Sensory Systems: Basics and Principles I

Bernd Wissinger
Molecular Genetics Laboratory
Institute for Ophthalmic Research

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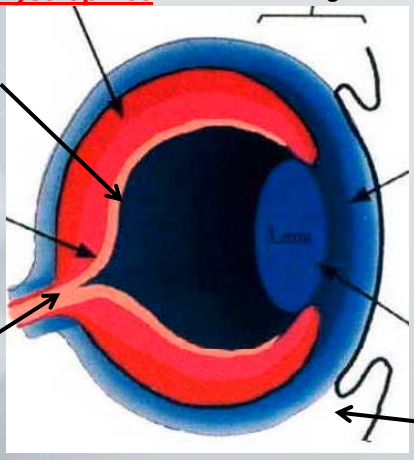
Genetic Diseases of the Eye

Retinal & RPE Dystrophies

Anterior Segment Malformations

Vitreoretinopathies



Corneal Dystrophies

Primary Glaucoma

Cataract

Optic Atrophies

Ocular Cancer:
- Retinoblastoma
- Uveal Melanoma

Ocular Mucle:
- Ptosis
- Ophthalmoplegia

Anophthalmos / Microophthalmia
Myopia / Hyperopia

Epidemiology - Terminology

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- **Prevalence**

  total number of cases of a certain disease in a defined population at a given time

- **Incidence**

  total number of new cases of a certain disease over a defined time period (typically one year) in a defined population

Epidemiology of Retinal Disorders

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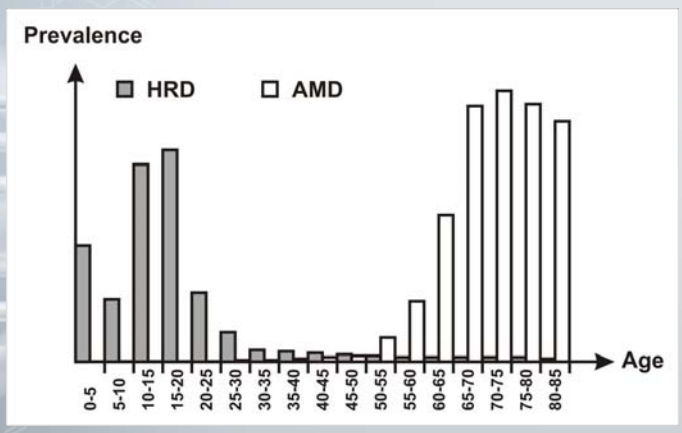
Hereditary Retinal Disorders

- **Prevalence:**
  1:1,490 (Northern France)
  (Puech et al., 1991, J Fr Ophthalmol 14: 153-164)
- **Prevalence in Children:**
  1:10,000 (Denmark),
  (Rosenberg, 1989, Doc Ophthalmol 73: 81-92.
- **Incidence (Legal Blindness; VA<1/50):**
  1:192,300 (Württemberg-Hohenzollern 1994
  (Krumpaszky et al., 1999, Ophthalmologica 213: 176-182)

Epidemiology of Retinal Disorders

Hereditary Retinal Disorders primarly affect Children and young Adults



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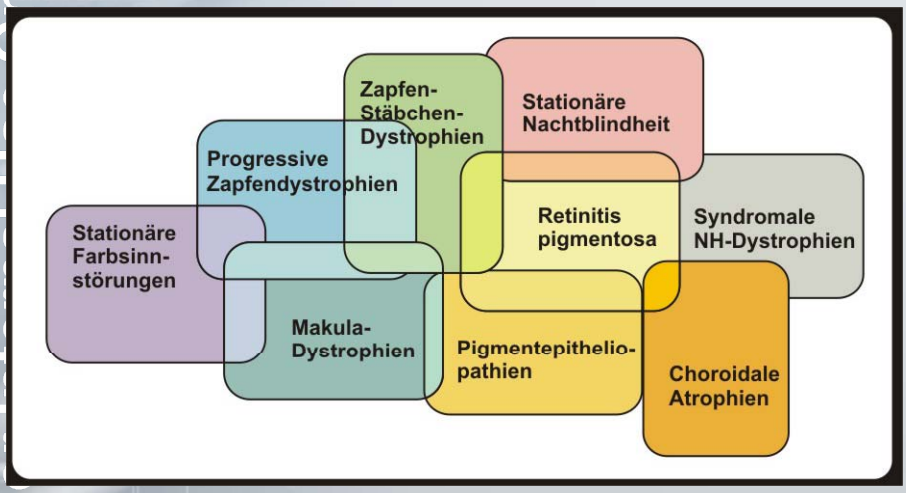
Variability of Hereditary Retinal Disorders



Variability of Hereditary Retinal Disorders

Main Clinical Parameters for Categorization

- **Course: Stationary <> Progressive**
- **Age of Onset: congenital <> late manifesting**
- **Localization: central <> peripheral**
- **Function: Rod <> Cone**
  Photoreceptor <> Synapse
  Neuroretina <> Ret. Pigment Epithelium
- **Morphology: Degeneration <> Dysfunction**
- **Symptomatic: non-syndromic <> syndromic**

Variability of Hereditary Retinal Disorders

Prevalence of Some Retinal Disorders

- **Retinitis pigmentosa**
  Prevalence: 1:2,500 – 1:4,000
  (Boughman et al., 1980, Am J Hum Genet 32: 223-235. Haim et al., 1992, Acta Ophthalmol 70: 178-186.)
- **Stargardts Macular Dystrophy**
  Prevalence: ~1:10,000
  (Blacharski, 1988, in Newsome: Retinal dystrophies and degenerations, Raven Press, NY, p.135-159.)
- **Achromatopsia**
  Prevalence: ~ 1:30,000 – 1:50,000
  (Francois, 1961, Heredity in Ophthalmology. CV Mosby)

Retinitis Pigmentosa

- Patient L.M., 36 years old, Locksmith
- Nightblind since late childhood, complains about progressive Visual Problems, worries about his driving license
- Visual Acuity: 0.5 / 0.4
- Visual Field: concentric reduced Sensitivity
- Color Vision: normal
- Electroretinography:
  - scotopic – sevrly reduced Responses
  - photopic – in lower normal Range
- Funduscopy: „bone-spicule“-like pigmentary Deposits in the Periphery, pale Optic Nerve Head narrow retinal vessels
- Light opaque Lense

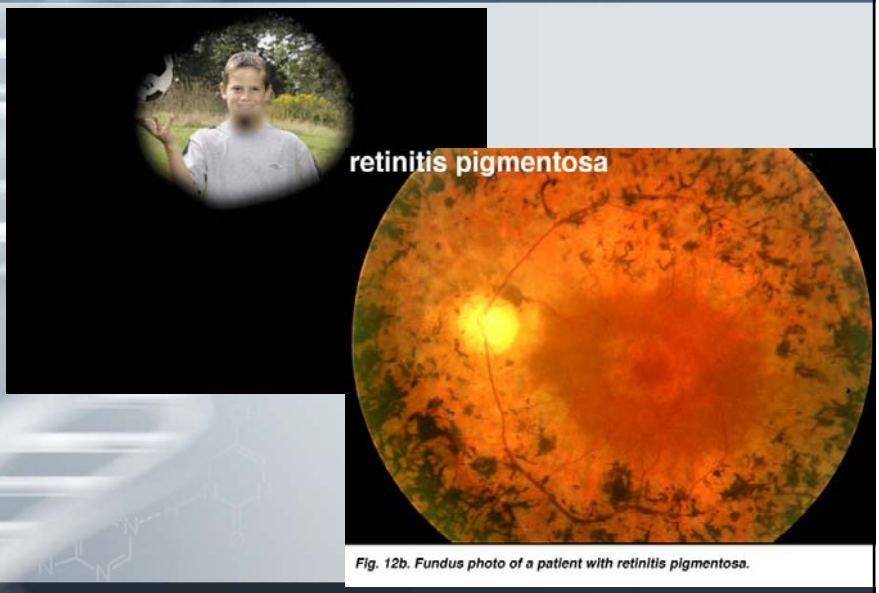


Fig. 12b. Fundus photo of a patient with retinitis pigmentosa.

Heterogeneity in Mode of Inheritance

Retinitis pigmentosa

- **~ 40-50% sporadic**
- **~ 20-30% autosomal recessive**
- **~ 10-25% autosomal dominant**
- **~ 6-18% X-chromosomal recessive**
- **Very rare: maternal, digenic**

Fishman 1978, Arch Ophthalmol 96: 822-826
Boughman et al., 1982, Am J Hum Genet 32: 223-235
Jay 1982, Br J Ophthalmol 66: 405-416
Hu 1982, Am J Med Genet 12: 51-56
Haim 2002, Acta Ophthalmol Scand 233: 1-34

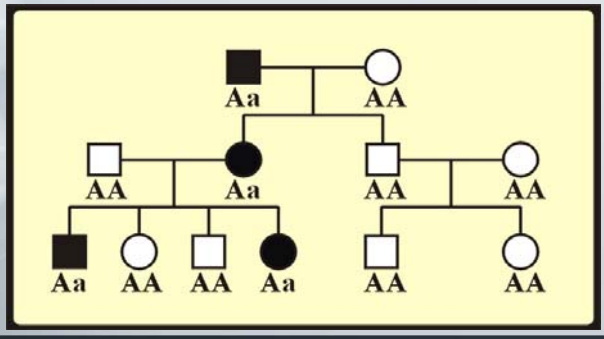
Modes of Inheritance

Autosomal Dominant

One mutant Allel (a) is sufficient for the Expression of the Trait (e.g. disorder)

- affects Males and Female with equal Frequency
- an Affected is always Child of an Affected
- 50% risk for each Child of an Affected

Affected male
Unaffected Male
Affected Female
Carrier Female



Examples ?

Modes of Inheritance

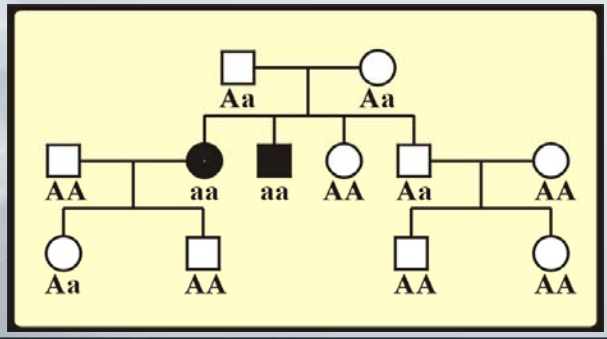
Autosomal Recessive

The Presence of two mutant Allels (a) are required for the Expression of the Trait (e.g. disorder)

- affects Males and Female with equal Frequency
- Parents are in typically unaffected (Heterozygote)
- 25% risk for each Child of a heterozygous Couple

Affected male
Unaffected Male
Affected Female
Carrier Female

Examples ?



Modes of Inheritance

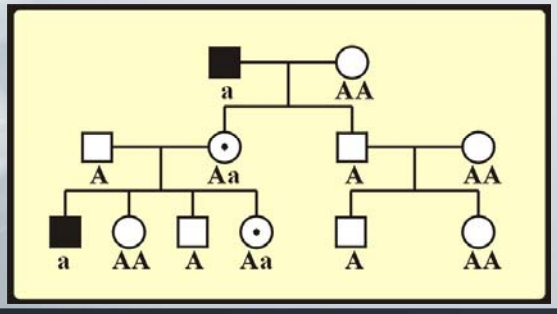
X-chromosomal Recessive

sole Presence of a mutant Allels (a) [in males; XY] or the Presence of two mutant Alleles [in Females, XX] is required for the Expression of the Trait (e.g. disorder).

- affects most exclusively Males
- no direct male-to-male Transmission
- 50% risk for each Son of a female Carrier

Affected male
Unaffected Male
Affected Female
Carrier Female

Examples ?



Modes of Inheritance

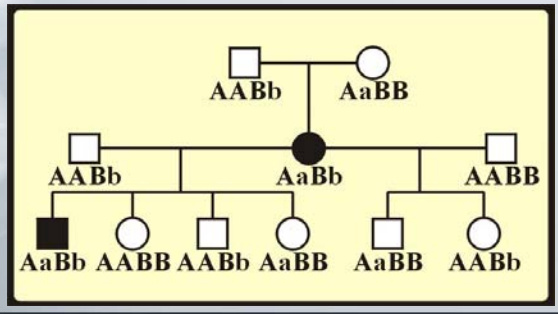
For Internal Use Only

Digenic (co-dominant)

The Presence of two mutant Allels (a, b) at independent Loci is required for the Expression of the Trait (e.g. disorder).

- affects Males and Females with equal Frequency
- an Affected may be Child of an Affected
- 25% risk for each Child of a double heterozygous Couple

Affected male
Unaffected Male
Affected Female
Carrier Female



Examples ?

Modes of Inheritance

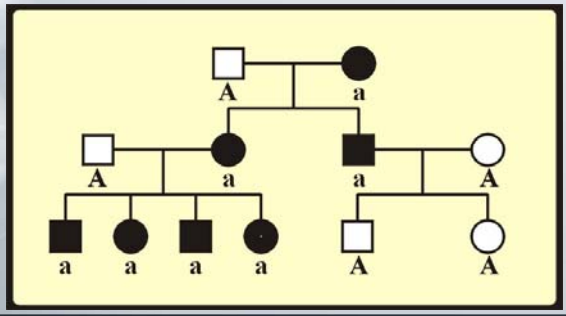
For Internal Use Only

Maternal (mitochonrial)

A mutant Allels (a) is required for the Expression of the Trait (e.g. disorder) and is exclusively transmitted via the Mother

- affects Males and Females with equal Frequency
- an Affected is always Child of an affected Mother
- no male-to-offspring Transmission
- 100% risk for each Child of an affected Mother

Affected male
Unaffected Male
Affected Female
Carrier Female



Examples ?

Pathology of the Eye: Genetics
Sensory Systems: Basics and Principles I

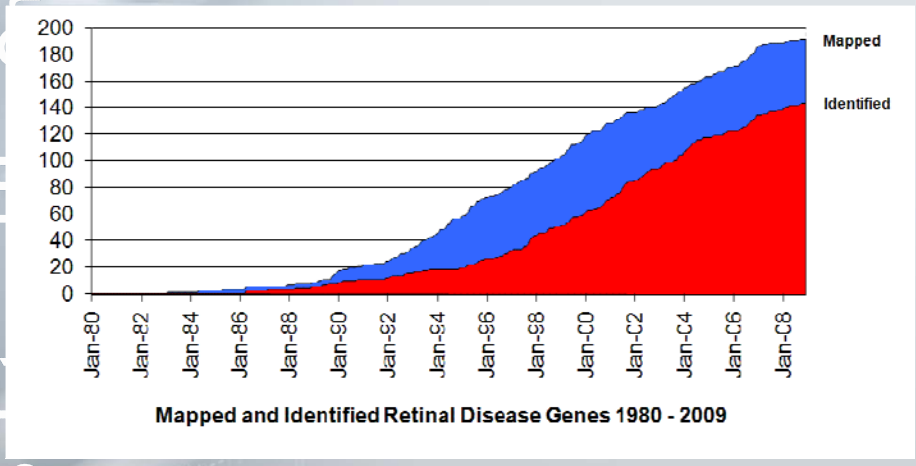
Heterogeneity in Mode of Inheritance

- **Congenital Stationary Nightblindness**
  X-L, AR, AD
- **Cone Dystrophy**
  X-L, AR, AD
- **Stargardt Macular Dystrophy**
  AR, AD
- **Leber Congenital Amaurosis**
  AR
- **Usher Syndrome**
  AR
- **Best Vitelliforme Macular Dystrophy**
  AD

Genetic Terminology

- **Locus**
  Physical or genetic Position of a Trait, Gene or DNA Sequence on a Chromosome.
- **Allele** (short for „allelomorph“)
  One of a series (two or more) of distinguishable Variants of a Locus on homologous chromosomes.
- **Genotype**
  (In a diploid Organism two copies of each Gene/DNA Element):
  Allelic Composition for a certain Gene or DNA Element on homologous chromosomes:
  Homozygous/Homozygosity – Identity of Alleles
  Heterozygous/Heterozygositiy – different Alleles
  (Hemizygous/Hemizygosity – only one Allele present)

Progress in Gene Mapping & Gene Identification



Mapped and Identified Retinal Disease Genes 1980 - 2009

Source: Retinal Information Network (RETNET), http://www.sph.uth.tmc.edu/Retnet/

Locus Heterogeneity – AD Retinitis Pigmentosa

Locus	*Chromosome*	*Gene*	*Prevalance*
RP4	3q22	RHO	15-26%
RP7	6p21	RDS	3-9%
RP1	8q12	RP1	3-6%
RP13	17q13	PRPF8	3-6%
RP11	19q13	PRPF31	5-10%(21% in UK)
RP18	1q21	PRPF3	1-3%
RP10	7q32	IMPDH1	2-5%
RP27	14q11	NRL	
	19q13	CRX	
	17q25	FSCN2	
RP17	17q23	CA4	
	1q22	SEMA4A	
RP37	15q23	NR2E3	
RP31	9q21	TOPORS	
RP42	7p15.3	KLHL7	
	2q11	SNRNP2000	
RP9	7p14	(PIM1K)?	

Locus Heterogeneity – AR Retinitis Pigmentosa

Gene	Chromosome	Gene	Chromosome
RHO	3q22	RGR	10q23
PDE6A	5q33	LRAT	4q32
PDE6B	4p16	TULP1	6p21(RP14)
SAG	2q37	MERTK	2q13
CNGA1	4p12	CRB1	1q31(RP12)
CNGB1	16q13	USH2A	1q41
RPE65	1p31(RP20)	NR2E3	15q23
RLBP1	15q26	CERKL	2q31(RP26)
ABCA4	1p22(RP19)	RPGRIP	14q11
NRL	14q11	RP1	8q12
EYS	6q12	IDH3B	20p13
PROM1	4p15	RBP3	10q11
SPATA7	14q31		

+ 4 mapped Loci (RP22, RP28, RP29, RP32)

Locus Heterogeneity – Usher Syndrome

Usher Syndrome (AR):
Typ 1: RP + congenital Deafness + Ataxia
Typ 2: RP + Deafness (HF)
Typ 3: RP + progressive Hearing Loss

Locus	Chrom.	Gene	Locus	Chrom.	Gene
~~USH1A~~	14q32	?	USH2A	1q41	Usherin
USH1B	11q13	MYO7A	USH2B	3p23	?
USH1C	11p15	Harmonin	USH2C	5q14	MASS1
USH1D	10q22	CDH23	USH2D	9q32	Whirlin
USH1E	21q	?			
USH1F	10q11	PCDH15	USH3A	3q21	Clarin-1
USH1G	17q24	SANS	USH3B	20q	?

But also: Unique Disease Loci

- **Stargardt Macular Dystrophy**
  AR ABCA4 1p21-p22
- **Best Vitelliforme Macular Dystrophy**
  AD VMD2 11q13
- **Sorsby Fundus Dystrophy**
  AD TIMP3 22q12-q13
- **Cone Dystrophy with Supernormal Rod Response**
  AR KCNV2 9p24

How to identify a „*Disease Gene*“ ?

- **Candidate Gene Analyses**
  Knowledge-based Approach, Hypothesis-driven
  Reverse Genetics: From Gene to Function (Malfunction)

Mutation Screening in Genes which based on their specific Function and/or Expression in the affected Organ/Tissue/Cell are considered as potential Cause of a certain Disorder.

For Instance: Genes for Components of the Phototransduction Cascade (Rhodopsin, Transducin, Phosphodiesterase, CNG-Channel), Genes involved in the retinal Vitamine A Metabolism and ~ transport (ABCA4, LRAT, RDH5)

How to identify a „*Disease Gene*“ ?

- **Linkage Analyses & Positional Cloning**
  Hypothesis-free Approach
  Forward Genetics: From Phenotype to Gene

Identification of a „Disease Gene“ based on its Location in the Genome.
Hypothesis-free Approach overcomes the (still) limited Knowledge of Gene Function, Cellular Processes & Disease Mechanisms.
„You can identify a Gene on which nothing is known or a Gene you never thought it might cause such a disorder“

For Instance: Genes for ubiquitously expressed Splicing Factors in AD Retinitis Pigmentosa.

How to identify a „*Disease Gene*“ ?

Steps in Linkage Analyses & Positonal Cloning

- **Linkage Analyses**
  - **Mapping the Disease Locus to a specific Chromosome or chromosomal Region**
  - **Searching for a Marker that shows a valid Pattern of Co-Inheritance with the Trait in questiuon.**
- **Refined Mapping of the Disease Locus through meiotic Breakpoint Mapping**
- **Mutation Screening in Genes that localize to the mapped chromosomal Region**

Linkage Analysis

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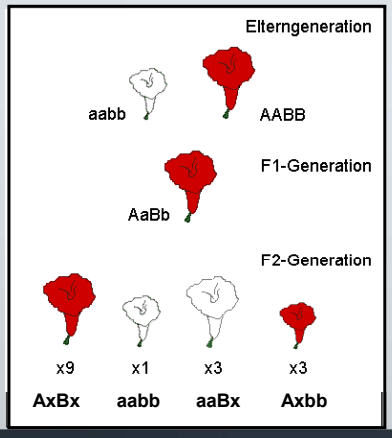
Mendel's Third Law (Principle of independent Assortment)

"Alleles for different traits are distributed to sex cells (offspring) independently of one another."

Recombination of traits will appear in the **F2 generation in predictable ratios.**

Trait 1: Flower Size

Trait 2: Flower Color



Linkage Analysis

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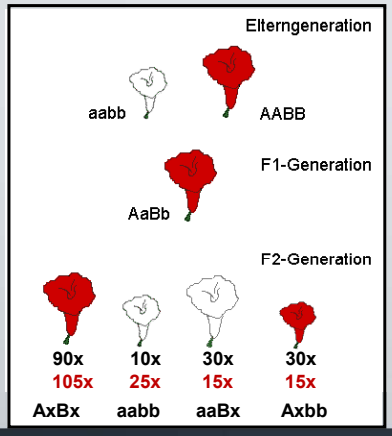
But: There are numerous examples in which the expected ratios of parental and recombinant phenotypes in F2 are not obtained.

>> The traits do not segregate independently.

Trait 1: Flower Size

Trait 2: Flower Color



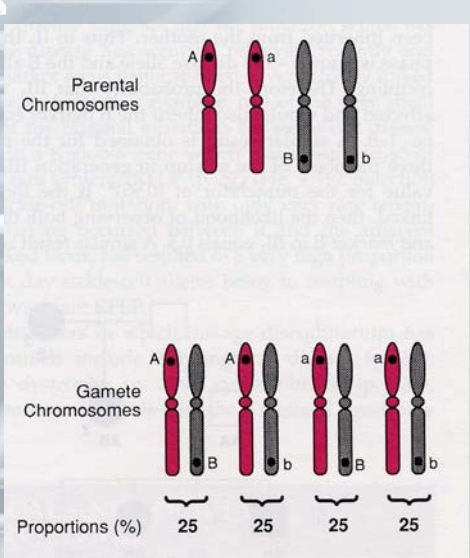
Expected (n=160):
Obtained:

Linkage Analysis

Chromosoms are the physical Units of Inheritance

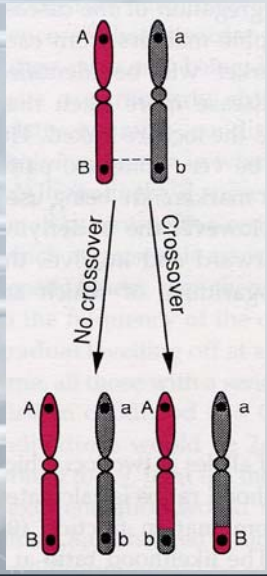
- **Genes (traits) localizing on different Chromosoms segregate independently.**
- **Genes (traits) localizing on the same chromosome segregate together (i.e. linkage)**

Linkage Analysis



Genes (traits) localizing on different Chromosoms segregate independently.

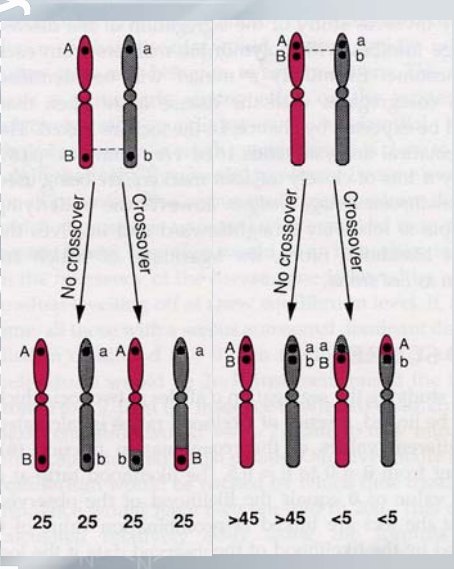
Linkage Analysis



Genes (traits) localizing on the same chromosome segregate together (i.e. linkage)

Recombination events between homologous Chromosoms during Meiosis (Crossover) break this Linkage.

Linkage Analysis



- **The Probability of a meiotic Recombination depends on the distance of the two Gene (Trait) Loci on the Chromosome.**
- **The smaller the distance - the lower the frequency of recombination.**
- **The frequency of recombination between two Loci is given in cM (centi-Morgan).**
  1 cM = 1% Recombination

Linkage Analysis

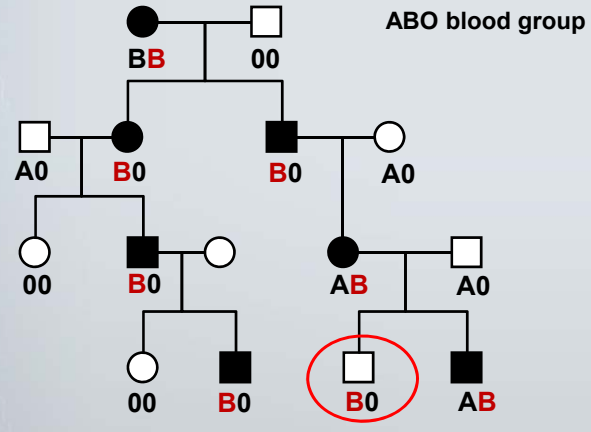
Linkage Analysis relies on both
i) the co-segregation of Traits
ii) the Occurence of Recombinations.

The Principle Question:
Am I able to establish a Linkage between a new, unknown Trait and a known test Trait ?

If I now the chromosomal Localisation of the test Trait, then – in case of Linkage – I can conclude that the unknown Trait also localizes to the same Chromosome

Linkage Analysis

Autosomal Dominant inherited Disorder



Disorder is linked to ABO blood group,
recombination frequency ~ 10%

Linkage Analysis

Today DNA Sequence Variations are used as Marker: Microsatellites, Single Nucleotide Polymorphisms (SNP)

Microsatellite marker D2S2311 on Chromosome 2q11

.....CATGAGCATGGAGTGTCTTGTAGTGCCGGAAAGTACTCAAAA
AGCACAATGTTGGGGGGCAAGTGGCGATAGGGCTTTCTACACAC
ACACACACACACACACACACACACACACACACACACACACACAC
GGAAGAAAATTAGAGGGACATAGCAACCAACCTGAAAGGGCTCC
CAATGGCCAAAACTGAAGCCATTTGGAAAACCAAATTTTTAAAT
TTAGTATTAGACTAT.....

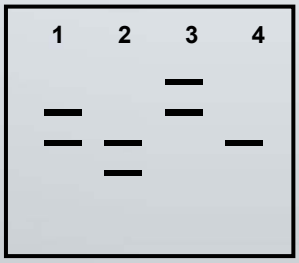
The number of CA-repeats in this sequence varies in the population !!!!

Linkage Analysis

Microsatellite marker D2S2311 on Chromosome 2q11

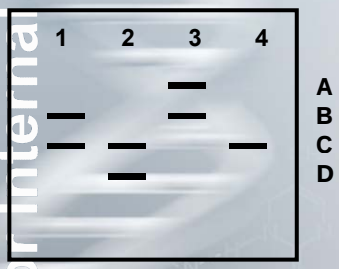
→
CATGAGCATGGAGTGTCTTGTAGTGCC
GGAAAGTACTCAAAAAGCACAATGTTG
GGGGGCAAGTGGCGATAGGGCTTTCTA
CACACACACACACACACACACACACAC
ACACACACACACACACACACACGGAAG
AAAATTAGAGGGACATAGCAACCAACC
TGAAAGGGCTCCCAATGGCCAAAACTG
AAGCCATTTGGAAAACCAAATTTTTAA
ATTTAGTATTAGACTATATCCCAA
←

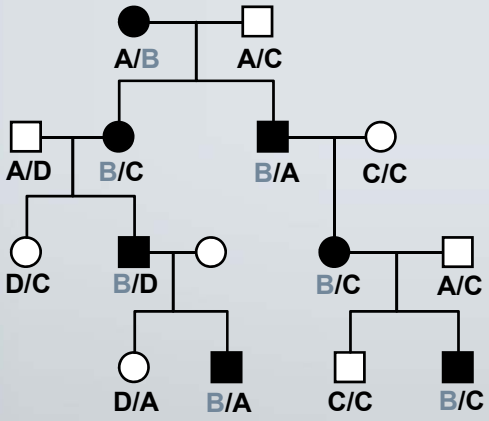
PCR amplification
v
Sizing of the DNA fragment on a gel



Linkage Analysis

Microsatellite Marker Segration in a Disease Pedigree





No Recombination !!: Θ = 0.0 – but how reliable is that ?

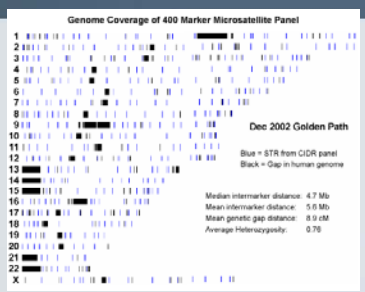
Linkage Analysis

Genome scans

STS: ~ 400 markers (ABI Prism)

Mean genetic distance 8.9 cM

Average heterozygosity 0.76

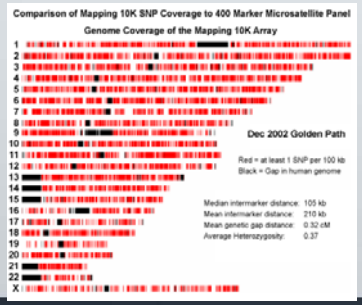


SNP: ~ 10,000 markers

Mean genetic distance 0.32 cM

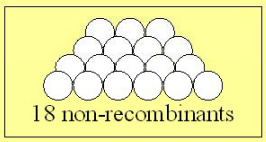
Average heterozygosity 0.37

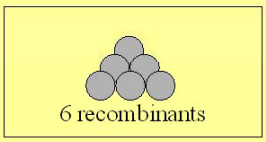
(Affymetrix 10K SNP array)



Linkage Analysis

How to estimate θ?

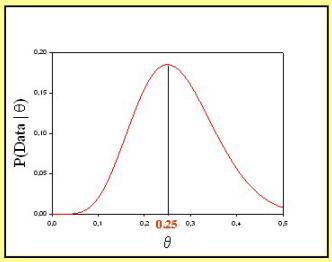




intuitively: $\hat{\theta} = \frac{6}{24} = 0.25$

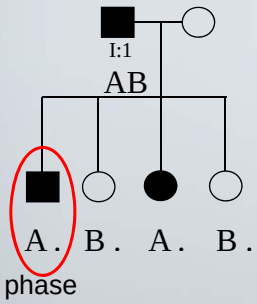
"maximum likelihood principle"

$P(Data|\theta) = \binom{24}{6} \cdot \theta^6 \cdot (1-\theta)^{18}$



Linkage Analysis

Statistical Considerations of Segregation Data



(Chance-)Probability: ½ x ½ x ½ = 1/8

Linkage Analysis

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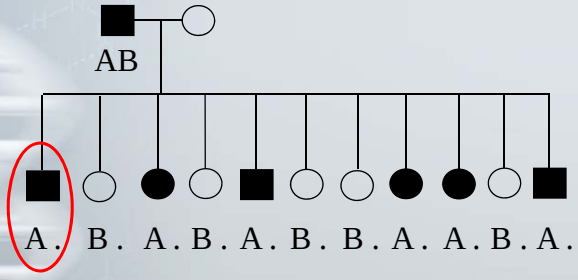
Statistical Considerations of Segregation Data



(Chance-)Probability: ½ x ½ x ½ x ½ x ½ x ½ x ½ x ½ x ½ x ½ = 1/1024

Linkage Analysis

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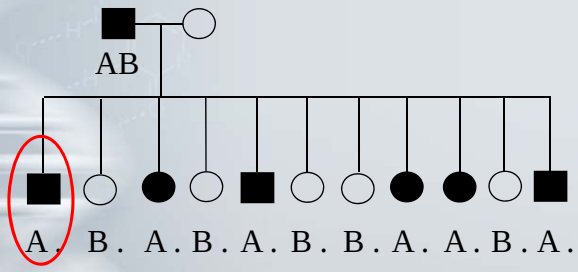
Statistical Calculations: LOD Scores



LOD score = logarithm of the odds ratio = $\log_{10}$ $\frac{\text{Likelyhood at } \theta}{\text{Likelyhood at } \theta = 0.5}$

Rule-of-thumb: For a dominant trait you need 11 informative Meioses to reach a LOD score of Z = 3.0 (significance threshold).

Linkage Analysis

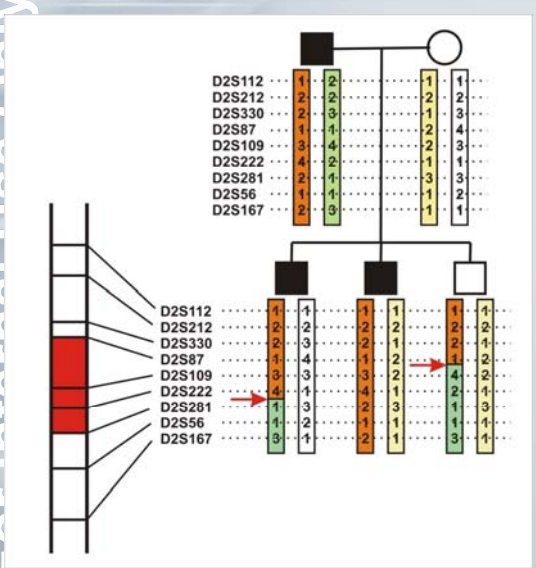
Table 1

Two-Point LOD Scores between the Polymorphic Markers Derived from Chromosome 10 and MBS in the Present Family

	LOD Score at θ =							
Locus	.00	.05	.10	.20	.30	.40	θ_{max}	LOD_{max}
D10S196	−∞	−1.01	.01	.68	.70	.41	.25	.74
D10S539	−∞	.48	1.28	1.68	1.42	.78	.20	1.68
D10S589	−4.65	1.74	2.14	2.08	1.54	.78	.14	2.20
D10S581	.51	4.47	4.33	3.52	2.36	1.04	.05	4.47
D10S557	2.38	2.26	2.09	1.64	1.10	.53	.00	2.38
D10S1241	2.86	2.77	2.60	2.02	1.23	.40	.00	2.86
D10S599	1.76	1.61	1.45	1.10	.74	.37	.00	1.76
D10S502	−5.42	.71	1.19	1.32	.98	.44	.17	1.35
D10S1670	−.21	2.74	2.87	2.59	1.92	1.01	.10	2.87
D10S522	−1.72	1.37	1.50	1.36	.98	.48	.11	1.51
D10S1646	−8.57	−1.50	−.79	−.11	.18	.21	.36	.22
D10S210	−.21	2.59	2.61	2.19	1.52	.76	.08	2.63
D10S1647	−1.08	1.62	1.81	1.67	1.23	.65	.12	1.82
D10S1678	−3.42	−.76	−.49	−.19	.00	.08	.40	.08
D10S1672	−.34	2.62	2.79	2.54	1.90	1.01	.11	2.79

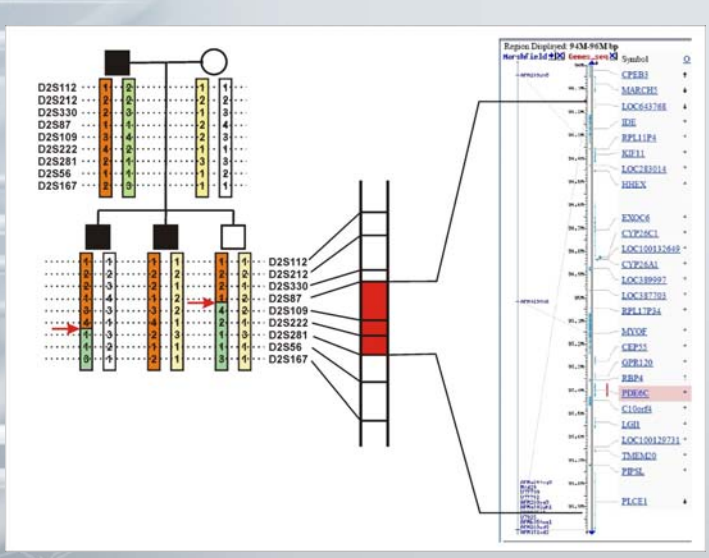
Example of a *Two Point LOD Score* - Table

Mapping of Meiotic Breakpoints



- **Reconstruction of the Phase of subsequent Marker Alleles**
- **Reconstruct Recombination Events**
- **Define Phase Segment that is shared by all Affecteds and absent in Unaffecteds**

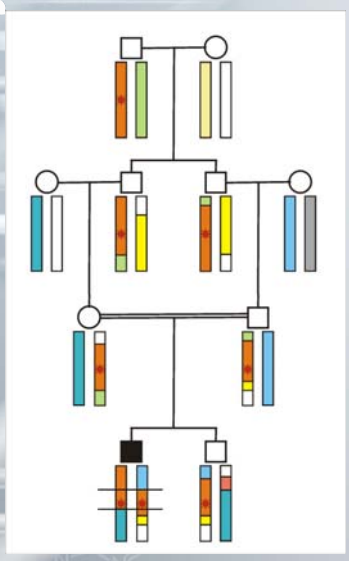
Translation into Physical Segment



Problems using Linkage Analysis

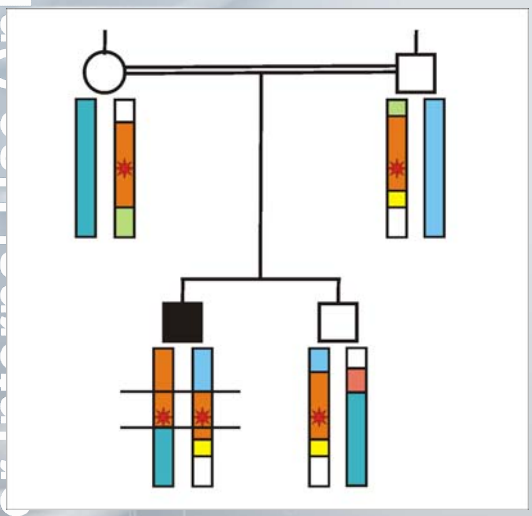
- To obtain a significant LOD Score large Families are required:
  e.g. 11 informative meiosis
  = 6 Affecteds for an autosomal recessive Trait
  = 4 Affecteds + 3 Unaffecteds
  = 3 Affecteds + 5 Unaffecteds
- Combining unrelated Families for Linkage Analysis is possible (additive)
- Locus Heterogenity will blast your results

Homozygosity Mapping



- For autosomal recessive Traits
- Can be applied on small Families or even single Patients
- Requires known or suspected Consanguinity of Parents
- Hypothesis: Patient is homozygous for a Mutation due to IBD (identity-by-descent)
- In addition to the Mutation the Patient is also homozygous for the flanking chromosomal region

Homozygosity Mapping



- Identify Regions of Homozygosity on the Genome
- High resolution SNP chips (e.g. 250k)
- Typically several Regions (on different chromsomes) of several Megabases

Mutations

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- **(Disease) Allele Heterogeneity**

Presence of multiple Alleles of a Gene that give rise to the same (non-wildtype) phenotype.

In the Context of „disease Genes“:
Different Mutations in a certain Gene cause the same Disorder.

This is the rule in Retinal Disorders and also in most other inherted Disorders

Mutations: CNGA3

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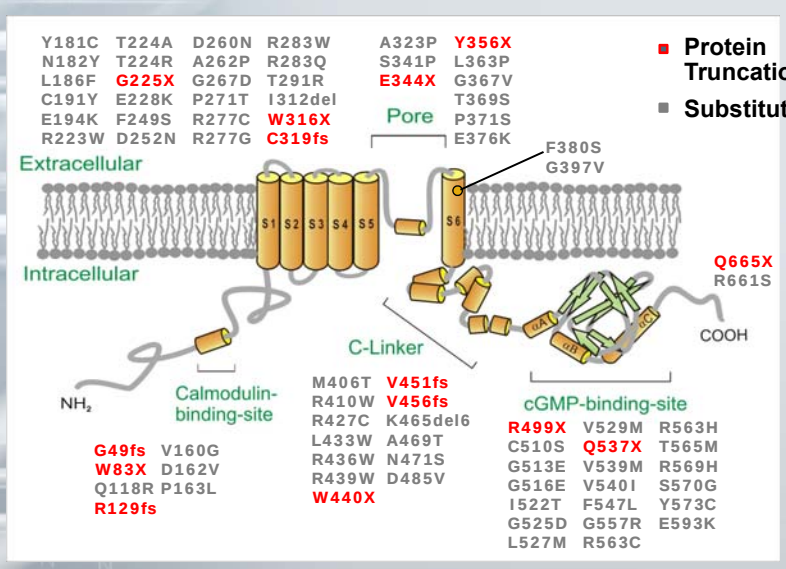
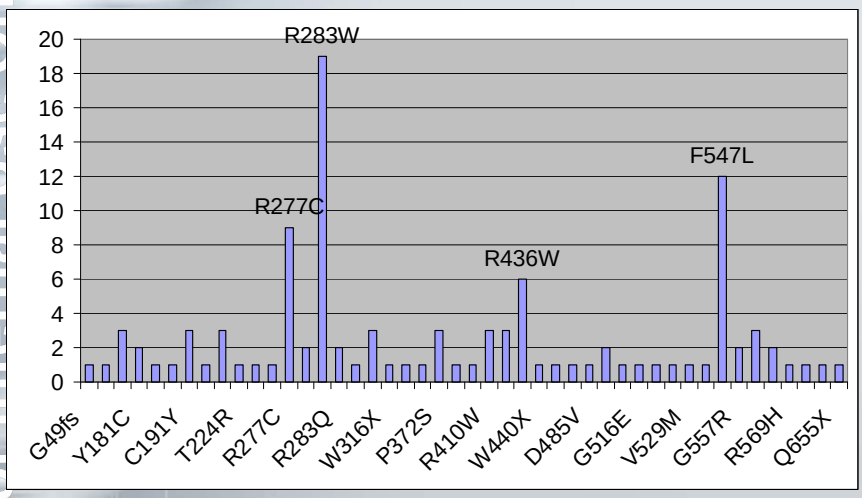


Frequency of Mutations: CNGA3



Types of Mutations

- ***Missense* Mutation**

= Amino Acid Substitution
-GGA- ➢ -AGA- = Gly ➢ Arg

- ***Nonsense* Mutation**

= Change into a Stop Codon
-GGA- ➢ -TGA- = Gly ➢ Stop

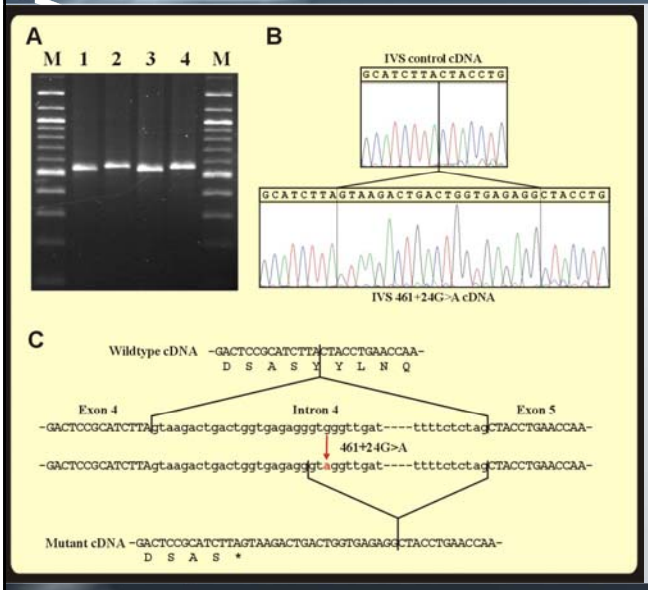
- **Deletion/Insertion**

= causes a Frameshift or the loss/gain of Amino Acid Residues
-GGA AGA GGA C- ➢ -GAA GAG GAC-
Gly –Arg -Gly ➢ Glu- Glu- Asp

- **Transcript Processing Mutations**

Missplicing of primary Transcripts
➢ Deletions/Insertions

Types of Mutations: Splicing Mutation



Example:
GNAT2 – Mutation in Achromatopsie

Activation of a cryptic Splice Site upon Mutation in the Intron (+24)

Dominant Mutations

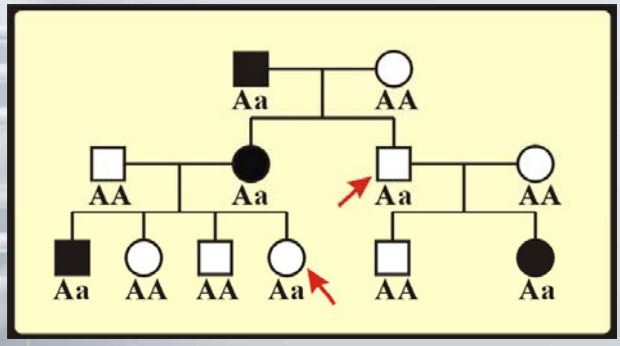
How to explain the dominant Effect of a Mutation

- **Haploinsufficiency**
  Single Gene Dose is not sufficient to maintain Function (whole Gene Deletions, early Stop Mutations)
- **Gain-of-Function**
  Mutant Protein with altered Function or Interaction:
  - permanent Activity, Mislocalization

Reduced Penetracne

- **Reduced Penetrance**

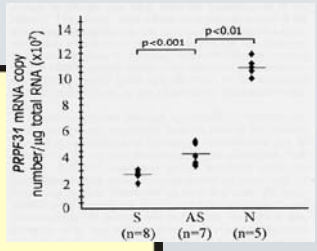
The Presence of a mutant Allele does not always lead to Expression of the Trait/Disease In Particualr in Dominant Disease

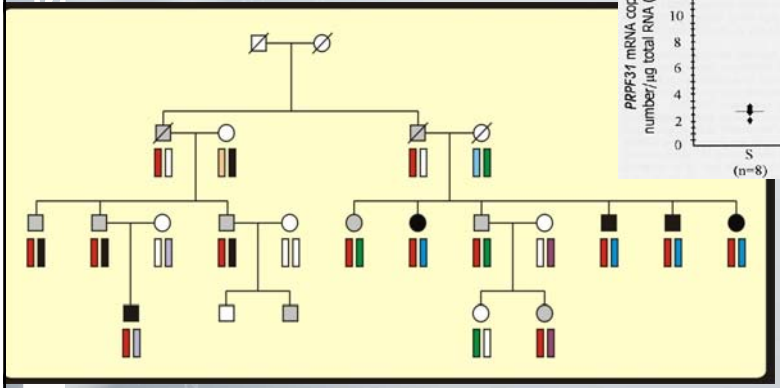


Reduced Penetracne

<u>Retinitis pigmentosa (AD):</u> PRPF31 (1115-1125del)
Vithana et al, 2003, Invest Ophthalmol Vis Sci 44: 4204-4209.

Expression Level of the WT-Allele



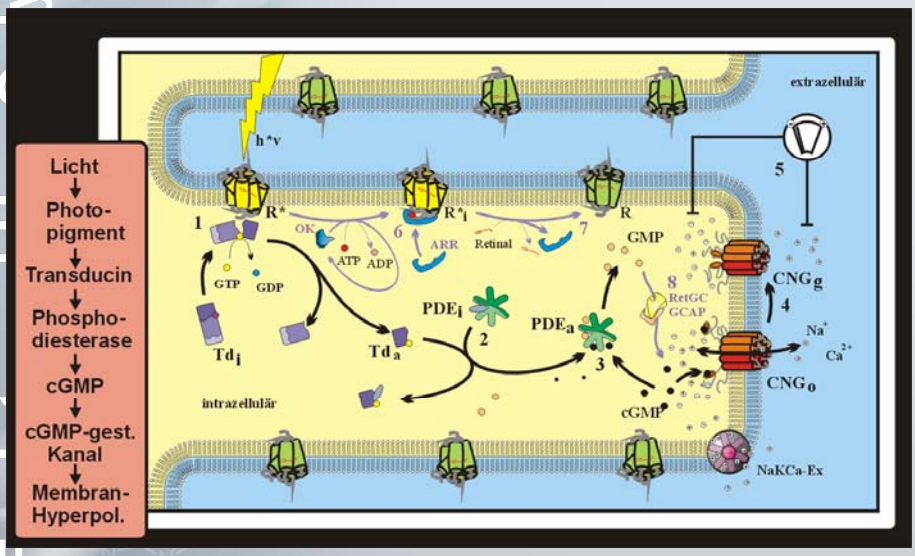


Function of Genes implicated in HRDs

Phototransduction Cascade

Rhodopsin	RHO	RP, CSNB
Cone Opsin	OPN1LW	Farbsehdefekte
Transducin	GNAT1	CSNB
C-Transducin	GNAT2	Achromatopsie
Phosphodiesterase	PDE6A	RP
	PDE6B	RP, CSNB
CNG-Kanal	CNGA1/B1	RP
C-CNG-Kanal	CNGA3/B3	Achromatopsie
Arrestin	SAG	RP, M.Oguchi
Rhodopsin Kinase	GRK1	M.Oguchi
Guanylatcyclase	GUCY2D	LCA, Cone-Rod Dys.
GC-Aktivator	GUCA1A	Cone Dystrophy

Function of Genes implicated in HRDs



Function of Genes implicated in HRDs

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Vitamine A – Metabolism and Transport

RE Metabolism	RPE65	LCA, RP
R'ol-Bindeprotein	RLBP1	Ret. punctata alb.
Retinoldehydrog.	RDH5	Fd. Albipunctatus
Retinoldehydrog.	RDH12	LCA, RP
Aktiv. G-Protein	RGR	RP
Lec.-R'ol Acyltr'fse	LRAT	LCA
Retinol-Flippase	ABCA4	Stargardt, CRD, RP
R'ol-Bindeprotein	RBP4	PE-Pathie

Function of Genes implicated in HRDs

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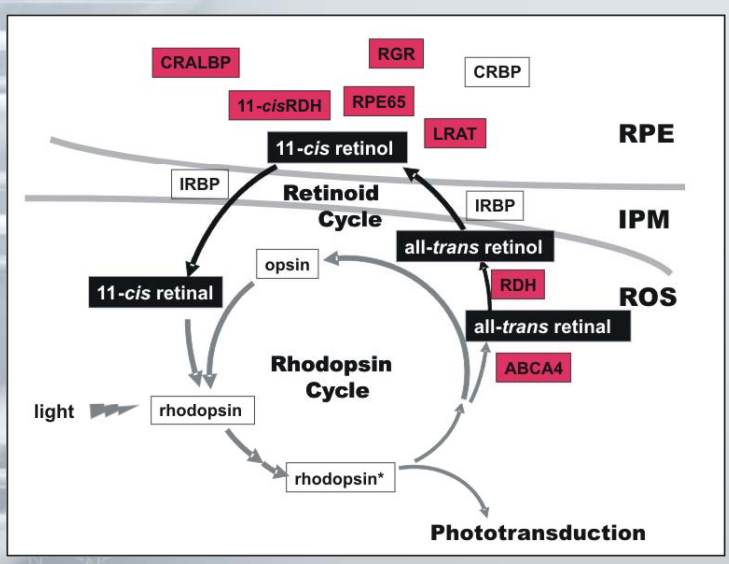


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