Short Report

Gonadal mosaicism as a rare cause of autosomal recessive inheritance


Autosomal recessive diseases are typically caused by the biparental inheritance of familial mutant alleles. Unusual mechanisms by which the recessiveness of a mutant allele is unmasked include uniparental isodisomy and the occurrence of a de novo chromosomal rearrangement that disrupts the other allele. Gonadal mosaicism is a condition in which a postfertilization mutation is confined to the gamete precursors and is not detected in somatic tissues. Gonadal mosaicism is known to give the impression of autosomal recessive inheritance when recurrence of an autosomal-dominant condition among offspring of phenotypically normal parents is observed. Here, we report an extremely rare event in which maternal gonadal mosaicism for a recessive mutation in COL4A4 caused the recurrence of Alport syndrome within a consanguineous family. Such rare occurrence should be taken into account when analyzing pedigrees both for clinical and research purposes.

Conflict of interest

Authors declare no conflict of interest.

The recessiveness of a mutant allele for an autosomal recessive disease gene usually keeps it unnoticed among carriers in the population until it is unmasked in an individual whose other allele is also mutated. The usual scenario is when there is biparental inheritance of the same or two different mutant alleles giving rise, respectively, to a homozygous or compound heterozygous individual. Typically, both mutant alleles are familial because it is highly improbable for a de novo event to specifically disrupt the same locus that harbors the other allele. Cancer is a notable exception where the recessiveness of a familial mutation in a tumor suppressor gene can be unmasked by a wide array of pathological de novo somatic processes that can disrupt the normal allele, but these are probably facilitated by the very presence of the familial mutant allele at the heterozygous state and that is why they are inherited as autosomal-dominant traits (1). Mutations in other genes, however, provide no such ‘facilitation’ so it remains exceedingly rare for the second allele to be disrupted by a de novo somatic event although this has been reported (2). Postzygotic de novo events limited to the germline (gonadal mosaicism) have also rarely been reported to contribute the second mutant allele, but these tend to be genomic rearrangements rather than point mutations. For example, 2% of patients with spinal muscular atrophy have inherited a familial mutation from one parent and a de novo genomic rearrangement from the other (3). Another example is the mapping of ALMS1 based on determining that one breakpoint in a patient with Alstrom syndrome and a balanced chromosomal translocation disrupted one ALMS1 allele while the other was inactivated by a familial point mutation (4).

Uniparental disomy is another uncommon condition in which the recessiveness of a given allele is unmasked by inheriting the same allele in two copies from one parent (isodisomy). Multiple reports of UPD as a cause of autosomal recessive diseases have been published and this possibility is usually considered when counseling individuals with an autosomal recessive disease making it imperative to always check the
carrier status of parents when dealing with a homozygous mutation (5, 6). Parents are also tested in the setting of compound heterozygous mutations in their offspring, but this is usually done to test segregation of the two mutations (in cis or in trans). In this report, we describe a very unusual consanguineous family in which three children inherited a familial COL4A4 mutation from the father and a gonadal mosaic mutation from the mother in the same gene. This report shows that gonadal mosaicism can rarely contribute to the inheritance of autosomal recessive diseases as well. Awareness of this rare possibility can have important research and clinical implications as we show in this report.

Subjects and methods

Human subjects

Both parents and all children were recruited after signing a written informed consent that is IRB-approved (KFSHRC RAC# 2070023). Patients had full clinical evaluation and renal biopsies to confirm the diagnosis of Alport syndrome pathologically. Blood was drawn in EDTA tubes for DNA extraction.

Autozygosity mapping

DNA samples from the three affected members were genotyped on the Axiom platform following the manufacturer’s instructions (Affymetrix, Santa Clara, CA). Homozygosity mapping was performed using autoSNPa by considering runs of homozygosity (>2 Mb) as surrogates of autozygosity given the consanguineous nature of the parents, followed by determination of the entire set of autozygosity blocks (autozygome) essentially as described before (7).

Exome sequencing

Exome capture was performed using TruSeq Exome Enrichment kit (Illumina) following the manufacturer’s protocol. Samples were prepared as an Illumina sequencing library, and in the second step, the sequencing libraries were enriched for the desired target using the Illumina Exome Enrichment protocol. The captured libraries were sequenced using Illumina HiSeq 2000 Sequencer. The reads were mapped against UCSC hg19 (http://genome.ucsc.edu/) by BWA (http://bio-bwa.sourceforge.net/). The SNPs and indels were detected by SAMTOOLS (http://samtools.sourceforge.net/).

Results

Clinical report

Index is 8.5-year-old girl who was referred for headache and high blood pressure for a duration of 8 months. She was treated with the aldomet and hydralazine. Her neonatal and past medical history was unremarkable. Clinical evaluation revealed stable and well-developed young girl with normal growth parameters. Urinalysis uncovered hematuria and proteinuria (+3 proteinuria, +2 blood, and microscopic urine examination revealed 5–10 RBCs/HPF and a few granular casts). Renal ultrasound showed highly echogenic kidneys. Electron microscopy on renal biopsy tissue showed glomerular basement membrane changes consistent with the diagnosis of Alport syndrome. She had progressive deterioration of her renal function and reached end stage renal disease 4 years later. Following a few months on dialysis, she received living unrelated renal transplant.

Patient 2 is the brother of index who presented with recurrent gross hematuria usually precipitated by upper respiratory tract infection and fever. He has hearing difficulties for the last 2 years prior to his presentation. Physical examination revealed normal blood pressure. Urinalysis revealed more than 100 RBCs/HPF and there were few granular and rbc casts. Renal ultrasound showed increased renal echogenicity consistent with intrinsic renal disease. In view of the positive family history for a younger sister who was diagnosed to have Alport syndrome, percutaneous renal biopsy was done which showed, by electron microscopy, changes consistent with Alport syndrome. Hearing tests revealed severe bilateral sensorineural deafness. Over the next 6 years he showed progressive deterioration of his renal function and received pre-emptive cadaver renal transplant.

Patient 3 is the younger sister of the index. She was brought when she was 3-years-old for evaluation of microscopic hematuria that was detected by urinalysis. Her past medical history was uneventful. Clinical examination revealed healthy looking child with normal growth parameters. Urinalysis showed +2 proteinuria and more than 50 RBCs/HPF, urine protein/creatinine ratio was increased 1.76 mg (normal less than 0.2). Renal ultrasound was normal.

On the basis of the confirmed family history of her two eldest siblings with Alport syndrome, clinical diagnosis of Alport syndrome was made and blood was sent for molecular genetics studies.

Autozygosity mapping

The recurrence of Alport syndrome among male and female offspring of double cousin parents made it highly likely that this is an autosomal recessive form of Alport syndrome, which can be caused by mutation in either COL4A3 or COL4A4. Because these genes are very large, we used autozygosity mapping on the index to prioritize the likely candidate. However, the autozygome of the index did not overlap with either gene. Subsequently, the other two patients were also checked and their autozygome did not overlap with these two genes either. Therefore, we proceeded with exome sequencing to identify the likely cause with the assumption it could represent a novel cause of Alport syndrome.
Identification of two novel COL4A4 mutations by exome sequencing

Exome sequencing on the index revealed >50,000 variants. However, we started the analysis by examining the two autosomal recessive Alport genes COL4A3 and COL4A4 as well as the X-linked gene COL4A5. To our surprise, two novel truncating mutations were identified in COL4A4 (COL4A4:NM_000092.4:exon29:c.2420del:p.(Gly807Valfs*62)) and COL4A4:NM_000092.4:exon15:c.914_930del:p.(Phe306Glyfs*118)). These two mutations were confirmed by Sanger sequencing and perfectly segregated with the disease status within this sibship.

Gonadal mosaicism contributed to autosomal recessive Alport syndrome

To test whether the two mutations are in cis or in trans, we tested segregation in both parents. While the father was heterozygous for one mutation (COL4A4:NM_000092.4:exon29:c.2420del:p.(Gly807Valfs*62)), the other mutation (COL4A4:NM_000092.4:exon15:c.914_930del:p.(Phe306Glyfs*118)) could not be detected in maternal blood. Mendelian check of the genomewide genotypes was consistent with the identity of both parents. The likely explanation of this, therefore, is maternal gonadal mosaicism (with or without somatic mosaicism which could not be ruled out). However, because paternal gonadal mosaicism remains theoretically possible, we examined the haplotype on which the COL4A4:NM_000092.4:exon15:c.914_930del:p.(Phe306Glyfs*118) mutation resides and found that all three patients have inherited this allele on the maternal haplotype confirming maternal gonadal mosaicism as the most likely source of this mutation (Fig. 1).

Discussion

When parents are consanguineous, homozygosity for a common ancestral recessive mutation is the likely cause of the recessive disease in their children. This likelihood correlates directly with the prevalence of

![Fig. 1](image)

Pedigree showing double-cousin parents with three children with Alport syndrome and their haplotype analysis (each parental haplotype is shown in a different color for easy tracking) using SNPs surrounding the COL4A4 locus. Note that all three affected children have the same maternal haplotype (shown in light blue) despite lack of mutation in maternal blood suggesting maternal germline mosaicism.
the disorder such that the rarer the disease is the less likely it is to be caused by two independently inherited alleles from the two consanguineous parents (8). Indeed, the odds are compelling in favor of a homozygous ancestral mutation as the cause of the very rare autosomal recessive form of Alport syndrome we observe in this family with double cousin parents. One may argue that the homozygosity scan performed on the index was unnecessary and sequencing ofCOL4A3 andCOL4A4 directly would have identified the unexpected compound heterozygosity without the need for exome sequencing. However, the very large size of these genes, the low cost of homozygosity scan in a lab such as ours where high throughput genotyping is performed routinely on a large scale encouraged us to consider this method as a screening tool before committing to direct sequencing (9).

Regardless of the methodology used, compound heterozygosity was eventually identified for two novel mutations inCOL4A4. This in itself is highly unusual in the setting of double consanguineous parents, but should serve as a reminder that excluding candidate genes by homozygosity scan, is not always infallible and is one potential pitfall of autozygosity mapping (7). Indeed, we have pursued exome sequencing in this family because lack of homozygosity at either of the autosomal recessive Alport loci suggested to us that we may be dealing with a novel autosomal recessive Alport locus. More surprising was the identification of gonadal mosaicism as the source of the maternal allele for this compound heterozygosity since this is highly unusual in the setting of autosomal recessive inheritance. This has important implications as interest grows in the use of next-generation sequencing to identify carrier status of recessive diseases in preconception carrier testing (10). Since healthy individuals carry a few pathological recessive alleles on average, it is inevitable that the two potential parents will be told of their carrier status of several disease genes, but if the two lists of genes do not overlap then there is usually a sense of safety. Our results suggest that the rare, but real, risk of gonadal mosaicism should be discussed when counseling such parents, in addition to considering baseline de novo rate in general. This is a reminder of the importance of professional genetic counseling because these rare occurrences need to be considered in the pretest preparation and posttest interpretation of the results. Our report also raises an interesting question about the assumption of UPD as an explanation of finding a homozygous mutation in the presence of only one affected parent; how many of these represent gonadal mosaicism? The answer remains unknown, but we believe this scenario is highly unlikely, simply because the odds are not in favor of the gonadal mutation affecting exactly the same nucleotide as in the other familial allele. Fortunately, study of the surrounding haplotype of the mutation should easily make the distinction between UPD and gonadal mosaicism in that highly unlikely hypothetical scenario.

In summary, we show that it is possible for the source of the second pathological allele in an autosomal recessive disease to be gonadal mosaicism and this should be taken into account when counseling parents where only one parent is a carrier.

References