Twins as a tool for evaluating the influence of genetic susceptibility in thyroid autoimmunity

Les jumeaux : un outil d’évaluation de l’influence de la susceptibilité génétique à l’auto-immunité antithyroïdienne

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Abstract

By means of large twin cohorts, it has been possible to provide relatively valid and unbiased data regarding the influence of genetic and to some extent epigenetic factors in the aetiology of thyroid autoimmunity. The comparison of concordance rates between monozygotic and dizygotic twins provides irrefutable evidence of a genetic component in the aetiology of both Graves’ disease and Hashimoto’s thyroiditis, as well as for harbouring thyroid autoantibodies. Biometric modelling shows that approximately 75% of the total phenotypic variance in autoimmune thyroid disease is due to genetic effects. Despite the well known gender difference in the prevalence of autoimmune thyroid disease, the analyses suggest that it is the same set of genes that operate in males and females. The lack of complete phenotypic concordance in monozygotic twin pairs indicates that also environmental and/or epigenetic factors are of importance. The impact of specific environmental and epigenetic exposures can be evaluated by investigating disease discordant twin pairs. Our studies show that skewed X chromosome inactivation is associated with clinically overt AITD but not with the presence of TPOAb in euthyroid individuals. It is now recognized that twin studies offer several features that uniquely enhance our ability to localize genes and understand their function. Future twin studies will incorporate information on genetic and epigenetic variation making it possible to quantify the precise effect of specific susceptibility genes and/or epigenetic variation on estimates of heritability.

Keywords: Twins; Autoimmune thyroid disease; Epigenetic; X chromosome inactivation

Résumé

L’étude de larges cohortes de jumeaux a rendu possible l’obtention d’informations relativement solides et non biaisées, sur l’influence de facteurs génétiques et jusqu’à un certain degré épigénétiques impliqués dans l’étiologie de l’auto-immunité antithyroïdienne. La comparaison des niveaux de concordances entre jumeaux mono- et di-zygotes apporte la preuve irréfutable de l’implication génétique dans l’étiologie de la maladie de Basedow, la thyroïdite de Hashimoto, et dans l’expression des auto-anticorps antithyroïdiens. Le modèle biométrique révèle qu’approximativement 75 % du total de la variance phénométique est lié à la génétique. En dépit de la prédominance féminine bien connue des maladies auto-immunes, les analyses suggèrent que c’est la même série de gènes qui intervient chez l’homme et chez la femme. L’absence de parfaite concordance phénométique dans les paires de jumeaux monozygotes indique aussi l’importance de facteurs environnementaux et/ou épigénétiques. L’impact de l’exposition aux facteurs environnementaux ou épigénétiques peut être évalué par l’investigation des paires de jumeaux discordants. Nos études démontrent que le biais d’inactivation du chromosome X s’associe aux maladies auto-immunes apparentes, mais non à la présence d’auto-anticorps anti-TPO chez les sujets euthyroïdiens. Il est maintenant reconnu que les études des jumeaux offrent différentes données utiles uniquement pour une meilleure connaissance de la localisation des gènes et de la compréhension de leurs fonctions. Les études futures sur les jumeaux incorporeront les informations sur les variations génétiques et épigénétiques. Elles rendront possibles la quantification d’un effet précis de la susceptibilité du gène et/ou de la variation épigénétique sur l’estimation de la transmission.

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Mots clés : Jumeaux ; Maladie thyroïdienne auto-immune ; Épigénétique ; Inactivation de l’X

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1. Introduction

The vast majority of human diseases, autoimmune thyroid disorders (AITD) being no exception, are complex traits with multiple genetic, epigenetic and environmental variables contributing to the development of the disease. AITD are common. The prevalence of clinically overt disease, such as Graves’ disease (GD) and Hashimoto’s thyroiditis (HT), is approximately 2% in women and 0.2% in men, while subclinical disease, reflected by the presence of thyroid autoantibodies (i.e. thyroid peroxidase antibodies [TPOAb] and thyroglobulin antibodies [TgAb]) in euthyroid individuals, is approximately 10 fold higher [1,2]. From a clinical point of view AITD cover a wide spectrum of phenotypes. Roughly, patients can be divided into those with hyperthyroidism – as seen in GD, those with hypothyroidism – reflecting HT, and into a large group of euthyroid subjects who harbour thyroid autoantibodies. Although these phenotypes are very different, it is generally believed that they share a number of common aetiological factors. Clearly, all three phenotypes frequently occur in different members of the same family [3,4] and some individuals progress from one phenotype to another. Moreover, around 15% of individuals with GD develop spontaneous hypothyroidism 10–15 years after treatment with a course of an antithyroid drug [5].

Although AITD’s are among the most common autoimmune disorders, their aetiology is still a puzzle. Despite a vast number of studies, the reason for development of AITD in certain individuals is still incompletely understood [3,6]. In fact, no single gene has been shown to be either necessary or sufficient for the development of AITD [3]. The same is certainly true for any suggested environmental factor [6,7].

The use of twins is a well established method for investigating the relative importance of genetic and environmental factors to traits and diseases [8]. In the following we will briefly describe the basic concepts of twin methodology, and thereafter highlight the results of recent twin studies investigating the impact of genetic and epigenetic factors in AITD.

2. Twin studies – basic concepts

2.1. Classic twin study

The classic twin study exploits that the twin individuals within each monozygotic (MZ) twin pair (also named identical) carry identical genes, and therefore any differences between them are solely due to the influence of the environment. In contrast, dizygotic (DZ, also named fraternal) twins share, as ordinary siblings, approximately 50% of their genes. It follows that differences between DZ twins are due to a combination of genetic and environmental factors. A higher degree of similarity (correlation or concordance) with respect to the presence of a given trait or disease among MZ twins than among DZ pairs can be taken as evidence of a genetic contribution to its aetiology [8]. Moreover, by applying quantitative analytical techniques to twin data, it is possible to separate and estimate the impact of genetic and environmental influence on the propensity to develop a given phenotype [8,9]. This provides an estimate of “heritability” which is a measure of the extent to which phenotypic variation in the population can be explained by genetic variation [8].

To derive a valid estimation of the heritability requires, however, that a number of issues are taken into account [10,11]. The most important being the assumption that the phenotype investigated is not influenced by gene–gene or by gene–environment interaction. Concerns have been raised about the validity of the classic twin methodology [12,13], since such interactions are likely present in most, if not all, phenotypes with a complex aetiology. However, when tested, twin methodology has been proven to be a robust and valid method [8,9].

2.2. Twin case-control study

The co-twin control method is well suited for studying the influence of specific risk factors in the development of traits and diseases. This method is based on the classic matched case-control design [14]. Using this approach, twin pairs who are discordant for a trait or disease are considered as matched pairs. In other words, when studying discordant twin pairs, the healthy co-twin serves as a control for the affected twin. Because the twin pairs, especially the MZ pairs, are matched for genetic, intrauterine and early childhood environment, this design controls for confounding by these factors. Thus, if the association between a risk factor and AITD is due to genetic confounding the association would be observed within DZ pairs, but not within MZ pairs.

3. Classic twin studies in autoimmune thyroid disease

There are only a few twin studies investigating the presence of clinically overt and well defined GD [15–17], HT [18], presence of thyroid autoantibodies [19,20], and distribution of TPOAb epitopic fingerprints [21] in population based twin cohorts. The results from these studies are summarized in table 1. Due to major differences in twin ascertainment, sample size, age and gender composition, definitions of phenotypes, evaluation of zygosity, as well as assays used for measurement of TPOAb and TgAb, it is very problematic to compare the individual studies. However, despite these differences, all studies have shown significantly higher degrees of similarity in MZ than in DZ twins, confirming the presence of genetic factors in the aetiology of these phenotypes. On the other hand, the fact that the concordance rates for GD, HT, as well as for presence of thyroid autoantibodies among MZ twins – were well below 100%, clearly demonstrates that environmental and/or epigenetic factors are also important.

Using structural equation modelling, we have found that 79% (95% CI, 38–90%) and 73% (46–89%) of the liability to the development of GD and thyroid autoantibodies (positive TPOAb and/or positive TgAb), respectively, is attributable to genetic factors [16,20]—this is the heritability estimate.

It is well known that AITD is associated with gender, with a five to 10 fold higher frequency in women [22]. The reason for this female predisposition to AITD is at present obscure. By including opposite sex twin pairs in the model-fitting analyzes...
of TPOAb and TgAb we have been able to evaluate whether this phenotype is influenced by the same genes in males and females [20]. The heritability for TPOAb was estimated to 61% (95% CI, 49–70%) and 72% (64–79%) in males and females, respectively. For TgAb concentrations, the estimates were 39% (24–51%) in males and 75% (66–81%) in females. The difference in the size of the heritability estimate between males and females was significant for TgAb, but not for TPOAb. Whether this reflects a genuine difference between males and females in the etiology of harbouring TgAb remains to be established. In addition, the analyzes indicated that, for both TPOAb and TgAb, it is the same set of genes that operate in males and females [20].

4. Twin case-control studies and epigenetic factors in autoimmune thyroid disease

Biometric twin modelling indicates that environmental factors can explain 21–27% of the phenotypic variation with respect toAITD [16,20]. In line with this, a large number of specific environmental factors have been associated with the development ofAITD, the most important being the level of iodine intake and cigarette smoking [6,7]. Naturally, the impact of all suggested environmental risk factors can be evaluated by means of disease discordant twin pairs. At present, however, only the impact of smoking [23], birth characteristics [19,24,25], and infection with Yersinia enterocolitica [26,27]. In summary, these studies show that smoking and Yersinia infection, but not birth characteristics, is associated with an increased risk of clinically overtAITD.

Also the impact of epigenetic phenomena such as DNA methylation, histone modification patterns and X chromosome inactivation (XCI) pattern can be investigated using the twin case-control method. In recent years, we have used this approach to evaluate the impact of skewed XCI in the aetiology ofAITD [28,29].

Using disease discordant twin pairs, we have examined whether skewed XCI is associated with clinically overt AIITD [28]. In that study, we found that the frequency of skewed XCI in female twins withAITD, GD and HT was 34%, 37% and 31%, respectively, which was higher than the prevalences in the corresponding twin control populations, 11% (p = 0.003), 14% (p = 0.045) and 8% (p = 0.057), respectively. Overall, skewed XCI was associated with an increased risk of developingAITD, odds ratio 9.0 (95% confidence interval 1.64 – 49.4), p = 0.022, suggesting a possible role of XCI in the aetiology of overtAITD among women. In accordance with these results we have also found a statistically significant positive association between the degree of XCI and the serum concentrations of TPOAb in euthyroid subjects [29]. In order to evaluate whether this association was causal or could be explained by the presence of genetic or environmental confounders we also analysed the association between XCI and TPOAb within MZ and DZ pairs. Despite similar distributions of XCI and TPOAb between MZ and DZ twins, stratification according to zygosity had a major impact on the results. The association was significant within the DZ pairs whereas the relationship was non-significant within the MZ pairs, indicating that genetic factors play a major role in the observed association. In other words, XCI per se does not seem to play a role in the aetiology of TPOAb. More likely, XCI and TPOAb are influenced by common genetic determinants (presence of genetic confounding). Whether this is also the case in clinically overtAITD remains to be clarified.

5. Conclusions

By means of large twin cohorts, it has been possible to provide relatively valid and unbiased data regarding the influence of genetic and epigenetic factors in the aetiology of thyroid autoimmunity.

The comparison of concordance rates between MZ and DZ twins provides convincing evidence of a genetic component in the etiology of GD, HT, thyroid autoantibodies (TPOAb and TgAb), as well as for harbouring the IDRA epitope of TPOAb. Biometric modelling shows that approximately 75% of the total phenotypic variance is due to genetic effects. Despite the well known gender difference inAITD, the twin analyzes suggest that it is the same set of genes that operate in males and females.

The comparison of the degree of XCI in twin pairs discordant for overtAITD shows that skewed XCI is associated with an
increased risk ofAITD. In contrast, the association between XCI and TPOAb in euthyroid subjects is most likely due to inheritance of common X-linked susceptibility genes (genetic confounding).

6. Research pointers

The challenge faced by research into the genetic susceptibility ofAITD is to identify genes and epigenetic factors of relative small effect against a background of substantial genetic and environmental variation. It is now recognized that twin studies offer several features that uniquely enhance our ability to localize genes and understand their function, as well as investigate the impact of epigenetic as well as environmental triggers [8]. A non-exhaustive list of research pointers is given below:

- to studyAITD in DZ twins, knowing their precise matching for age, common family environment and background environmental variation, provides a means to enhance the power of conventional strategies to detect genetic influence via linkage and association;
- the unique genetic matching ofMZ twin pairs enables an accurate assessment of the pattern of differential gene usage through comparison of gene expression in MZ pairs that are discordant forAITD;
- twins have the potential to provide information on the ways in which genes and environmental factors interact. This can be approached through modelling of twin data from pairs that are discordant for environmental exposures;
- intervention studies in twins provide a means for assessing differential responses to environmental stimuli;
- extension of the classic twin study to involve multiple variables allows assessment of the degree to which the correlation between the circulating levels of TPOAb and TgAb (or between GD and HT) is influenced by the same set of genes (genetic pleiotropy);
- a comparison ofMZ twin pairs is an ideal design for studying epigenetic modifications. MZ twins are genetically identical individuals, and epigenetic differences between MZ twins would lead to discordance of the twins. Investigation of epigenetic differences (or similarities) such as DNA methylation and histone modification patterns in MZ twins may improve our understanding of a specific phenotypic outcome. Furthermore, by comparing the epigenetic status of MZ twins and DZ twins, it would be possible to estimate inherited and acquired epigenetic contributions.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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