CASE REPORT   Reproductive genetics

Gonosomal mosaicism for an NFI deletion in a sperm donor: evidence of the need for coordinated, long-term communication of health information among relevant parties

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BACKGROUND: Screening of gamete donors can reduce but cannot eliminate the risks for medical problems in donor-conceived offspring. We present a case of gonosomal mosaicism discovered in an anonymous sperm donor after receiving two reports of neurofibromatosis type 1 (NFI) in donor-conceived offspring, to illustrate that long-term, systematic investigation of health issues in donors and offspring can be invaluable to the welfare of these individuals.

METHODS: A repeat physical evaluation and ophthalmology examination were performed on the donor. DNA samples were examined by RT-PCR fragment analysis, multiplex ligation-dependent probe amplification (MLPA) and targeted array-comparative genomic hybridization (aCGH).

RESULTS: Gonosomal mosaicism for a deletion mutation in the NFI gene was identified in 20% of sperm and a smaller percentage of lymphocytes.

CONCLUSIONS: Long-term communication of medical information among donors, recipients and donor-conceived offspring is beneficial for the health management of all parties. Development of a secure, coordinated data system is critical to achieving this goal. Recommendations are provided for management and communication of critical information based on this experience.

Key words: gamete donor / neurofibromatosis / NFI / mosaicism / genetic counseling

Introduction

Applicants to California Cryobank’s (CCB) semen donor program are required to have a complete physical examination by a contracted physician and a family medical history risk assessment by a CCB genetic counselor. Karyotype analysis and carrier screening for cystic fibrosis, spinal muscular atrophy and hemoglobinopathies are performed on all applicants, in accordance with professional guidelines [Watson et al., 2004; Practice Committee for the American Society for Reproductive Medicine (ASRM), 2008; American Association of Tissue Banks (AATB), 2008; Prior, 2008]. Additional evaluations are performed if indicated by the applicant’s family history or ethnic background (Gross et al., 2008). Although the risks for birth defects can never be eliminated, this screening process can help to reduce the risk for medical problems in donor-conceived offspring.

Long-term reporting of health information by gamete donors and donor-conceived offspring can also be instrumental in the health management of these individuals (Maron et al., 2009; Callum et al., 2010). CCB has had an internal system for documentation and follow-up on medical issues in donors and offspring for over 25 years. This system allows for continued eligibility determination regarding distribution of a donor’s specimens and for informing recipient families and donors...
about health management issues. In this report, we describe how
long-term follow-up and coordinated communication of information
contributed to accurate diagnosis and counseling about recurrence
risks due to mosaicism for neurofibromatosis type 1 (NF1).

NF1 is one of the most common Mendelian disorders, occurring in
approximately 1/2500–1/3500 live born individuals. It is character-
ized by the development of nerve sheath tumors throughout the
body, with significant clinical variability in the onset and severity of
symptoms even among family members who carry the same genetic
mutation. It is inherited in an autosomal dominant manner and
~50% of cases are believed to result from de novo mutations due
to the absence of clinical findings in the parents by physical exami-
ation and/or negative molecular results in DNA from parental
blood samples when the causative mutation is known. Clinical diagno-
sis can be established in an individual who meets at least two of the
following diagnostic criteria, as determined by the National Institute
of Health (NIH): six or more café-au-lait spots (CALs); two or
more neurofibromas of any type, or at least one plexiform neuro-
fibroma; axillary and/or inguinal freckling; optic nerve pathway
lesion; two or more Lisch nodules; or a distinctive osseous lesion
(Ruggieri and Huson, 2001; Radtke et al., 2007; Jett and Friedman,
2010). The NIH criteria have traditionally been considered to be
both highly specific and highly sensitive in adults with NF1 and a clinical
diagnosis is often sufficient for disease management and counseling
regarding recurrence risk. Molecular testing is available for confirm-
ation of an NF1 diagnosis, but testing is complex due to the large
size of the NF1 gene and the lack of common mutations for this dis-
order. As such, molecular confirmation may be considered unneces-
sary for clinical management in the absence of reproductive needs
(Jett and Friedman, 2010). However, there is increasing evidence
that molecular confirmation of an NF1 diagnosis is essential for accu-
crate counseling and management of an affected individual and his or her
relatives because NF1 has a high somatic mutation rate (Ruggieri and
Huson, 2001), and because recent reports indicate that nearly half of
individuals with SPRED1 mutations, which are associated with Legius
syndrome, have signs that fulfill the NIH criteria for NF1 diagnoses
(Messaia et al., 2009).

Case Report

We received a report that a donor-conceived offspring was diagnosed
with NF1 based on clinical findings. The biological mother of the
affected child was negative for signs of NF1 on physical examination.
The donor had been determined to be eligible for the donor program
based on the results of his physical examination, laboratory
tests and genetic evaluation, which were unremarkable. The child’s
diagnosis was initially thought to represent a de novo mutation.

A short time later a second report was received involving another
offspring from the same donor and a different recipient; the child did
not have a formal diagnosis but exhibited findings suggestive of NF1.
The two reports suggested a high likelihood of, at the very least,
‘gonadal’ mosaicism for an NF1 mutation in the donor, and increased
risk for NF1 in other offspring of this donor. Distribution of the
donor’s specimens was immediately discontinued. CCB genetic coun-
selors attempted to contact all CCB clients who had used specimens
of this donor to discuss these reports. During this process, two ad-
ditional clients reported that their children from this donor had been
diagnosed with NF1. Genetic testing records were obtained and con-
irmed the presence of the same NF1 mutation in both children.

The donor underwent repeat physical and ophthalmological exa-
iminations with attention to signs of NF1. The evaluations were negative
for developmental disability, freckling, neurofibromas, scoliosis and
Lisch nodules of the iris by slit lamp examination. Four hyper-
pigmented cutaneous lesions were observed on the donor’s back: a
brownish-gray nevus measuring 4 × 6 cm between the scapulae, a
nevus measuring 1.5 × 2 cm over the lower thoracic spine, a nevus meas-
uring 3 × 5 cm over the lower lumbar spine and a nevus meas-
uring 2 cm in diameter on the upper part of the buttocks. (The
donor declined to provide clinical photographs).

Comprehensive molecular evaluation using RT–PCR fragment anal-
ysis, multiplex ligation-dependent probe amplification (MLPA) and
targeted array-comparative genomic hybridization (aCGH), revealed
an intragenic deletion involving exons 11-23.1 of the NF1 gene (i.e.
c.1642−?_3974+?del) in ~20% of sperm cells (ratio of 0.75–0.9
after normalization for eight probes covering the deleted region).
This multi-exon deletion was below the detection threshold of MLPA
and aCGH in the blood cells, but was detectable using RT–PCR, as
the boundaries of this deletion reside within the region spanned by
the primers used for RT–PCR of the exons 1-27b and a shorter frag-
ment is preferentially amplified by PCR. The presence of this specific
intragenic multi-exon deletion was confirmed at the gDNA level
using breakpoint-spanning PCR, showing the identical breakpoints
in the sperm and blood cells, confirming gonosomal mosaicism for an
NF1 deletion in the donor. The detailed molecular characterization
of this deletion will be reported separately (unpublished data).

Discussion

Gamete facilities strive to screen donors to minimize health problems
in donor offspring. However, all donors carry genetic variants, which
increase susceptibility to specific disorders, and these risks cannot
be eliminated. Furthermore, common human traits such as CALs
are present in gamete donors and donor applicants are unlikely to
be excluded based on the finding of a few CALs in the absence of
underlying disease. It would be difficult to define specific findings
for donor exclusion since CALs alone will vary in number, size and distrib-
ution in each individual evaluated, and often represent isolated birth
marks of no clinical significance (Tekin and Bodurtha, 2001). As such,
it is critical that all concerned parties are informed about and under-
stand that there are significant limitations to identifying health risks for
gamete donors and donor-conceived individuals due, in part, to
changes in family medical histories over time and the delayed onset
and recognition of many medical conditions.

Some recipients may believe that, due to these limitations, all
gamete providers are required to collect and distribute long-term
health updates on their donors. They may not be aware of the
limits to the services available from gamete providers or the difficulty
of trying to obtain this information (Ethics Committee for the ASRM,
2009). In addition, the ability of the gamete provider or any organiza-
tion to manage health updates and the associated risk information
requires contributions from all individuals involved in this process.
The participation of gamete providers is necessary to facilitate
contact with the donors. The contributions of the recipient families
are also essential as indicated in this case. Specifically, (i) the donor
was not recognized as having a significant risk for gonadal mosaicism until the second offspring report was received; (ii) the low-level mosaicism would have been much more difficult to detect or have been refractory to detection using gDNA-based techniques if the causative mutation in affected offspring had not been identified previously and (iii) it was essential that the families were willing to share their medical records to facilitate molecular studies on the donor’s specimens.

Despite the benefit of communication and follow-up investigations, this case and our previous experiences indicate that up to 50% of health problems in donor-conceived individuals are unreported (unpublished observations). Informal discussions with clients have revealed some common reasons for underreporting including: privacy regarding donor conception; assumption that the medical issue is unrelated to the donor or inherited from the mother’s side; and, belief that a physician reported the issues in question. Clients may not consider that their pediatricians may not report their child’s findings because of incomplete information about the donor or the donor program from which specimens were obtained for that child’s conception. Even obstetricians, whom clients often assume are responsible for birth reporting, may lack this information unless they have medical records from the inseminating provider, and because they may not be informed of later-onset symptoms or diagnoses. Additionally, physicians may assume that the parent has contacted the gamete facility. Incomplete reporting limits the donor facilities’ ability to contribute to long-term healthcare and distribution of medical information that may be relevant to the offspring’s or donors’ health.

In response to these issues, the following are advised as the minimum requirements prior to the distribution of gametes from any individual:

- Individual consultations with donor applicants for collection and evaluation of detailed family medical histories [Qureshi et al., 2005; Committee on Genetics, The American College of Obstetricians and Gynecologists (ACOG), 2011].
- Genetic testing on the donor, including, at minimum, those tests that should be offered if the donor is planning his or her own pregnancy according to the recommendations of the American College of Medical Genetics and/or ACOG (Watson et al., 2004; Fletcher and Bocian, 2006; Practice Committee for ASRM, 2008; Prior, 2008; Gross et al., 2008), or local professional body.
- Education of recipients as to their donor’s medical history and genetic screening results, and the limitations of those evaluations.
- Communication to recipients about their donors’ availability, or lack thereof, for additional medical and genetic screening evaluations and future health updates.
- Recommendation that every recipient pursue a personal genetic consultation prior to any reproductive procedures (Committee on Genetics for ACOG, 2011) so that the recipient may consider genetic screening for those disorders that are appropriate based upon his or her personal family medical history and ethnic background. Preconception evaluations can be critical for assisting the recipient in selecting a donor, especially if the recipient screens positive as a carrier for a disorder that is inherited in an autosomal recessive manner. The preconception evaluations allow the recipient more opportunity to select a donor who has had negative carrier screening for the disorder in question, or to identify a donor who is available for additional screening to reduce risks to the health of the offspring. Not all donors are available for reciprocal carrier screening following a positive screening result in the recipient. This can be particularly problematic for the recipient if embryos have been created or if there is an ongoing pregnancy.
- Consultations with the donors and recipients should be performed by a trained professional knowledgeable in medical genetics.

As illustrated, in order to fully contribute to the health of the families involved, documentation and communication of health information must continue after collection and distribution of gametes. It requires coordinated reporting by and communication among the various participants in the donor contract. It also requires secure storage and accurate interpretation of reported information. We recommend the following to achieve these ideals:

- A systematic, confidential system for collection and investigation of reports of birth defects and the long-term health of donors, donor-conceived persons and their biological relatives.
- Assessment of the information reported and interpretation of the relevant risks for other biologically related individuals by knowledgeable healthcare professionals.
- An established structure for communicating health updates and risk information to individuals to whom it may be relevant.
- Complete traceability of gamete distribution records, or preferably, accurate documentation of birth records and periodically updated client contact information.

The goals outlined above are likely to be widely supported, but the process for achieving these goals and the responsibilities of the various parties, especially of gamete providers, are less well defined. If gamete providers invest in the long-term health of these individuals, this may help to reduce the aura of mistrust often directed toward these organizations. Such transparency may also aid in reducing miscommunication of medical information and the relevant risks to other recipient families, which is known to occur through social networks.

We recognize the many limitations of gamete providers to offer some of these services internally, which is why we support the development of an independent donor health resource for confidential reporting of health updates about donors, donor-conceived individuals and their biological relatives. It is a resource that must be managed by knowledgeable healthcare professionals and which requires support and input from donors, recipients and organizations involved in donor gamete agreements. This network will reduce the burden on gamete providers to be the sole keeper and distributor of shared health information.

Pediatricians, obstetricians, genetic counselors and other providers should encourage recipients of donor gametes to inform donor facilities or the appropriate registry of their children’s births, medical issues and updated contact information to facilitate this process. Increased awareness of the benefits of sharing health information may improve reporting; however, reporting to the donor facility is also limited if they lack a formal system for documenting and investigating these reports. Collective development of this service may be the optimal solution.
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