Prior Molecular Diagnostic Accuracy and Age of Disease Onset Variation in the CRC-SCA, a Multicenter Study of Spinocerebellar Ataxias

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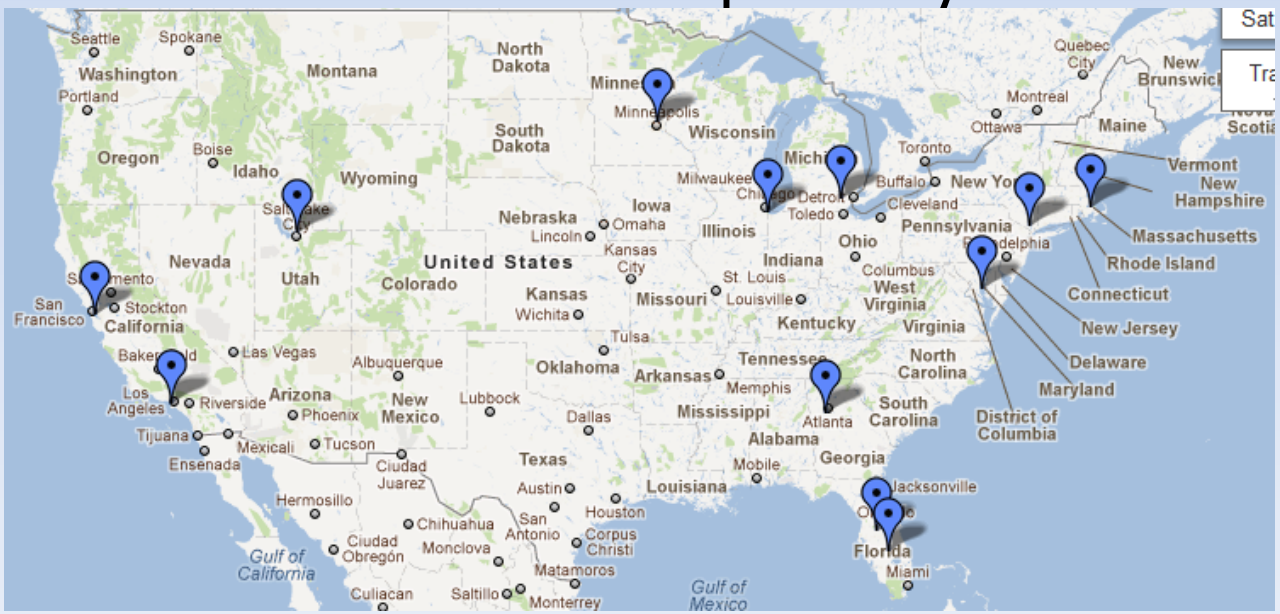
Study Design

- Nationwide study – 12 Centers
  - One central IRB
- 4 study related clinic visits every 6 months.
- Study Type: Observational
- Blood collection – Tissue Repository



Recruitment

- **Eligibility**
  - Age : 6 years and older
  - Sex : Both
- **Criteria**
  - Inclusion Criteria:
    - Presence of symptomatic ataxic disease
    - Molecular diagnosis of SCA 1, 2,3,or 6 affected family member
    - Willingness to participate
  - Exclusion Criteria:
    - Known recessive, X-linked and mitochondrial ataxias
    - Lack of SCA 1, 2, 3 or 6 by DNA testing

Center	SCA1	SCA2	SCA3	SCA6	Total
University of Chicago	8	7	11	16	42
Columbia University	3	5	2	0	10
Emory University	12	14	22	4	52
Harvard Medical School	8	2	19	3	32
John Hopkins Medical University	0	7	8	10	25
University of California Los Angeles	3	12	26	13	54
University of California San Francisco	2	1	3	1	7
University of Florida	11	9	11	13	44
University of Michigan	8	2	6	3	19
University of Minnesota	0	4	7	4	15
University of South Florida	4	5	10	6	25
University of Utah	0	2	0	0	2
Total Enrolled	59	70	125	73	327

Study Design

- Genetic Testing
  - Verification of diagnosis
    - No disclosure of misdiagnosis
    - Continuation in study
  - Age of Onset disease modifiers –
    - ***DRPLA ,FRDA, HD, HD2, KCNC3*, RAI1, SBMA, SCA10, SCA12, SCA17*** **and** ***SCA7***

Methods

- **DNA Extraction**
  - Standardized protocol
- **Genotyping**
  - 15 genes (16 repeats)
  - Multiplex PCR followed by capillary electrophoresis with internal standards
- **Quality Control**
  - 2 CEPH DNA samples (1332-02 and 1347-02) included in every run/marker
  - Re-genotyping and Sanger Sequencing - 10% of samples

Multiplex PCR

Gene name	Primer Name	Dye	Amplicon size
*KCNC3**	3A-F 3B-R	PET	170
*KCNC3**	2A-F 2B-R	VIC	120
SCA1	Rep1 Rep2	FAM	223
SCA3-MJD	MJD25 MJD52	NED	215

Gene name	Primer Name	Dye	Amplicon size
SCA2	SCA2A SCA2B	PET	220
SCA7	SCA7C SCA7D	VIC	297
DRPLA	B37F B37R	NED	140
SBMA	BMAF SBMAR	FAM	270

Gene name	Primer Name	Dye	Amplicon size
SCA6	SCA6F SCA6R	VIC	184
SCA17	SCA17A SCA17B	NED	245
HD	HD1-new HD3	FAM	103
SCA10	attct-L attct-R	PET	250

Gene name	Primer Name	Dye	Amplicon size
RAI1	SCZ15 SCZ16	FAM	181
HD2	L237-1 L237-2	VIC	241
SCA12	SCA12-F SCA12-R	NED	350
FRDA	GAA-F GAA-R	PET	350

Results

Quality control

1st stage:

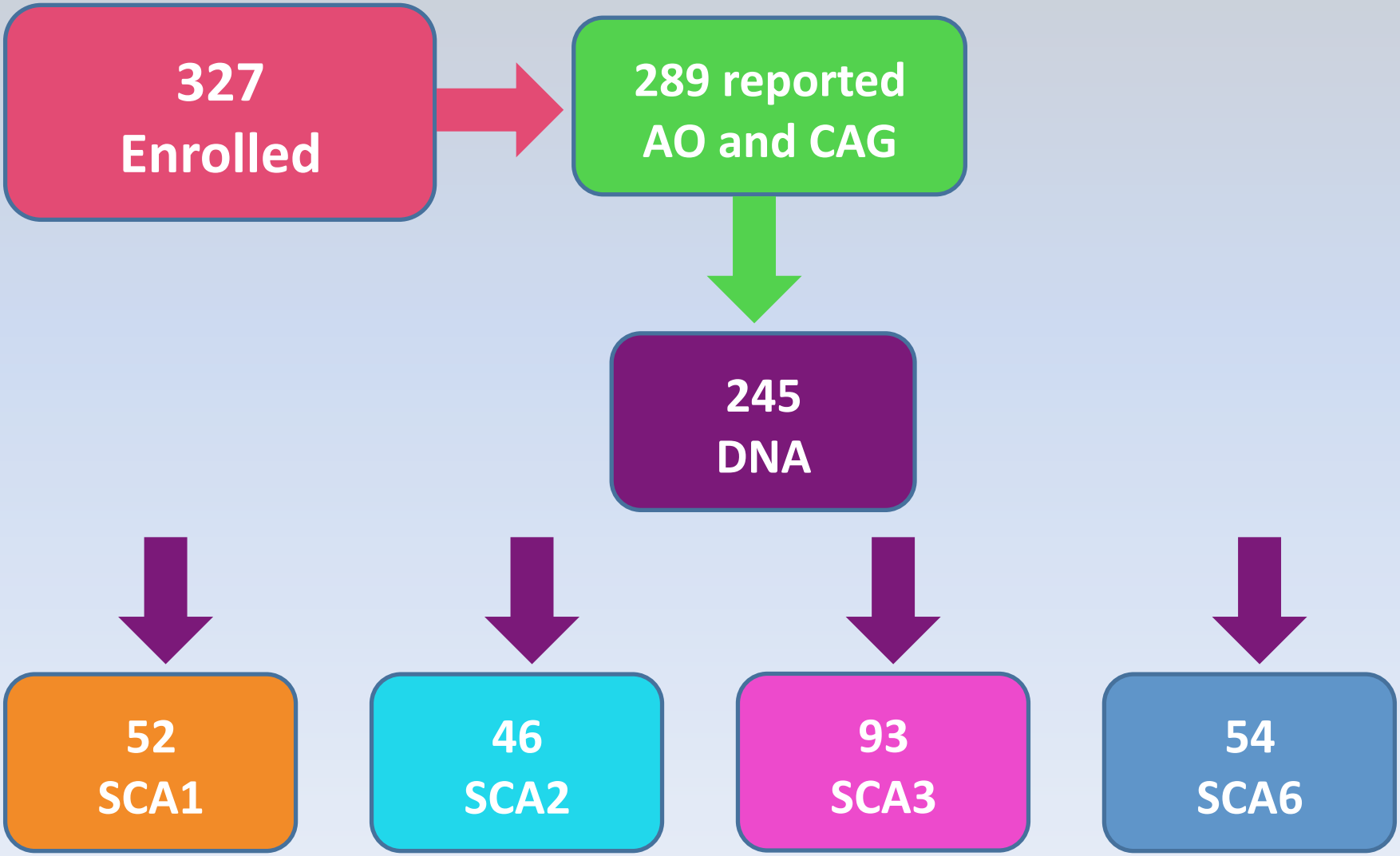
- Re-genotyping of 10% randomly chosen samples
- For 25 of 25 samples 100% concordance of 1st and 2nd multiplex genotype.

2nd stage:

- Sequence analysis of 10% of samples:
  - 98.8% concordance with repeat number determined by fragment sizing
  - 7 samples differed by 1-3 repeats; all had long mutant SCA3 alleles.

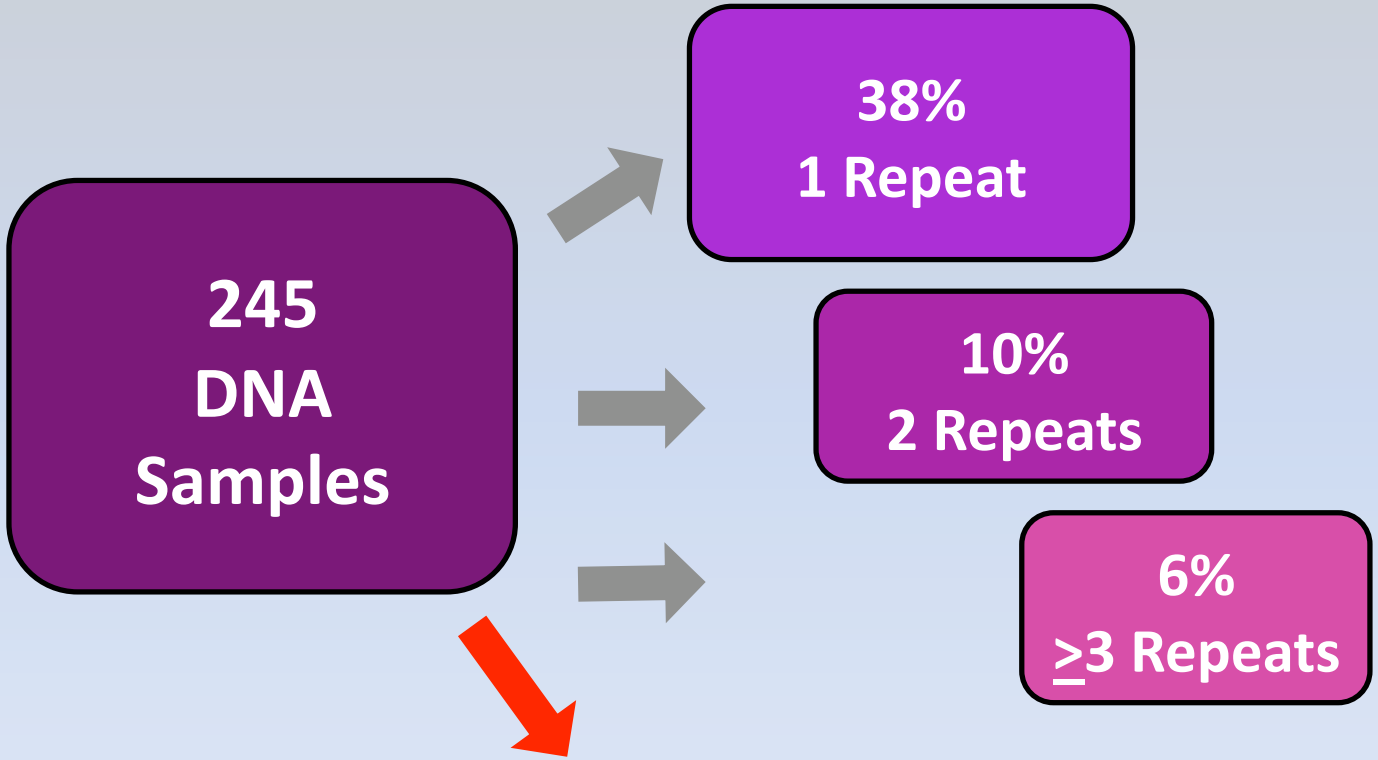
Results

Distribution of Mutant Genotypes

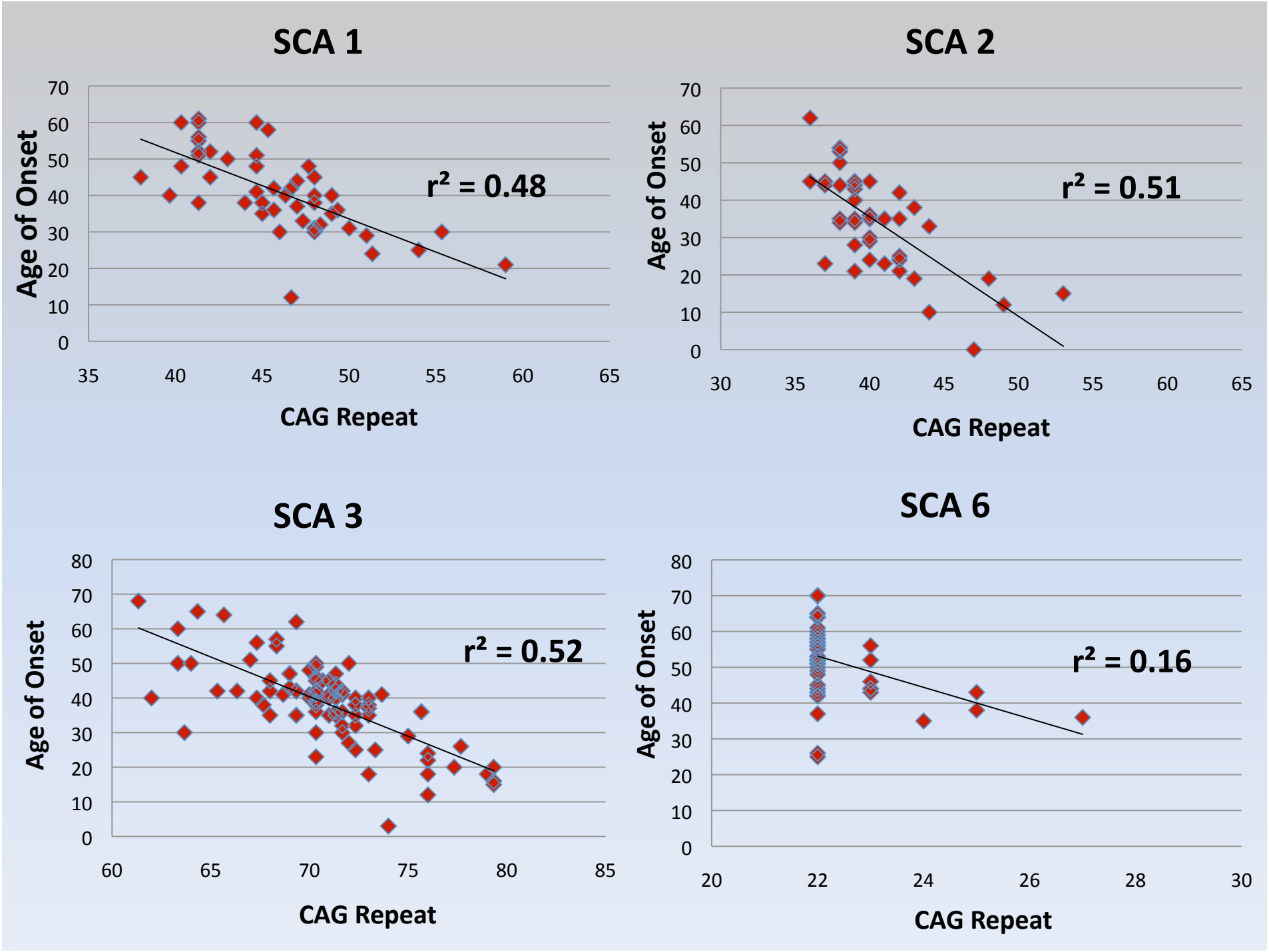


Results

Comparison with reported Genotypes



No mutant expansions found: **4**


SCA 1
Age of Onset
CAG Repeat
r² = 0.48
SCA 2
Age of Onset
CAG Repeat
r² = 0.51
SCA 3
Age of Onset
CAG Repeat
r² = 0.52
SCA 6
Age of Onset
CAG Repeat
r² = 0.16

CRCA-SCA (n = 238)

	SCA1 (n = 47)	SCA2 (n = 45)	SCA3 (n = 92)	SCA6 (n = 54)
Age at onset, y, mean (SD)	41.13 (11.17)	35 (11.93)	38 (11.75)	52 (10.35)
Normal Allele Range	27-36	22-29	14-36	11-16
Mutant Allele Range	38*-59	36-53	61-79	22-27
r^2	**0.48**	**0.51**	**0.52**	**0.16**

Dutch-French Cohort[1] (n = 699)

	SCA1 (n = 138)	SCA2 (n = 166)	SCA3 (n = 342)	SCA6 (n = 53)
Age at onset, y, mean (SD)	35.5 (10.6)	34.6 (13.3)	39.5 (11.6)	49.2 (9.8)
Normal Allele Range	26-37	15-29	3-35	6-14
Mutant Allele Range	40-69	35-58	58-82	21-28
r^2	**0.63**	**0.80**	**0.70**	**0.56**

*Sequence verified, 1 Warrenburg et al 2005

Conclusions I

- Overall, high concordance of 1st genotyping and 2nd retyping.
- Minor differences between PCR genotyping and resequencing only for long mutant SCA3 alleles.
- Overall **diagnostic** accuracy very good,
  but
  Four patients (~1.5%) without presumed SCA genotype.

Conclusions II

- Inverse correlations between AO and CAG repeat lengths weaker than previously reported.
- Possible explanations:
  - Age of Onset Determination in a multi-center study
  - Geographic distribution
  - Ethnic diversity,
  - Exclusion of 1st and 2nd degree relatives.

Collaborators

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