Controversies in pediatric glaucoma surgical treatment - taking both sides!
Sharon F Freedman (Durham NC), Alex V Levin (Philadelphia PA), Ken K Nischal (London, UK)

I. Introduction/Relevance: Optimal management of pediatric glaucoma remains controversial, especially for refractory cases. Many of our surgical interventions have both up- and down-sides for young children, and the "right procedure" may vary depending upon the surgeon, institution, and child.

II. "To Mitomycin or not to Mitomycin - that is the question" – use of mitomycin during trabeculectomy surgery
   A. "To Mitomycin" - Pro – Ken Nischal
      1. Indications for trabeculectomy in children (age, glaucoma type, prior surgeries)
      2. Surgical steps and tricks for mitomycin – augmented trabeculectomy in children
      3. Success with mitomycin trabeculectomy in children – how the drug has helped!

   B. "Not to Mitomycin" – Con – Alex Levin
      1. Contraindications for trabeculectomy in children (age, glaucoma type, other factors).
      2. Dangers specific to mitomycin augmentation of trabeculectomy in children
         - thin blebs, leaks, infection, cataract, etc.
      3. Life long risks
   C. Rebuttal/comments (Freedman moderator)

III. "Can I do anything I want to? – the ethics of applying new “adult” surgical glaucoma procedures in children"
   A. Yes – Freedman - how else will be get new tools to use for these poor kids?
      1. Consider use of medications for glaucoma in children – none of them was tested in children, but we used them and learned about them as we went – examples of timolol and brimonidine...
      2. Consider use of glaucoma drainage devices in children
      3. Consider use of the Schlemm canal fiberoptic for trabeculectomy in children
   B. No – Levin! It is not ethical to apply unproven, new technology to a vulnerable population without proof or a research protocol.
   C. Rebuttal (Nischal moderator) if needed (likely will skip this part because you will be so persuasive!)

IV. "Forks in the road to Schlemm canal – comparing various angle surgeries"
   A. How I do it – Levin
      1. Goniotomy – indications and brief tips of technique
      2. What I do when the view is not clear – endoscopic goniosurgery
   B. How I do it – Freedman
      1. Goniotomy vs. trabeculotomy
      2. 360-degree iScience "otomy"
      3. Special cases – uveitis and early onset aphakic glaucoma
   C. How I do it – Nischal
      1. My angle surgery of choice
      2. Why I like combined “otomy-ectomy” and how I do it

V. "Plumbing 101 – what tube do I use"
   A. My choices for tubes and my most common problem with them - Levin
      1. Indications for a glaucoma drainage device in my practice – who gets one?
      2. My preferences – what I use, where I put the tube, tips for surgery
      3. My least favorite problem after this surgery
   B. Same as above - Freedman
   C. Comments (Nischal moderator)

VI. "License to kill – options for cyclodestruction"
   A. The case for cryotherapy or transscleral diode cyclodestruction – Nischal
      1. Indications for cyclodestruction
      2. Why it is better to stay "outside the eye"
      3. How to minimize dread complications of phthisis/retinal detachment/hypotony
   B. The case for endoscopic diode cycloablation –Freedman
      1. Indications for cyclodestruction
      2. You must see where you are going!
      3. How to minimize complications
   C. Comments (Levin moderator)

VI. Panel Discussion– wrap-up with audience participation
**Group A**
- Tumor ≤ 3 mm in height
- Tumor ≥ 2 DD (3mm) from fovea AND ≥ 1 DD (1. mm) from optic nerve
- NO vitreous seeding
- NO retinal detachment

**Group B**
- Tumor mm in height
- Retinal detachment ≤ 5 mm from edge of tumor OR no retinal detachment
- NO vitreous seeding

**Group C**
- Local, fine limited seeds (i.e. treatable by plaque)
- AND/OR
- Retinal detachment up to 1 quadrant

**Group D**
- Diffuse vitreous and or subretinal seeding and or
- Massive non discrete endo e ophytic disease
- E ophytic disease and retinal detachment 1 quadrant up to total retinal detachment

**Group E**
Presence of any one or more of :
- Irreversible neovascular glaucoma
- Massive intraocular haemorrhage
- Anterior to anterior vitreous face
- Aseptic orbital cellulitis
- Tumour touching the lens
- Diffuse infiltrating retinoblastoma
- Phthisis or prephthisis
### International Classification (extraocular): proposed

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 0</td>
<td>Patient treated conservatively</td>
</tr>
<tr>
<td>Stage I</td>
<td>Eye enucleated: complete histological resection</td>
</tr>
</tbody>
</table>
| Stage II | Eye enucleated: microscopic residual tumour risk  
  i.e invasion of:  
  - optic nerve  
  - choroid (massive >3mm)  
  - anterior chamber  
  - sclera |
| Stage III | Regional extension  
  a. Overt orbital disease  
  b. Preauricular or cervical node extension |
| Stage IV | Metastatic disease  
  a. Haematogenous metastasis, no CS  
    1. Single  
    . Multiple  
  b. CS extension  
    1. Prechiasmatic  
    . CS mass  
    . Leptomeningeal disease |
Chemotherapy for Retinoblastoma

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Indications for chemotherapy in the management of intra-ocular retinoblastoma

Unilateral retinoblastoma

a) Neoadjuvant chemoreduction prior to local therapy. Chemotherapy may be used in localised tumours grade B or C in which there is a reasonable prospect of salvage of the globe and/or visual or in more advanced tumours in an eye thought to have potential for useful vision. In certain situations, such as tumours involving the macula, it may be possible to avoid the use of local therapy in order to preserve vision although most cases will require some local therapy once the tumour has shown maximal shrinkage.

b) Post-primary enucleation or adverse histological features including:
- retrolaminar invasion of optic nerve
- disease in the anterior chamber
- deep choroidal invasion:

Management of unilateral RB following primary enucleation:

Unilateral RB enucleation

Histology review

No adverse histological features

No further treatment
Observe

Adverse histological features See above

4 cycles of JOE chemotherapy
**Bilateral retinoblastoma**

Management of bilateral tumours must be tailored to the individual case. The aim of treatment is to attempt to retain use of vision in at least one eye. In cases of advanced bilateral tumours this may involve enucleating the eye containing the most advanced tumour and using chemotherapy to reduce the tumour in the remaining eye rendering it amenable to local therapy. Chemotherapy may also reduce tumours bilaterally facilitating local therapy and thus avoiding the need for enucleation.

**Metastatic retinoblastoma**

The vast majority of patients present with intraocular retinoblastoma for which the 5-year survival is in excess of 95%. However, retinoblastoma may metastasize either locally or to distant sites (bone, CNS, liver), a situation more commonly seen in the developing world when access to health care results in later disease presentation. Metastatic RB has a very poor prognosis with conventional therapy and is highly lethal. Recent use of aggressive multi-modality therapy including conventional chemotherapy, high-dose chemotherapy with autologous stem cell rescue and radiation has improved the outlook and improved the prospect of cure in a limited number of patients.
Chemotherapy Protocols
The first-line chemotherapy agents used in the management of retinoblastoma are vincristine, carboplatin and etoposide. All or a combination of these agents, are generally used around the world as first-line agents.

**UK Protocol for first line chemotherapy (JOE)**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dosage</th>
<th>Administration</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vincristine</strong> day 1</td>
<td>1.5 mg m^2 or children 10 kg</td>
<td>Intravenous bolus total dose 2 mg</td>
</tr>
<tr>
<td><strong>Carboplatin</strong> day 1</td>
<td>600 mg m^2 or children 10 kg</td>
<td>Infuse over 1 hour</td>
</tr>
<tr>
<td><strong>Etoposide</strong></td>
<td>300 mg m^2 or children 10 kg</td>
<td>Infuse over 4 hours</td>
</tr>
</tbody>
</table>

Each course given at approximately 21 day intervals, when the neutrophils are \( > 1 \times 10^9 / l \) and platelets \( > 100 \times 10^9 / l \).

**Recommendations for children under 10kg in weight:**
- Less than 6 months of age: 50% of calculated dose by body surface area
- 6 months to 1 year of age: 75% of calculated dose by body surface area
- Over 1 year of age: 100% of calculated dose by body surface area

Indications for second-line chemotherapy include:
- Recurrent intra-ocular RB following first-line chemotherapy.

There are a number of therapeutic options for children who have an intraocular relapse after prior treatment with standard first-line chemotherapy. These include cryotherapy, laser, carboplatin monotherapy, chemothermotherapy, scleral plaque radiotherapy, second-line chemotherapy or external beam radiotherapy. Children with vitreous disease or relapsed tumours not suitable for focal therapy and for whom radiotherapy is the last option, should be considered for second-line chemotherapy. The presence of vitreous disease is also an indication for sub-conunctival carboplatin.
b Second line chemotherapy is also indicated as adjuvant therapy following enucleation of an eye which has failed standard first line chemotherapy (Vincristine, Etoposide and Carboplatin).

**UK Protocol for Second line Chemotherapy (IVAd)**

<table>
<thead>
<tr>
<th>Drug:</th>
<th>Vincristine Day 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dosage:</td>
<td>1.5 mg m² or children 10 kg</td>
</tr>
<tr>
<td>Administration:</td>
<td>Intravenous bolus ma total dose 2 mg</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Drug:</th>
<th>Doxorubicin (Adriamycin) Days 1 + 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dosage:</td>
<td>25 mg m² dose or children 10 kg</td>
</tr>
<tr>
<td>Administration:</td>
<td>Infuse over 4 hours</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Drug:</th>
<th>Ifosfamide Days 1 + 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dosage:</td>
<td>os amide 3 gm m² dose or children 10 kg</td>
</tr>
<tr>
<td>Administration:</td>
<td>Infuse over 3 hours</td>
</tr>
</tbody>
</table>

Recommendations or children under 10 kg in weight See above

There are very few children requiring chemotherapy for progressive or recurrent disease following both first and second line therapy. These children must be managed on an individual basis and the risks associated with further chemotherapy examined against the likely benefit.

**Late Effects of Therapy for Retinoblastoma**

Survivors of RB face long term consequences associated with both the cancer and its treatment. These include:

a Chemotherapy-related late effects

The risks of chemotherapy associated late effects are directly related to the treatment received and most agents have known toxic effects some of which may not become manifest until later in life. Each of the agents used in retinoblastoma requires specific long-term surveillance.
The potential late adverse effects of chemotherapy for RB are:
Carboplatin – auditory and renal dysfunction
Etoposide – secondary leukaemia
Vincristine – peripheral neuropathy
Ifosfamide – secondary leukaemia, gonadal dysfunction and renal / bladder dysfunction
Doxorubicin – cardiac dysfunction

Standard UK protocols for the management of late chemotherapy toxicity are available on-line at www.cclg.org.uk

b) Radiotherapy related late effects
In view of the significant late effects associated with external beam radiotherapy, it now tends to be reserved or salvage treatment of RB if frontline therapies have failed. The side effects include:
  - Eye related problems dependent on technique: cataract formation, retinal changes, lacrimal gland dysfunction
  - Bone and soft tissue hypoplasia
  - Pituitary dysfunction
  - Second malignant neoplasms within and on the edge of the radiation field

c) Psychosocial and visual late effects
Many children treated for RB will have long term visual impairment which may range from monocular vision secondary to unilateral enucleation to blindness. Some children are registered partially sighted or blind and many require additional support in mainstream school or specialised schooling. The impact of this on the child’s psychosocial development including educational performance, mobility, independence, social functioning, peer acceptance and self-esteem must be considered. Long term follow-up of survivors of RB should include multidisciplinary access to specialised nurses, psychology, play therapy and social work.
Second Malignancies

In the heritable type of retinoblastoma there is a high risk that a second, non-ocular, cancer will develop later in life. This increased risk is associated with the radiation used in treating retinoblastoma and the presence of the RB1 gene mutation which increases the risk of subsequent cancers. In the non-heritable type there will also be a, much smaller, increased risk for patients exposed to radiation to treat their retinoblastoma.

On the basis of research studies in Great Britain and the Netherlands we calculate that every 100 people surviving heritable retinoblastoma:

- 3 will develop cancer by age 10
- 8 “ “ “ “ 20
- 11 “ “ “ “ 30
- 20 “ “ “ “ 40
- 30 “ “ “ “ 50

Thus, there is a high risk of a second cancer among people with heritable retinoblastoma. However, every 100 people in Great Britain about 35 will eventually develop a cancer.

The types of second cancer seen in RB are: osteosarcomas, rhabdomyosarcoma, fibrosarcoma, leiomyosarcoma, brain tumours, melanoma and some epithelial tumours such as lung and bladder cancer.

The CCLG Retinoblastoma working group in the UK have developed guidelines for the long term follow-up of RB patients. The frequency, duration and nature of long-term follow up for patients treated for retinoblastoma is dependent upon whether:

- RB is hereditary or non-hereditary
- Cytotoxic treatment was received or the retinoblastoma, particularly exposure to radiotherapy or chemotherapy
- Radiotherapy was received
- Extent of visual impairment

These will be available soon on the Children’s Cancer and Leukaemia Group (CCLG) website www.cclg.org.uk
Intra-ophthalmic arterial chemotherapy for the treatment of Retinoblastoma

Background

Intra-arterial chemotherapy has the potential benefit of delivering chemotherapy locally, in high concentrations to enhance the therapeutic effect while minimising systemic toxicity. Since 1993 semi-selective intra-arterial chemotherapy has been used routinely in Japan and a similar technique but using direct intra-ophthalmic artery catheterisation with modern micro-catheters has been used by the team in New York since 2006. It has growing acceptance within the international retinoblastoma community and reports indicate that ocular event-free survival at 2 years event being enucleation or BR is 67% or all eyes, 75% or eyes undergoing AC as primary treatment and 63% or eyes that had ailed previously with systemic chemotherapy or BR.

Indications:

Bilateral Retinoblastoma

1. In a child who has had one eye enucleated and the remaining eye has potential or vision but has ailed 1st line therapy - prior salvage therapy chemotherapy having been included in either the 1st line or salvage therapy and where A Melphalan is considered the best therapeutic option.

2. In a child with both eyes in situ, where the eye to be treated has a reasonable prospect or vision having ailed 1st line therapy - prior salvage therapy chemotherapy having been included in either the 1st line or salvage therapy and where A Melphalan is considered the best therapeutic option.

Unilateral Retinoblastoma

1. A chemotherapy may be considered in situations where a unilaterally aected eye with potential or vision, has ailed irst line conservative therapy which has included chemotherapy, but still has potential or vision and where A Melphalan is considered the best therapeutic option.
The potential risks associated with IA chemotherapy include:

- Risks of a general anaesthetic
- Bleeding from the arterial puncture, haematoma or arterial thrombus resulting in compromised circulation to the lower limb.
- There is a very small risk that the procedure may compromise cerebral circulation resulting in cerebrovascular accident.
- Mild myelosuppression following intra-arterial chemotherapy
- Children may experience swelling around the eye, flushing of the skin around the eye and forehead, transient 3rd nerve palsy and droopiness of the eyelid.
- A number of children experience a drop in the vision in the treated eye which may be temporary or in a small number of cases permanent. Occasionally total loss of vision in the affected has been reported.

There have been reports of children experiencing hypotension and bradycardia during the procedure prior to the infusion of the melphalan. This seems to be related to mechanical instrumentation of the internal carotid artery and responds to adrenaline promptly.
Overview of clinical and molecular genetic management in Retinoblastoma

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Introduction

Retinoblastoma, although rare acting 1 in 20,000 children, is often cited as a paradigm for our understanding and management of cancer genetic disorders.

It has long been recognised as a dominantly inherited disorder when present in the familial form. However, most cases are not apparently inherited and these are unilateral and non-inherited in at least 60% of cases or bilateral and de novo in the remainder 1,2.

Familial and bilateral cases are almost invariably due to an inherited mutation and have an earlier age of diagnosis to unilateral cases (mean age 8 months compared to 25 months). Examination of this epidemiological data led Knudson to postulate the two hit hypothesis of tumour development 3. This was subsequently confirmed when the retinoblastoma gene was cloned by Friend et al in 1986 4.

Subsequent molecular analysis of germline and tumour DNA in unilateral cases confirmed that approximately 15% of these cases are inherited and that the remainder have usually have two identifiable mutations confined to the tumour and absent in the blood. Hence these are likely to be somatic mutations not present in the affected individual’s germline with a low risk of vertical transmission.

This knowledge and data is compatible with the empiric data on recurrence risk in families if due consideration is given to the fact that a small proportion of cases will exhibit mosaicism in the tissues of affected children or in the gonadal tissue of parents.

The significance of mosaicism will be illustrated and its management implications discussed. We also will discuss whether novel or improvement in molecular techniques such as allelic specific amplification or next generation sequencing should modify our current screening protocols 6,7 or that DNA may influence our prenatal and perinatal management.
10 Points on the Genetic Management of Retinoblastoma Cases

After the identification of any child with retinoblastoma the following processes need to be considered (see appendix 1):

1. Is there a family history indicating autosomal dominant inheritance? Be careful to consider non-penetrance in unilateral cases.

2. A unilateral case is very early onset (<12 months) or multifocal might this case subsequently present as a bilateral case and hence be a heritable form of Rb.

3. Consider appropriate genetic testing. Chromosomes are certainly indicated if there is evidence of developmental delay, dysmorphic features or a history of miscarriage. Chromosome analysis will not detect more subtle molecular changes and there is debate on whether they are indicated in the absence of the 3 features above. For inherited or bilateral cases molecular analysis of the germline is always indicated. For unilateral cases post enucleation start with somatic tumour studies. If no tumour tissue is available then it is usually to request germline studies.

4. If the results of paragraph 3 above are negative consider role of linkage testing in the family. This may allow exclusion of individuals from surveillance programmes.

5. For families with informative molecular results work back through each relevant branch of the family until their risk is excluded and they are identified as at population risk.

6. Consider the possibility of mosaicism in unilateral cases and unaffected parents of bilateral cases. This is important so not to miss offspring risk from gonadal mosaicism.

7. Molecular tests should be instigated in all cases, where possible, as informative results will potentially identify all individuals at risk of developing or transmitting Rb. Informative results will also typically exclude a larger number of individuals who do not need screening.

Failure to pursue genetic testing, whether by the family or doctor, has significant implications:

a. at risk patients may be overlooked, a particular risk in low penetrant families.

b. many patients may receive unnecessary surveillance, at avoidable cost to the family or healthcare system. This usually is greater than the cost of molecular testing.

c. unnecessary surveillance is associated with avoidable morbidity, and even a small risk of anaesthetic mortality.

There are molecular analysis should be seen as cost effective and standard best practice and not a selectable “add on”.

Only if molecular investigation is uninformative, <10% of bilateral disease or unilateral enucleations, should empiric data be used or definitive counselling of families and instigating surveillance. Empiric data is attached (appendix 2 – modified from Musarella and Gallie 1 7 10 and A practical guide to human cancer genetics 2).
As the detection rate of molecular testing increases ever nearer to 100% with techniques such as allelic specific PCR or next generation sequencing the residual risk of “non informative cases” due to mosaicism decreases. There are therefore needs to be an iterative evidence based and informed debate of the protocols for screening.

Updated 21/2/2011

References

1 Website www.geneclinics.org


7 Anna Rohlin, Josephine Wernersson, vonne ngwall, ei Wiklund, an B rk, Margareta Nordling. Parallel sequencing used in detection of mosaic mutations: comparison with our diagnostic DNA screening techniques. Human Mutation 30 1012-1020 200


Appendix 1

Algorithm for the clinical and molecular genetic management of Retinoblastoma
Empiric risk data for relatives of isolated case of retinoblastoma

Modified from Musarella and Gallie (1987) and A Practical Guide to Human Cancer Genetics (Ed Hodgson, Eng, Foulkes and Maher)

<table>
<thead>
<tr>
<th>Nature of Retinoblastoma in proband</th>
<th>Relationship to Proband</th>
<th>Likelihood of being a gene carrier</th>
<th>Likelihood of developing a retinoblastoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilateral</td>
<td>M Twin</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Bilateral</td>
<td>Offspring</td>
<td>50</td>
<td>45</td>
</tr>
<tr>
<td>Bilateral</td>
<td>Sibling</td>
<td>5</td>
<td>2.7</td>
</tr>
<tr>
<td>Bilateral</td>
<td>Offspring unaffected sibling</td>
<td>0.5</td>
<td>0.27</td>
</tr>
<tr>
<td>Bilateral</td>
<td>First cousin</td>
<td>0.05</td>
<td>0.027</td>
</tr>
<tr>
<td>Unilateral</td>
<td>M Twin</td>
<td>10</td>
<td>5.4</td>
</tr>
<tr>
<td>Unilateral</td>
<td>Offspring</td>
<td>7.5</td>
<td>5.7</td>
</tr>
<tr>
<td>Unilateral</td>
<td>Sibling</td>
<td>0.0</td>
<td>0.4</td>
</tr>
<tr>
<td>Unilateral</td>
<td>Offspring unaffected sibling</td>
<td>0.0</td>
<td>0.04</td>
</tr>
<tr>
<td>Unilateral</td>
<td>First cousin</td>
<td>0.00</td>
<td>0.004</td>
</tr>
</tbody>
</table>

The risk of developing a retinoblastoma was calculated assuming a 90% penetrance in gene carriers which is probably an underestimate.

Any full sibling other than M twin. Risk for half sibling would be expected to be half this figure.
Appendix 3

PROTOCOL FOR SCREENING SIBLING AND OFFSPRING OF PATIENTS WITH RETINOBLASTOMA

Dilated fundus examination
- within a week of delivery
- Monthly to 6 months of age
- 2 monthly to 2 year of age
- 3 monthly to 3 years of age
- 4 monthly to 4 years of age
- 6 monthly to 7 years of age

Screening to be discontinued only if genetic testing results show a patient does not carry the RB mutation.

Where Rb mutation is not proven in parent, patient should be advised to seek genetic counseling at age 16.

Proband genetic testing should be repeated if not up to date.

A sibling is a proven gene carrier but no tumor identified their surveillance would continue 6 monthly to age 10 as affected retinoblastoma cases
Appendix 4

Definitions of Some Relevant Genetic Terms

Molecular and Genetic Terms and Definitions

**Sequencing** – method or reading individual base pairs within a gene. Considered the gold-standard or mutation detection. Will usually sometimes not detect whole gene deletions, whole on deletions or mutations within introns.

**SSCP (single strand conformational polymorphisms) and heteroduplex analysis** – these are methods of scanning genes or “small mutations”. These mutations are detected by changes in the rate of migration of the altered gene sequence through gels. The detection rates are variable but less than sequencing.

**MLPA – multiplex ligation dependent probe amplification** – this method is useful for the detection of large intragenic deletions and duplications.

**Linkage analysis** – method of tracking markers through family members to try and identify regions of genetic material cosegregating with a disease or phenotype. This does not identify a mutation but can identify or exclude gene carriers indirectly. The analysis requires DNA and knowledge of the phenotype from more than one person in a family.

**Recombination** – rearrangement of genetic material between homologous chromosomes so that two linked markers or a phenotype and a marker are no longer cosegregating on the same chromosome. The closer the markers are situated, the less likely a recombination event is to occur. Recombination results in an error rate which should be quoted in linkage studies.

**Chromosome analysis or karyotype analysis** – this is a separate genetic test from mutation studies. The analysis will only detect large chromosomal deletions, duplications or re-arrangements and not mutations within a gene.

**Segregation analysis** – analysis of the inheritance of phenotype or genotype to see if it is manifesting in a mendelian fashion, if this is dominant or recessive and also if autosomal or linked.

**Free fetal DNA (ffDNA) –** DNA from a fetus is leaked in small amount into the maternal circulation. This is usually detectable in increasing amounts from about 7 weeks but has a half-life of 6 hours so is rapidly cleared after delivery. The paternal contribution of this DNA is different from mothers and therefore paternally inherited mutations should be detectable in mothers blood.

**Mutation Types**

**Missense mutation** – change of the amino acid sequence but a full length protein is still usually produced. It can be difficult to determine if a missense mutation is truly
pathogenic. Supporting evidence of pathogenicity includes showing such changes are de novo in an isolated case, or is co-segregating with the phenotype in a family or has previously been reported in other families. Functional studies may help determine significance in some cases.

**Nonsense mutation** – sequence change which introduces a stop codon into a gene and results in a truncated protein, these are usually pathogenic.

**Frameshift mutation** – sequence change which affects 1 or more base pairs but not a multiple of three so the reading frame of the gene is disrupted. These mutations will alter amino acids and result in an abnormal stop codon. They are usually pathogenic.

**Splice site mutation** – a gene sequence change in the region of the exon/intron boundary. The DNA is usually processed so the introns are spliced out and the exons joined. Failure to recognise the boundary can lead to introns being erroneously spliced out. These changes can be difficult to detect if the mutations are in intronic regions. They can also be difficult to determine if they are pathological and some of the same mechanisms described under missense mutations may be used.

**RNA analysis** – analysis of RNA rather than DNA, the former of which is technically more difficult and has only limited availability in non-research settings. Can be useful to investigate splice site mutations and on skipping.

**Other Genetic Terms**

**Penetrance** – the frequency a phenotype is manifest with a particular genotype

**Expressivity** – the variable nature or severity of a phenotype with a specific genotype.

**Mosaicism** – the presence of a mutation in some cells but not all cells in a multicellular organism. This is important to understand as an Rb mutation could be present in both retina but not necessarily detectable in the blood. Such a mutation could, theoretically, also be present in the gonads although not the blood.

**Gonadal mosaicism** – presence of a mutation in some or all of the gonadal cells but not all cells within a multicellular organism. It is important to be aware the possibility as a mutation not detected in the blood may still be present in the gonads and thus transmitted to the next generation.
Protocol for retinoblastoma screening

A. Bilateral proband: only member of family affected

B. Unilateral proband: only member of family affected
When the RB1 mutation(s) are not known, the % risk for the relatives of retinoblastoma patients to develop retinoblastoma can be estimated to be high (red) or moderate (turquoise).

Modified from Figure 11. A. Valenzuela, H. S. L. Chan, E. Héon, B. L. Gallie
A Language for Retinoblastoma: Guidelines Through Standard Operating Procedures
Pediatric Retina. Reynolds J, Editor. Kind permission from Prof Brenda Gallie, Hospital
for Sick Children, Toronto.

**Key**

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<th>Color</th>
<th>Description</th>
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<tr>
<td>Black</td>
<td>Patient with retinoblastoma</td>
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<tr>
<td>Red</td>
<td>Relative of retinoblastoma patient with high risk of developing retinoblastoma</td>
</tr>
<tr>
<td>Turquoise</td>
<td>Relative of retinoblastoma patient with moderate risk of developing retinoblastoma</td>
</tr>
</tbody>
</table>
Protocols for Retinoblastoma

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Follow up Protocol for patients

During treatment
Chemotherapy is typically given over 6 cycles at 3-4 weekly intervals.

During Chemotherapy + local therapy
very 3-4 weeks or most cases. The timing of UA and treatment is coordinated to precede the next cycle of chemotherapy. This allows the maximum effect of each cycle to occur so that focal treatment can be more effectively performed, and also allows time for the blood counts to recover, making GA and local treatment safer.

Review every 2-3 weeks
• if no chemotherapy is used - i.e. cases with very small tumours that can be managed with local treatment alone.
• or very large tumours where it may not be possible to treat the entire tumour at each sitting
• or rapidly growing macular tumours.

Following enucleation –
All cases receive a 1-2 week socket check in clinic.
Following this-
Unilateral - 6 week UA followed by artificial eye fitting
Bilateral - Receive 3-4 weekly UAs or the other eye. Artificial eye fitting 6 weeks post op.

Following successful treatment

UA or Clinic depending on co-operation

Unilateral – every 2 months or 6 months
• every 3 months or 12 months
• every 4 months or 12 months
• every 6 months to age 10

Aim to transfer care to local ophthalmologist after 12 months disease free.
**Bilateral**
- every 2 months or 12 months
- every 3 months or 12 months
- every 4 months or 12 months
- every 6 months to age 16

Aim or local follow up after 24 months disease free

The transition from EUAs is often gradual, initially alternating between UA and clinic or a few visits before switching to clinic only.

**Screening of the immediate family**

All siblings and both parents should have undus examination at the time of proband diagnosis

**Offspring and siblings of parents with retinoblastoma**

See genetics handout

**References**
PRESENTATION AND DIFFERENTIAL DIAGNOSIS

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INCIDENCE

1:23,000 live births - 40 – 45 new cases per year UK
- 10 – 200 new cases per year USA
Represents 3 of childhood cancers

35% - 40% Bilateral
- 15 ve amily history
- 5 New germline mutation
- Mean age presentation is 13 months

60% - 65% Unilateral
- 15 have germline mutation
- Mean age presentation is 24 months

PRESENTATION

eukocoria yellow white pupillary re le 60 - 65
Strabismus 30 - 35

Acute in lammation
Pseudohypopyon tumour tissue in the anterior chamber
Rubeosis giving rise to ac uired heterochromia
Buphthalmos traocular spread
Raised intracranial pressure

DIFFERENTIAL DIAGNOSIS OF LEUKOCORIA

Artefact (commonest)
O a is lash photographs

Anterior Segment disease

Cataract
Corneal opacity
Hypopyon

Posterior Segment disease

Coat’s disease

Coat’s disease, an exudative retinal vasculopathy is the commonest other diagnosis in cases with suspected retinoblastoma. Important differentiating features between the two include

<table>
<thead>
<tr>
<th>Feature</th>
<th>Coat’s</th>
<th>RB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinal reflex</td>
<td>Yellow reflex (xanthocoria)</td>
<td>White reflex (leucocoria)</td>
</tr>
<tr>
<td>Vitreous</td>
<td>Clear</td>
<td>May have seeds</td>
</tr>
<tr>
<td>Examination and USG B scan</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Large dilated tortuous vessels</td>
<td>Often present in periphery in one or more quadrants</td>
<td>Vessels may be dilated but less likely to be tortuous, and usually not localised to a quadrant</td>
</tr>
<tr>
<td>Retinal detachment</td>
<td>Usually total detachment with subretinal exudation</td>
<td>Retina may not be detached, there may be multiple tumours, and scattered subretinal seeds</td>
</tr>
</tbody>
</table>

Persistent foetal vasculature - PFV or PHPV - often associated with dragged ciliary processes visible through the dilated pupil, and slight microphthalmos.

Retinal dysplasia - Norries disease

Coloboma
Retinopathy of prematurity
continental Pigmenti
Myelinated nerve fibres
Retinal detachment Retinal old scars
Retinoschisis
Organising vitreous haemorrhage
Melanoma
Epiretinal membrane

Infection / Inflammation

Toxocariasis

To oplasmosis
Intermediate Uveitis
Tuberculous Sarcoid granuloma

Neoplasia

*Isolated Retinal / optic nerve hamartoma*
*Syndromal retinal hamartoma*
*Metastatic malignancy*

Medulloepithelioma

Other

*Juvenile xanthogranuloma*
*Morning glory syndrome*

**INVESTIGATIONS**

**Old photographs**
It is helpful if the parents can bring old photographs that might demonstrate a white reflex or squint at the first visit.

**Imaging**
CT scan is best avoided due to the radiation dose which may potentiate the development of second cancers in germline cases. Ultrasound B scan might be helpful in making the diagnosis by demonstrating mass lesions with calcium in the posterior segment lesions. MRI is safer than CT scan, and indicated if the child has papilloedema or symptoms or raised intra-cranial pressure to rule out pineal lesions and or intracranial tension

**Blood tests**
For genetic testing
For routine haematology Full blood count etc
For routine blood chemistry Serum Creatinine, electrolytes etc
Torsional Diplopia \textsuperscript{1,2}

- Binocular diplopia
  - Typically associated with vertical diplopia most commonly with 4\textsuperscript{th} Cranial Nerve Palsy
  - Diplopic image is often twisted or blurred
  - Often worse in down gaze

- History of head trauma

- Diplopia response on prism offset
  - “Fuzzy”
  - “Twisted”
  - “Diagnol”

- Amblyoscope Exam
  - Measure the amount of torsion present
  - Can offset torsion to test for fusional response

\textsuperscript{1} Woo SJ, Seo JM, Hwang JM; Clinical characteristics of cyclodeviation. Eye (Lond) 2005; 19 (8):873-8.

“Retinal” Diplopia\textsuperscript{3,4}

- Vertical diplopia, eliminated with occlusion of either eye
- Worse at distance
- Worse under photopic conditions
- Monocular visual disturbances, not relieved with pinhole
  - Metamorphopsia
  - Blur
  - Distortion
  - Illusory movement of fixation target
- Horizontal phoria or mismatch between subjective complaints and objective findings
- Fair to good stereopsis
- Poor sensory fusion
  - Diplopia relieved only temporarily with prism
  - “Eats up” vertical prism over time
- + Lights-on, lights-off test


Paradoxical Diplopia Secondary to Anomalous Binocular Correspondence

- Binocular diplopia
  - Typically horizontal (crossed or un-crossed)
  - Diplopic image is often in the direction opposite than that expected (given alignment)
  - Often intermittent
  - Often worse in dim illumination
- History of constant, childhood-onset strabismus
- Diplopia response on fovea-to-fovea sensory tests
  - Diplopia with prism offset of deviation on red filter, Bagolini, or Worth 4-dot tests
  - Diplopia response on after-image test
- Amblyoscope Exam
  - Sensory angle ≠ Motor angle
  - Presence of "pseudo-fovea"
  - Presence of suppression scotoma that may be in unexpected location (given alignment)

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Central Disruption of Fusion/ Horror Fusionis\textsuperscript{7,8}

- **Central Disruption of Fusion**
  - CNS basis for loss of pre-existing fusion ability
  - Can involve loss of sensory fusion, loss of motor fusion or loss of both sensory and motor fusion

- **Horror Fusionis**
  - Absence of central fusion combined with absence of central suppression usually associated with childhood strabismus or longstanding unilateral loss of vision

- **Important to diagnose / rule out pre-operatively when considering EOM surgery to treat diplopia**

- **Who is at risk**
  - Visually mature patients (adults/teenagers) with:
    - Longstanding constant deviation (HF)
    - Sensory deviation secondary to longstanding unilateral loss of vision, now with VA recovered (HF)
    - Constant diplopia with history of head injury associated with loss of consciousness (CDF)
    - Constant diplopia and acquired CNS lesion/mass (usually midbrain, rare) (CDF)

- **Evaluation**
  - Prism neutralization with red filter
  - Amblyoscope
    - More sensitive
    - Can neutralize cyclodeviations

\textsuperscript{7} Wendell ME, Mets MB, Wilson H: Horror Fusionis Revisited. \textit{Amer Orthopt J} 1986;36:146-150.
Monocular Diplopia\textsuperscript{9,10}

- Can be physiologic or pathologic
  - External irregularities of the eyelids or cornea
  - Uncorrected irregular corneal astigmatism (most common)
  - Early keratoconus
  - Lenticular changes
  - Macular cystoid changes
  - Large neurologic scotomas with bisect fixation
  - Intracerebral hemorrhage or tumors in or near area 19
- Acquired, never congenital
- Complaints of second “shadow” image
  - Primary image is always darker and better defined than the secondary image
  - Typically images are vertically displaced, but can be horizontal
  - Usually worse or more noticeable in the distance
- Diplopia still present after one eye has been occluded
- Diplopia relieved with pinhole test
- Small to no phoria present on cover test
- Fair stereopsis
- Mildly decreased vision in the eye with the symptoms
- Retinoscopy is best diagnostic tool

American Orthoptic Council Workshop:  
When the Patient Sees Double and the Doctor Sees Nothing  
A Guide to Double Vision  
AAPOS 37th Annual Meeting – San Diego, CA  
April 1, 2011

I. Who is at risk for double vision?  
   a. Patients with refractive error  
      i. Uncorrected refractive error  
      ii. Aniseikonia  
   b. Patients with strabismus  
      i. Acquired strabismus  
         1. Secondary to trauma  
         2. Secondary to systemic disease  
         3. Decompensation of phoria  
      ii. Loss of or change in suppression  
         1. Fixation Switch Diplopia due to change in refractive error or visual acuity  
         2. Aggressive anti-suppression exercises  
         3. Loss of suppression due to emotional or physical trauma  
      iii. Sensory strabismus  
         1. Poor visual acuity in one eye  
         2. Significant visual field loss in one or both eyes  
   c. Patients with macular or paramacular pathology  
      i. Retinal “wrinkle” or pucker  
      ii. Retinal membrane  
      iii. Traction  
   d. Patients with significant visual field loss in one or both eyes  
      i. Advanced glaucoma  
      ii. Sensory visual pathway disease  
   e. Patients with an abnormal fusion mechanism  
      i. Disrupted sensory fusion mechanism in the primary or secondary visual cortex  
         1. Central loss of fusion due to brain injury or prolonged monocular deprivation  
         2. Maldevelopment of sensory fusion mechanism due to infantile strabismus or visual deprivation (i.e.: congenital cataract)  
      ii. Disrupted motor fusion mechanism (vergence system) in the tertiary cortex, motor cortex, or brainstem\(^1\)  
         1. Acquired (i.e.: Parkinson’s Disease)  
         2. Congenital (i.e.: Cerebral Palsy)  

II. The Exam  
   a. History  
      i. Does the double vision disappear when you cover one eye?

\(^1\) These patients typically have strabismus, though it is often intermittent.
1. Yes: binocular diplopia
2. No: monocular diplopia

ii. What event coincided the onset of your double vision?
1. Trauma to head or face
   a. Was there a loss of consciousness?
2. Illness
   a. Recent diagnosis of systemic disease?
      i. Diabetes
      ii. Multiple Sclerosis
      iii. Thyroid dysfunction
      iv. Neoplasm
   b. Acute illness (“flu”)
3. Change in glasses and/or optical correction
   a. Change in prescription strength
   b. New bifocals or new type of bifocal
   c. Glasses to contact lenses (or contacts to glasses)
   d. Refractive surgery
4. Change in visual activities
   a. Increase in frequency of night driving
   b. Increase in amount of near work
5. Other symptoms
   a. Headache
   b. Vertigo
   c. Visual confusion (two different images superimposed in the same location)
   d. Loss of depth perception

iii. Is the double vision constantly present, intermittent, or variable?
1. Is the double image always there, but image changes location in space?
2. Does the double image appear and disappear, but remain in the same location relative to fixation?

iv. Do you have a history of strabismus, “lazy eye”, or glasses wear as a child?²

v. Is there a position or visual circumstance in which you do NOT see double?
   1. Patient may not be aware of his/her head posture
   2. Check old photos (i.e.: driver’s license) for evidence of pre-existing head posture

b. Visual Acuity
   i. Which is the dominant eye?
   ii. Does the dominant eye have the better acuity?

c. Refractive Error
   i. Refractometry

² If the patient has no history of diplopia, he/she may not associate the childhood strabismus with a new onset double vision and fail to mention it.
1. Patients with history of amblyopia or childhood-onset strabismus may need objective refractometry (retinoscopy) and/or cycloplegic refractometry
d. Motility
   i. Is there a field or gaze of single vision?
      1. Is the patient truly binocular in this field?
   ii. Determine if manifest strabismus is present in critical gaze positions using cover-uncover test
      1. Driving position (primary position at distance)
      2. Reading position (down gaze at near)
      3. Computer position (primary position at arm’s length)
      4. Any other gaze position that is occupationally relevant to patient
e. Binocularity
   i. Define characteristics of diplopia
      1. Test technique
         a. Place red filter over better-seeing eye
         b. Direct gaze to light source or lighted acuity chart (single letter)
         c. Dim room lights
      2. Where is the red image, relative to the white? (If diplopia is binocular, second image should always be in the field opposite to that where the eye is pointing.)
         a. If eye is in towards the nose (esotropia), then image from that eye is temporally displaced.
         b. If eye is out towards ear (exotropia), then image from that eye is nasally displaced.
         c. If eye is up (hypertropia), then image is down.
         d. If eye is down (hypotropia), then image is up.
         e. Presence of pre-existing anomalous fusion mechanism is indicated if:
            i. Patient is diplopic, but unable to localize second image in space
            ii. Location of double image “doesn’t make sense” given type of deviation
   ii. Assess fusion potential
      1. Add increasing prism with apex toward the direction of the misalignment until images fused (good fusion potential)
         a. Esotropia: base-in
         b. Exotropia: base-out
         c. Hypertropia: base-down
         d. Hypotropia: base-up
      2. If patient unable to fuse with any combination of prisms and red filter (poor fusion potential)
         a. Turn on room lights, remove red filter, keep prisms in place. Does the patient now see single?
b. Rule out cyclotropia with double Maddox rod test or amblyoscope

c. Rule out anomalous fusion mechanism
   i. Amblyoscope Exam
   ii. Bagolini Lenses
   iii. After Image

III. The Clinical Characteristics of Diplopia

a. Retinal Diplopia
   i. Vertical diplopia eliminated with occlusion of either eye
   ii. Worse at distance
   iii. Worse under photopic conditions
   iv. Monocular visual disturbances, not relieved with pinhole
      1. Metamorphopis
      2. Blur
      3. Distortion
      4. Illusory movement of fixation target
   v. Horizontal phoria or mismatch between subjective complaints and objective findings
   vi. Fair to good stereopsis
   vii. Poor sensory fusion
      1. Diplopia relieved only temporarily with prism
      2. “Eats up” vertical prism over time
   viii. +Lights-on, lights-off test

b. Central Loss of Fusion
   i. CNS basis for loss of pre-existing fusion ability
   ii. Can involve loss of sensory fusion, loss of motor fusion, or loss of both sensory and motor fusion
   iii. Those at risk include Visually mature patients with:
      1. Constant diplopia with history of head injury associated with loss of consciousness
      2. Constant diplopia and acquired CNS lesion/mass (usually midbrain, rare)
   iv. Amblyoscope is best diagnostic tool

c. Horror Fusionis
   i. Absence if central fusion combined with absence of central suppression usually associated with childhood strabismus or longstanding unilateral loss of vision
   ii. Those at risk include visually mature patients with:
      1. Constant deviation with/without diplopia with unclear history
      2. Longstanding constant deviation
      3. Sensory deviation secondary to longstanding unilateral loss of vision, now with recovered vision
   iii. Amblyoscope is best diagnostic tool

d. Torsion

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3 This requires special sensory testing and equipment. Patient should be referred to an orthoptist.
i. May or may not have associated vertical misalignment

ii. Sometimes worse when reading
   1. Can be worse in down gaze

iii. Complains images are blurred or “shadowed”
   1. Relieved with either eye occluded

e. Monocular
   i. Causes may be physiologic or pathologic and include
      1. External irregularities of the eyelids or cornea
      2. Uncorrected irregular corneal astigmatism
      3. Early keratoconus
      4. Lenticular changes
      5. Macular Cystoid changes
      6. Large neurologic scotasmas with bisect fixation
      7. Intracerebral hemorrhage or tumors in or near area nine-teen

ii. Complains of second “shadow” image
   1. Primary image is always darker and better defined than the secondary image
   2. Typically images are vertically displaced, but can be horizontal
   3. Usually worse or more noticeable in the distance

iii. Acquired, never congenital

iv. Diplopia still present with either eye occluded

v. Diplopia often relieved with pinhole test

vi. Small to NO phoria present on cover test

vii. Fair stereopsis

viii. Mildly decreased vision in eye with symptoms

ix. Retinoscopy is best diagnostic tool

f. Paradoxical
   i. Secondary to anomalous binocular correspondence

   ii. Binocular diplopia
      1. Typically horizontal (crossed or un-crossed)
      2. Diplopic image is often in the direction opposite than the expected (given alignment)
      3. Often intermittent
      4. Often worse in dim illumination

   iii. History of constant, childhood-onset strabismus

   iv. Diplopia response on fovea-to-fovea sensory tests
      1. Diplopia with prism offset of deviation on red filter, Bagolini, or Worth-4-dot tests
      2. Diplopia response on after-image test

   v. Amblyoscope Exam
      1. Sensory angle Motor angle
      2. Presence of “pseudo-fovea”
      3. Presence of suppression scotoma that may be in unexpected location (given alignment)
IV. The Management
a. Optical
   i. Best for patients with
      1. Refractive error, already wearing optical correction
      2. Straight eyes
      3. Monocular or binocular diplopia
      4. Strongly dominant eye
   ii. Options
      1. Refractive blur of non-dominant eye
         a. “over-plus” non-dominant eye
      2. Alter plane of refractive correction
         a. Glasses to contact lenses or reverse
      3. Change bifocal type
         a. Progressive to D-seg, or reverse
      4. Contact Lens
         a. Better for irregular corneal astigmatism

b. Orthoptic
   i. Best for patients with
      1. Intermittent strabismus
      2. Good fusion potential
      3. Good health and highly motivated
   ii. Options
      1. Teach suppression
      2. Improve fusional vergence amplitudes

   c. Occlusion
      i. Best for patients with
         1. Double vision and straight eyes
         2. Poor fusion potential
         3. Strabismus, but poor candidate for other therapies
      ii. Options
         1. For those who don’t already wear glasses
            a. “Pirate patch”
            b. Occlusive contact lens
            c. Adhesive patch
         2. For those who wear glasses
            a. Frosted adhesive tape
            b. Clear nail polish
            c. Bangerter filter
            d. Min lens

d. Prism
   i. Best for patients with

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4 Teaching fusion typically results in permanent diplopia and is NOT recommended.
1. Diplopia due to small angle strabismus (< 12∆)
2. Good fusion potential
3. Comitant deviation that is similar at distance and near fixation
4. Refractive error, already wearing glasses

ii. Options
   1. Fresnel temporary press-on prisms
   2. Ground-in to spectacles
   3. Displaced optical centers (only possible if refractive error is high)

e. Surgery
   i. Best for patients with
      1. Diplopia due to large angle strabismus or torsion
      2. Incomitant deviation
      3. Good fusion potential
   ii. Options: depend on type of strabismus
Further Reading


