How to analyse known genes and how to find new genes

EUROSCA Clinico-Genetic Training

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Part 1: How to analyse known genes

1. Indications for genetic testing in ataxia patients
2. Which test, which panel to choose
3. Methods for genetic testing in SCA
4. Clinical pitfalls, diagnostic pitfalls

ad 1) Indications for genetic testing

- Although several genetic subtypes manifest with rather typical clinical features and display a rather uniform time course (i.e. retinopathy in SCA7 or onset after the 5th decade in SCA6), the inter-individual variance is hampering close phenotype-genotype correlations. Clinical phenotypes are not pathognomonic for any SCA mutation.
- To date 7 coding CAG repeat expansion (DRPLA, SCA1, SCA2, SCA3, SCA6, SCA7, and SCA17), 4 non-coding repeat expansions (SCA8, SCA10, SCA12, and FMR1/FXTAS), and four genes with missense-mutations (SCA5 (SPTBN2-gene), SCA13 (KCNC3-gene), SCA14 (PRKCG-gene) and SCA27 (FGF14-gene)) are causative for dominant ataxias. Including those loci with distinct linkage in dominant ataxia families, considerable heterogeneity exists.
- Epidemiological data characterize SCA1, SCA2, SCA3, SCA6, and SCA7 being the most prevalent worldwide with SCA3 causing ≈ 25% and SCA7 causing ≈3% of all SCA mutations (T. Bird in www.geneclinics.org).

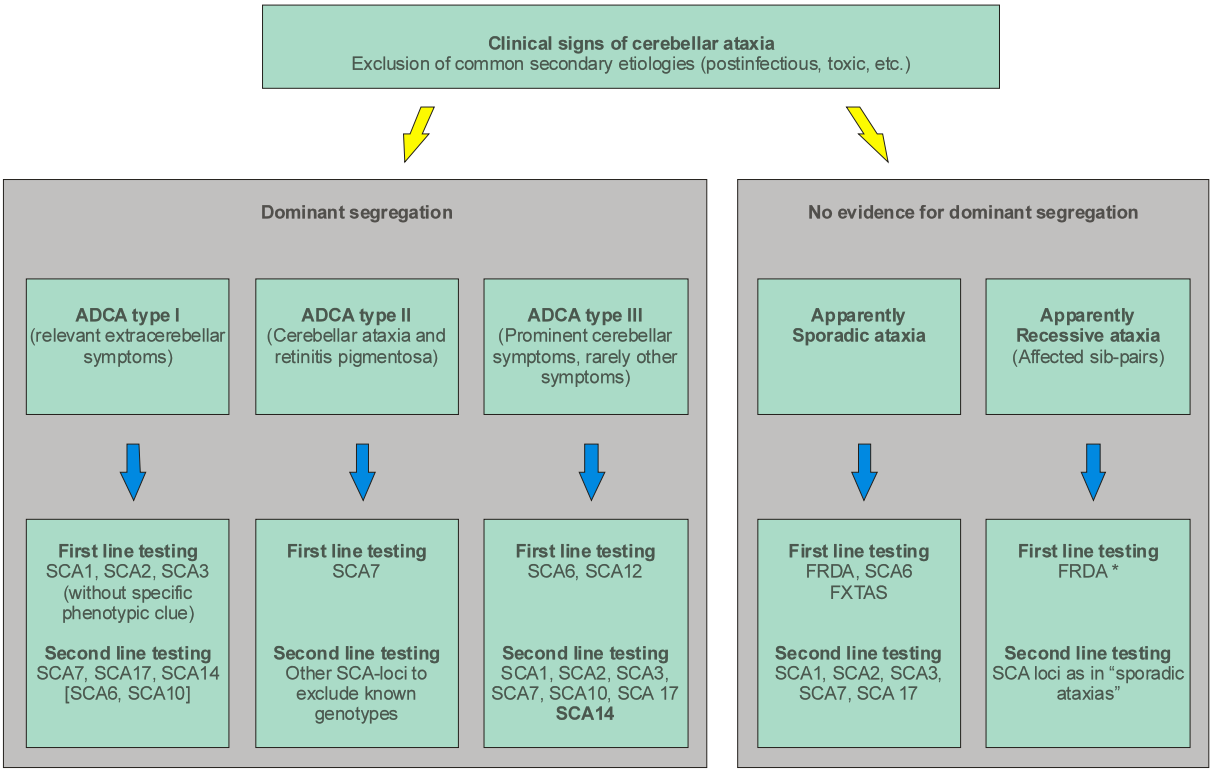
ad 2) Which test, which panel to chose

- In order to decide which genotypes to test, the family history of the proband is most important (**Figure 1**). Assessment should include questions about
    - Dominant segregation?
    - Age at onset in affected family members?
    - Origin of the family?
    - Persons at-risk (in those families genetic counselling should take place before genetic testing is performed)

Annotations concerning
SCA8: There is reduced penetrance of the expanded CTG alleles, therefore demonstration of a repeat expansion does not prove causality of the variant. SCA8 should be tested in ADCA families, but genotyping of sporadic ataxia patients or predictive and prenatal is not advised.
SCA10, SCA12: Up to now there is no evidence for European SCA10 and SCA12 families (although the U.S. SCA12 family has been of German descent). SCA10 and SCA12 should be tested in ADCA families. Other indications (i.e. sporadic ataxia patients with epilepsy) are very speculative.
SCA14 is relatively rare in European ADCA families (≈ 1%). Genetic testing is quite expensive as point mutations have to be discovered. Thus, genetic is very time-consuming and other SCA mutations should be excluded first. In families segregation analysis of flanking markers might be useful.

FXTAS (Fragile-X tremor / ataxia syndrome) is relatively prevalent in "sporadic" male ataxia patients older than 50 years. Hagerman et al. defined diagnostic criteria for the syndrome.
FGF14: Up to now there is no evidence for positive families in Europe (> 100 index cases tested). Genetic testing is quite expensive and time-consuming. Therefore other SCA loci should be excluded first.
SCA4: In Japan, a promoter variant in the puratrophin-1 gene segregates in all ADCA families mapped to chromosome 16q. This variant has been excluded in the German SCA4 family so that this locus is still under debate.
SCA5: Although mutations in SPTBN2 have been demonstrated in one French and one German family, no prevalence data for unmapped ADCA families is available. To date, genetic testing is only available on research basis.
SCA13: The same is true for KCNC3 mutations in SCA13.

Figure 1: Flowchart illustrating (2) how to select appropriate genotypes for genetic testing in ataxia patients (from Schöls et al). For sporadic ataxia patients data in Abele et al. and Futamura et al.



* Other genotypes for recessive ataxias should be considered

Predictive testing

Predictive testing means *testing at-risk persons for the family-specific mutation, who do not show any signs of the disease*.
Several genetic societies and lay-organisations have worked out recommendations for predictive testing (for example see the Hereditary Disease Foundation,

www.hdfoundation.org). To exemplify the cornerstones of their recommendations, physicians are ask

- Not to test minors
- To enable genetic counselling for the probands prior to drawing a blood sample
- To consider psychological support for the proband
- To set-up a schedule for actions, i.e. to allow the proband to have enough time to cope with the situation
- As prenatal testing is very uncommon, this issues exclusively should be dealt by geneticists

ad 3) Methods for genetic testing

- Usually PCR based tests are applied. Therefore EDTA-blood is most suitable for DNA preparation of the proband (alternatively autopsy tissue or buccal swaps are applicable, but especially the latter contributes a considerable risk of sample contamination).
- For all loci where the SCA mutation is known (SCA1, SCA2, SCA3, SCA6, SCA7, SCA8, SCA10, SCA12, SCA14, SCA17, FGF14, FMR1/FXTAS, FRDA [and other recessive ataxia genes], only the index case / the proband is tested. SCA5 and SCA13 testing have not yet entered clinical routine.
- In those loci, where the SCA mutation is not yet known several family members are needed to perform linkage analysis. To date isolated cases can not be assessed for SCA4, SCA11, SCA15, SCA18, SCA19, SCA21, SCA22, SCA23, SCA24, SCA25, and SCA26. Caution must paid, as linkage analysis is never diagnostic / unambiguous.

ad 4) Clinical pitfalls, diagnostic pitfalls

- Be aware that >90% of the rare testing errors / misdiagnoses occur because samples have been mixed up
    - Always draw two independent blood samples per patient
    - Involve genetic laboratories which test these samples independently
    - If only one sample has been investigated, always validate pathological results with a second sample
- PCR testing might miss giant repeat expansions due to allelic drop out. For genotypes where large expansions are frequent (SCA10, FMR1/FXTAS, FRDA) additional testing strategies (genomic southern blot, triple primer PCR; Cagnoli et al.) should be provided by your genetic laboratory.
- In Germany ≈ 10% of all laboratories participating in quality measures failed to detect all pathological genotypes (especially SCA3 has been problematic). Genetic laboratories should participate in local or European quality management measures and should process positive controls for all genotypes they test for.

Part B: How to find new genes

5. Clinical recommendations
6. Statistical recommendations
7. Principles of linkage analyses
8. Strategies in small families
9. EUROSCA resources

ad 5) Clinical recommendations

- In order to allow successful positional cloning (see below) significant clinical work has to precede genetics:
- Large families have to be collected, where not only affected family members but also unaffected sibs are very important.
- For all affected family members conclusive clinical data has to be collected.

ad 6) Statistical recommendations

The main measure for linkage is the LOD-score, which can be modelled for existing pedigrees. In general the higher LOD-score a family can contribute, the better a new disease gene can be detected in this family. Usually families with less than 5 affected members are too small for linkage analysis.

ad 7) Principles of linkage analyses

- The most powerful genetic technique is termed *"positional cloning"* aiming to link clinical data with genomic markers ("linkage"), persuing "fine mapping" by extending existing families or add further families, and to disclose the disease-causing mutations in the candidate gene by direct sequencing.
- *Linkage analysis* calculates the probability whether a specific genetic marker (microsatellite or Single Nucleotid Polymorphism SNP) is directly linked with a phenotype or not. LOD-scores numerate this likelihood in a logarithmic scaling. Scores greater 3 are indicative that a disease-causing genetic variation is in proximity of the genetic marker which has been investigated.
    - The genome-wide marker sets used are either microsatellites (there is a commercial panel of 384 markers covering the whole human genome) or SNPs (there are genome-wide SNP panels with up to 100.000 loci).
- High-throughput genotyping results have to be converted in haplotypes in order to assess possible recombinations within the candidate gene region.

ad 8) Strategies in small families

- Phenotypes have to be compared with large, linked families
- Genomic marker analysis should allow to confer these families to one (several) ADCA loci.

- But remind, that rather low LOD-scores do not exclude other chromosomal regions as candidate region.

ad 9) EUROSCA resources

- The EUROSCA Clinico-Genetic project is lead by Alexis Brice, INSERM, Paris
- Major tasks are
    - Collection of European ADCA families with unknown mutations
    - At least 5 affected families members
    - EUROSCA runs facilities for high-throughput genotyping, and has bundled biostatistical expertise for small families
- Contact: brice@ccr.jussieu.fr

Selected references

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