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Clinical outcomes after preimplantation genetic diagnosis of patients with Corino de Andrade disease (familial amyloid polyneuropathy)

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KEY MESSAGE

Current treatments for familial amyloidosis only delay the fatal outcome. The disease can only be eradicated by avoiding the birth of new cases. High live birth rates following PGD are reported in this study, indicating that the disease itself, liver transplantation or tafamidis treatment do not negatively impact clinical outcomes.

A B S T R A C T

The aim of this study was to determine whether patients with transthyretin-related hereditary amyloidosis (V30M), after transplantation or under tafamidis treatment, have normal gamete reproductive capacity. A retrospective analysis was carried out of all preimplantation genetic diagnosis (PGD) cycles performed in patients with the V30M mutation. The groups analysed were: total cases with V30M, female cases with V30M and male cases with V30M. Detailed demographic, stimulation, embryological, clinical and newborn outcomes were evaluated. Comparisons revealed that patients have a high likelihood of achieving a live birth per PGD treatment cycle (48%). This is the first large report on patients with the V30M mutation treated with PGD.

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The high rate of live birth obtained should represent a strong stimulus for patients to use PGD as it proved to be effective and safe. As a neurodegenerative disease that leads to death, it is of maximum importance that it could be eradicated using PGD in order to definitively avoid the transmission of the disease.

Introduction

Familial amyloid polyneuropathy was first described by Corino de Andrade in northern Portugal (Andrade, 1952; Andrade et al., 1969) and is currently named transthyretin-related hereditary amyloidosis (V30M). It is an autosomal-dominant neurodegenerative disorder characterized by progressive sensory, autonomic and motor neuropathy that leads to death 10–20 years after the onset of symptoms (Ando et al., 2013; Sousa et al., 1995).

The disease is caused by a single nucleotide mutation (Saraiva et al., 1984, 1985) in the transthyretin gene (Tsuzuki et al., 1985; Wallace et al., 1985). The transthyretin protein (TTR) transports plasma thyroxin (Ingbar, 1963) and retinol-binding protein/vitamin A (Zhang et al., 2012). It is mainly produced by hepatocytes, but is also synthesized in the retinal pigment epithelium, ventricular choroid plexus and Schwann cells (Murakami et al., 2010; Schreiber et al., 1990). TTR does not cross the blood–brain barrier, with cerebrospinal fluid concentrations being much higher than in plasma (Saraiva et al., 1985).

TTR is composed of four monomers (Kanda et al., 1974) and the mutation causes tetramer dissociation into monomers, which precipitate in loose connective tissue of neurons and progressively self-assemble into amyloid fibrils (Quintas et al., 2001). Monomers interact with the membrane of Schwann cells and endoneurium capillaries, triggering calcium influx. High calcium intracellular concentrations promote an increase in free oxygen radical levels, membrane lipid peroxidation and apoptosis, which elicit progressive demyelination, neuron loss and nerve ischemia (Hou et al., 2007; Sousa and Saraiva, 2003; Teixeira et al., 2006).

Disease prevention is crucial, as most individuals are heterozygous and no toxic amyloid deposition occurs until adulthood, with patients diagnosed at reproductive ages (Ando et al., 2013; Saraiva et al., 1984; Sousa et al., 1995). Current treatments are mainly directed towards symptom relief (Ando et al., 2013; Lobato and Rocha, 2012), but others have been developed to halt the progression of the disease. These include liver transplantation (Ando et al., 2013; Furtado, 2000); tafamidis, a stabilizer agent that prevents TTR monomer dissociation (Coelho et al., 2013a), and gene therapy with small interference RNA to block translation of the mutated mRNA (Coelho et al., 2013b). However, none of these therapies are able to cure the disease. As it is a neurodegenerative disease that leads to death, it is of maximum importance to eradicate it, and at present only PGD can definitively avoid transmission of the disease. The present work presents PGD cycles performed for this disease, with a detailed analysis of demographic, stimulation, embryological, clinical and newborn outcomes.

Materials and methods

Ethics approval

According to the National Law on Medically Assisted Procreation (Laws 2006) and the National Council on Medically Assisted Procreation guidelines (CNPMA, 2015), databases were used following patient informed and written consent.

Patients

Since 2000, 266 PGD cycles have been performed at CGR. Of these, 47 cycles were from Portuguese patients presenting familial amyloid polyneuropathy. These patients were studied, diagnosed, treated and are being followed up at the Research Centre of Corino de Andrade, which is the reference centre in Portugal. Patient carriers of the V30M mutation who underwent PGD treatments were retrospectively studied. For this, they were divided into the following groups for comparisons: total carriers (Total-V30M), female carriers (Female-V30M) and male carriers (Male-V30M). The information for each patient is presented in **Supplementary Table S1**, including whether the patients were asymptomatic (those known to have the mutation but not yet showing symptoms) or symptomatic (those who had undergone liver transplantation or were taking tafamidis).

Karyotype analysis

The karyotype was obtained using G-banding, which included the analysis of at least 30 metaphases from peripheral blood lymphocytes, according to an established protocol (Rooney and Czepulkowski, 1997).

Testicular biopsy procedure

Andrology evaluation and testicular procedures were performed by an expert urologist according to standard methods (Bujan et al., 1989; Madureira et al., 2014). Testicular aspiration or open testicular biopsy for sperm extraction was performed under spermatic cord block (Gorgy et al., 1998; Li et al., 1992). For tissue preparation, small testicular seminiferous tubule fragments were fragmented and squeezed and the resultant fluid fraction treated according to a previously described protocol using erythrocyte-lysing buffer and enzymatic digestion with DNase and collagenase (Crabbé et al., 1998; Sousa et al., 2002). The testicular volumes were normal, the epididymis were also normal and the vas deferens were present. No postoperative complications were observed. Retrieved testicular sperm was frozen with Sperm Freezing Medium or CryoSperm (Origio, Måløv, Denmark), aspirated into labelled straws (Cryo Bio Systems, Saint-Ouen-sur-Iton, France), sealed, left over liquid nitrogen (LN_2) vapour (30 min) and finally immersed and stored in LN_2.

Stimulation protocol

Women underwent controlled ovarian hyperstimulation with a gonadotrophin-releasing hormone short antagonist multiple-dose flexible protocol in the large majority of cases (Cetrotide [cetrorelix], Merck Serono Europe, Geneva, Switzerland; Orgalutran [ganirelix], Organon, Oss, the Netherlands), and the long agonist protocol (Suprefact [buserelin], Sanofi Aventis, Frankfurt, Germany) in other cases. For stimulation, recombinant follicle-stimulating hormone (Puregon,

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