Hereditary angioedema in 2 sisters due to paternal gonadal mosaicism

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CLINICAL IMPLICATIONS

We report the occurrence of type I hereditary angioedema in two sisters with unaffected parents caused by paternal gonadal mosaicism. Gonadal mosaicism is a rather uncommon genetic phenomenon, but nevertheless, correct diagnosis is important since it may affect further family planning.

Keywords: C1 esterase inhibitor; hereditary angioedema; mosaicism; SERPING1 mutation; diagnosis

Word count: 1167 words

To the editor,

Hereditary angioedema (HAE) is a heritable disorder that is characterized by recurrent, circumscribed, non-pitting, non-pruritic, often painful subepithelial swellings of sudden onset, that generally fade during 48-72 hours (1). Patients with HAE experience angioedema because of a defective control of the plasma kinin-forming cascade. Type I and type II HAE are autosomal dominant conditions resulting from heterozygous mutations in the *SERPING1* gene that encodes the serpin peptidase inhibitor (C1 esterase inhibitor – "C1-INH")(1). Type I HAE is characterized by low serum levels of C1-INH. In type II HAE, serum levels of C1-INH are normal or even elevated but the protein is dysfunctional. Deletions, nonsense or frameshift mutations in *SERPING1* usually result in HAE type I. Type II HAE is rather caused by missense mutations in *SERPING1* leading to a dysfunctional C1-INH protein (2). Mutations usually reside in exon 8 that codes for the reactive site of the protein.

Here we report a new missense mutation associated with type I HAE in two sisters with unaffected parents. We show that the father has gonadal mosaicism and discuss the genetic counselling issues related to parental germline mosaicism.

Table 1 displays the clinical and laboratory findings in the family members in 2015. Both affected sibs had low C4, C1-INH function and C1-INH plasma concentrations, consistent with a type I HAE. The parents and non-affected sister displayed normal C4 and C1-INH values. The oldest daughter (proband) had her first bouts in 2009, at age 17 years, after initiation of a contraceptive containing ethinylestradiol 20 µg (Yaz®, Bayer, Diegem, Belgium). These bouts mainly involved extremities, labia majora and intestines. For 5 years, the diagnosis of a HAE was overlooked and she presented with repetitive oedema that only subsided when her oestrogen-containing contraceptive was switched to a 68 mg etonogestrel implant (Implanon

NXT®, Organon, AB Oss, The Netherlands), which is a progesterone analogue. The implant was removed because of local discomfort and replaced by a contraceptive containing oestradiol 1.5 mg (Zoely, Teva, Haarlem, The Netherlands). Soon after, oedema's reoccurred until we diagnosed type I HAE in mid-2015 and when she became asymptomatic after switching her contraceptive to desogestrel 75 µg (Cerazette®, Organon). Meanwhile, in 2014, at age of 17 years, her youngest sister also developed spontaneous oedema attacks, mainly of lips, extremities and of the viscera. Main triggers appeared to be stress and start of a contraceptive containing 35 µg ethinyl estradiol (Diane 35®, Bayer, Diegem, Belgium).

In the affected sisters, the *SERPING1* gene was analysed. Bidirectional Sanger sequencing of the seven coding exons and their intron-exon boundaries as well as MLPA analysis of the eight exons (SALSA MLPA P243 SERPING1, MRC-Holland) were performed on gDNA isolated from blood lymphocytes to maximize the chances for identifying a mutation. Analysis revealed the heterozygous presence of a single nucleotide change in exon 3, predicted to result in a threonine to isoleucine substitution at residue 179 ([NM_000062.2] c.536C>T;p.Thr179Ile) of the protein. This specific missense mutation has not been reported in databases of normal variation (ExAC, 1000 genomes, GoNL, gnomAD) but was previously identified once in our cohort of 50 patients analysed because of HAE. According to various prediction programs (Polyphen-2, Mutation Taster, SIFT – GRCh37 build), this missense mutation is considered as likely pathogenic. To prove the pathogenicity of this nucleotide change, we investigated the segregation of the mutation among unaffected relatives. The nucleotide change was not found in the lymphocytes of the parents, hereby adding more evidence that the change is a pathogenic missense mutation. Furthermore, we genotyped 30 SNPs surrounding the mutation in both affected sisters and their parents (Table 1, repository file). This analysis revealed the same haplotype in both sisters, supporting the hypothesis of parental gonadal

mosaicism and excluding non-paternity. Subsequently, we examined the sperm of the father. This analysis clearly showed the presence of the p.T179I mutation in a fraction of the sperm cells (figure 1). The mutation was undetectable in his lymphocytes which explains the absence of clinical signs and laboratory abnormalities.

The occurrence of an autosomal dominant disorder in more than one child from unaffected parents should prompt the clinician to consider the possibility of somatic or gonadal mosaicism in one of these parents, certainly when non-paternity has been excluded. Mosaicism refers to the presence in an individual of normal and abnormal cells that are genetically distinct but are derived from a single zygote. Somatic mosaicism refers to the presence of the mutation in some of the somatic cells whereas gonadal (germline) mosaicism indicates that the mutation is restricted to gonadal tissue (3, 4). These mutations occur after fertilization and during postzygotic growth of the embryo. Most individuals with somatic mosaicism will show no or few clinical signs of the disorder, depending on the percentage and nature of the somatic cells carrying the mutation. By definition, individuals with gonadal mosaicism are clinically normal.. In both instances, there is an increased risk for having multiple affected offspring. This will depend on the percentage of germ cells affected by the mutation, which for obvious reasons cannot be determined accurately. The consideration of somatic and gonadal mosaicism is therefore important for genetic counselling.

Somatic and gonadal mosaicism are uncommon but have been reported in several genetic disorders such as neurofibromatosis type 1, osteogenesis imperfecta and Duchenne muscular dystrophy. Its occurrence may be underestimated in HAE since 25-30% of the cases have so-called new or “de novo” *SERPING1* mutations without obvious family history. Furthermore, gonadal mosaicism has been reported in 2 unrelated families with HAE (5, 6). Somatic

mosaicism may be more frequent than we suspect since it can be missed using conventional Sanger sequencing of blood DNA. The newly technologies of second generation sequencing are more sensitive to detect somatic mosaicism because of the massively parallel sequencing approach allowing more deep sequencing (4).

The missense mutation we report here, is located at the beginning of the Serpin domain in a moderately conserved part of the gene. HAE types I and II are characterized by a high allelic heterogeneity with almost each family carrying their own "private" mutation in the *SERPING1* gene. The clinical severity of the disease does not seem to be strictly related to the type of mutation. Although missense mutations (usually in exon 8) are characteristic of type II HAE, missense mutations can also be found in type I HAE. They are usually located in less conserved regions, including exon 3, as is the case in this family (7, 8). In a rather small group of patients with type I HAE, Speletas *et al*. (9) found that missense mutations were associated with a significantly later disease onset and a lower probability of manifesting HA attacks before the 10^{th} year of age, as is exemplified by this family.

In conclusion, we report here a family with parental gonadal mosaicism for a novel missense mutation in SERPING1 detected in 2 affected sisters with HAE type I. This finding is important since it has implications for further family planning. Individuals with germline mosaicism should be counselled about the increased risk of having multiple affected children.

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