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LETTERS

Fabry or not Fabry – a question of ascertainment

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Fabry disease, an X-linked sphingolipidosis,[1] is the most common lysosomal storage disease in Norway.[2] Affected individuals may be offered intravenous enzyme replacement therapy, every second week at a cost of about € 200 000 per year for an average adult. To prevent organ damage, early treatment is probably the best. Accordingly, Fabry disease screening of patients with renal failure, cardiomyopathy, stroke and small-fiber neuropathy has been advocated.[3] If Fabry disease is detected, the family can be offered diagnostic follow-up and treatment of mutation carriers.

Recently, the diagnosis of Fabry disease in both transplanted kidneys from a 16-year-old male traffic victim was published.[4] Kidney graft biopsy samples at the time of transplantation and at 3 and 12 years later showed large and constant amounts of Fabry-specific lipid deposits, mainly in the podocytes. Furthermore, the alpha-galactosidase A activity in a donor plasma sample at the time of transplantation was 0.3 nkat/l (normal range 2.3–9.9), that is, the same level as in classical Fabry disease males.[4] Fourteen years later (this study), a likely missense mutation of an evolutionary conserved isoleucine residue, c.593C>T [p.I198T], was found in *GLA*, the alpha-galactosidase A gene, in an archived blood sample (Table 1). This amino acid substitution has a well-established pathogenic potential and constitutes about 1% of all missense changes in the Human Gene Mutation Database (http://www.hgmd.org). Retrospectively, there were no indications of clinical disease, that is, no history of pain in hands or feet, and no records of poor sweating or unexplained fever episodes. However, it is well known that clinical symptoms of Fabry disease can be minimal or remain unnoticed at young age, also in males.

Thirteen years after the kidney transplantation, a diagnostic follow-up of the donor's family was initiated. The boy's mother and the mother's three sisters, now 54–64 years old, were diagnosed with from 10 to 61% of lower normal enzyme activity levels in blood leukocytes (from 2.2–13.4 μkat/kg protein in the presence of *N*-acetylgalactosamine inhibitor, normal range 23–38), as commonly found in Fabry heterozygotes (Table 1). His mother also had elevated plasma and urine globotriaosylceramide (GL-3) levels. However, as in her son, Fabry-related symptoms were lacking, and our baseline Fabry investigations gave normal findings (Table 1). Diagnostic kidney biopsies, performed in two of the mother's sisters, revealed completely normal findings in one and only a single cell (podocyte) with myelin figures in the other. All the sisters' 11 children (9 boys and 2 girls) were healthy and asymptomatic.

The index patient certainly fulfilled major diagnostic criteria for a Fabry disease diagnosis – but would he have become clinically affected? The family history suggests otherwise: the maternal grandmother was *GLA* c.593C>T-negative, and the maternal grandfather had died at an age of 91 years after a life as a healthy man. Even though confined germline mosaicism for a *GLA* mutation could be an explanation for his lack of symptoms,[5] we find it more likely that this previously unknown *GLA* missense change is non-penetrant. The major reason for this is the normal kidney biopsies in two of his daughters. Characteristic morphological changes in the kidneys are unequivocal findings in both male and female Fabry patients, from early childhood and onward.[6] In addition, the *a priori* likelihood that nine grown-up sons of female *GLA* mutation carriers have always been completely asymptomatic, if there really is Fabry disease in the family, is <1%.

The prevalence of such biochemically true-positive but clinically false-positive alleles in metabolic enzymes is unknown. We suggest calling them as fringe alleles. Such alleles are most likely found upon screening of low-risk group, like in general newborn screening. In Fabry disease, pharmaceutical companies advocate low threshold for testing and family follow-up. This family exemplifies that diagnostic skepticism may be warranted if clinical support of a metabolic condition is lacking. In the era of expensive orphan drug therapy for an increasing number of metabolic disorders, this letter is meant as a word of caution against the prevailing view that is reminiscent of a Robbie Williams hit: Let me ascertain you!

Table 1 Findings in female carriers of the *GLA* missense change

	Sister 1 (donor's mother)	*Sister 2*	*Sister 3*	*Sister 4*
GLA c.593C>T [p.Ile198T]	+	+	+	+
Blood α-gal A activity (μkat/kg prot, ref. 22–36)	2.2	3.4	6.3	13.4
Plasma GL-3 (μmol/l, ref. 1.6–3.3)	3.9	2.9	2.3	3.0
Urine GL-3 (ref. <10 μmol/mol creat)	11	0.26	0.37	0.16
GFR (ml/min/1.73 m^2)	89	79	109	105
U-microalbuminuria (mg/nmol creat, ref. < 2.5)	0.6–0.7	0.6–1.0	1.1–1.7	0.8–1.2
EKG	*N*	*N*	*N*	*N*
LV mass (g/m$^{2.7}$, ref. 18–44)	60	37	38	38
Kidney biopsy	n.d.	n.d.	*N*	*N*
Cerebral MRI with MR–angio	~*N*	*N*	~*N*	~*N*
Skin angiokeratomas	none	none	none	none
Eye examination (eg cornea verticillata)	*N*	*N*	*N*	*N*
Audiometry	*N*	*N*	*N*	*N*
Thermotest (for thin-fiber neuropathy)	*N*	*N*	~*N*	~*N*
Offspring	2 boys	2 boys/1 girl	3 boys	2 boys/1 girl
Other findings	Hypertension	Healthy	Healthy	Healthy

Abbreviations: +, 'present'; *N*, normal; ~*N*, nearly normal (minimal changes); n.d., not done; ref., reference interval.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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The causality of *de novo* copy number variants is overestimated

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The application of array CGH or chromosomal microarrays is causing a revolutionary change in clinical genetics and especially cytogenetics, as it enables the genome wide identification of submicroscopic copy number variations (CNVs).[1] Given the significant increase in diagnostic yield compared with conventional karyotyping in patients with intellectual disability (ID) and the technical ease of use, the technique is now recommended as a first tier diagnostic test for patients with ID and/or multiple congenital anomalies (MCA).[2,3] Arrays not only enable detection of disease-causing CNVs in patients with ID/MCA, but also in patients with isolated heart defects, neurological diseases and psychiatric disorders. Therefore, besides pediatricians and clinical geneticists, more and more other medical specialists request array analysis arrays.[4–7] In addition, there is a rapid implementation of array CGH in prenatal diagnosis.[8–11]

For a small subset of CNVs, the association with an ID/MCA phenotype is beyond doubt. However, many CNVs detected using high-resolution arrays remain private, or the functional relationship with the phenotype is, at best, vague. To enable genotype–phenotype correlations, databases collecting phenotypes and genotypes have been established.[3,12,13] Although those databases have been successful in establishing a functional relationship for recurrent CNVs, a genotype–phenotype relationship has yet to be established for a majority of rare CNVs. Variable expressivity and reduced penetrance often confound significant associations to be made and is especially challenging for rare variants.[1] Due to the difficulty of associating CNVs with a phenotype, several reports provide guidelines on how clinical laboratories can interpret array results. If a clear association between the phenotype under investigation and the CNV is lacking, a series of steps are guiding the interpretation of the clinical significance. In all guidelines, a rule of thumb is that *de novo* CNVs, not occurring in normal individuals, are considered causal for the abnormal phenotype.[1,3,14–16]

During our screen of patients with mental retardation and developmental disorders, we identified several private *de novo* CNVs. Previously, we reported a 250 kb *de novo* deletion in C20orf133, nowadays known as *MACROD2*, in a patient with Kabuki syndrome.[17] A highly conserved region of C20orf133, likely to have a role in chromatin or chromosome biology, was deleted, and the gene was shown to be expressed in mice in the tissues affected in Kabuki syndrome. Although screening of 62 Kabuki syndrome patients failed to identify mutations in C20orf133, the disorder was hypothesized to be genetically heterogeneous.[17,18] Recently, mutations were identified in *MLL2* in 33 out of 50 Kabuki syndrome patients.[19] Sequencing of this gene in the patient with the C20orf133 deletion, shows the presence of a *de novo* mutation in *MLL2*.[20]

Most recently, we had a similar experience with another *de novo* CNV. During a screen of patients with ID and eye disorders, we identified a *de novo* 86.5 kb deletion in a patient referred because of an eye malformation associated with mental retardation. The deletion harbored only a single gene, *AMBRA1*. As the gene is expressed in the neural retina and brain, and mice knock-outs result in exencephaly,[21] the deletion was considered a likely cause for the observed phenotype. To further establish the clinical relationship between the gene and the phenotype, morpholino knockdowns were performed in zebrafish, which resulted in eye coloboma as well as equilibrium defects. On the basis of these observations and during the preparation of a manuscript, a more detailed phenotypic description of the patient was requested. The patient was characterized by bilateral coloboma, anosmia, disturbance of the equilibrium and ID, a CHARGE like phenotype. Once the diagnosis of CHARGE was uttered, a mutation analysis of *CHD7* was instigated.[22] A *de novo* mutation was identified causing a splice site mutation, thus disrupting the *CHD7* gene.

Although smaller modifier effects of the deletions on the phenotype cannot be excluded, the overall phenotype can in both cases be explained by *de novo* point mutations rather than to *de novo* CNVs. Note that CNV detection in both cases was performed on DNA extracted from blood and not on cell cultures. The latter are known to accumulate chromosomal rearrangements and hence, its use for CNV detection should be avoided for clinical diagnosis. We believe that these two case reports are representative of a larger number of misinterpretations that are currently made in diagnostic laboratories