A 20 year history of clinical and genetic study of thyroid autoimmunity in a Tunisian multigenerational family: Evidence for gene interaction

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ABSTRACT

Autoimmune thyroid diseases (AITD), which include Hashimoto thyroiditis (HT), Graves’ disease (GD) and primary idiopathic myxoedema (PIM), are recognized by their clinical and genetic heterogeneity. In this study, we have carried on a global approach gathering 20 year genetic and clinical data on a Tunisian multigenerational family (Akr). Our purpose was search for a combined genotype involved in AITD susceptibility using 33 gene polymorphisms. The Akr pedigree is composed of more than 400 members distributed on 10 generations. Clinical follow-up was performed by appreciation of the thyroid gland and measurement of both thyroid hormone and auto antibody levels. We used FBAT software to test for association between gene polymorphisms and AITDs. Clinical follow-up has showed that the number of AITD patients has increased from 25 to 78 subjects subdivided on 51 cases of GD, 22 PIM and 5 HT. Concerning genetic analysis, our study has revealed new gene association when compared with our previous analysis (considering single genes). Thus, PTPN22, TG and VDR gene polymorphisms have became associated with p-values ranging from $4.6 \times 10^{-2}$ to $4 \times 10^{-3}$ when considered with other.
genes on the same chromosome; giving evidence for gene interaction. The most significant association was found with the MHC region \((p = 7.15 \times 10^{-4})\). Moreover, and among gene polymorphisms explored, our analysis has identified some of them as AITD biomarkers. Indeed, PDS gene polymorphisms were associated with either exophthalmia or goiter \((p\)-values from \(10^{-2}\) to \(10^{-3}\)). In conclusion, our study gives evidence for gene interaction in AITD development confirming genetic complexity of these diseases.

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Introduction

Autoimmune thyroid diseases (AITD) (MIM 608173) are among the most common human autoimmune diseases, with a population prevalence of 2% in iodide-sufficient regions (Vanderpump, 2011). They include a number of conditions that share common cellular and humoral immune responses targeted at the thyroid gland. AITD include Graves’ disease (GD) (MIM 275000) and Hashimoto’s thyroiditis (HT) (MIM 140300). AITD are characterized by: i- the infiltration of the thyroid by T and B cells that are reactive with thyroid antigens and ii- the production of thyroid autoantibodies with the resultant clinical manifestations.

Biometric twin modeling showed that approximately 75% of the total phenotypic variance in AITDs is because of genetic effects (Brix and Hegedüs, 2012). Thus, genes known to play a role in AITD include mainly two types: i- immunoregulatory genes such as major histocompatibility complex (HLA), cytotoxic T lymphocyte associated 4 (CTLA4) (MIM 123890), CD40 (MIM 109535), vitamin D receptor (VDR) (MIM601769) and ii- genes involved in thyroid physiology such as thyroglobulin (TG) (MIM 188450), thyroid stimulating hormone receptor (TSHR) (MIM 603372), and Solute Carrier Family 26, Member 4 (PDS) (MIM 605646).

An additional class of genes implicated in AITDs is the protein tyrosine phosphatase (PTP) (PTPN2 (MIM 176887) and PTPN22 (MIM 600716)).

In a recent study, we have given evidence for a polygenic model corresponding to AITD where the different genes contribute each only a small risk of disease (Bougacha-Elleuch et al., 2011). Thus, if we consider the CTLA4 gene which is among the most replicated gene in AITD susceptibility, the corresponding relative risk is of just 1.3–1.7. On the other hand, despite the huge number of studies investigating genetic associations between genetic variants and AITDs, the evaluation of combinations of SNPs has been less commonly addressed. In literature, there is evidence that combinations of SNPs can be strongly associated with complex diseases than individual SNPs (Wan et al., 2009). In this regard, Wei et al. have given evidence for a greater effect of a combination of 17 SNP on chromosome 2q33 on GD pathogenesis (Wei et al., 2011). Moreover, the hypothesis of gene–gene interaction in AITDs is poorly investigated.

In the last decade, we have conducted both candidate genes and whole genome scan analysis of AITDs in a multigenerational family (Akr) (reviewed in 6). We have revealed genetic association with several susceptibility genes (Bougacha-Elleuch et al., 2012). Their contribution in AITD pathogenesis was of a middle magnitude. All these studies were dealing with the association of a “single” gene and neither of them has considered a “pool” of candidate genes. Giving the genetic complexity of AITD, we have carried on a global approach gathering all genetic and clinical data on Akr family. Our purpose was search for a combined genotype involved in AITD susceptibility.

In the present study, we give results of a 20 year clinical and biological follow-up of Akr family members. We have also searched for possible association of a combined genotype/haplotype in 33 explored gene polymorphisms with regard to AITD affection, as well as for a cumulative effect of several alleles from different associated genes. We report novel gene polymorphism associations giving evidence for gene interaction in AITD.

Subjects and methods

Subjects

In 1990 a follow-up survey of patients in the department of Endocrinology of CHU Hedi Chaker in Sfax, Tunisia, indicated the existence of numerous cases from the same region. The interview of the affected as well as their relatives has permitted establishment of the complete pedigree (Akr family). The Akr family members were
subjected to a regular clinical and biological follow-up since 1990. Up to 2010, more than 50 visits were made by a group of endocrinologists and geneticists. Sera from affected and unaffected members were tested for FT4 and TSH hormone levels, as well as for the presence of anti-thyroid peroxidase (TPO) and anti TG antibodies. The diagnosis of GD was based on the presence of biochemical hyperthyroidism, as indicated by a decrease in TSH, an increase in T4 levels and positive anti thyrotropin receptor, anti TPO and/or anti TG antibodies, in association with diffuse goiter or the presence of exophthalmia. HT was diagnosed by documented clinical and biochemical hypothyroidism requiring thyroid hormone replacement and presence of TPO auto-antibodies, with or without antibodies to TG, in the presence of a palpable goiter. Primary idiopathic myxoedema was diagnosed by the presence of hypothyroidism requiring T3 or T4 replacement. Patients with PIM have an atrophic gland.

Methods

In our previous studies, we have conducted candidate gene analysis ofAITD in Akr family. Investigated genes were those involved in both thyroid physiology and immune response (Table 1).

Statistical analysis

We used the FBAT software (Laird et al., 2000) to test for association between the gene polymorphism and the disease under “biallelic”, “additive” and “recessive” model. Meanwhile, the global haplotype tests of association were performed under “multiallelic” mode in HBAT. This test was conducted also to screen a pool of traits and markers in order to select the most “promising” combination of traits interacting with markers.

| Gene   | Localization | Studied polymorphisms       | Reference                  |
|--------|--------------|------------------------------|----------------------------|
| TNF RII| 1p36.22      | rs1061622                    | Kammoun-Krichen et al., 2008|
| PTPN22 | 1p13.2       | rs2476601                    | Chabchoub et al., 2006     |
| IL1A   | 2q13-21      | rs1800587, rs16944           | Kammoun-Krichen et al., 2007|
| IL1B   |              | rs1143634                    |                            |
| IL1RN  | 6p21         | VNR                          | Bougacha-Elleuch et al., 2004|
| HLA-A  |             | HLA-A                        |                            |
| HLA-B  |             | HLA-B, rs1800629 (CA)n       |                            |
| TNF    |              |                              |                            |
| HLA-DR |             | HLA-DR                       | Hadj-Kacem H et al., 2003  |
| HLA-DQ |             | HLA-DQ                       |                            |
| PDS    | 7q22.3       | D7s501, D7s496, D7s2459, D7s692|                            |
| Tg     | 8q24.21      | D8s1801, D8s1712, D8s558, rs180223, rs853326, rs853304, Tgms2, rs2076740, D8s529, D8s1796, D8s1710 | Belguith-Maalej et al., 2008 |
| VDR    | 12q13.11     | rs2228570, rs1544410, rs731236 | Maalej et al., 2008        |
| TNFRI  | 12p31.31     | (GT)17(GA)n, rs767455        | Kammoun-Krichen et al., 2008|
Results

Clinical data

The Akr family members have been subjected to a regular follow-up between 1990 and 2010. This continual survey has revealed an increasing number of affected members. The number of AITDs patients has increased from 25 to 78 (Fig. 1). The pedigree is currently composed of more than 400 members (including 78 affected individuals) distributed on 10 generations. The number of GD patients increases over the period mainly between 2004 and 2007. Concerning autoimmune hypothyroidism (AH), it has the same feature except a small decrease in the patient’s number between 2006 and 2007 due to the fact that many cases of GD were misdiagnosed earlier.

In 2010, AITD were diagnosed in 78 subjects (19.5% of Akr explored members) subdivided on 51 cases of GD and 27 cases of AH. This latter consists of 22 cases of PIM and 5 HT. All of these HT patients had hypothyroidism, except for one with a euthyroid state. Mean age of patients was 37 years (range, 7–70 years), sex ratio (F/M) was 1.68. Clinical and biological features of these patients are summarized in Table 2.

Among Akr patients, there were 13 subjects (10.74%) who were unaffected and have developed AITD later. The AH was more frequent seen (77% of the cases), while Graves’ disease was found in only 23% of the cases. In addition, 5 subjects had a goiter and/or positivity of anti thyroid antibodies meaning that they are predisposed to develop AITD. Their mean age was 25.25 years (19–31) and sex ratio (F/H) was 1.5.

Genetic analysis

Genotypes of 246 Akr members (68 affected and 117 unaffected members) were collected. They correspond to the 33 polymorphisms previously investigated in 14 genes involved in immunoregulatory or thyroid physiology (Table 1).

Statistical analysis was performed using FBAT. Akr pedigree was subdivided into 14 pedigrees including 76 nuclear families (Fig. 2). The different models were examined and the better scores were retained.

In a first step, we have considered two types of files: the first consists of markers belonging to the same chromosome (6 files were then generated) and the second encompasses all markers.

In a second step, we were interested in previously reported associated genes. Therefore, the corresponding associated allele was considered against all the remaining alleles.

For all these kinds of files, we have looked for association under three disease models: i- AITDs model: where AH and GD patients were considered as affected; ii- GD model in which we have considered only GD as affected and finally iii- AH model where only AH patients were considered as affected.

In all cases, we have looked for both single and haplotype associations. The most significant results found under this analysis are recapitalized in Table 3.

As we can see, it is clear from our analysis that there are improved results compared with previous analysis (considering single genes). Thus, in chromosomes 1, 8 and 12 the corresponding gene polymorphisms
PTPN22, Tg and VDR respectively) have became associated with p-values ranging from 0.046 to 0.004 and that only under a particular disease model, which is in general AITD model. Moreover, the D7S2459 microsatellite marker in PDS gene region was not associated before (Hadj Kacem et al., 2003) and in the current analysis it has given evidence for association with its 6th allele (Table 3). Concerning the MHC region, it shows the most significant association. Thus, the HLA-DR gene has given improved p-value when considering the AH disease model \( p = 7.15 \times 10^{-4} \). The remaining genes have not shown any improvement compared with previous analysis. Using HBAT, we did not find any associated haplotype in a given chromosome.

In a second step, we have looked for any correlation between the different AITD traits (age at onset, goiter …) and individual genotypes for the 33 studied markers, using FBAT. Our analysis has given evidence for association between 5 gene polymorphisms and the explored disease traits (Table 4). Moreover, we reported particular association between D7S501 on one side and GD, exophthalmia on the other side \( (p = 0.0016 \) and \( p = 0.015 \) respectively). On the other hand, D7S501 and D7S2459 have given evidence for association with goiter \( (p = 0.009 \) and \( 0.029 \) respectively).

### Discussion

In the present study, we report results of a continual survey during 20 years of the Akr family members. To the best of our knowledge, Akr family with such high prevalence of AITDs is among the rare families in the world. Indeed, this large pedigree is issued from a restricted number of founders, and consequently, it could be considered as a genetically homogeneous sample.

This family lives in an area belonging to the governorate of Sidi Bouzid, which is located in the middle east of Tunisia. It covers 35,279 ha and the number of habitants was estimated at 33,979 in 2004 by the National Institute of Statistics. This multigenerational family is characterized by its high consanguinity. Thus, the mean of consanguinity was estimated at 3% in Akr family and 2.1% in the studied district. This latter value is nearly the same reported in the governorate of Sidi Bouzid. Indeed, in the Tunisian population, the consanguinity rates differ significantly between the different governorates. Particularly, the governorate of Sidi Bouzid has the highest rate \( (2.13\%) \) (Chalbi and Zakaria, 1998).

Moreover, and according to our previous epidemiological study, conducted in Akr district, the prevalence of AITD in the studied region was about 43.64‰ \( (59.91\% \) and 29.72‰ for women and men respectively). The prevalence of GD and AH was 23.31‰ and 21.33‰ respectively. The estimated incidence of AITD was 7.21 per 1000 inhabitants in the studied region (Bougacha-Elleuch et al., 2011). Therefore, with such high frequencies, AITD constitute a public health problem in this region particularly with its low life level. Indeed, the rural population was estimated at 84%. In this regard, this survey was particularly interesting for this “poor” district. In fact, and thanks to this clinical follow-up, Akr district inhabitants were regularly examined by endocrinologists and the corresponding treatment was regularly delivered to affected members. Moreover,

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| Table 2 | Clinical, demographic and biological features of Akr affected members. |
|---------|-------------------------------------------------------------|
|         | PIM | HT | GD |
| Number  | 22  | 5  | 51 |
| Age at onset (years)\(^a\) | 42.38 (9–65) | 38.8 (33–50) | 29.84 (7–70) |
| Sex ratio (F/M) | 2.66 | 4 | 1.32 |
| Goiter | 0 | 0.6 | 0.58 |
| Homogeneous | – | 0.67 | 0.7 |
| Heterogeneous | – | 0.33 | 0.1 |
| Multi nodular | – | 0 | 0.2 |
| Exophthalma | 0 | 0 | 0.51 |
| Associated diseases | 0.27 (T2D, GJS, CGN, Vitiligo) | 0.4 (T2D, CGN) | 0.15 (T1D, GJS, Addison, polycythemia, vitiligo) |
| Treatment | | | |
| ATS | 0 | 0 | 0.60 |
| LT4 | 0.86\(^b\) | 0.8\(^b\) | 0 |
| IRA | 0 | 0 | 0.23 |
| Surgery | 0 | 0 | 0.17 |

\(^a\) Mean age with the age range between cotes.

\(^b\) The remaining patients were lost sight.
this clinical survey has permitted an early diagnosis for many “predisposed” individuals (Akr and nonAkr family members). In this respect, 100 individuals affected with AITD were diagnosed in this region during the survey. They are subdivided on 78 from Akr family (Table 2) and 22 (14 AH and 8 GD) belonging to other 5 families living in the same district (unpublished results). All these patients have regularly benefitted of a clinical follow-up. On the other hand, and thank to this tight survey, we have revealed 2 multigenerational and consanguineous families harboring 40 cases with congenital hypothyroidism. These families are currently under clinical and genetic investigation (unpublished results). Therefore, the studied district, located at the middle east of Tunisia, could be considered as a “hot spot” region for thyroid defects.

It is already clear that AITD cover a spectrum of phenotypes. From a clinical point of view, patients can be categorized into those with hyperthyroidism, reflected by GD, those with hypothyroidism—mirrored by AH, and into a large group of euthyroid subjects who harbor thyroid auto antibodies. Both GD and AH and euthyroid subjects with positive thyroid auto antibodies, are characterized by lymphocytic infiltration of the thyroid gland accompanied with both humoral and cellular immune system activations. In particular, this is so during the active phase of disease, when thyroid auto antibodies and activated T cells are presented in the circulation (Weetman, 2001). Moreover, all three phenotypes are frequently seen in different members of the

Fig. 2. Akr family pedigree. ■ ● Affected members. □ ○ Unaffected members. A: The full Akr pedigree. B: The 14 pedigrees extracted from Akr family.
same family (Tomer et al., 2003; Taylor et al., 2006) and quite commonly, a transition from one disease to another is seen. This could be explained by a common genetic background for these phenotypes. This observation was already seen in Akr family. Indeed, and in parallel with confirmed patients, 121 control members of the Akr family were followed during 20 years. Clinical and biological investigations have revealed 13 subjects (10.74%) who were euthyroid, with a positivity of anti-Tg and/or anti-TPO auto antibodies and a possible presence of a goiter. Later, they have developed AITD. The AH was more frequent seen (77% of the cases), while GD was found in only 23% of the cases. Moreover, we have depicted 5 other members who are currently still euthyroid with a positivity of anti-Tg and/or anti-TPO auto antibodies and a goiter. Given familial history of AITD in our case, the number of euthyroid persons who have developed AITDs was more important than that reported in a prospective study in Netherlands (Effraimidis et al., 2011). In this latter, authors described a natural transition from euthyroid to overt autoimmune hypo or hyperthyroidism in 51 cases among 790 women after a follow-up of 5 years.

Given the complex nature and the existing evidence for genetic heterogeneity of AITD, and in order to reduce genetic heterogeneity, we have targeted our genetic study to an exceptional multigenerational family (Akr). Thanks to this large pedigree, we have already, during the last decade, explored a variety of candidate genes (reviewed in 6).

On the other hand, it’s widely accepted that complex diseases are not controlled simply by an individual gene or DNA variation but by their combination. In literature, there is paucity in the field of gene interaction. Among few studies on AITD, it has been already reported a combination of 17 SNPs in the 2q33 region associated with GD (Wei et al., 2011). Concerning Akr family, all candidate gene studies performed were pointed to investigation of single genes. In this regard, our current study is the first one which was interested in a global genetic analysis. Thus, VDR and PTPN22 genes have previously given evidence for lack of association when analyzed individually (Maalej et al., 2008; Chabchoub et al., 2006). When, we have

Table 3
Significant statistical findings reported with FBAT analysisa.

| Chromosome | Marker | Allele | Disease model | p-value | Gene |
|------------|--------|--------|---------------|---------|------|
| 1          | rs2476601 | 1      | AITD (1)      | 0.025   | PTPN22 |
|            | HLA-DR    | 1      | AITD          | 0.034   | HLA-DR |
|            |           | 1      | GD            | 0.014   |      |
|            |           | 7      | HT            | 0.0009  |      |
|            |           | 1      | HT (b)        | 0.000715|      |
| 7          | D7S501    | 2      | GD            | 0.025   | PDS  |
|            | D7S2459   | 6      | AITD (1)      | 0.027   |      |
| 8          | DBS1801   | 5      | AITD (1)      | 0.046   | Tg   |
|            | DBS5558   | 10     | GD (1)        | 0.045   |      |
|            | Tgms2     | 6      | GD (1) (recessive) | 0.004  |      |
|            | DBS1710   | 4      | AITD (1) (recessive) | 0.019  |      |
| 12         | rs2228570 | 1      | HT (1) (recessive) | 0.024  | VDR  |
|            | rs731236  | 1      | AITD b        | 0.031   |      |
|            |           | 1      | AITD (1) (recessive) | 0.015  |      |

(1): association was reported only under this model.

a In this table, are mentioned only improved results compared with previous analysis.

Table 4
Significant associations reported between AITD traits and studied gene polymorphisms.a

| Chromosome | Marker   | Gene   | p-value |
|------------|----------|--------|---------|
| 1          | rs2476601| PTPN22 | 0.076   |
| 2          | rs1143634| IL1B   | 0.010   |
| 6          | rs1800629| TNF    | 0.0012  |
| 8          | DBS1710  | Tg     | 0.019   |
| 12         | rs731236 | VDR    | 0.02    |

a In this table are mentioned only positive associations reported with all studied AITD traits.
considered genotypes of other gene polymorphisms located on the same chromosome (Table 1), the situation has changed. In fact, PTPN22 and VDR genes have reached association threshold when analyzed together with TNFRII and TNFRI genes respectively. These findings support the hypothesis that there is interaction between these candidate genes in AITD susceptibility. Such kind of analysis is poorly performed in AITD.

Another issue is that in the majority of our previous studies, investigation of the studied polymorphisms has not interested all the family members. Indeed, only nuclear families harboring affected and unaffected individuals were included. Considering the large pedigree, we were able to use all the genotype information. This was particularly interesting for the Tg gene. In a previous study, there was no significant linkage between AITDs and Tg gene polymorphisms (Belguith-Maalej et al., 2008). However, in the current study, we report genetic association mainly with two microsatellite markers (Tgms2 and D8S1710) with the most significant with Tgms2 and only under the GD disease model \( p = 4 \times 10^{-3} \). Moreover, we could explain this association by clinical evolution of Akr family members. Indeed, Tg gene polymorphisms were explored using DNA from 87 members whose only 43 were affected.

In our analysis, in addition to the different statistical models used, we have reanalyzed genetic association based on the previous associated alleles, and therefore all studied polymorphisms were considered as biallelic ones (associated versus nonassociated alleles). This kind of analysis has particularly given better fit for HLA-DR12 allele which has yielded a \( p \)-value of \( 7 \times 10^{-4} \) (the corresponding \( p \)-value was \( 9 \times 10^{-3} \)).

When considering results of all studied genes in Akr family, it’s clear that the most significant association was with MHC i.e. TNF-308 gene polymorphism (rs1800629) and HLA-DR12 allele. These findings are in good agreement with results of a genome-wide association scan in AITDs which have shown that MHC class II region genes were most tightly linked to susceptibility (Burton et al., 2007). However, a recent study found “a novel and major association of HLA-C in Graves’ disease that eclipses the classical HLA-DRB1 effect” (Simmonds et al., 2007). In all cases, the MHC region remains among the important loci involved in AITD susceptibility.

Besides our analysis focusing on genetic association based on genotypes of the 33 studied polymorphisms, we have also looked for possible association between AITD traits and these markers, using FBAT. Our analysis has given evidence for association between 5 gene polymorphisms and the different explored disease traits (Table 4) meaning that these polymorphisms could be considered as “biomarkers” for AITDs in general. Moreover, we reported particular association between D7S501 and D7S2459 on one side and goiter on the other side (\( p = 0.009 \) and 0.029 respectively). This result argues our previous report showing that D7S2459 marker located in PDS gene was strongly associated with HT which is characterized with presence of goiter (Hadj Kacem et al., 2003). Such finding could be based on a hypothesis that PDS gene polymorphisms could be deemed as a particular “biomarker” for goiter development. This is not surprising given that PDS gene was primarily involved in Pendred Syndrome whose main feature is goiter with deafness (Everett et al., 1997).

Despite a huge number of studies dealing with the identification of genes in AITD, the harvest with respect to number of replicated genes and/or genetic markers has been modest (Simmonds and Gough, 2011; Weetman, 2009) Even taken together, the replicated genes/genetic markers account only for 10–20% of the heritability in AITDs. The situation for the multigenerational and consanguineous Akr family is not so different. Indeed, we have conducted several candidate gene analyses in addition to a genome scan and we have not yet depicted a “strong” gene conferring an important susceptibility to AITD. These findings argue again our hypothesis that there are no major genes in AITD susceptibility previously reported (Bougacha-Elleuch et al., 2011). Moreover, it seems likely that additional AITD-specific genes remain to be discovered and virtually certain that AITD genetic architecture involves gene–gene interactions.

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References

Vanderpump, M.P., 2011. The epidemiology of thyroid disease. Br. Med. Bull. 99, 39–51.

Brix, T.H., Hegedüs, L., 2012. Twin studies as a model for exploring the aetiology of autoimmune thyroid disease. Clin. Endocrinol. (Oxf.) 76 (4), 457–464.

Bougacha-Elleuch, N., Ben Arab, S., Rebai, A., et al., 2011. No major genes in autoimmune thyroid diseases: complex segregation and epidemiological studies in a large Tunisian pedigree. J. Genet. 90 (2), 333–337.

Wan, X., Yang, C., Yang, Q., et al., 2009. MegaSNPHunter: a learning approach to detect disease predisposition SNPs and high level interactions in genome wide association study. BMC Bioinforma. 10, 13.

Wei, B., Peng, Q., Zhang, Q., et al., 2011. Identification of a combination of SNPs associated with Graves’ disease using swarm intelligence. Sci. China Life Sci. 54 (2), 139–145.

Bougacha-Elleuch, N., Mnif, M., Charfi, N., et al., 2012. Hashimoto’s Disease. In: Springer, Drahomira (Ed.), A New Look at Hypothyroidism, pp. 69–90.

Laird, N.M., Horvath, S., Xu, X., 2000. Implementing a unified approach to family-based tests of association. Genet. Epidemiol. 19 (Suppl. 1), S36–S42.

Chalbi, N., Zakaria, D., 1998. Modèles de famille, endogamie e consanguinité apparente en Tunisie Essais de mesure (French). Fam. Popul. 1, 39–59.

Weetman, A.P., 2001. Determinants of autoimmune thyroid disease. Nat. Immunol. 2 (9), 769–770.

Tomer, Y., Ban, Y., Concepcion, E., et al., 2003. Common and unique susceptibility loci in Graves and Hashimoto diseases: results of whole-genome screening in a data set of 102 multiplex families. Am. J. Hum. Genet. 73 (4), 736–747.

Taylor, J.C., Gough, S.C., Hunt, P.J., et al., 2006. A genome-wide screen in 1119 relative pairs with autoimmune thyroid disease. J. Clin. Endocrinol. Metab. 91 (2), 646–653.

Effraimidis, G., Strieder, T.G., Tijssen, J.G., et al., 2011. Natural history of the transition from euthyroidism to overt autoimmune hypo-or hyperthyroidism: a prospective study. Eur. J. Endocrinol. 164 (1), 107–113.

Maaelej, A., Petit-Teixeira, E., Chabchoub, G., et al., 2008. Lack of association of VDR gene polymorphisms with thyroid autoimmune disorders: familial and case/control studies. J. Clin. Immunol. 28 (1), 21–25.

Belguith-Maalej, S., Hadj Kacem, H., Rebai, A., et al., 2008. Thyroglobulin polymorphisms in thyroid patients with autoimmune thyroid diseases (AITD). Immunobiology 213 (7), 577–583.

Everett, L.A., Glaser, B., Beck, J.C., et al., 1997. Pendred syndrome is caused by mutations in a putative sulphate transporter gene (PDS). Nat. Genet. 17 (4), 411–422.

Simmonds, M.J., Howson, J.M., Heward, J.M., et al., 2007. A novel and major association of HLA-C in Graves’ disease that eclipses the classical HLA-DRB1 effect. Hum. Mol. Genet. 16 (18), 2149–2153.

Burton, P.R., Clayton, D.G., Cardon, L.R., et al., 2007. Association scan of 14,500 nonsynonymous SNPs in four diseases identifies autoimmunity variants. Nat. Genet. 39 (11), 1329–1337.

Belguith-Maalej, S., Belguith-Salhi, N., Rebai, A., et al., 2009. Interleukin gene polymorphisms in Tunisian patients with autoimmune thyroid diseases. Cytokine 43 (2), 110–113.

Simmonds, M.J., Gough, S.C., 2011. The search for the genetic contribution to autoimmune thyroid disease: the never ending story? Brief. Funct. Genom. 10 (2), 77–90.

Weetman, A.P., 2009. The genetics of autoimmune thyroid disease. Horm. Metab. Res. 41 (6), 421–425.

Kammoun-Krichen, M., Bougacha-Elleuch, N., Makni, K., et al., 2008. A potential role of TNF receptor gene polymorphisms in autoimmune thyroid diseases in the Tunisian population. Cytokine 43 (2), 110–113.

Kammoun-Krichen, M., Bougacha-Elleuch, N., Makni, K., et al., 2007. Association analysis of interleukin gene polymorphisms in autoimmune thyroid diseases in the Tunisian population. Eur. Cytokine Netw. 18 (4), 196–200 (Dec).

Bougacha-Elleuch, N., Rebai, A., Mnif, M., et al., 2004. Analysis of MHC genes in a Tunisian isolate with autoimmune thyroid diseases: implication of TNF—308 gene polymorphism. J. Autoimmun. 23 (1), 75–80.