

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 I (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 RTPCR 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 (NFI).

NFI is one of the most common Mendelian disorders, occurring in approximately 1/2500–1/3500 live born individuals. It is characterized 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 examination and/or negative molecular results in DNA from parental blood samples when the causative mutation is known. Clinical diagnosis 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 neurofibroma; axillary and/or inguinal freckling; optic nerve pathway tumor; 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 NFI and a clinical diagnosis is often sufficient for disease management and counseling regarding recurrence risk. Molecular testing is available for confirmation of an NFI diagnosis, but testing is complex due to the large size of the *NFI* gene and the lack of common mutations for this disorder. As such, molecular confirmation may be considered unnecessary for clinical management in the absence of reproductive needs (Jett and Friedman, 2010). However, there is increasing evidence that molecular confirmation of an NFI diagnosis is essential for accurate counseling and management of an affected individual and his or her relatives because NFI 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 NFI diagnoses (Messiaen et al., 2009).

Case Report

We received a report that a donor-conceived offspring was diagnosed with NFI based on clinical findings. The biological mother of the affected child was negative for signs of NFI 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 NFI. The two reports suggested a high likelihood of, at the very least, 'gonadal' mosaicism for an *NFI* mutation in the donor, and increased risk for NFI in other offspring of this donor. Distribution of the donor's specimens was immediately discontinued. CCB genetic counselors attempted to contact all CCB clients who had used specimens of this donor to discuss these reports. During this process, two additional clients reported that their children from this donor had been

diagnosed with NFI. Genetic testing records were obtained and confirmed the presence of the same *NFI* mutation in both children.

The donor underwent repeat physical and ophthalmological examinations with attention to signs of NFI. The evaluations were negative for developmental disability, freckling, neurofibromas, scoliosis and Lisch nodules of the iris by slit lamp examination. Four hyperpigmented 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 measuring 3 × 5 cm over the lower lumbar spine and a nevus measuring 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 analysis, multiplex ligation-dependent probe amplification (MLPA) and targeted array-comparative genomic hybridization (aCGH), revealed an intragenic deletion involving exons 11–23.1 of the *NFI* 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 fragment 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 *NFI* 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 distribution 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 understand 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 organization 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 was planning his or her own pregnancy according to the recommendations of the American College of Medical Genetics and/or ACOG (Watson *et al.*, 2004; Pletcher 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 allows 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 up-dated 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.

Authors' roles

All authors were involved in this investigation including critical discussions about management of the case and/or contacting and counseling the donor and recipients and/or performing the genetic analysis. All authors also reviewed or contributed content to the manuscript.

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Conflict of interest

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