADELQK------PGVRL-WGSEAPPSQDTSVTGLVEKLTVQDG
D.rerio	XP_002666488.1	148	KRNLTQMADELQK------PGVRL-WGSEAPPSQDTSVTGLVEKLTVQDG
D.melanogaster	NP_648378.1	150	RRDLSEFADEDQA--KAAKQS-----GEEEEKAVHR-----SGG----HG
A.gambiae	XP_001688657.1	150	RRDLSELVNNSDR--PPAERNELNDVGGDDRIITNR-----QKGPVVLPH
C.elegans	NP_501392.1	148	RRDLAKWAEERNI--EPADREAYTTAKTTDDKKKNR-----S--------
A.thaliana	NP_196882.1	148	RKNLKGKQNHHHA----------H
O.sativa	NP_001054011.1	149	RKPLRQPQPKKHH----------H

Results, effect on TSC2, inhibitor of the mTOR, which promotes cell proliferation

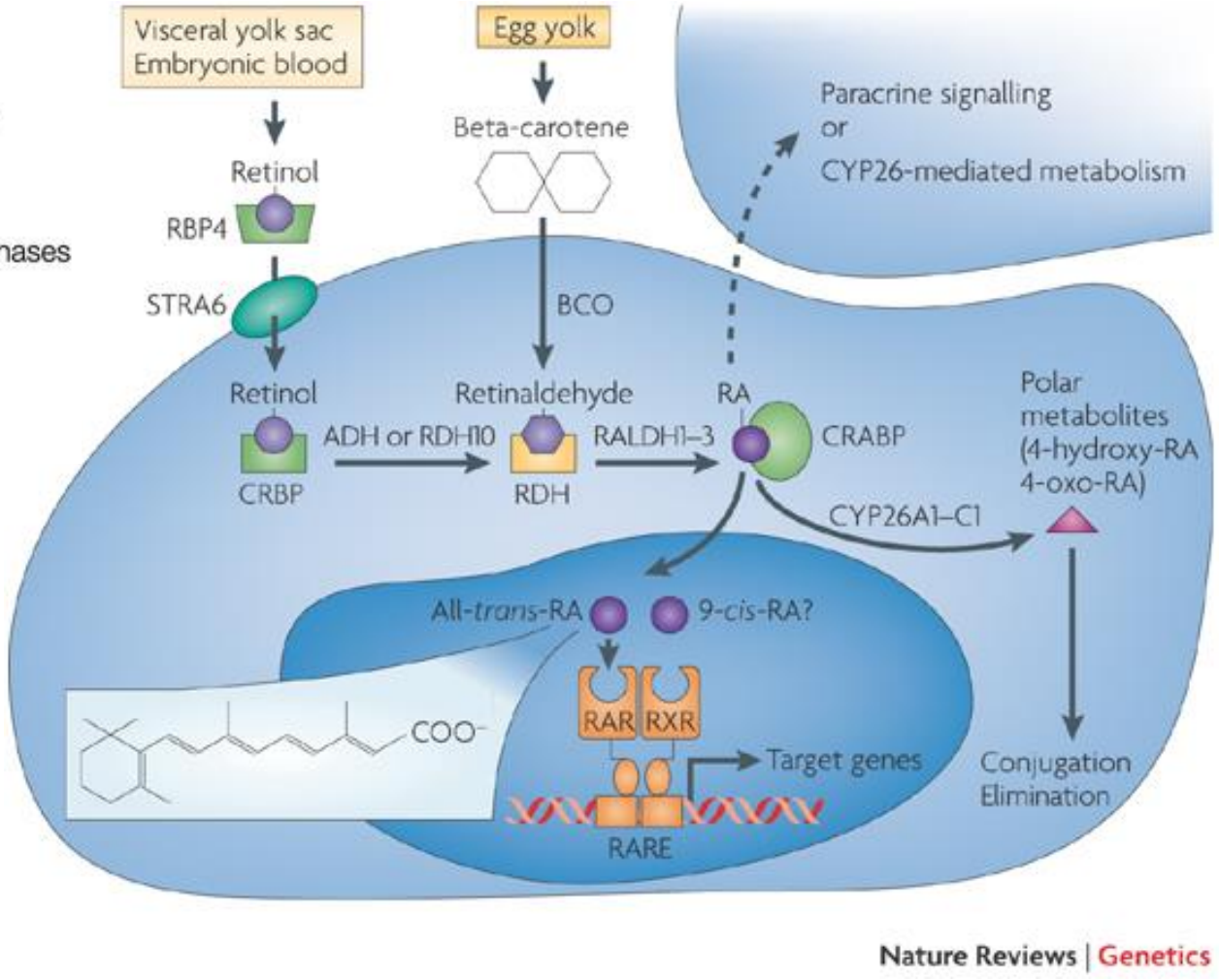




Suggests c.471 +2T→A mutation truncated exon 8 and leads to the loss of TSC2 binding and a reduction of TSC2 protein levels.

Retinoic Acid Signaling Pathways

Vitamin A (retinol)
↓ Alcohol dehydrogenases/ retinol dehydrogenases
Retinal
↓ Retinaldehyde dehydrogenases
all-*trans*-RA, 9-*cis*-RA
↓ CYP26A1, CYP26B1
4-oxo-RA, 4-OH-RA, 5,8-epoxy-RA



Affymetrix gene expression array showed significantly reduction of STRA6 expression in all three affected male patients' fibroblast cells.

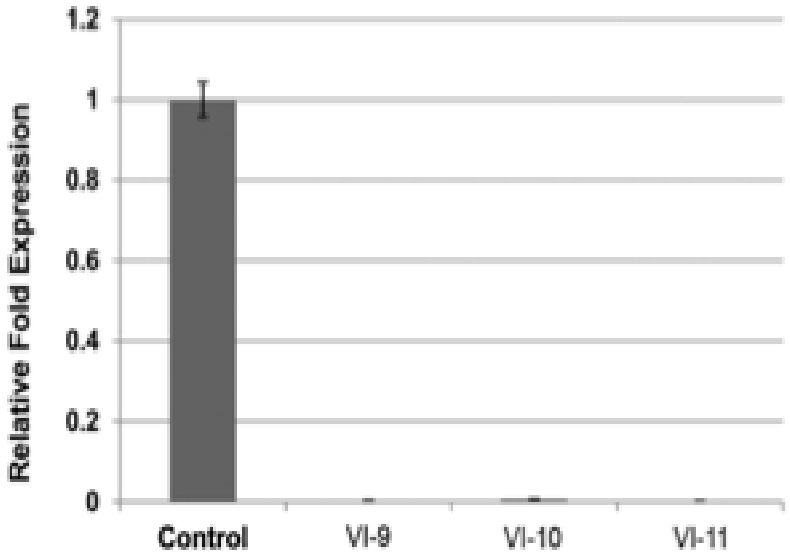
Results, 3H-retinol uptake assay

STRA6 binds the RBP4-retinol complex and mediates cellular uptake of vitamin A, which is the precursor to retinoic acid, a developmental morphogen. The RBP4 disease-associated mutations abolish STRA6's vitamin A uptake activity.

In humans, mutations of STRA6 (15q24.1) results in Matthew-Wood syndrome, characterized by anophthalmia or severe microphthalmia, and pulmonary hypoplasia or aplasia, etc.

A

qRT-PCR of STRA6 expression



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