# PRE-IMPLANTATION GENETIC DIAGNOSIS FOR INHERITED EYE DISEASES

**Hlavatá L.[1,2], Ďuďáková Ľ.[2], Trková M.[3], Soldátová I.[3], Skalická P.[2,4], Kousal B.[2,4], Lišková P.[2,4]**

**[1]Jan Lešťák Eye Clinic Ltd.**
**[2]Institute of Hereditary Metabolic Disorders, 1st Faculty of Medicine, Charles University Prague and General University Hospital Prague**
**[3]GENNET s.r.o., Prague**
**[4]Department of Ophthalmology, Charles University Prague and General University Hospital Prague**

***The authors of the study declare that no conflict of interest exists in the compilation, theme and subsequent publication of this professional communication, and that it is not supported by any pharmaceuticals company.***

Doc. MUDr. P. Lišková, MD, Ph.D.
Ústav dědičných metabolických poruch
1. lékařská fakulta UK a VFN
Ke Karlovu 2
128 00 Praha 2
petra.liskova@lf1.cuni.cz
http://ocnigenetika.lf1.cuni.cz

## SUMMARY

**Objective:** Preimplantation genetic diagnosis (PGD) is an established application of genetic testing in the context of *in vitro* fertilization. PGD is an alternative method to prenatal diagnosis which aims to prevent the transmission of an inherited disorder to the progeny by implanting only embryos that do not carry genetic predisposition for a particular disease. The aim of this study is to provide an overview of eye disorders for which PGD has been carried out.
**Methods:** The European literature search focused on best practices, ethical issues, risks and results of PGD for inherited eye disorders.
**Results:** PGD is performed for a number of ocular disorders; a prerequisite for its application is however, the knowledge of a disease-causing mutation(s). The main advantage of this method is that the couple is not exposed to a decision of whether or not to undergo an abortion. Qualified counselling must be provided prior to the PGD in order to completely understand the risk of disability in any child conceived, consequences of disease manifestation, and advantages as well as limitations of this method. In the group of non-syndromic eye diseases and diseases in which ocular findings dominate, PGD has been performed in European countries for aniridia, choroideremia, congenital fibrosis of extraocular muscles, Leber congenital amaurosis, ocular albinism, retinitis pigmentosa, X-linked retinoschisis, Stargardt disease, blepharophimosis-ptosis-inverse epicanthus syndrome and retinoblastoma. Sexing for X-linked or mitochondrial diseases has been carried out for blue cone monochromatism, choroideremia, familial exudative vitreoretinopathy, Leber hereditary optic neuropathy, macular dystrophy (not further specified), Norrie disease, X-linked congenital stationary night blindness, X-linked retinoschisis and nystagmus (not further specified).
**Conclusion:** In recent years, there has been an increase in potential to use PGD. The spectrum of diseases for this method has widened to include severe inherited eye diseases.

**Key words:** preimplantation genetic diagnosis; monogenic eye diseases; in vitro fertilization

*Čes. a slov. Oftal., 72, 2016, No. 5, p. 167–171*

## INTRODUCTION

Pre-implantation genetic diagnosis (PGD) is an established application of genetic testing. It represents a preventive method, which can be used within the framework of assisted reproduction to prevent the transmission of a monogenous hereditary disease or chromosomal abnormality to progeny (4, 13).

The basis of in vitro fertilisation (IVF) is the extracorporeal fertilisation of an egg by a sperm and the subsequent implantation of the embryo into the womb of the mother. In the case of PGD this step is preceded by the performance of a genetic laboratory examination, the results of which assist in selecting suitable embryos for transmission (14). An essential prerequisite for the application of PGD in monogenic diseases is knowledge of the cause of the pathology on the level of DNA. This method is not appropriate for complex pathologies, even if with a significant genetic predisposition. PGD may be used not only for detecting the transmission of a specific genetic disorder, but also for determining deletions and duplications of chromosomes in embryos where one of the parents is a carrier of a balanced chromosomal aberration (translocation, inversion). A further alternative to PGD is pre-implantation genetic screening (PGS) for embryos of parents in whom it is not known as to whether they have suffered from a specific genetic defect, but have a higher risk of producing an embryo with an incorrect number of chromosomes (repeated miscarriages, higher age, repeated failures of IVF, undergone chemo/radiotherapy) (12).

PGD cannot be substituted for prenatal diagnosis, which is a set of diagnostic measures with the aim of distinguishing or eliminating molecular, chromosomal, morphological, structural and functional disorders of an embryo or foetus during pregnancy (3). On the basis of concrete results, the doctor instructs the parents and recommend the further procedure. The final decision then rests with the parents as to whether the mother will carry up to birth a foetus in which a certain defect or genetically conditioned pathology has been diagnosed, or decide to terminate the pregnancy.

## OBJECTIVE

The objective of our study was to present a summary overview of ocular pathologies for which PGD is performed.

## METHODS

Literary research focusing on consultancy, methodology, ethical aspects, risks and legislation for the performance of

PGD with the aim of preventing the transmission of monogenically conditioned ocular pathologies.

## RESULTS

**Consultation before PGD**

Before the actual performance of PGD, carriers of genetic abnormalities must be provided with a qualified consultation by a doctor with a specialised qualification in the field of medical genetics, in accordance with the provision of Act no. 373/2011 Coll, Sections 28-29. The objective of this consultation is to explain the principle, benefit, reliability and potential risks of PGD, including unexpected findings. Patients should fully understand the technical limitations of PGD, the risk of error, should be engaged in a discussion as to whether PGD is necessary, and subsequently sign an informed consent form.

Common indications for PGD are structural chromosomal aberrations or monogenically conditioned pathologies, under certain circumstances it is also possible to consider also indicating for HLA genotyping. The selection of sex with the aim of balancing a family is generally considered controversial and is not performed in the Czech Republic. Pathologies without a determined genetic cause and testing of certain phenotype traits such as eye colour are entirely inappropriate for indication of PGD (5).

**Methodology of PGD**

A fundamental prerequisite for the application of PGD is knowledge of the mutation causing the given disease. In the case of ocular pathologies this condition cannot always be met, since a range of genes, e.g. for retinitis pigmentosa remain unknown, or upon genetic screening mutations of unknown significance are detected, in which the pathogenesis is difficult to detect and requires complex functional studies.

A detailed description of the individual steps of PGD and the methods used, including their advantages and disadvantages, was recently summarised in two publications in Czech language (15, 19). In short, after IVF embryos are cultivated up to the stage of blastocytes, on the 5-6th day of development of the embryo a trophoectoderm biopsy is performed, at which 4-10 cells are obtained. Afterwards full genome amplification of their DNA is performed. In the case of pathologies with monogenic heredity, methods of indirect genetic diagnosis are mostly used. At present these are haplotype analysis (PCR method), using short term repeat as markers (2) and karyomapping (array method), using single nucleotide polymorphism as markers (18). In both method the haplotype (specific set of markers) is determined in the section comprising the gene. A condition for PGD is a genetic examination of both partners and a direct relative with the genetically confirmed disease. Embryos which carry a common allele(s) (in recessive hereditary pathologies it is possible to accept carrying of a single allele with pathogenic mutation following informed discussion with the parents) with an afflicted relative (usually parent or sibling), identified using haplotyping, are excluded.

In both methods both testing for the presence of a mutation using comparison of haplotypes is performed, as well as examination of the number of chromosomes of the embryo. The advantage of karyomapping is that we can perform both tests simultaneously; in the case of the PCR method the haplotype analysis in “healthy embryos” is supplemented by the method of sequencing of a new generation in order to detect variations in the number of copies (10).

In the case of pathologies with X-linked inheritance, couples may also select the sex. In this case array or haplotype analysis is performed, in which the sex of the embryo is determined.

**PGD in the case of genetically conditioned ocular pathologies**

At present PGD is used for a range of ocular pathologies with a significant functional impact on visual functions.

PGD is considered above all by couples in whom one or both have a genetically conditioned autosomally dominant disorder, or couples who have already given birth to a disabled child.

The most common indication is retinoblastoma with autosomally dominant heredity (25). With regard to the fact that the causal gene RB1 (retinoblastoma 1) codes protein important for regulation of the cell cycle, patients with a hereditary form of retinoblastoma also have an extremely high risk of subsequent incidence of primary tumours, most often leiomyosarcoma, osteosarcoma and skin melanoma (17). In the case of this disease PGD was first successfully applied in 2003 in a family in which the father and daughter suffered from bilateral retinoblastoma, and at the same time a causal substitutional mutation was identified in the gene RB1. PGD was used in the couple's second and third pregnancy and led to the birth of two healthy siblings (27).

In the group in which neither the father nor the mother suffer from an ocular pathology but have one or more visually impaired children with an autosomally recessive hereditary disease, and wish to have further children without visual impairment, a case of application of PGD is documented in the literature for example in which both parents were carriers of one deletion of the gene RPE65 (Retinal Pigment Epithelium-Specific Protein 65kDa) and already had two children with Leber congenital amaurosis. Following the implantation of a healthy embryo and one embryo with a mutated allele, healthy twins were born (9).

It is occasionally possible to encounter a request for pre-conception screening of a partner for the presence of mutations also in couples in whom one of the partners is diagnosed with an autosomally recessive hereditary ocular pathology. Despite the fact that the risk of conception of a progeny with the same pathology is virtually negligible provided that the partners are not mutually related, i.e. only fractionally higher in comparison with the healthy population, in the case of certain diseases these requests are not entirely unjustified. For example, in the

case of gene ABCA4 (ATP-Binding Cassette, Sub Family A member 4), mutations of which condition autosomally recessive hereditary Stargardt disease (1), it is estimated that in the regular European population 1 in 870 of the population could be a carrier of pathogenic mutations (20). A case of a couple was published, in which the man manifested typical symptoms of Stargardt disease, whereas an ocular examination of his partner did not detect any abnormalities. A genetic examination for the gene ABCA4 was indicated for both partners. Both pathogenic mutations were determined in the male. In the female one mutation with the origin of Stargardt disease was also determined, which increased the risk of transmission to the progeny in this specific couple to 50%, as a result of which PGD was recommended, leading to the birth of a healthy progeny (23).

In X-linked pathologies the risk of transmission of a genetic strain for a specific disease to both sexes is 50%, however, in men the pathology is generally expressed, whereas women are often carriers without a clinical finding, or their finding is only minimal without an impact upon quality of life. In this case embryos of female sex are recommended for transfer. In the case of certain ocular pathologies such as retinitis pigmentosa, however, it is necessary to keep in mind that women may manifest a wide spectrum of phenotype findings from none to severe, including total blindness, mostly manifesting themselves one or two decades later than in men (16).

The European Society of Human Reproduction, which regularly evaluates data from European centres, stated in its report that in 2010 the total number of performed cycles with a pre-implantation genetic examination was 5 651, of which 2 978 cycles were within the framework of PGS and 2 673 cycles were within the framework of PGD. Of ocular pathologies the largest number of cycles were performed with the aim of preventing the transmission of retinoblastoma with autosomally dominant type of heredity, followed by Stargardt disease and oculocutaneous albinism with autosomally recessive type of heredity. In the case of X-linked pathologies selection of sex was chosen in a number of cases a prevention of transmission of the pathology. The most frequent indication was retinitis pigmentosa, followed by Leber hereditary optic neuropathy. An enumeration

**Table 1** Monogenic eye diseases and syndromic disorders with severe ocular involvement for which PGD was performed in 2010 in Europe.

| Type of inheritance | Disease | Method of embryo selection | Number of PGD cycles |
|---|---|---|---|
| Autosomal dominant | Aniridia | PCR | 2 |
| | Von Hippel-Lindau syndrome | PCR | 11 |
| | Congenital fibrosis of the extraocular muscles | PCR | 3 |
| | Marfan syndrome | PCR | 34 |
| | Retinitis pigmentosa | PCR | 1 |
| | Retinoblastoma | PCR | 14 |
| | Stickler syndrome, type 2 | PCR | 2 |
| | Blepharophimosis, ptosis and epicanthus inversus syndrome | PCR | 3 |
| Autosomal recessive | Leber congenital amaurosis, type 8 | PCR | 1 |
| | Ocular albinism | PCR | 4 |
| | Stargardt disease | PCR | 7<br>1 |
| X-linked | Blue cone monochromacy | sexing | 1 |
| | Chorioideremia | PCR | 1 |
| | | sexing | 2 |
| | Familial exudative vitreoretinopathy | PCR | 1 |
| | Macular dystrophy | sexing | 1 |
| | Norrie disease | PCR | 1 |
| | Retinitis pigmentosa, type 3 | PCR | 2 |
| | X-linked retinitis pigmentosa | sexing | 6 |
| | X-linked congenital stationary night blindness | sexing | 1 |
| | X-linked infantile nystagmus | PCR | 2 |
| | X-linked nystagmus | sexing | 1 |
| | X-linked retinoschisis | sexing | 1 |

PCR – polymerase chain reaction; PGD - preimplantation genetic diagnosis

of all ocular pathologies for which PGD was performed in 2010 from 62 European centres that are part of the PGD consortium, is presented in table 1 (7).

Websites of foreign centres which are not a part of the European consortium state provision of PGD for a range of other ocular diseases or syndromic pathologies where the ocular finding may be one of the dominant features, e.g. achromatopsia, autosomally dominant hereditary atrophy of the optic nerve, Bardet-Biedl syndrome, Best's disease, microphthalmus, neurofibromatosis type I, Senior-Loken syndrome, Usher syndrome (11).

According to information obtained on the Google search engine by entering the key words "pre-implantation genetic diagnosis", PGD is performed in a minimum of nine centres in the Czech Republic. The only detailed publicly available annual report of the Gennet company from 2012 states that PGD was performed due to the following indications of severe ocular pathologies or diseases in which the ocular symptomatology may predominate in its significance: posterior polymorphous corneal dystrophy, X-linked retinoschisis, Hippel-Lindau syndrome and Marfan syndrome (26).

**Ethical aspects of PGD in relation to ocular pathology**

In addition to the fundamental ethical aspects such as selection of embryos and thereby the selection of health characteristics (22) or the possibility of intentional selection of sex of the child motivated by the socio-cultural background (21), the use of PGD in ophthalmology raises also further ethical issues. PGD should be indicated in the case of serious pathologies with an early manifestation. However, the boundary between the pathologies that can be considered serious and those that cannot is not entirely clear. Furthermore, it is also necessary to take into consideration the individual situation within the family, the cultural and socio-economic context. A range of hereditary diseases may have a highly variable age of manifestation, some lead to a diminution of visual functions as late as in adulthood, whilst the current development in science offers hope that targeted therapies may be developed for a range of hereditary ocular pathologies.

**Risks of PGD**

In addition to the general risks in connection with the process of IVF, PGD also has its risk of "error", which is generally stated within the range of 2-5% (8). For this reason it is recommended during pregnancy to verify the result of PGD by prenatal diagnosis, i.e. by examination from the chorionic villi or amniotic fluid. At the same time it is necessary to be aware that in PGD we observe only a specific hereditary disease, the risk of which is known within the family. Children born after PGD may therefore have other genetic pathologies not observed by us. However, we reduce the risk of transmission of an embryo with chromosomal aneuploidy (e.g. Down's syndrome) upon the use of karyomapping, by simultaneously conducting an examination of the number of chromosomes. It is possible to detect aneuploidy also with the use of data from new generation sequencing, which is performed after haplotype analysis in healthy embryos for the purpose of detecting a monogenic pathology.

**Legislation of PGD**

The economically developed European countries that under certain conditions have legalised use of PGD include Belgium, Denmark, Finland, France, Greece, the Netherlands, Norway, Portugal, Austria, Spain and the United Kingdom. PGD is indicated in the case of monogenically conditioned pathologies, in some countries also for chromosomal abnormalities and X-linked pathologies. HLA typing in the sense of selection of an immunocompatible embryo in order to save a relative is permitted in nine of the above countries, including the Czech Republic. Due to the strict legislation, in Germany PGD is restricted only to examination from a biopsy of the polar body, which enables very limited diagnostic possibilities. Similarly in Switzerland testing is permitted on polar bodies only in the case of serious and incurable pathologies, regulation in PGD in this country is in the stage of further review (6).

In the Czech Republic genetic testing is regulated by Act no. 373/2011 Coll., sections 28-29. Laboratories performing PGD should abide by Recommended Procedure no. 4 (Recommendation on pre-implantation genetic laboratory examination) as presented on the website of the Society of Medical Genetics and Genomics. The website describes the indications for which PGD and PGS are recommended by a doctor with a specialised qualification in the field of medical genetics (24).

## CONCLUSION

Although PGD as a preventive method does not enable therapy, it can be used to select an embryo without a genetic strain for a certain pathology, thereby eliminating the risk of transmission of a disease to further generations. By contrast with prenatal diagnosis, the couple is not exposed to the decision on whether to terminate an already commenced pregnancy. Indication of PGD for genetically conditioned ocular pathologies requires a highly individual approach. A range of patients in whom visual impairment is manifested progressively during the course of life do not perceive their condition as sufficiently handicapping as to consider PGD for this indication. On the other hand, some patients, despite an objectively less severe deficit of visual functions perceive their situation and the psychological consequences in connection with treatment or repeated examination as sufficiently serious for PGD to represent a clear choice.

The study was supported by grants GAUK 4315/2015, SVV UK 260256/2016, UNCE 204011 and AZV 16-32341A.

## LITERATURE


1. **Allikmets, R., Singh, N., Sun, H., et al.:** A photoreceptor cell-specific ATP-binding transporter gene (ABCR) is mutated in recessive Stargardt macular dystrophy. Nat Genet, 15(3); 1997: 236–246.
2. **Altarescu, G., Zeevi, DA., Zeligson, S., et al.:** Familial haplotyping and embryo analysis for Preimplantation genetic diagnosis (PGD) using DNA microarrays: a proof of principle study. J Assist Reprod Genet, 30(12); 2013: 1595–1603.
3. **Bodurtha, J., Strauss, JF.:** Genomics and Perinatal Care. N Engl J Med, 366(1); 2012: 64–73.
4. **Braude, P., Pickering, S., Flinter, F. et al.:** Preimplantation genetic diagnosis. Nat Rev Genet, 3(12); 2002: 941–955.
5. **Brezina, PR., Kutteh, WH.:** Clinical applications of preimplantation genetic testing. BMJ, 350; 2015: g7611.
6. Council of Europe: Background document on preimplantation and prenatal genetic testing. [cit. 28.7.2016]. Dostupné na: http://www.coe.int/t/dg3/healthbioethic/Activities/07_Human_genetics_en/INF(2015)6%20e%20dpi%20dpn.pdf.
7. **De Rycke, M., Belva, F., Goossens, V., et al.:** ESHRE PGD Consortium data collection XIII: cycles from January to December 2010 with pregnancy follow-up to October 2011. Hum Reprod, 30(8); 2015: 1763–1789.
8. **Dreesen, J., Destouni, A., Kourlaba, G., et al.:** Evaluation of PCR-based preimplantation genetic diagnosis applied to monogenic diseases: a collaborative ESHRE PGD consortium study. Eur J Hum Genet, 22(8); 2014: 1012–1018.
9. **Ekmekci, CG., Tac HA., Gulum N., et al.:** Birth of a healthy twin after preimplantation genetic diagnosis for Leber congenital amaurosis (RPE65 gene). Reprod Biomed Online, 26, Supplement 1; 2013: S38–S39.
10. **Fiorentino, F., Bono, S., Biricik, A., et al.:** Application of next-generation sequencing technology for comprehensive aneuploidy screening of blastocysts in clinical preimplantation genetic screening cycles. Hum Reprod, 29(12); 2014: 2802–2813.
11. Genesis Genetics: What we test for. [cit. 28.7.2016]. Dostupné na: http://genesisgenetics.org/pgd/what-we-test-for/
12. **Ginsburg, ES., Baker, VL., Racowsky, C., et al.:** Use of preimplantation genetic diagnosis and preimplantation genetic screening in the United States: a Society for Assisted Reproductive Technology Writing Group paper. Fertil Steril, 96(4); 2011: 865–868.
13. **Handyside, AH., Xu, K.:** Preimplantation genetic diagnosis comes of age. Semin Reprod Med, 30(4); 2012: 255–258.
14. **Harton, GL., De Rycke, M., Fiorentino, F., et al.:** ESHRE PGD consortium best practice guidelines for amplification-based PGD. Hum Reprod, 26(1); 2011: 33–40.
15. **Hüttelová, R., Kleibl, Z., Řezáčová, J., et al.:** Předpoklady pro preimplantační genetickou diagnostiku (PGD) u nosičů mutací v nádorových predispozičních genech. Klin Onkol, 22(Suppl); 2009: S69–S74.
16. **Kousal, B., Skalicka, P., Valesova, L., et al.:** Severe retinal degeneration in women with a c.2543del mutation in ORF15 of the *RPGR* gene. Mol Vis, 20; 2014: 1307–1317.
17. **MacCarthy, A., Bayne, AM., Brownbill, PA., et al.:** Second and subsequent tumours among 1927 retinoblastoma patients diagnosed in Britain 1951-2004. Br J Cancer, 108(12); 2013: 2455–2463.
18. **Natesan, SA., Bladon, AJ., Coskun, S., et al.:** Genome-wide karyomapping accurately identifies the inheritance of single-gene defects in human preimplantation embryos in vitro. Genet Med, 16(11); 2014: 838–845.
19. **Putzová, M., Pecnová, L., Hulvert, J., et al.:** Preimplantační genetická diagnostika monogenně podmíněných chorob – její možnosti, úskalí a první úspěchy v České republice. Čes-slov Pediat, 63(11); 2008: 626–633.
20. **Riveiro-Alvarez, R., Aguirre-Lamban, J.,. Lopez-Martinez, MA, et al.:** Frequency of *ABCA4* mutations in 278 Spanish controls: an insight into the prevalence of autosomal recessive Stargardt disease. Br J Ophthalmol, 93(10); 2009: 1359–1364.
21. **Sharp, RR., McGowan, ML., Verma JA., et al.:** Moral attitudes and beliefs among couples pursuing PGD for sex selection. Reprod Biomed Online, 21(7); 2010: 838–847.
22. **Shenfield, F.:** Ethical aspects of pre-implantation genetic diagnosis. Obstet Gynaecol Reprod Med, 18(11); 2008: 312–313.
23. **Sohrab, MA., Allikmets, R., Guarnaccia, MM., et al.:** Preimplantation Genetic Diagnosis for Stargardt Disease. Am J Ophthalmol, 149(4); 2010: 651–655.
24. Společnost lékařské genetiky a gemoniky: Doporučení k preimplantačnímu genetickému laboratornímu vyšetření. [cit. 28.7.2016]. Dostupné na: https://www.slg.cz/stitky/doporuceni
25. **Vogel, F.:** Genetics of retinoblastoma. Hum Genet, 52(1); 1979: 1–54.
26. Výroční zpráva GENNET [cit. 24.8.2016]. Dostupné na: http://docplayer.cz/8121097-Obsah-uvodni-slovo-vedouciho-lekare-5-iv-preimplantacni-diagnostika-pgd-8-v-postnatalni-laboratorni-diagnostika-10-i-uvod.html.
27. **Xu, K., Rosenwaks, Z., Beaverson, K., et al.:** Preimplantation genetic diagnosis for retinoblastoma: the first reported liveborn. Am J Ophthalmol, 137(1); 2004: 18–23.