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# Preimplantation genetic diagnosis using fluorescent *in situ* hybridization for cancer predisposition syndromes caused by microdeletions

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**BACKGROUND:** Neurofibromatosis type 1 (NF1) and Von Hippel-Lindau (VHL) are dominantly inherited late onset cancer predisposition syndromes caused by mutations in the respective tumor suppressor genes (TSGs) *NF1* and *VHL*. Less frequently TSGs are partially or fully deleted. Preimplantation genetic diagnosis (PGD) for cancer predisposition can be applied to select against the mutant allele in carrier couples. However, microdeletions within a single cell can, at present, not be detected by molecular diagnostic methods usually applied for PGD of monogenic disorders.

**METHODS:** We performed PGD using interphase fluorescent *in situ* hybridization (FISH) on single blastomeres for three couples of which the women carried a microdeletion. One patient had the recurrent 1.4 Mb microdeletion covering *NF1*, a second suffered from an intragenic *NF1* deletion and the last had a deletion of *VHL*.

**RESULTS:** In total, seven PGD cycles were carried out for these couples, which resulted in the delivery of a healthy twin for the *VHL* microdeletion carrier.

**CONCLUSIONS:** FISH-based PGD is a straightforward approach to detect (micro)deletions in single blastomeres. It seems likely that the number of conditions for which PGD-FISH is beneficial will increase rapidly with the advent of high-resolution arrays.

**Key words:** neurofibromatosis type 1 / Von Hippel-Lindau / PGD / FISH

## Introduction

Neurofibromatosis type 1 (NF1; MIM# 162200), also known as Von Recklinghausen disease, is a common autosomal dominant disorder with a birth incidence of 1 in 3000–3500 (Huson, 1989). In 95% of NF1 individuals, a mutation is found in the *NF1* gene (*NF1)* (Messiaen *et al*., 2000). *NF1* is a tumor suppressor gene (TSG) (Legius *et al*., 1993) located on chromosome 17q11.2 and ~350 kb in length, with a 8.5 kb coding region comprised of 60 known exons. *NF1* encodes a 2.818 amino acid peptide, called neurofibromin (Wallace et *al*., 1990). Five percentage of NF1 patients have an *NF1* microdeletion (Clementi *et al*., 1996; Kluwe *et al*., 2004). Patients with gross *NF1* microdeletions frequently show a more severe clinical phenotype with more neurofibromas at an earlier age, a lower average IQ (Descheemaeker *et al*., 2004), facial dysmorphies and an increased risk for the development of malignant peripheral nerve sheath tumors (De Raedt *et al*., 2003). Two recurrent types of *NF1* microdeletions have been described (type I and II). Type I *NF1* microdeletions are the most prevalent, have a length of 1.4 Mb and are formed by an interchromosomal recombination during meiosis I between misaligned flanking paralogous sequences, called NF1REPs (Lopez-Correa *et al*., 2001, De Raedt *et al*., 2006). Type II *NF1* microdeletions are typically smaller (1.2 Mb) and are mitotic in origin (intrachromosomal deletion) (Steinmann *et al*., 2007). Type II microdeletions are mosaic, resulting in a less severe clinical phenotype in comparison with constitutional *NF1* microdeletions (Mensink *et al*., 2006).

Von Hippel-Lindau (VHL; MIM# 193300) is a relatively rare disorder inherited in an autosomal dominant trait with a high penetrance

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(Hes et al., 2000). The birth incidence is estimated at 1 in 36 000 (Maher et al., 1991). The syndrome is characterized by a predisposition for hemangioblastomas in the retina and the central nervous system (CNS), pheochromocytoma, renal cell carcinoma and pancreatic tumors (Hes et al., 2000). The basis of the familial inheritance is a germline mutation in the VHL TSG gene located on chromosome 3p25-26 (Latif et al., 1993). *VHL* is ∼10 kb in length containing three exons with an open-reading frame of 852 nucleotides coding for 284 amino acids (Gnarra et al., 1994). More mutations, microdeletions and microinsertions are found, compared with large deletions (4–380 kb) (Maher et al., 1996). Partial or full deletions of *VHL* may result in absence of pheochromocytoma and a preponderance of CNS hemangioblastoma (Hes et al., 2000).

In autosomal dominant syndromes caused by loss of TSG activity, such as VHL syndrome and NF1, individuals inherit one normal and one mutant copy of the TSG. As a consequence of a second hit, those patients are predisposed to a wide array of tumors. Accordingly, VHL and NF1 are considered as late onset cancer predisposition syndromes.

The usefulness of preimplantation genetic diagnosis (PGD) for late onset genetic disorders, for which the mutations are known, including those caused by germline mutations in TSGs, has been proven before, especially for *NF1* and *NF2* mutations (Verlinsky and Kuliev, 2002; Verlinsky et al., 2002; Robertson, 2003). Li-Fraumeni syndrome, determined by *TP53* mutations, was the first inherited cancer predisposition syndrome for which PGD resulted in the birth of a healthy child (Verlinsky et al., 2001). Due to the large number of known *NF1* mutations, the development of mutation-specific single-cell protocols is impractical. Therefore, recently, a multiplex PCR for microsatellite markers linked to the *NF1* gene was performed to trace carrier embryos (Spits et al., 2005). Since a small proportion of NF1 and VHL patients carry submicroscopic deletions, they may benefit from a fluorescent *in situ* hybridization (FISH)-based PGD test. We have developed PGD using interphase FISH for the detection of microdeletions in *NF1* and *VHL*.

# Materials and Methods

## Case reports

All three patients were known to our genetic center because of NF1 ('Patients 1 and 2') or VHL ('Patient 3'). Following adequate counseling, both partners of each couple signed an informed consent which was approved by the ethical committee of the hospital. Karyotyping was performed on G-banded metaphase spreads of cultured lymphocytes using conventional methods and showed a normal result for both partners of each couple.

'Patient 1' was a 26-year-old woman. As a young child, she presented with multiple café-au-lait spots, a delayed motor development and learning disabilities. Based on these findings, the diagnosis of NF1 was suspected. Her parents and siblings were unaffected. Later on, she developed Lisch nodules, cutaneous neurofibromas and a coarse face. She has a low normal IQ. FISH using PAC clone RPCI5-926B9 located in *NF1* showed a *de novo* deletion. The exact size of the deletion was further delineated with FISH and PCR (Fig. 1). FISH revealed clones RPCI3-409L16, RPCI5-962N3 and RPCI1-23C4 to be deleted, whereas PCI5-901E1 and RPCI5-984G23 were normal. Fragments from both NF1REPa and NF1REPc (Fig. 1) were then simultaneously and non-specifically amplified during the same PCR reaction and paralogous sequence variants were sequenced at several sites throughout the low copy repeats. Both breakpoints were respectively identified in the recombination hotspot of paralogous recombination site 2 (De Raedt et al., 2006), more specifically in NF1REPa between the positions 141988 and 142996 from reference sequence AC005562 (Fig. 1) and in NF1REPc between the positions 132502 and 133513 from reference sequence AC090616 (Fig. 1), demonstrating that the patient is carrying the recurrent 1.4 Mb *NF1* microdeletion. RPCI5-926B9 was used for PGD.

'Patient 2' was a 26-year-old woman with NF1. She was diagnosed in childhood because of multiple café-au-lait spots and axillary and inguinal freckling. There is no family history of NF1. She was surgically treated at the age of 24 years because of a diffuse cutaneous neurofibroma in the lumbar region and on the left shoulder. She again underwent surgery at the age of 27 years because of painful subcutaneous nodular neurofibromas at the peroneal site of the left lower leg. There was no evidence of malignancy. This woman has a teaching degree and there is no evidence of learning difficulties. FISH analysis with PAC RPCI5-926B9 showed a normal result. Further multiplex ligation-dependent probe amplification (MLPA) mutation analysis on Epstein–Barr virus (EBV) transformed cells revealed an intragenic deletion from exon 2 till exon 28 (∼170 kb) within *NF1*.

Clone P1-9 that spans ∼65 kb of *NF1*, including exon 2–11 (Leppig et al., 1996), was used for PGD (Fig. 1).

'Patient 3' was a 28-year-old woman and had a familial history of VHL caused by a total deletion of the *VHL* gene. Presymptomatic genetic testing by FISH using a cosmid of the *VHL* gene showed that she was a carrier of the familial VHL deletion, although she had no symptoms of VHL yet. The

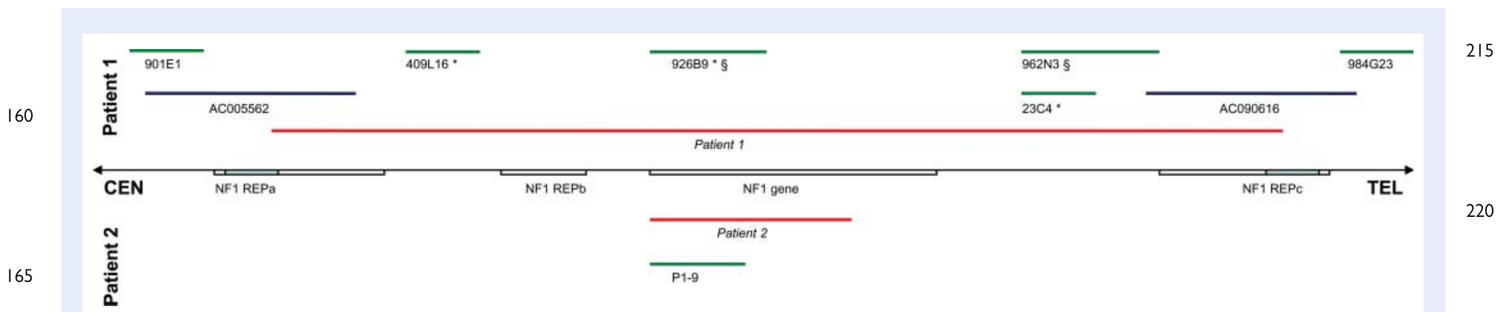


**Figure 1** The NF1 region on chromosome 17 (cen = centromere; tel = telomere) with in red the size of the deletion of 'Patients 1 and 2'.
Open boxes show the position of the NF1 gene and reps, light blue boxes indicate the position of the recombination hotspots. The BAC and PAC clones used for FISH are drawn in green, with asterisks (**) indicating deleted clones, while § are showing the clones that were used for FISH-PGD. The BAC clones used for PCR are drawn in dark blue.

deletion was confirmed with MLPA and haplotype segregation based on analysis with a set of polymorphic dinucleotide repeats. BAC RP11-382A21, covering the VHL microdeletion, was used as a FISH probe for PGD.

## Ovarian stimulation, oocyte retrieval, insemination and embryo transfer

All three patients were stimulated using gonadotrophins (Menopur®; Ferring, Kiel, Germany or Gonal-F; Merck-Serono, Geneva, Switzerland) during a long protocol. The follicular response was monitored by regular gynecological ultrasound measurements and peripheral blood measurements for estradiol. Ultrasound-guided oocyte aspiration was carried out 35 h after i.m. injection of 10 000 IU of human chorionic gonadotrophin (hCG) (Pregnyl®; Organon, Oss, The Netherlands). Oocytes were fertilized using conventional IVF. Normal fertilization was assessed by the presence of two pronuclei 16–20 h after insemination. On Days 2 and 3 after fertilization, embryo development was evaluated according to the number of blastomeres, the percentage of fragmentation and the symmetry of the blastomeres. Embryo transfer was performed on Day 4 after fertilization. Fourteen to sixteen days after oocyte retrieval, β-hCG was tested and was found positive if ≥25 IU/l. Supernumerary embryos of sufficient morphological quality that were genetically normal for the investigated region were cryopreserved.

## Embryo culture, biopsy and blastomere fixation

All embryos were cultured in single (six cycles; Life Global medium, ON, Canada) or sequential (one cycle; Cook, Sydney, Australia) media. Before biopsy, embryos were first incubated in $Ca^{2+}/Mg^{2+}$-free medium (Life Global medium and Cook, Sydney, Australia, respectively).

On Day 3 after fertilization, all ≥6 cell stage embryos that had <25% fragmentation were biopsied using a non-contact, 1.48-μm diode laser system (Fertilase®; MTG, Aldorf, Germany) coupled to the inverted microscope. Two blastomeres were gently aspirated from each embryo. The embryos were immediately transferred to fresh medium; while the aspirated blastomeres were separately washed twice with culture medium to remove possible oil remnants. Nuclei were fixed on Superfrost plus microscope slides (LaboNord, Templemars, France) with 0.01 N HCl/0.1% Tween 20 solution as described previously (Melotte *et al.*, 2004). Finally, slides were washed in 1× phosphate-buffered saline for 5 min and dehydrated by sequential washing in 70%, 90% and 100% ethanol, respectively.

## Probe preparation and FISH analysis

PGD was performed by FISH using locus-specific probes. PAC and BAC DNA was isolated by Nucleobond AX (Machery-Nagel, Düren, Germany) and directly labeled by the Random Prime Labelling System (Invitrogen, Carlsbad, CA, USA) in spectrum orange (SO) or green (SG) (Abbott laboratories, IL, USA). Labeling reactions were purified by the QIAquick PCR cleanup kit (Qiagen, Hilden, Germany) and eluted with TE buffer (10 mM Tris and 1 mM EDTA; pH7.5) (TEKnova, Hollister, CA, USA). Fifty nanograms of labeled probe DNA combined with 100 μg cot-1 DNA (Invitrogen) were dried and dissolved in 5 μl hybridization mixture [50% formamide, 2× standard sodium citrate buffer (SSC) and 10% sodium dodecyl sulfate].

Slide pretreatment, co-denaturation, hybridization and post-hybridization washing steps were performed as described previously (Melotte *et al.*, 2004). Briefly, 1 μl of probe was applied to the slide, covered with a cover slip (10 mm diameter) and sealed with rubber cement. Nuclei and probe were denatured simultaneously on a hot plate at 75°C for 5 min. Hybridization was allowed to take place overnight in a humid chamber at 37°C. After hybridization, excess or non-specific bound probe was removed by subsequent washes in 0.4× SSC/0.3% Igepal CA-630 (Sigma Aldrich) (73°C for 2 min), 2× SSC/0.1% Igepal CA-630 [room temperature (RT) for 1 min] and 2× SSC (RT for 1 min) followed by dehydration through ethanol series. After drying, the slides were mounted in Vectashield anti-fade medium (Vector Laboratories, Peterborough, UK) containing 2.5 ng/ml 4′,6-diamidino-2-phenylindole (DAPI; Boehringer Ingelheim GmbH, Ingelheim, Germany). Nuclei were examined using an Axioplan 2 microscope (Zeis NV, Zaventem, Belgium).

The quality of the probe mixtures was tested on nuclei derived from stimulated blood lymphocytes from both partners. For each probe, the number of signals was counted in 100 nuclei. Individual analysis of the probes revealed two signals in at least 96% of the cells for RPCI5-926B9 (SO), RPCI5-962N3 (SO), P1-9 (SO), RP11-50C4 (SG), RP11-382A21 (SG) and LSI 21 (21q22) (SO) (Abbott laboratories, IL, USA) (Fig. 1). During the PGD of ‘Patient 1’ no control probe was used. For ‘Patients 2 and 3’, we decided to add a control probe describing the ploidy state of the embryo. For this, we used a self-labeled telomere 17q for ‘Patient 2’ and a commercial probe LSI 21 for ‘Patient 3’. Combination of the probes P1-9 and telomere 17q resulted in 95% hybridization efficiency for ‘Patient 2’, whereas for ‘Patient 3’ we found in 96% of the cells two clear signals for RP11-382A21 combined with LSI 21.

# Results

## PGD results

‘Patient 1’, carrying a 1.4 Mb *NF1* microdeletion, went through three IVF-PGD cycles. FISH using RPCI5-926B9 showed either a single signal in each blastomere consistent with a deletion (Fig. 2), two signals in each blastomere consistent with a normal diploid embryo (Fig. 2), or a different number of signals in both blastomeres in which case the embryo was considered to be mosaic for the *NF1* locus. The PGD results are summarized in Table 1.

During the first IVF-PGD cycle, six embryos were biopsied: three embryos were diagnosed as normal for the investigated region, one embryo carried the deletion and one embryo was mosaic (with two signals in one blastomere and one signal in the second), whereas the last embryo remained without diagnosis, due to the presence of a degraded nucleus in one cell and an anucleated second cell. One of the embryos considered to be normal for the NF1 locus was transferred, but no pregnancy was obtained. The other two normal embryos were used for cryopreservation.

During the second cycle, eight embryos were suitable for biopsy. Two embryos were normal, four carried the deletion (two of them had only one nucleus to analyze) and two embryos were mosaic. The first mosaic embryo showed one normal blastomere with two signals, while the second blastomere was binucleated with one signal in both nuclei. In the second mosaic embryo, three signals were observed in one cell, while the nucleus of the second blastomere was degraded. Both normal embryos were transferred, but no hCG was detected.

In the third cycle, 10 embryos were biopsied. Five embryos were considered to be normal, two carried the deletion. The remaining three embryos were mosaic abnormal: one was multinuclear and mosaic, the second embryo showed four distinct signals in one cell and no signal in the second cell and in the last embryo three signals

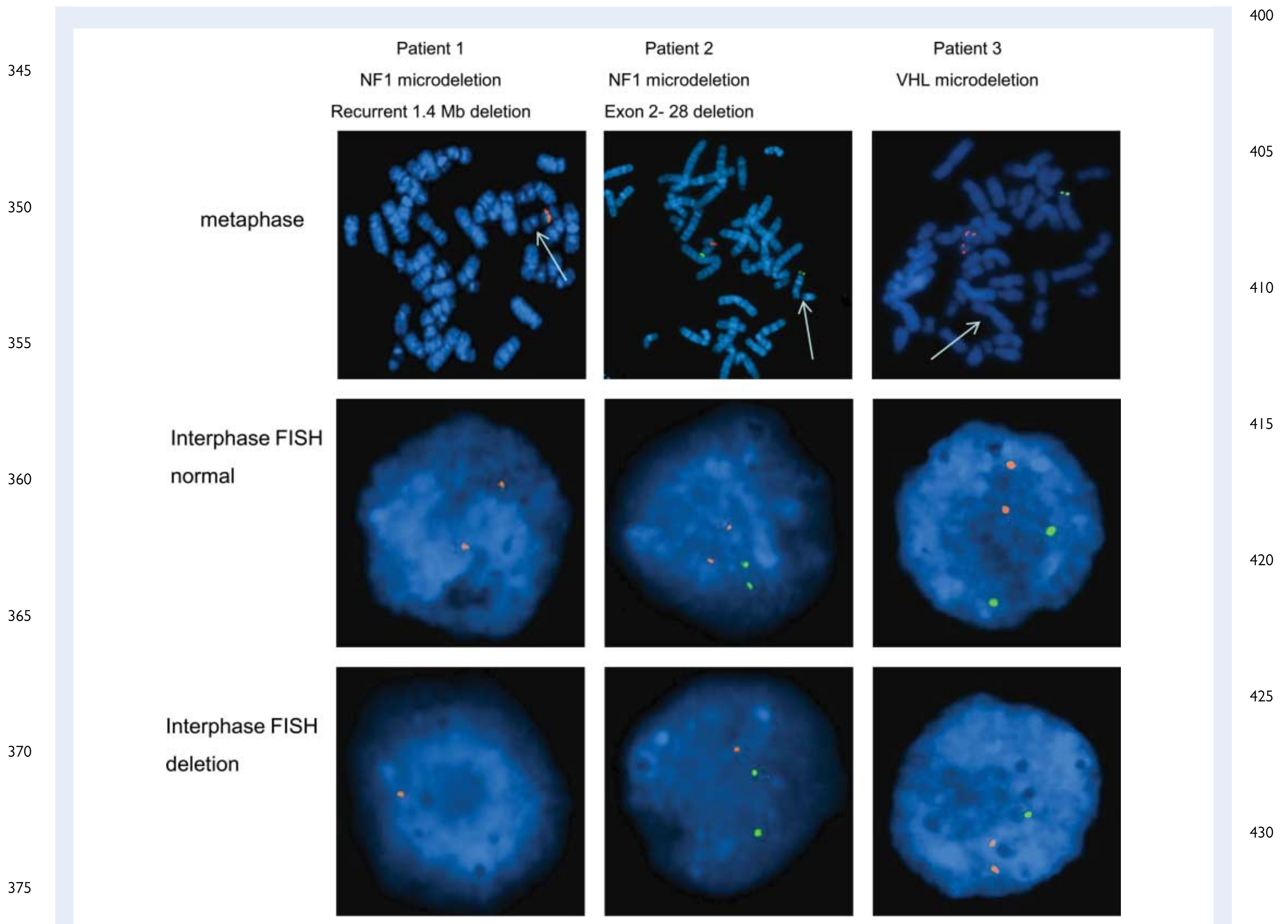


**Figure 2** FISH preparation and results.

For 'Patient 1' RPCI5-926B9 labeled in SO was used for PGD. For 'Patient 2' we used P1-9 in SO as a probe in *NF1* and the telomeric probe 'tel 17q' labeled in SG as a control probe. For 'Patient 3', BAC RP11-382A21 (SG) was used to detect deletions of *VHL* and LSI 21 (SO) was used as a control probe. The metaphase spreads (row 1) confirmed that RPCI5-926B9, P1-9 and 382A21 were respectively located at 17q11.2 ('Patients 1 and 2'), and at 3p25-26 ('Patient 3'). The affected chromosomes were indicated with an arrow. In the interphase nuclei (rows 2 and 3), we observed two signals in the non-affected nuclei (row 2) and only one signal for the investigated locus in case of a microdeletion (row 3).

(two bigger and one smaller signal) were detected in both blastomeres. Since a duplication of *NF1* was not expected to occur, the FISH analysis was repeated with probe RPCI5-962N3, also located in the microdeletion region. With this probe, only two signals were visible. Hence, the smaller signal observed during the first analysis was considered to be a false-positive signal. Two out of five normal embryos were transferred, without hCG detection, whereas the other three were cryopreserved.

After three unsuccessful PGD treatments, a spontaneous pregnancy ensued. The couple underwent amniocentesis and analysis, using the same probe as for PGD showing an unaffected fetus.

For 'Patient 2', carrying an intragenic *NF1* microdeletion, clone P1-9 was selected for FISH to detect the deletion. In order to detect aneuploidy and hybridization failure, we used the 17q subtelomeric probe, RP11-50C4 as a control. If for both probes two signals were obtained in each nucleus, the embryo was considered normal (Fig. 2), if for the control probe two signals were obtained, but for the microdeletion probe only a single signal, the embryo carried the microdeletion (Fig. 2). If for the control probe only a single, or three or four signals were obtained, the embryo was considered, respectively, monosomic, trisomic or tetrasomic for chromosome 17.

In the first cycle, 17 embryos were biopsied on day 3. Two embryos were normal, whereas eight embryos carried the deletion (three of them had only one nucleus to analyze). One embryo was monosomic and two were trisomic for chromosome 17. Furthermore, two embryos were mosaics since one cell with two and one cell with three signals for both the telomeric and the *NF1*-deletion-specific probe were detected. Additionally, two mosaics had one blastomere

**Table I** Summary of PGD results

| Patient | Cycle | Oocytes | Fertilized oocytes | Embryos biopsied | PGD unaffected (normal) | PGD affected (deletion) | PGD abnormal | No diagnosis | Embryos transferred | Cryo | Outcome |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 16 | 15 | 6 | 3 | 1 | 1 | 1 | 1 | 2 | No hCG |
| | 2 | 28 | 16 | 8 | 2 | 4 | 2 | 0 | 2 | 0 | No hCG |
| | 3 | 32 | 28 | 10 | 5 | 2 | 3 | 0 | 2 | 3 | No hCG |
| 2 | 1 | 29 | 22 | 17 | 2 | 8 | 7 | 0 | 1 | 1 | No hCG |
| | 2 | 15 | 7 | 5 | 1 | 2 | 2 | 0 | 1 | 0 | No hCG |
| 3 | 1 | 14 | 12 | 10 | 1 | 6 | 3 | 0 | 1 | 0 | No hCG |
| | 2 | 9 | 8 | 8 | 3 | 4 | 1 | 0 | 2 | 0 | hCG detected (twin) |

with two and one with a single signal for only one of the probes. One of the normal embryos was transferred, whereas the other one was used for cryopreservation.

In the second cycle, five out of seven embryos could be used for biopsy. One embryo was normal, two embryos carried the deletion and one embryo was monosomic for chromosome 17. One embryo was multinuclear and showed a mosaic abnormal result. No hCG was detected after single embryo transfer.

For 'Patient 3', carrying a *VHL* microdeletion, BAC clone 382A21 was used to detect the deletion. As a control probe, LSI 21 was used (Fig. 2). On day 3 after fertilization, respectively 10 and 8 embryos were biopsied in the first and second cycle. In the first cycle, one embryo was considered normal, since two signals for both probes were detected in both nuclei, while four embryos carried the deletion, since only a single signal was observed for the *VHL* region in both blastomeres. One embryo suffered a trisomy 21, whereas another one was normal for chromosome 21, but had no signals for the VHL probe. Furthermore, two embryos carried the deletion in one cell and had no diagnosis in the second cell because of a degraded nucleus. One embryo was mosaic abnormal in both nuclei. The normal embryo was transferred, but no hCG was detected.

In the second cycle, we found one embryo with a trisomy 21, four embryos carried the deletion (three of them with two cells analyzed), while three embryos were considered to be normal since both nuclei showed two distinct signals. Two of the three normal embryos were transferred, which resulted in a twin pregnancy. Instead of prenatal diagnosis, the couple opted for post-natal testing. Therefore, we performed FISH on buccal cells using the VHL cosmid, which confirmed the absence of the *VHL* deletion in the boy and the girl.

# Discussion

We have performed PGD using FISH for two carriers of an *NF1* microdeletion and one carrier of a *VHL* deletion. To our knowledge, this is the first time FISH-based PGD for cancer predisposition syndromes has been performed. This approach resulted in a twin pregnancy giving rise to two healthy children for the VHL carrier, whereas no pregnancy was obtained through PGD in either of the *NF1* deletion carriers. Subsequently, one of the *NF1* microdeletion carriers delivered a baby without NF1 following a natural conception. Microdeletion syndromes occur relatively frequently. Recent research has shown a variable penetrance and expressivity of several microdeletion syndromes (Hannes *et al*., 2008), which result in familial inheritance. This approach may thus benefit other deletion carriers.

Reports using FISH-based PGD for microdeletions syndromes are rare. FISH-based PGD has thus far only been reported on a mildly affected DiGeorge patient with a 22q11 microdeletion, as well as for carriers of deletions in the dystrophin gene (Xp21) causing the Duchenne and Becker muscular dystrophy (Iwarsson *et al*., 1998; Malmgren *et al*., 2006). This paucity of reports using FISH-based PGD is surprising considering the relatively high frequency of pathogenic copy number changes. Thus far, microdeletions have been analyzed by an indirect linkage analysis, e.g. multiplex PCR for microsatellite markers (Spits *et al*., 2005). For each family, polymorphic markers need to be established and indirect marker analysis carries the risk that a recombination may occur between the markers and the deletion, thus causing a carrier embryo to be selected. FISH probes for

recurrent microdeletions can be reused in other deletion carriers. It seems likely that the number of families that can benefit from FISH-based PGD will increase in the years to come. Array-based analysis of genomes is now revealing large frequencies of copy number variations in both healthy individuals (Redon *et al*., 2006), as well as in patients with developmental anomalies (Veltman *et al*., 2003; Shaw-Smith *et al*., 2004; Menten *et al*., 2006; Thienpont *et al*., 2007). With the discovery of a number of copy number variants that can cause autosomal recessive, X-linked (Froyen *et al*., 2007) or imprinted disorders (Breckpot *et al*., 2008), it seems plausible that the number of FISH-based PGDs will increase in the near future.

PGD is a new technology. The impact of mosaicism in early cleavage stage embryos on PGD is unclear. Consequently, it is still a matter of debate whether the biopsy of two rather than one blastomere would reduce the IVF success rate (Michiels *et al*., 2006; Goossens *et al*., 2008). In this study, we opted to analyze two blastomeres to increase the accuracy. In 50 out of 64 (78.1%) cases, a FISH result was obtained in both nuclei. In the other embryos, no diagnosis was possible (1 of 64, 1.6%) or only a single blastomere could be analyzed (13 of 64, 20.3%). A negative FISH result was either due to a split FISH signal that confused its designation as a single or two signals or due to a degraded nucleus in at least one cell. Moreover, we used a control probe for the 'Patients 2 and 3' to be able to detect the ploidy state of the embryo. Since whole chromosome copy number changes as well as changes in the overall ploidy status (haploidy, triploidy, etc.) are well known in early cleavage stage embryos and can cause misdiagnosis in the search for a microdeletion region, 7 out of the 40 embryos (17.5%) from 'Patients 2 and 3' were scored abnormal based on the ploidy state of the control probe and thus were not transferred. On the other hand, 12 out of 64 embryos (18.8%) were mosaic and thus abnormal, which confirms the mosaic nature of cleavage stage embryos.

The increasing access to new technologies, such as PGD, that utilize genetic testing to guide reproductive choices raises important ethical, social and legal issues that must be addressed to ensure the most responsible translation of these new technologies to clinical practice (Offit *et al*., 2006). The medical indications of assisted reproductive technologies for adult-onset cancer predisposition syndromes, such as NF1 and VHL syndrome, remain to be defined (Offit *et al*., 2006). On the basis of the American Medical Association, the ethics committee of the American Society of Reproductive Medicine, as well as the European medical ethics societies, the main criteria for allowing PGD are the likely severity of the tumor, its penetrance, the probability of its occurrence, the (later) age of onset and the possibility of preventive surgery, early detection and effective treatment. It is obvious that PGD for cancer predisposition is an acceptable approach for couples at risk, such as NF1 or VHL microdeletion carriers.

# Author's Role

E.V. and C.M. designed, prepared and executed the FISH experiments and wrote the manuscript. S.D. and T.D. performed IVF and embryobiopsy. H.B. sequenced the breakpoint of 'Patient 1'. J.P.F. and E.L. performed the genetic counseling of the patients. J.R.V. supervised. All authors read the manuscript critically.

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# References

Breckpot J, Takiyama Y, Thienpont B, Van Vooren S, Vermeesch JR, Ortibus E, Devriendt K. A novel genomic disorder: a deletion of the SACS gene leading to spastic ataxia of Charlevoix-Saguenay. *Eur J Hum Genet* 2008;**16**:1050–1054.

Clementi M, Boni S, Mammi I, Favarato M, Tenconi R. Clinical application of genetic polymorphism in neurofibromatosis type 1. *Ann Genet* 1996; **39**:92–96.

De Raedt T, Brems H, Wolkenstein P, Vidaud D, Pilotti S, Perrone F, Mautner V, Frahm S, Sciot R, Legius E. Elevated risk for MPNST in NF1 microdeletion patients. *Am J Hum Genet* 2003;**72**:1288–1292.

De Raedt T, Matthew S, Heyns I, Brems H, Thijs D, Messian L, Stephens K, Lazaro C, Wimmer K, Kehrer-Sawatzki H *et al*. Conservation of hotspots for recombination in low-copy repeats associated with the NF1 microdeletion. *Nat Genet* 2006;**38**:1419–1423.

Descheemaeker MJ, Roelandts K, De Raedt T, Brems H, Fryns JP, Legius E. Intelligence in individuals with a neurofibromatosis type I microdeletion. *Am J Med Genet A* 2004;**131**:325–326.

Froyen G, Van Esch H, Bauters M, Hollanders K, Frints SG, Vermeesch JR, Devriendt K, Fryns JP, Marynen P. Detection of genomic copy number changes in patients with idiopathic mental retardation by high-resolution X-array-CGH: important role for increased gene dosage of XLMR genes. *Hum Mutat* 2007;**28**:1034–1042.

Gnarra JR, Tory K, Weng Y, Schmidt L, Wei MH, Li H, Latif F, Liu S, Chen F, Duh FM *et al*. Mutations of the VHL tumour suppressor gene in renal carcinoma. *Nat Genet* 1994;**7**:85–90.

Goossens V, De Rycke M, De Vos A, Staessen C, Michiels A, Verpoest W, Van Steirteghem A, Bertrand C, Liebaers I, Devroey P *et al*. Diagnostic efficiency, embryonic development and clinical outcome after the biopsy of one or two blastomeres for preimplantation genetic diagnosis. *Hum Reprod* 2008;**23**:481–492.

Hannes FD, Sharp AJ, Mefford HC, de Ravel T, Ruivenkamp CA, Breuning MH, Fryns JP, Devriendt K, Van Buggenhout G, Vogels A *et al*. Recurrent reciprocal deletions and duplications of 16p13.11: the deletion is a risk factor for MR/MCA while the duplication may be a rare benign variant. *J Med Genet* 2009 (in press).

Hes F, Zewald R, Peeters T, Sijmons R, Links T, Verheij J, Matthijs G, Leguis E, Mortier G, van der Torren K *et al*. Genotype–phenotype correlations in families with deletions in the von Hippel-Lindau (VHL) gene. *Hum Genet* 2000;**106**:425–431.

Huson SM. Recent developments in the diagnosis and management of neurofibromatosis. *Arch Dis Child* 1989;**64**:745–749.

Iwarsson E, hrlund-Richter L, Inzunza J, Fridstrom M, Rosenlund B, Hillensjo T, Sjoblom P, Nordenskjold M, Blennow E. Preimplantation

genetic diagnosis of DiGeorge syndrome. *Mol Hum Reprod* 1998; **4**:871–875.

Kluwe L, Siebert R, Gesk S, Friedrich RE, Tinschert S, Kehrer-Sawatzki H, Mautner VF. Screening 500 unselected neurofibromatosis 1 patients for deletions of the NF1 gene. *Hum Mutat* 2004;**23**:111–116.

Latif F, Tory K, Gnarra J, Yao M, Duh FM, Orcutt ML, Stackhouse T, Kuzmin I, Modi W, Geil L *et al*. Identification of the von Hippel-Lindau disease tumor suppressor gene. *Science* 1993; **260**:1317–1320.

Legius E, Marchuk DA, Collins FS, Glover TW. Somatic deletion of the neurofibromatosis type 1 gene in a neurofibrosarcoma supports a tumour suppressor gene hypothesis. *Nat Genet* 1993;**3**:122–126.

Leppig KA, Viskochil D, Neil S, Rubenstein A, Johnson VP, Zhu XL, Brothman AR, Stephens K. The detection of contiguous gene deletions at the neurofibromatosis 1 locus with fluorescence *in situ* hybridization. *Cytogenet Cell Genet* 1996;**72**:95–98.

Lopez-Correa C, Dorschner M, Brems H, Lazaro C, Clementi M, Upadhyaya M, Dooijes D, Moog U, Kehrer-Sawatzki H, Rutkowski JL *et al*. Recombination hotspot in NF1 microdeletion patients. *Hum Mol Genet* 2001;**10**:1387–1392.

Maher ER, Iselius L, Yates JR, Littler M, Benjamin C, Harris R, Sampson J, Williams A, Ferguson-Smith MA, Morton N. Von Hippel-Lindau disease: a genetic study. *J Med Genet* 1991;**28**:443–447.

Maher ER, Webster AR, Richards FM, Green JS, Crossey PA, Payne SJ, Moore AT. Phenotypic expression in von Hippel-Lindau disease: correlations with germline VHL gene mutations. *J Med Genet* 1996; **33**:328–332.

Malmgren H, White I, Johansson S, Levkov L, Iwarsson E, Fridstrom M, Blennow E. PGD for dystrophin gene deletions using fluorescence *in situ* hybridization. *Mol Hum Reprod* 2006;**12**:353–356.

Melotte C, Debrock S, D'Hooghe T, Fryns JP, Vermeesch JR. Preimplantation genetic diagnosis for an insertional translocation carrier. *Hum Reprod* 2004;**19**:2777–2783.

Mensink KA, Ketterling RP, Flynn HC, Knudson RA, Lindor NM, Heese BA, Spinner RJ, Babovic-Vuksanovic D. Connective tissue dysplasia in five new patients with NF1 microdeletions: further expansion of phenotype and review of the literature. *J Med Genet* 2006;**43**:e8.

Menten B, Maas N, Thienpont B, Buysse K, Vandesompele J, Melotte C, de Ravel T, Van Vooren S, Balikova I, Backx L *et al*. Emerging patterns of cryptic chromosomal imbalance in patients with idiopathic mental retardation and multiple congenital anomalies: a new series of 140 patients and review of published reports. *J Med Genet* 2006; **43**:625–633.

Messiaen LM, Callens T, Mortier G, Beysen D, Vandenbroucke I, Van Roy N, Speleman F, Paepe AD. Exhaustive mutation analysis of the NF1 gene allows identification of 95% of mutations and reveals a high frequency of unusual splicing defects. *Hum Mutat* 2000;**15**:541–555.

Michiels A, Van Assche E, Liebaers I, Van Steirteghem A, Staessen C. The analysis of one or two blastomeres for PGD using fluorescence in-situ hybridization. *Hum Reprod* 2006;**21**:2396–2402.

Offit K, Kohut K, Clagett B, Wadsworth EA, Lafaro KJ, Cummings S, White M, Sagi M, Bernstein D, Davis JG. Cancer genetic testing and assisted reproduction. *J Clin Oncol* 2006;**24**:4775–4782.

Redon R, Ishikawa S, Fitch KR, Feuk L, Perry GH, Andrews TD, Fiegler H, Shapero MH, Carson AR, Chen W *et al*. Global variation in copy number in the human genome. *Nature* 2006;**444**:444–454.

Robertson JA. Extending preimplantation genetic diagnosis: the ethical debate. Ethical issues in new uses of preimplantation genetic diagnosis. *Hum Reprod* 2003;**18**:465–471.

Shaw-Smith C, Redon R, Rickman L, Rio M, Willatt L, Fiegler H, Firth H, Sanlaville D, Winter R, Colleaux L *et al*. Microarray based comparative genomic hybridisation (array-CGH) detects submicroscopic chromosomal deletions and duplications in patients with learning disability/mental retardation and dysmorphic features. *J Med Genet* 2004;**41**:241–248.

Spits C, De Rycke M, Van Ranst N, Joris H, Verpoest W, Lissens W, Devroey P, Van Steirteghem A, Liebaers I, Sermon K. Preimplantation genetic diagnosis for neurofibromatosis type 1. *Mol Hum Reprod* 2005; **11**:381–387.

Steinmann K, Cooper DN, Kluwe L, Chuzhanova NA, Senger C, Serra E, Lazaro C, Gilaberte M, Wimmer K, Mautner VF *et al*. Type 2 NF1 deletions are highly unusual by virtue of the absence of nonallelic homologous recombination hotspots and an apparent preference for female mitotic recombination. *Am J Hum Genet* 2007;**81**:1201–1220.

Thienpont B, Mertens L, de Ravel T, Eyskens B, Boshoff D, Maas N, Fryns JP, Gewillig M, Vermeesch JR, Devriendt K. Submicroscopic chromosomal imbalances detected by array-CGH are a frequent cause of congenital heart defects in selected patients. *Eur Heart J* 2007;**28**:2778–2784.

Veltman JA, Jonkers Y, Nuijten I, Janssen I, van der Vliet W, Huys E, Vermeesch J, Van Buggenhout G, Fryns JP, Admiraal R *et al*. Definition of a critical region on chromosome 18 for congenital aural atresia by arrayCGH. *Am J Hum Genet* 2003;**72**:1578–1584.

Verlinsky Y, Kuliev A. Preimplantation diagnosis for diseases with genetic predisposition and nondisease testing. *Expert Rev Mol Diagn* 2002; **2**:509–513.

Verlinsky Y, Rechitsky S, Verlinsky O, Xu K, Schattman G, Masciangelo C, Ginberg N, Strom C, Rosenwaks Z, Kuliev A. Preimplantation diagnosis for p53 tumour suppressor gene mutations. *Reprod Biomed Online* 2001; **2**:102–105.

Verlinsky Y, Rechitsky S, Verlinsky O, Chistokhina A, Sharapova T, Masciangelo C, Levy M, Kaplan B, Lederer K, Kuliev A. Preimplantation diagnosis for neurofibromatosis. *Reprod Biomed Online* 2002;**4**:218–222.

Wallace MR, Marchuk DA, Andersen LB, Letcher R, Odeh HM, Saulino AM, Fountain JW, Brereton A, Nicholson J, Mitchell AL *et al*. Type 1 neurofibromatosis gene: identification of a large transcript disrupted in three NF1 patients. *Science* 1990;**249**:181–186.

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