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LETTERS

Pre-implantation genetic diagnosis in pulmonary arterial hypertension due to *BMPR2* mutation

To the Editors:

Pulmonary arterial hypertension (PAH) is a rare and severe condition that may present as familial/heritable disease [1–3]. In recent years, there have been considerable advances in the management of PAH and disease-specific therapies have improved survival rates [3–5]. Nevertheless, PAH remains a devastating disease with progressively debilitating symptoms and high mortality even in the modern management era [6, 7].

Familial cases of PAH have been detected since the 1950s [1, 2]. Before the availability of modern genetic tools, studies of the genealogies demonstrated that familial PAH segregated as an autosomal dominant trait, thus leading to a 50% chance of inheriting the disease allele. More recently, mutations in the bone morphogenetic protein receptor 2 gene (*BMPR2*) have been detected in 70–80% of familial cases [1, 8, 9]. The *BMPR2* mutations have an incomplete but variable penetrance (on average, 10–20% of mutation carriers develop PAH, but it can be much higher in some families). A genetic anticipation phenomenon (characterised by a younger age at PAH diagnosis in subsequent generations) has been demonstrated in familial PAH, with an increased risk of cases occurring in children and young adults. Recently, we have shown that *BMPR2* mutation carriers with PAH present with more severe haemodynamic compromise at diagnosis, which is ~10 yrs earlier than noncarriers, leading to premature disability and death [8, 9]. Thus, there is an urgent need for new diagnostic techniques for families with *BMPR2* mutation carriers. As stated by HARPER and SENGUPTA [10], the current reproductive options for these couples are to remain childless, have no genetic testing on any pregnancy (reproductive chance), undergo pre-natal or pre-implantation genetic diagnosis, have gamete donation, or adopt. The present clinical research report describes the first successful *BMPR2* mutation pre-implantation genetic diagnosis in a family with several paediatric and adult severe PAH cases.

A couple with an extensive family history of PAH caused by a *BMPR2* mutation (c.1472G>A; p.Arg491Gln) (fig. 1) was referred to our institute to discuss pre-implantation genetic diagnosis. The husband's father (II.7) developed PAH at the age of 28 yrs and died 11 yrs later despite lung transplantation. An aunt (II.2) and an uncle (II.5) developed fatal paediatric PAH (death occurred at the ages of 13 and 16 yrs, respectively). Another uncle (II.10) and his son (III.14) were also treated for severe PAH that was diagnosed at the ages of 61 and 7 yrs, respectively. Moreover, the grandfather (I.1) died suddenly when he was 56 yrs of age, presumably of PAH. The husband (III.7) carried the *BMPR2* mutation but had neither clinical nor echocardiographic evidence of PAH. Analysis of the genetic tree (fig. 1) indicated that *BMPR2* mutation penetrance was high in this family. The future mother had no personal or familial history of PAH. *BMPR2* gene mutations are so rare in the general population that she would be incredibly unlikely to possess a *BMPR2* mutation, and she was not tested for this mutation. In these circumstances, the couple asked for pre-implantation genetic diagnosis and provided written informed consent to undergo the procedure. This request was evaluated and approved by a multidisciplinary committee (CDPN-DPI, Paris, France; October 9, 2008).

The *in vitro* fertilisation protocol started with transvaginal ultrasound-guided oocyte retrieval under general anaesthesia after ovarian stimulation. After oocyte denudation, intracytoplasmic sperm injection was performed. Once fertilisation was achieved, the zygotes were cultured in specific culture media (ISM1™ and ISM2™; Medicult, Lyon, France) until day 3. The embryo biopsy was performed in ISM2™ medium using a no-contact laser (Fertilase; MTGMedical Technology Vertriebs-GmbH, Altdorf, Germany) for zona drilling. It was carried out on cleaved, day 3 embryos selected on morphological criteria. Embryos that presented at least six cells and <30% of anucleated fragments were submitted to biopsy. For each embryo, a small volume of rinsing medium was transferred

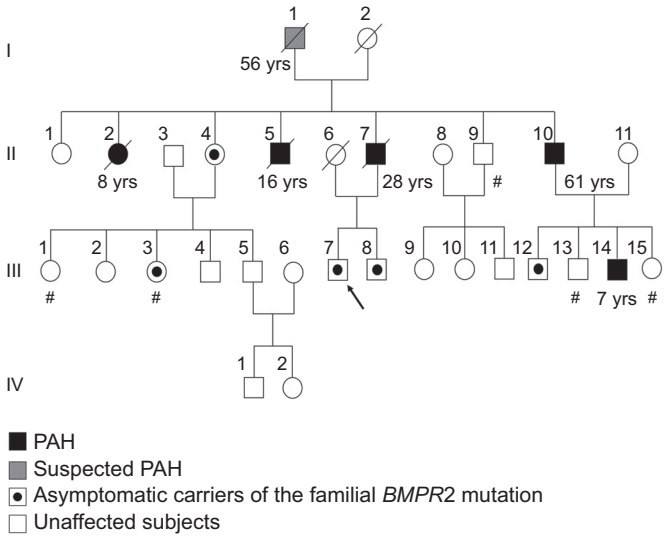

FIGURE 1. Family tree of the subject asking for pre-implantation genetic diagnosis (III.7; arrow). Ages in years correspond to age at pulmonary arterial hypertension (PAH) diagnosis. *BMPR2*: bone morphogenetic protein receptor 2. #: noncarriers of the familial *BMPR2* mutation.

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similarly to the blastomeres and used as a negative control. Genetic analysis results were obtained on day 4 and unaffected embryos were selected for embryos transfer.

20 oocytes were submitted to intracytoplasmic sperm injection resulting in 12 embryos, of which seven were suitable for biopsy. Two blastomeres were systematically aspirated for each embryo and a successful genetic analysis was obtained for all. Two embryos were unaffected and five carried the *BMPR2* mutation. An unaffected embryo was implanted, leading to a successful pregnancy and the birth of a healthy child who was not carrying the deleterious *BMPR2* mutation.

Pre-natal diagnosis allows the detection of an *in utero* fetus that carries a mutation causing a serious disease. If the mutation is found, a medical abortion can be proposed. By contrast, pre-implantation genetic diagnosis is medically assisted reproduction with selection and implantation of embryos that do not carry the deleterious mutation, thus avoiding the distress of a medical abortion. These techniques are used in many other diseases but they are controversial in conditions where penetrance is not 100% and in late-onset disease, such as heritable PAH [1, 10]. Due to the psychological impact of abortion on parents, especially in the setting of an incompletely penetrant genetic disease, our group has been in favour of pre-implantation genetic diagnosis in selected heritable PAH families after multidisciplinary discussion.

We considered that the family asking for pre-implantation genetic diagnosis was a good candidate for this procedure. First, the mutation penetrance was high in that family, emphasising the risk of other PAH cases in the offspring of *BMPR2* mutation carriers. Secondly, the mutation was carried by the husband; we are currently hesitant to offer pre-implantation genetic diagnosis in female *BMPR2* mutation carriers, because of the possible risk of occurrence of the disease in a pregnant patient and because the effect of ovarian stimulation in females with a *BMPR2* mutation is currently unknown.

In conclusion, we suggest that pre-implantation genetic diagnosis may be considered in selected families with familial/heritable PAH, which remains a dramatic and incurable disease.

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