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Allan-Herndon-Dudley syndrome (AHDS) in two consecutive generations caused by a missense MCT8 gene mutation. Phenotypic variability with the presence of normal serum T3 levels.

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Abstract

Allan-Herndon-Dudley syndrome (AHDS), an X linked condition, is characterized by severe intellectual disability, dysarthria, athetoid movements, muscle hypoplasia and spastic paraplegia in combination with altered TH levels, in particular, high serum T3 levels. Mutations in the *MCT8* gene coding for the monocarboxylate thyroid hormone transporter 8 have been associated with AHDS. Here we describe a family with the presence of a *MCT8* gene mutation, p.A224T, in three consecutive generations. In two generations its presence was detected in the hemizygous state in two males with neurological abnormalities including mental retardation, axial hypotonia, hypertonia of arms and legs and athetoid movements. One of them presented normal thyroid hormone levels. Mutation was also detected, although in the heterozygous state, in three females showing thyroid hormone levels in the normal range. Our results show the difficulty of distinguishing AHDS from patients with X-linked intellectual disability solely on the basis of clinical features and biochemical tests, and we advise screening for *MCT8* mutations in either young or older patients with severe intellectual disability, axial hypotonia/dystonia, poor head control, spastic paraplegia, and athetoid movements even when they have normal thyroid hormone profiles.

1. Introduction

Allan-Herndon-Dudley syndrome (AHDS; MIM 309600) is one of the first X-linked intellectual disability (XILD) syndromes reported [1] and was among the first XILD conditions to be genetically mapped [2]. Most patients share the severe neurological deficit and the markedly elevated serum T3 and low T4 and rT3 levels. The neurological phenotype in most patients includes central hypotonia with poor head control, which evolves into spastic quadriplegia; inability to sit, stand or walk independently; severe intellectual disability; and absence of speech [3]. After the identification of *MCT8* mutations in patients diagnosed with AHDS, the relationship between

MCT8 deficiency and AHDS could be established [4]. More than 45 families have been described with hemizygous affected males carrying mutations in the *MCT8* gene [5]. The mutations range from large deletions, resulting in the loss of one or more exons, to smaller frame-shift causing deletions or insertions, to missense or nonsense mutations. Recently we described a patient of Sardinian origin with a severe form of AHDS due to a novel insertion mutation in *MCT8*, associated with a peculiar thyroid hormone phenotype [6]. Here we report another Sardinian family with two patients in two consecutive generations affected by AHDS and carrying a missense mutation in the *MCT8* gene, with one of the patients presenting normal thyroid hormone levels.

2.Clinical Report

2.1 Patient 1 (IV-2, Fig.1)

An 8-month old boy was evaluated because of severe hypotonia from early life and global developmental delay. His parents were not consanguineous. The pregnancy was uncomplicated but cesarean delivery was performed at week 36 because of maternal hypertransaminasemia. Birth weight was 2.9 kg (50th percentile), length was 49 cm (50-75th percentile) and head circumference 32.5 cm (-2SD). APGAR score was 9-10. At first presentation at the age of 8 months, he showed generalized hypotonia, hyperreflexia, clonus and Babinski reflexes. The patient has a healthy brother who is four years younger. He was revaluated at age 7 years. His height was at 50th percentile, weight at 10th centile and head circumference at -2SD. He presented severe developmental delay, axial hypotonia with neck drop and difficulty in supporting the head, and muscle tone and deep tendon reflexes increased in both lower and upper limbs. He also showed poor muscle and fat mass, plagiocephaly, slightly myopathic facies, widow's peak and mild dorsolumbar scoliosis (Figure 2). He was not able to walk independently but he could walk a few steps with orthotic devices. He was not able to speak but he had some nonverbal interaction with the examiner. He didn't show athetoid movements, nor seizures. His cardiovascular examination was

normal with heart rate of 86/min. Fundoscopic examination, abdominal ultrasound, electromyography and brainstem auditory, visual and sensory evoked potentials were normal. Electroencephalographic studies studies were normal and magnetic resonance imaging of the brain performed at 3 years old showed generalized delayed myelination. Biochemical investigations showed normal haematological and blood chemical values, with no metabolic abnormalities in blood and urine. Results of endocrine studies were normal except for altered thyroid function with high serum free T3 level at 6.34 pg/ml (NR 1.4-5), low free T4 levels at 0.75 ng/dl (NR 0.8-2), normal basal serum TSH at 1.69 mUI/ml (NR 0.4-4.0), high serum sex hormone-binding globulin (SHBG) at 145 nmol/L (NR 13-71) and no detectable thyroid autoantibodies (TG-A and TPO-A) (Fig. 1). Standard TRH test carried out using i.v. administration of 7 mg/kg of thyrotropin-releasing hormone (Protirelin, Ferring Arzneimittel GmbH, Germany) was in the normal range (Table 2). Thyroid ultrasound and CT scans were normal. High-resolution chromosome, FISH analysis of the subtelomeric regions of all chromosomes, molecular *FMR1, MECP2* gene analysis and DNA methylation analysis for AS region were normal.

2.2 Patient 2(III-1, Fig.1)

The maternal uncle of patient 1 also showed a neurological disorder of early onset. Generalized hypotonia and poor head control were obvious by the age of 3 months. He also presented paroxysmal dyskinesia with version of the head to the back, opening of the mouth and tonic stretching of the arms and legs and additional tonic-clonic convulsions. He was evaluated at age 36 years (Figure 3). He showed normal height, weight and head circumference. His neurological findings were: severe intellectual disability with psychiatric symptoms, self-destructive behaviour, bruxism, drooling, severe dysarthria, truncal hypotonia, limb hypertonia, hyperreflexia, clonus, Babinski reflexes and choreo-athetoid movements of the face, trunk and extremities. He never walked and his speech was limited to a few words. He presented scoliosis, dorsal kyphosis, contractures of the knees and ankles and specific craniofacial features: elongated face with midface

hypoplasia, prominent mandible and cupped, large ears. In addition he showed gastroesophageal reflux. Fundoscopic examination and cardiologic evaluation were normal with heart rate of 75/min. Abdominal and thyroid ultrasound were normal. Magnetic resonance imaging of the brain was normal except for the report of a hypophyseal enlargement. Laboratory examinations showed normal haematological and blood chemical values and no metabolic anomalies were detected. Thyroid function tests showed FT3 at 3.93 pg/ml (NR 1.4-5), FT4 levels at 0.76 ng/dl (NR 0.8-2), basal serum TSH at 0.74 mUI/ml (NR 0.4-4.0), normal sex hormone-binding globulin (SHBG) at 64.60 nmol/L (NR 13-71) and no detectable thyroid autoantibodies (TG-A and TPO-A). Standard TRH test carried out using i.v. administration of 500mg of thyrotropin-releasing hormone (Protirelin, Ferring Arzneimittel GmbH, Germany) was in the normal range (Table 2).

3. Methods

Mutation analysis of *MCT8* gene was carried out. After obtaining informed consent, genomic DNA of the proband, the maternal uncle and other family members (IV-2, IV-3, III-1, III-2, III-3 , III-4, II-2, II-4, II-6, II-7, I-2, in Figure 1) was extracted from peripheral lymphocytes by standard methods. Mutation detection was performed by direct sequencing of the 6 amplified exons of the *MCT8* gene using appropriate pairs of primers (primer sequences available upon request) as previously described [6].

4. Results

Since the clinical findings as well as the TH abnormalities suggested AHDS, the *MCT8* gene was tested for mutations. Sequence analysis revealed the presence of a nucleotide substitution c.670 G->A in exon 2 resulting in a missense mutation p.A224T in the hemizygous state in the proband IV-2 and the maternal uncle III-1 (Figure 1) while it was found in the heterozygote state in his mother III-3, maternal aunt III-4 and grandmother II-2 and his healthy brother IV-3 was negative (Figure 1). The mutation was confirmed by DNA sequencing in both directions. This is a non conservative substitution replacing replacing a hydrophobic by a neutral polar residue located in the second

putative Trans-membrane domain (TMD). Subsequently, the mutation search was extended to other family members (II-4, II-6, II-7, I-2) in whom no mutation was detected.

5. Discussion

In this study we report a family with two patients affected by AHDS in two consecutive generations with a mutation in the *MCT8* gene. The c.670 G->A, p.A224T missense substitution detected in hemizygosity in patients IV-2, III-1 (Figure 1) replaces an hydrophobic with a neutral polar residue located in the second putative TMD. Family genetic analysis showed the presence of p.A224T mutation in II-2 but not in I-2 suggesting that it has occurred *de novo* in II-2 (Figure 1). Another mutation p.A224V has been previously described in the same amino acid position [7]. Functional studies on the mutant p.A224V protein showed a reduced uptake and subsequent metabolism of T3 *in vitro* [7]. The suggested mechanism is a mislocalization of the mutant p.A224V protein that is distributed mostly in the cytoplasm, resulting in inhibition of trafficking to the plasma membrane [7]. Based on these data we suggest a similar mechanism for p.A224T in the pathogenesis of AHDS in our cases. Mutation p.A224T has also been reported in another case of AHDS with severe hypotonia at birth and severe spastic quadriplegia within the first year of life [8]. Both patients of the present report show clinical features of AHDS with a relatively mild phenotype (Table 1). Both have normal auxological parameters (stature, weight and cranial circumference), presence of speech that is limited to a few words, but do not present swallowing and feeding difficulties.

Patient 1, 7 years old, unlike patient 2 hasn't yet experienced seizures, movement disorders and gastroesophageal reflux, probably because these features are more common and more pronounced in adult life although they have also been described in infancy and childhood [9]. Magnetic resonance imaging of the brain showed generalized delayed myelination in patient 1 at 3 years and normal imaging of the brain in patient 2 as reported in other AHDS patients, suggesting that *MCT8* gene mutations may be responsible for a severe myelination delay rather than a permanent

myelin defect [5]. Myelination defect characterizes Pelizaeus-Merzbacher disease, notable by nystagmus and impaired motor development within the first months of life, followed by ataxia, dystonia, dysarthria, and progressive spasticity. A recent paper has reported a group of 53 families with male members affected by a severe form of undetermined hypomyelinated leukodystrophies [10]. Mutations in the *MCT8* gene were detected in 11% of cases, in a group of patients characterized by Pelizaeus-Merzbacher-Like disease and showing unusual improvement of myelination with age, suggesting a role of MCT8 protein in myelin production and axon-glial interactions [10]. These data suggests that in patients with severe intellectual disability, dystonia, leukodystrophy and progressive spasticity, *MCT8* genetic testing should be performed.

Patient 1 (IV-2 Figure 1) showed high FT3, low FT4, normal TSH and high levels of SHBG suggesting a state of peripheral hyperthyroidism in the liver. The thyroid phenotype of Patient 2 (III-1, Figure 1) was different characterized by FT3 normal values in four different serum measurements, low FT4, and normal TSH and SHBG levels. To date, there are only two previous reports describing two patients with AHDS, an adult and a child, who had serum FT3 levels in the upper normal limit [10,11]. These findings might suggest that the abnormal thyroid parameters are less evident in adult patients affected by AHDS. Alternatively, there may be casual fluctuations of the FT3 values within the normal range. Based on these data we can infer that high FT3 level is a well-recognized but not essential marker for diagnosis of AHDS in patients with XLID. Standard TRH test induced normal TSH secretion in both patients (Table 2), FT3 and FT4 values were slightly increased but in the normal range in patient III-1 at the end of test while in the younger patient (IV-2) there was a larger increase at 120' as compared to the elevated basal values. Serum FT3 and TSH levels were normal in three female carriers (II-2, III-3 and III-4, Figure 1), a borderline FT4 value was found in II-2 and a normal one was found in III-3 and III-4. Serum TG-A and TPO-A were not detected in all the carriers (Figure 1). Clinically, the female carriers had normal phenotype, including normal growth and head circumference, and normal cognitive

functions.

In conclusion, these data confirm phenotypic variability existing in AHDS that could be also observed within the same family, probably depending on the age of the patient.

Ours results show the difficulty to distinguish AHDS from patients with XLID on the basis of only the clinical and biochemical features and we advise screening for *MCT8* gene mutations in either young or older patients with severe intellectual disability, axial hypotonia/dystonia, poor head control, spastic paraplegia, and athetoid movements even with normal thyroid hormone profile.

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Legends

Figure 1. Pedigree and thyroid evaluation of the analyzed family

Figure 2. Appearance of patient 1 showing limb hypertonia and elbow and knee contractures.

Figure 3. Appearance of patient 2 showing elongated face with bitemporal narrowing, central balding, large ears and dystonic posturing of the hands.

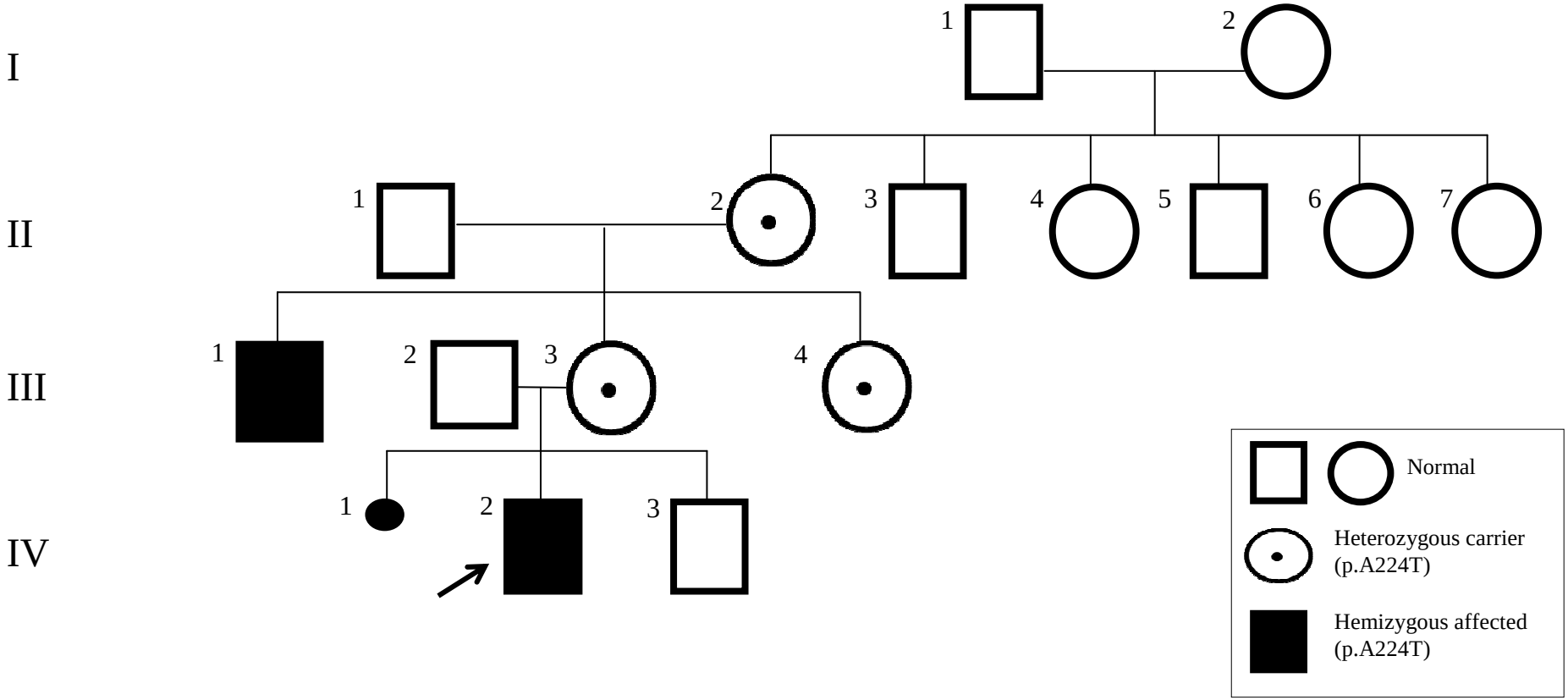
Table I. Summary of phenotypic findings detected in the patients as compared to those reported in the literature.

Clinical findings	**Clinical findings reported**[a]	**Patient IV-1**	**Patient III-1**
Microcephaly	+(10%)	-	-
Short stature	+(17%)	-	-
Low weight	+(66%)	-	-
Congenital hypotonia	+(100%)	+	+
Muscle hypoplasia	+ (88%)	+	+
Narrow, long face	+(75%) (adulthood)	-	+
Myopathic appearance	+(9%)	+	+
Cupped ears	+(38%)	+	+
Scoliosis	+(53%)	+/-	+
Pectus excavatum	+(58%)	+	-
Contractures	+(59%)	+	+
Hyperreflexia/clonus/Babinski	+(94%)	+	+
Limited speech	+(69%)	.+	+
Absent speech	+(31%)	-	-
Ataxia	+(60%)		
Non Ambulatory	+(38%)	+	+
Valgus/everted feet	+(77%)	-	-
Abnormal hand positioning	+(81%)	+	+
Undescended testes	+ (8%)	+	+
Seizures	+(19%)	-	+
Paroxysmal dyskinesias provoked by passive movement of body or limb position	+ (n.a.)	-	+
Athetoid and dystonic movements	+ (n.a.)	-	+
Severe mental retardation	+(n.a.)	+	+
Gastroesophageal reflux	+(n.a.)	-	+

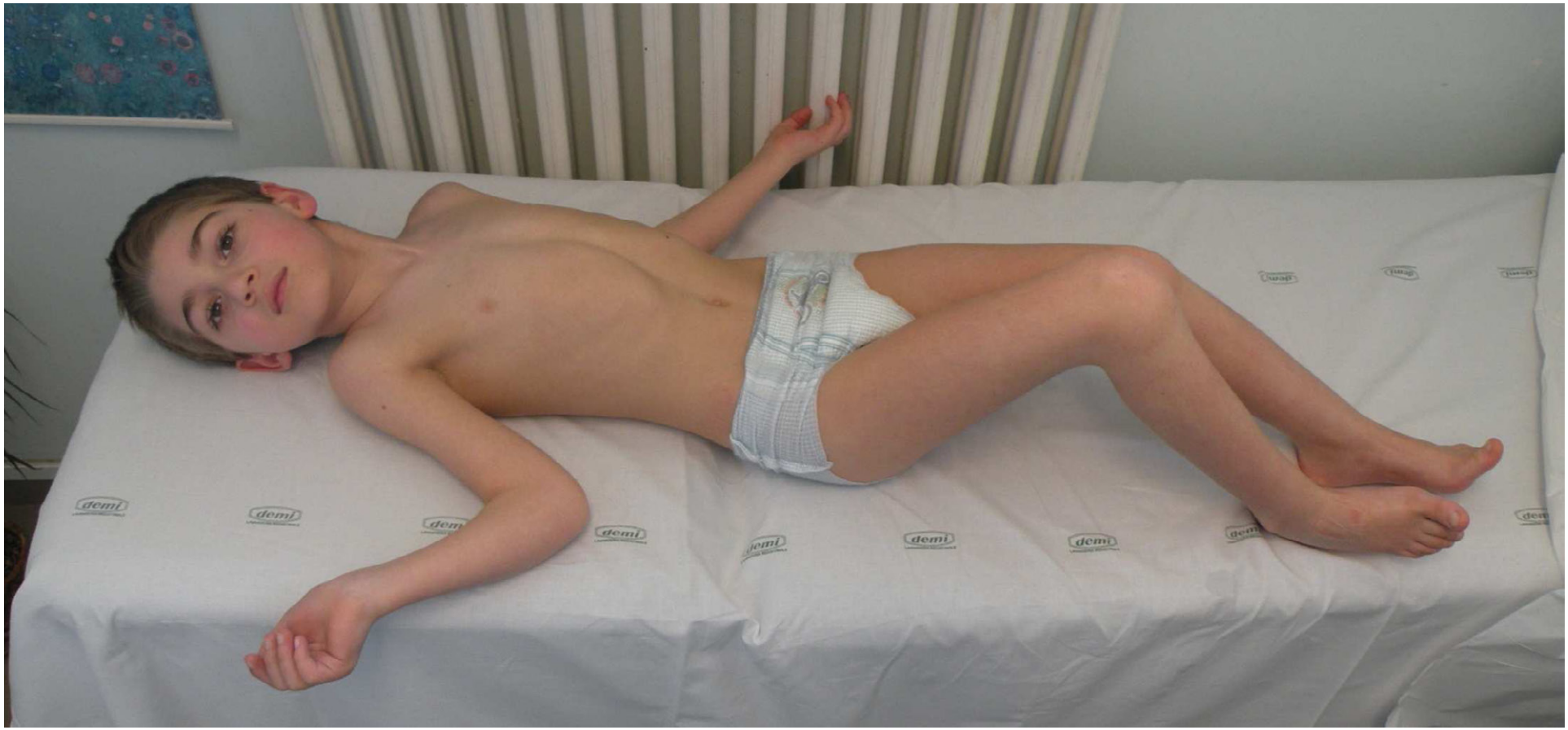
[a] Clinical characteristics were reported from reference 9, n.a. : not available

Table 2. Results of TRH test in the analyzed patients

Patient n° III.1					
TRH test	0’	30’	60’	90’	120’
TSH (NR 0.4-4.0 mUI/ml)	1.58	9.87	7.10	5.73	3.83
FT3 (NR 1.4-5.0 pg/ml)	3.17	2.73	3.49	4.24	4.98
FT4 (NR 0.8-2.0 ng/dl)	0.57	0.58	0.63	0.80	0.82
Patient n° IV.2					
TRH test	0’	30’	60’	90’	120’
TSH (NR 0.4-4.0 mUI/ml)	1.15	5.00	3.40	1.87	2.30
FT3 (NR 1.4-5.0 pg/ml)	6.26	6.80	8.80	8.70	9.30
FT4 (NR 0.8-2.0 ng/dl)	0.48	0.49	0.57	0.80	0.73



Thyroid status in members of the Sardinian family with AHDS							
N°	Genetic status	Age (years)	Free T 3 (pg/ml)	Free T 4 (ng/dl)	TSH (mUI/ml)	SHBG (nmol/L)	TG-A/TPO-A
II.2	Heterozygote	57	4.03	0.78	0.82		-/-
II.4	Normal	62	3.34	1.04	2.41		
III.1	Affected	36	3.93	0.76	0.74	64.60	-/-
III.2	Normal	41	3.41	0.98	0.88	21.1	-/-
III.3	Heterozygote	35	4.23	1.04	0.77		-/-
III.4	Heterozygote	31	3.49	0.89	1.88		-/-
IV.2	Affected	7	6.34	0.75	1.69	145	-/-
IV.3	Normal	2	4.3	1.15	1.20	153	-/-
Reference range			1.4-5.0	0.8-2.0	0.4-4.0	13-71	-/-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