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APPRECIATION OF SHORT REPEATS NUMBER IN NEUROGENETIC DISORDERS DIAGNOSTIC

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SUMMARY

Key words: SCA, short repeats, Friedreich, ataxia.

There are known many hereditary ataxias caused by short repeats expansions, most prominent of them being Huntington disease, Friedreich ataxia and a vast number of spinocerebellar ataxias (SCA's).
These ataxias are very similar by clinical signs, being sometimes almost phenocopies, so a molecular-genetic diagnostic method is the only option for differential diagnostics.
There is also a well-known effect called genetic anticipation, when some alleles with a quite large, but still normal number of repeats may further expand in next generations. It puts potential children of people harboring these alleles at risk of disease development. And it is a cause why a screening of affected child's parents and their siblings is necessary.
A large number of publications shows that number of repeats most frequently directly correlates with age of disease onset and speed of its progression. So, counting short repeats number may give not only diagnostic, but prognostic information for affected persons and their relatives.
In this publication we propose a diagnostic approach based on a combination of agarose gel AFLP and capillary electrophoresis based fragment analysis to detect short repeats expansion and calculate total repeats number for multiple forms of SCA and Friedreich ataxia. As a test group a selection of patient's DNA with proposed diagnosis of SCA or Friedreich ataxia stored in LGMU biobank was used.

REZUMAT

Cuvintele-cheie: SCA, repetari scurte, Friedreich, ataxia.

Exista multe ataxii ereditare cauzate de expansiile a repetarilor scurte, dintre ele cele mai cunoscute sunt boala Huntington, ataxie Friedreich si ataxii spinocerebelare (SCA).
Aceste ataxii sunt foarte similare in simptoame clinice fiind aproape fenocopii una de alta, asadar investigatiile molecular-genetice sunt o singura optiune pentru un diagnostic diferential.
Este cunoscut si asa numit efectul anticiparii genetice, cand un alel cu un numar larg, dar normal de copii a repetarii tinde spre largirea numarului de copii a repetarii scurte in urmatoarele generatii. Acest fapt supune copii potentiali a acestor oameni riscului de dezvoltarea bolii corespunzatoare. Și aceasta este cauza necesetatii screeningului de părinții a copiilor afectati și rudelor lor.
Un număr mare de publicații arată că numărul de repetări cel mai frecvent se corelează direct cu vârsta de debut a bolii și viteza progresiei acesteia. Așadar, calcularea numarului de repatari scurte are nu numai un rol diagnostic dar și prognostic pentru pacienți și rudele lor.
În aceasta publicație noi propunem o metoda diagnostica bazata pe AFLP în gel de agaroza și analiza fragmentilor bazata pe electroforeza capilara pentru a detecta expansie repetarilor scurte și de a calcula un numar total a repetarilor pentru multe forme a SCA și ataxie Friedreich. Ca un grup de test a fost utilizata o selecție de ADN a pacientilor cu propunerea dioagnozei de SCA sau ataxie Friedreich care au fost stocate în banca biologica LGMU.

РЕЗЮМЕ

Ключевые слова: СЦА, короткие повторы, атаксия, атаксия Фридрейха.

Существует множество наследственных атаксий, вызываемых экспансией коротких повторов, самыми известными из которых являются хорея Хантингтона, атаксия Фридрейха и спиноцеребеллярные атаксии. Данные атаксии имеют крайне схожие клинические проявления, являясь почти что фенокопиями друг друга. Таким образом, молекулярно-генетические исследования играют ключевую роль в их дифференциальной диагностике.

Существует также явление, известное как эффект предожидания, проявляющийся в дальнейшей экспансии однажды удлинившихся аллелей повторов у следующих поколений. Этот факт полвергает потенциальному риску заболевания детей носителей данных аллелей. Также это обуславливает необходимость скрининга родителей больных детей и их родственников на предмет нахождения предрасположенных к экспансии аллелей.

В большом количестве публикаций подмечается также частая зависимость возраста дебюта и скорости прогрессирования заболеваний от количества коротких повторов в связанном с заболеванием гене. Таким образом, для пациентов и их родственников подсчёт количества повторов имеет не только диагностическое, но и прогностическое значение.

В данной публикации, мы предлагаем метод диагностики, основанный на комбинации AFLP анализа в агарозном геле и фрагментного анализа посредством капиллярного электрофореза для определения экспансии коротких повторов и подсчёта количества повторов как таковых у больных с подозрением на различные формы СЦА и атаксию Фридрейха. В качестве тестовой группы использовались образцы ДНК пациентов с предполагаемым диагнозом СЦА или атаксии Фридрейха, хранившиеся в биобанке Лаборатории Молекулярной Генетики Человека Института Матери и Ребёнка, Кишинёв, Молдова.

BACKGROUND

Exists a group of vary clinically similar diseases called spinocerebellar ataxias, which includes about 60 different pathologies with different patterns of inheritance. In this group can be included and Friedreich ataxia, being very similar in clinical signs. About 12 of those diseases are caused by short repeat expansions, making this mechanism of pathogenesis very significant to diagnose SCA's and Friedreich ataxia [1-11]. Data of some of those diseases are shown in table 1 below.

As it is shown in table 1, the most frequent mechanism of pathology is polyglutamine tract expansion leading to resulting protein misfolding, aggregation and cellular damage induced by aggregates. This mechanism was first studied in Huntington disease [12], but is very common in a large number of neurological diseases.

Another mechanism is "missplicing" due to excessive repeat expansion in splicing region of introns. This mechanism is responsible for majority cases of Friedreich ataxia [11].

One more interesting mechanism of pathogenesis is Repeat-Associated-Non-ATG-Translation (RAN-translation). In this case, pathogenesis is due to that fact, what in real life not only ATG codon may be the starting one, but other codons are much weaker starting codons. So, after

Table 1. ***Data about some of short repeat caused ataxias. A.D. - autosomal dominant, A.R. - autosomal recessive patterns of inheritance.***

Disease	Affected gene	Normal repeats number range	Repeats number in pathology	Repeat sequence	Inheritance pattern
SCA1	*ATXN1*	6 - 35	49 - 88	CAR	A.D.
SCA2	*ATXN2*	14 - 32	33 - 77	CAR	A.D.
SCA3	*ATXN3*	12 - 40	55 - 86	CAR	A.D.
SCA6	*CACNA1A*	4 - 18	21 - 30	CAR	A.D.
SCA7	*ATXN7*	7 - 17	38 - 120	CAR	A.D.
SCA8	*ATXN8/ATXN8OS*	16 - 37	110-250	CTG	A.D.
SCA10	*ATXN10*	10–32	280–850 — low penetrance 850–4500 — full penetrance	ATTCT	A.D.
SCA12	*PPP2R2B*	7 - 41	43 - 51	CAG	A.D.
SCA17	*TBP*	25 - 42	47 - 63	CAR	A.D.
SCA36	*NOP56*	3 - 14	650 - 2500	GGCCTG	A.D.
Friedreich ataxia	*FXN1*	7 - 34	90+	GAA	A.R.
Spinobulbar muscular atrophy	*AR*	4 - 34	35 - 72	CAR	X-linked

expansion of their number, probability to initiate protein translation from a wrong place increases. This mechanism is particularly responsive for SCA3 [13] and DM-1 [15] pathogenesis and completely responsive for SCA8 [14, 15]. Yet another mechanism of pathogenesis is promoter methylation due to high-GC repeat expansion. This mechanism is responsible for SCA12 development [16].

METHODS

To develop primers for diagnosis we used BLAST (for reference gene sequence search and primer alignment) [18], Unipro UGENE (for modeling PCR product sequence) [19] and IDT Oligoanalyzer (to calculate PCR conditions) [20] software.

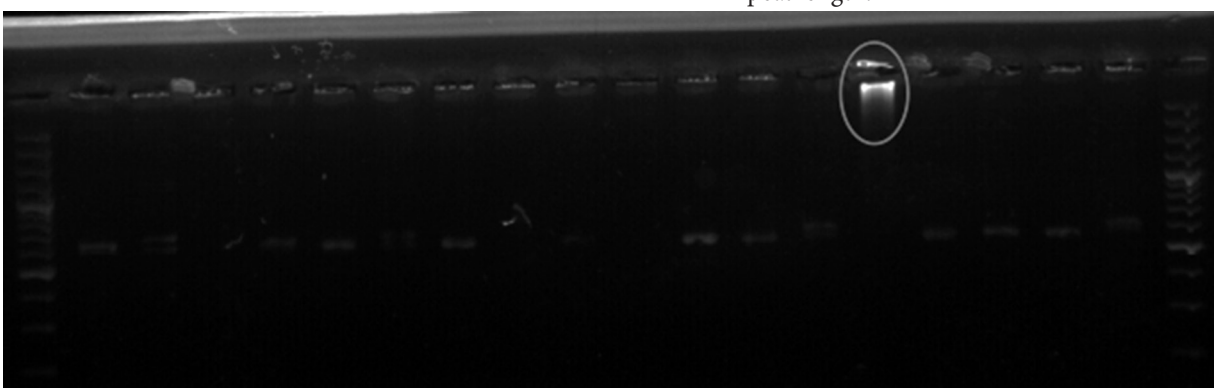
PCR primers were labeled with FAM, VIC or PET dye to be detected by capillary electrophoresis. Currently, we can't publish their sequences, because they are in the process of patenting.

At first step, a PCR with labeled primers was performed. To detect very expanded alleles we used classic agarose gel AFLP (Amplified Fragment Length Polymorphism) method (Pic. 1). Very long alleles detected at this step were not examined by capillary electrophoresis (it can handle alleles with length up to 1200 base pairs only) and were considered unconditionally pathogenic, because repeat number in them always corresponds to pathogenic variants (see table 1).

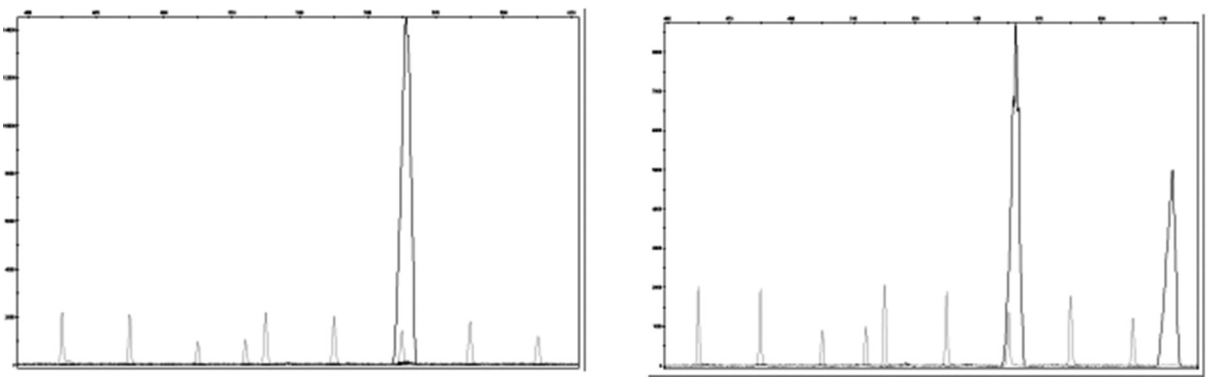
At the next step, smaller-size allele PCR products were separated and detected by capillary electrophoresis (Pic. 2) in denaturing conditions. Multiplexed mixes were also tried (Pic. 3).

Knowing PCR product length, a number of repeats may be calculated upon formula:

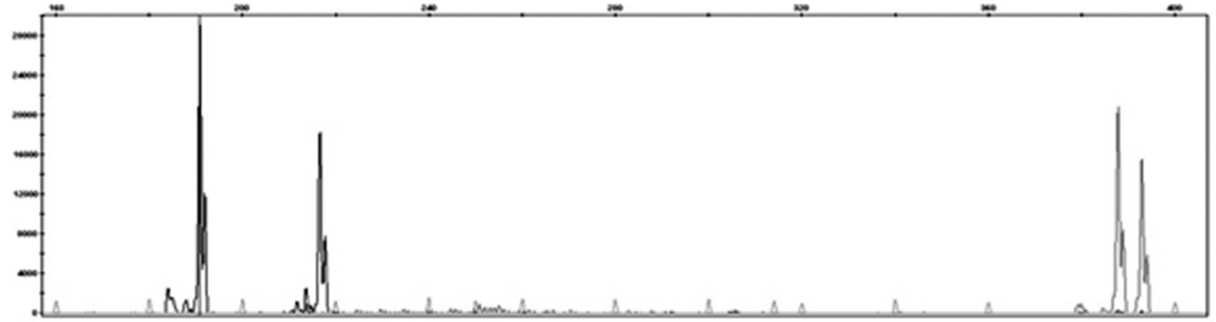
$N=\frac{L_a-L_{nr}}{L_{sr}}$, where N — repeat number, L_a — PCR product length, L_{nr} — length of non-repeat sequence in PCR product, L_{sr} — single repeat length.



Picture 1. ***Very large repeat number PCR product is detected through AFLP (encircled).***



Picture 2. ***An example of fragment analysis picture for homozygous (left) and heterozygous (right)*** samples.



Picture 3. ***A sample of multiplexed fragment analysis, FAM and VIC channels are used.***

Results and discussions. 46 samples from biobank were analyzed for repeat number in FXN1, TBP, ATXN3 and ATXN10 genes. This selection of samples included those 16 samples of Friedreich ataxia patients that were reported by Sacara et al. In 2012 [21]. Totally, Sacara et al. reported 30 registered cases of SCA and 16 cases of Friedreich ataxia in the Republic of Moldova prior year 2012.
Results for FXN1 GAA repeat number are shown in table 2.

Repeat number	Allele number
indefinite	18
3	8
5	7
6	15
7	28
15	1
16	2
17	1
18	6
19	2
20	1
24	1
>=1500	2

Table 2. ***A number of alleles with different repeat number in studied group. Indefinite are considered alleles from samples with no amplification or low quality of separation through capillary electrophoresis.***

However, only one case of Friedreich ataxia was confirmed. It may be caused by similarity of Friedreich ataxia clinical signs with a number of spinocerebellar atxias mentioned above, by rare mutation causing Friedreich ataxia (SNP instead of short repeat expansion) or by samples degradation over time (see further).
The most frequent alleles are with 6 and with 7 GAA repeats. Some of samples did not amplified, what may be caused or by SNP in primer binding loci, or by sample degradation over time. Amplification of those samples will be repeated later.
For ATXN3 most ubiquitous allele contained 20 repeats; for ATXN10 — 13 and for TBP — 35 and 37 repeats.
In future, a large group of non-affected persons will be studied to establish different allele frequency in general population.

CONCLUSIONS

We succeeded in development of molecular-genetic diagnostic for several short-repeat caused ataxias and studied a group of 46 patients to detect different allele frequency.
A single patient with Friedreich ataxia was confirmed, what may be due the fact it has very similar clinical signs with SCA's and could not be differentiated just clinically or in other patients it may be caused by a more rare mutation than GAA repeat expansion.
The most frequent allele for FXN1 gene is the one with 7 GAA repeats, what corresponds quite well with data obtained previously by Cossee et al. [17] Less frequent but very common one one was with 6 GAA repeats.
The most ubiquitous alleles for TBP gene are close to upper limit of normal repeats number range (see tab. 1). It seems that further research of TBP allele repeat number range in population of the Republic of Moldova may be necessary.
Further research of spinocerebellar ataxias caused by repeat expansions is needed.

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