alloprotease involved in protein quality control.

Objectives: To identify the gene causing SCA28 and to demonstrate its pathogenic role.

Methods: AFG3L2 gene was analysed with a dHPLC/direct sequencing approach, in the original SCA28 family and in our large collection of families and patients with spinocerebellar ataxia negative for mutations in known SCA genes. Functional studies were performed in a yeast cellular model. Expression studies were performed in human and rat central nervous system.

Results: We have found that AFG3L2 is the disease-causing gene. We have identified 9 heterozygous AFG3L2 missense mutations in the original kindred and in other 8/325 (~2.5%) unrelated SCA families. Interestingly, concurrent heterozygosity for a recessive paraplegin mutation negatively modulated the phenotype in one family. All the AFG3L2 mutations are located in functional domains of the protein at highly conserved amino acids. Structural modelling in the eubacterial FtsH protease indicates that they may affect substrate interaction. AFG3L2 protein and transcript were found to be highly and selectively expressed in cerebellar Purkinje cells. Expression of normal and mutant AFG3L2 homocomplex in m-AAA-deficient yeast cells demonstrate that the mutations cause respiratory deficiency and proteolytic defect. Preliminary phenotypic analysis of the patients harbouring a mutation in the SCA28 gene showed a relatively uniform ADCA1-type clinical presentation characterized by slowly-progressive cerebellar ataxia with cerebellar atrophy and a frequent occurrence of oculomotor dysfunction and pyramidal signs.

Conclusion: This work identifies AFG3L2 as a novel cause of dominant neurodegenerative disease and indicates an essential role of the mitochondrial AFG3L2 homocomplex in protecting the cerebellum against neurodegeneration [Italian Ministry of Health grant (ex art 56) to FT].

EARLY-ONSET SCA2 : CLINICAL AND GENETIC CONSIDERATIONS

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Materials/Methods: The patient is a girl of 16 months, born after an unventful pregnancy with an 8/9 Apgar score at birth. After 6 months of age she stopped growing both in weight and height. Her current weight is 6400 g. At 7 months of age delayed neuropsychomotor development in different areas (posture, balance, tone, vision and hearing) was first noted. At 16 months she is still unable to sit unsupported, finalistic voluntary movements are poor. Diffuse hypertonia but normal tendon reflexes were also found. She has difficulties at following sources of light and to fix her gaze on objects. Defence reflexes are also absent. Pupils are symmetric and reactive to light. Both flash ERG and flicker PEV are reduced in amplitude. At fundoscopy irregular diffuse pigmentation of the retina has been detected. A brain MRI revealed severe atrophy of brainstem and cerebellum. Her father is a 33-year-old man who developed signs of cerebellar ataxia after age 30, with no definite diagnosis. His mother had died of a similar disorder at age 58. He had fathered another girl, now six years old, and apparently in good health.

Results: In the father the genetic analysis revealed a CAG expansion in SCA2 gene in a typical range of the adult-onset disease (CAG 14±2 e 51±2). In the daughter a large CAG expansion (CAG >90) was detected only by means of the Fluorescent Repeat-Primer PCR Assay (3).

Discussion/Conclusions: Extreme CAG expansions in SCA2 have been observed in rare cases associated to infantile onset, usually transmitted through affected fathers [1,2]. Consequently the clinical picture of early-onset SCA2 is still poorly defined and the occurrence of such a possibility is not widely acknowledged among neuropediatricians. Infantile SCA2 is generally an extremely severe disease characterized by psychomotor retardation, cerebellar atrophy, retinopathy and early mortality. Such a diagnosis should be considered, when there is a family history of dominantly transmitted cerebellar ataxia, and this possibility taken into account in the genetic counseling of at risk subjects. Moreover for the molecular analysis appropriate techniques should be used in order to avoid false negative results in affected children.

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A CASE OF SPINOCEREBELLAR ATAXIA TYPE 17 (SCA 17)

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Introduction: SCA 17 is a dominant neurodegenerative disorder characterized by ataxia, cognitive decline, dystonia and parkinsonism. The disease is caused by a triplet repeat expansion mutation within TATA binding protein (TBP). Here we report a case of SCA 17 case with an atypical phenotype.

Case report: A 69-year-old woman reported a history of 4 years characterized by clumsiness of left upper limb over four years with increasing disability. Two years after symptoms onset slowly progressive speech disorder, dysphagia, urine incontinence and gait and balance disorders with reduction of postural reflex developed. When she was first admitted to our clinic (May 2008), at her neurological examination the patient showed facial hypomimia, gait disturbance, marked dysarthria, rigidity and bradykinesia of four limbs predominant on the left, rest tremor and torsional dystonia of the upper left limb. No cognitive decline was observed. Routine biochemistry, haematological tests (including thyroid function test, ceruloplasmin test, vitamine E, tumor markers), electrocardiogram, echocardiography, autonomic function testing were all normals. The response to levodopa has poor. Brain magnetic resonance imaging revealed pons atrophy, cortical cerebellar atrophy, "hot cross bun sign" in pons. To investigate the nigrostriatal system integrity, we performed a cerebral SPECT with 123I-FP-CIT that showed reduced uptake. The cardiac MIBG scintigraphy however, resulted normal. A family history of Parkinson's disease was reported (paternal uncle). A wide genetic testing was performed including SCA 1-3, SCA 6- 8, SCA 17, LRRK2, NACP. Genetic test were all normal, except for SCA 17 that revealed an expanded allele with 44 CAG repeats and one normal allele with 35 CAG repeats.

Discussion and conclusion: Here we report a patient with clinical picture suggestive of a parkinsonism type multiple system atrophy – parkinsonian variant. The genetic testing performed in this patient a pathological expanded fragment of TBD gene allowing a diagnosis of spinocerebellar ataxia (SCA) type 17. This case expand current phenotype associated with SCA 17.

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SPINOCEREBELLAR ATAXIA TYPE 15: CLINICAL AND MOLECULAR-GENETIC FEATURES OF TWO ITALIAN FAMILIES

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Background: SCA15 is known as a pure cerebellar ataxia characterized by onset between 10 and 50 yrs, very slowly progressive gait ataxia, dysarthria, titubation, postural or action tremor of hand, neck and trunk, mild hyperreflexia, gaze-evoked nystagmus, and impaired vestibulo-ocular reflex. MRI shows a marked cerebellar atrophy, mainly in the upper vermis, whereas the brainstem is not involved. Type 1 inositol 1,4,5-triphosphate receptor (ITPR1) is reported as the SCA15 causing gene. At present, six families are described worldwide: five carry a partial deletion of ITPR1 and one a missense mutation.

Materials and Methods: We examined a four generation Italian family segregating an autosomal dominant form of cerebellar ataxia, and found a positive linkage at the SCA15 locus. In this family and in 43 familial SCA index cases, we performed a gene-dosage sensitive quantitative PCR (qPCR) to search for ITPR1 gene deletion, using a probe mapping in ITPR1 exon 3.

Results: We identified two families with a deletion that was further characterized by a custom array-CGH spanning 3 Mb centred on the ITPR1 gene. In one family, the deletion extended over ~238 Kb including ITPR1 exons 1–39, while in the second it spanned ~447 Kb and comprised SUMF1 exons 1–7 and ITPR1 exons 1–59. In these two SCA15 families, clinical data were available for 10 patients and brain MRI for six. The disease onset between 25 and 72 yrs (50 ± 17 yrs, mean ± SD); the first symptoms were unsteadiness, gait ataxia and dysarthria, variably associated with nystagmus, hyperreflexia and Babinsky sign, dysmetria, dysphagia and mild cognitive impairment. Notably four patients present facial dyskinesias that were never reported in SCA 15. In all affected subjects MRI showed cerebellar vermis atrophy with a mild involvement of the hemispheres in some individuals.

Discussion: Our results further support ITPR1 gene as causative of SCA15, and describe two novel families in Italy. Overall, the phenotype mainly overlapped known SCA15 cases. However, our data show that this disease may have a greater variability in the age at onset (25–72 yrs) and in the phenotype than previously described. The variable disease expression mimicked anticipation in one family.

Conclusions: The presence of slowly progressing, relatively ipurei cerebellar ataxia combined with predominant vermis atrophy at the MRI suggests SCA15. In these cases the search for ITPR1 deletions by qPCR is mandatory and simple.

SINGLE-CENTER, OPEN-LABEL, SEQUENTIAL TRIAL TO TEST THE EFFICACY, SAFETY AND TOLLERABILITY OF EPOETIN ALFA IN PATIENTS WITH FRIEDREICH'S ATAXIA

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Background: Friedreich's ataxia (FRDA) is an inherited recessive disorder characterised by progressive neurological disability. FRDA is the consequence of frataxin deficiency. Although several drugs have been proposed, there is no available treatment. Recently it was shown that erythropoietin (EPO) administration increases frataxin expression in lymphocytes of FRDA patients. Two clinical trials showed that Epoetin-beta is able to increase frataxin levels between 20%–30% in peripheral blood mononuclear cells (PBMCs) of patients treated with 5000 IU t.i.w. or 2000 IU t.i.w. respectively.

Objective: The aim of the project is to assess the *in vivo* effect of Epoietin-alpha on frataxin expression in PBMCs. Secondary endpoints are echocardiography indexes and treatment safety.

Methods: Inclusion criteria were molecular diagnose of FRDA, aged 18-50. Exclusion criteria were treatment with Idebenone, wheelchair bound patients, renal, hepatic or haematological diseases, history of arterial or venous thrombosis, arterial hypertension, pregnancy or breastfeeding. Patients were treated with 600 IU/Kg s.c. of Epoetin-alpha and frataxin levels were determined at time 0, 24, 48, and 96 hours, 7, 15, 30, and 60 days. 30 days after the last visit, patients were treated with 1200 IU/Kg s.c. of Epoetin-alpha. Endpoints were determined as for the fist administration. Frataxin was dosed in PBMCs with lateral flow immunoassay.

Results: Patients showed a very small and non-significant acute increase of <10% (first administration) and 20% (second administration) of frataxin in PBMCs at 24–48 hours after treatment. On the opposite, a long term increase of frataxin was noted. 3 months after treatment with the first single dose frataxin levels were increased of 43% ($p<0.05$), and six months after the second dose was administered levels reached a 83% increase ($p<0.01$). All patients showed at least an increase of ≥20% at one time point. Transferrin saturation decreased of 44% and 43.2% of baseline value 7 days after each injection. Main echocardiography indexes were not modified after treatment. Although patients showed a subjective improvement in strength and resistance to exercise, no change on neurological scales was noted.

Conclusions: We confirm the previous observation that EPO is able to increase frataxin expression in PBMCs. In our study frataxin increased up to 83% at 6 months after two high doses of Epoetin alfa, and all patients responded to treatment. Acute treatment with EPO was safe and well tolerated.

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OUTCOME AFTER TWO YEARS OF ENZYME REPLACEMENT THERAPY (ERT) IN 29 PATIENTS WITH LATE-ONSET TYPE II GLYCOGENOSIS (GSDII)

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Objective: To evaluate the effects of human recombinant acid alfa-glucosidase (rhGAA) in late-onset GSDII. Type II Glycogenosis (GSDII), is a lysosomal storage disorder due to acid alfa-glucosidase (GAA) deficiency. The infantile phenotypes are responsive to rhGAA enzyme replacement therapy (ERT). Recently, rhGAA became available for the