WOLFRAM SYNDROME (DIABETES INSIPIDUS, DIABETES MELLITUS, OPTIC ATROPHY AND DEAFNESS [DIDMOAD]: SIMILAR PHENOTYPES BY DIFFERENT GENETIC MECHANISMS

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Objective: Wolfram Syndrome (WS) is an autosomal recessive neurodegenerative disorder characterized by non autoimmune diabetes mellitus, optic atrophy, diabetes insipidus and deafness or “DIDMOAD” (Diabetes Insipidus, Diabetes Mellitus, Optic Atrophy, and Deafness). WFS1 gene is located on the short arm of chromosome 4. WS prevalence is 1/770,000 live births, with 1/354 carrier frequency.

Research design and methods: We evaluated 6 Italian children from 5 unrelated families. Genetic analysis for WS was performed by PCR amplification and direct sequencing.

Results: Mutation screening revealed five distinct variants: one novel mutation (c.1346C>T; p.T449I) and four previously described, all located in the exon 8.

Conclusions: Phenotype/genotype correlation is difficult and the same mutation gives very different phenotypes. Severely inactivating mutations result in a more severe phenotype than mildly inactivating ones. Clinical follow-up showed the progressive syndrome seriousness.
Wolfram Syndrome includes non autoimmune diabetes mellitus and optic atrophy occurring within the first decade, followed by diabetes insipidus and deafness (1). Additional characteristics are ureterohydronephrosis, neuropsychiatric and endocrinological impairment and seldom powdered cataract and retinopathy (2). Mortality is about 65% before 35 years, due to central respiratory failure and renal failure (1). The gene involved (WS1) was identified in 1998 on chromosome 4p (3). WS1 spans 33.4-kb of genomic DNA and includes 8 exons: 1st is noncoding, exons 2-7 are coding exons and 8th is 2.6 kb long (3). WS1 mRNA encodes a 890 aminoacid polypeptide, with nine putative transmembrane domains and a 100 kd molecular mass. WS1 mRNA is expressed in heart, brain, placenta, lung and pancreas; WS1 transcripts were detected in liver, skeletal muscle and kidney. WS1 protein is an endoglycosidase H-sensitive membrane glycoprotein that localizes in the endoplasmic reticulum (ER), where regulates membrane trafficking, protein processing, with a crucial role in β-cells death, through apoptotic pathway (4).

RESEARCH DESIGN AND METHODS

We evaluated 6 Italian patients (2 males, 4 females) with WS from 5 different families. We performed brain nuclear magnetic resonance (NMR) to assess posterior pituitary and brain structures (5), endocrinologic evaluation, ultrasonography and intravenous urography to detect renal abnormalities (6).

Genomic DNA for WS1 gene mutation screening was obtained after written informed consent. The WFS1 gene coding region was analyzed by PCR amplification and direct sequencing using primers and methods as previously described (7). Sequences were compared with human genomic and cDNA WSF1 sequences (GenBank accession n° AF084481) and changes in the nucleotides were checked against published polymorphisms and mutations. Each sequence alteration was confirmed by sequencing both DNA strands of two independent PCR products.

RESULTS

Mutation screening revealed a total of five distinct variants, including one novel mutation (c.1346C>T; p.T449I) and four described variants (c.1230_1233delCTCT, c.1362_1377del16, c.1328G>T, IVS6+16G>A). Two patients (i.e. case 1: a male patient with a compound heterozygous mutation [S443I] + [IVS6+16G>A] and case 2: a female patient carrying an homozygous mutation c.1362_1377del16) have been already subject of pubblication by our group (8). All the mutations were in exon 8.

In particular case 3 is a male patient with homozygous mutation at the nucleotide c.1362_1377del16 showed the most severe phenotype, and at age of 11 acute respiratory failure. Brain NMR revealed brain stem, cerebellum, medulla and pons atrophy (Fig. 1a), reduced high signal intensity from the posterior pituitary and from the optic nerve (Fig. 1b). Urinary tract infections were followed by kidney insufficiency. Renal scintigraphy showed left obstructive hydronephrosis at the pyelo-uretheral junction. Urodynamic study showed high bladder pressure and confirmed hydronephrosis. Atonic bladder with emptying problems was followed by radical cystectomy with ileal duct when 19 year-old. Interestingly, the other patient carrying the same mutation did not show any respiratory involvement up to now. Her main clinical complication was less severe kidney insufficiency, otherwise non requiring surgery.

The other described male patient ([S443I] + [IVS6+16G>A]) showed a less
severe phenotype, characterized only by diabetes mellitus and optic atrophy, without diabetes insipidus or kidney damage.

The female patient homozygous for mutation c.1230_1233delCTCT leading to V412fsX440, (case 4) showed all the clinical features of the syndrome. In family n° 5 a new mutation, c1346C>T (ACC>ATC codon change) leading to pT449I aminoacid change, was detected, and heterozygous in consanguineous parents. Cases 5 and 6 were two sisters (mutation c.1346C>T; p.T449I) from the same family and sex but still different phenotype: the older has urethral involvement, and severe anorexia; the younger had microalbuminuria but no neurological and urinary tract involvement.

CONCLUSIONS

Severe respiratory complications (the “severe phenotype”) (9) was observed in one boy. A severe respiratory involvement led to the diagnosis of WS in adult patient (10). Neurological dysfunctions are responsible for apneic and hypopneic spells during sleep.

Urinary tract involvement including hydrourether, detrusor-sphincter dyssynergia and detrusor overactivity occurs in up to 90% of patients, in adolescence or adulthood, and might be due to neuronal degeneration (6). No correlation has been found between the bladder dysfunction and presence or duration of other manifestations, suggesting that bladder dysfunction may be a primary rather than a secondary manifestation of the syndrome (6).

Mutational studies in patients with WS reported a wide spectrum of mutations distributed throughout the coding sequence of the WS1 gene.

WS1 protein in vivo is organized as a tetramer which originates a membrane Ca^{2+} channel of the ER and lack of function of WS1 determines apoptotic input signaling (11). In spite of these great improvements in the study of the physiological role of WS1, it is still difficult to establish a phenotype/genotype correlation (9). WS1 expression was detected in both pancreatic β-cells and the limbic system of mice. Moreover, using immunohistochemical methods, strong WS1 expression was found in the hippocampus and cerebellum of mice. Using a specific rat insulinoma cell line and fractionated mouse brain tissue, wolframin localization into the endoplasmic reticulum was confirmed.

The only predicting information that genetic analysis can give regards the difference between severely inactivating (such as premature stop codon from insertion or deletion) and mildly inactivating mutations (such as missense mutations). Patients homozygous for missense mutation seem to have a better prognosis than patients carrying severely inactivating mutation. Even in our study, although clinical symptoms are different, patients showing more severe features have a severely inactivating mutation (9).

All the mutations described are located in exon 8, corresponding to the transmembrane region and carboxy tail of wolframin protein (9). This is in agreement with other studies in Italian and world-wide populations. Phenotype/genotype correlation is difficult: the same mutation gives different phenotypes both in related and unrelated. Severely inactivating mutations seem to give a more severe phenotype than mildly inactivating mutations.
REFERENCES
Fig. 1: Brain magnetic resonance of case 3 WS patient:
1a: Brain stem atrophy
1b: Optic nerve atrophy