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Complex HLA association in paraneoplastic cerebellar ataxia with anti-Yo antibodies

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ABSTRACT

Anti-Yo paraneoplastic cerebellar degeneration (PCD) is a devastating autoimmune complication of gynecological cancers. We hypothesized that as for other autoimmune diseases, specific HLA haplotypes are associated. We conducted high resolution HLA typing of Class I/Class II in 40 cases versus ethnically matched controls. Three cases with anti-Yo antibodies and peripheral neuropathy were also included. We detected protective effects of DPA1*01:03∼DPB1*04:01 (OR = 0, p = 0.0008), DRB1*04:01∼DQA1*03:03(OR = 0, p = 0.0016) and DPA1*01:03∼DPB1*04:01 (OR = 0.35, p = 0.0047) overall. Increased DRB1*13:01∼DQA1*01:03∼DQB1*06:03 was also found in PCD ovarian cases (OR = 5.4, p = 0.0016). These results suggest differential genetic susceptibility to anti-Yo per cancer and with a primary HLA Class II involvement.

1. Introduction

Paraneoplastic neurological syndromes (PNS) are rare immune diseases triggered by cancer (Giometto et al., 2010). Paraneoplastic cerebellar degeneration (PCD), the most common PNS, is a sub-acute ataxia caused by an extensive Purkinje cell death associated with specific circulating autoantibodies (Abs), occurring in the context of a cancer. Approximately half of PCD are associated with anti-Yo (also called cerebellar degeneration-related protein CDR2/CDR2L or PCA1) antibodies (Yo-Ab) (Venkatraman and Opal, 2016, Graus and Dalmau, 2012, Eichler et al., 2013), making Yo-PCD the most common PCD subtype although very rare disease. These cases are generally associated with gynecological cancers such as ovarian (Venkatraman and Opal, 2016; Greenlee and Brashear, 1983; McKeon et al., 2011; Peterson et al., 1992; Cao et al., 1999; Drlicek et al., 1997; Zaborowski et al., 2015), breast (Tanriverdi et al., 2013, Rojas-Marcos et al., 2012, Dorn et al., 2003, Waterhouse et al., 1991, Ogita et al., 2008, Plantone et al., 2011) and, more rarely endometrial cancers (Rana et al., 2012; Panegyres and Graves, 2012; Brock et al., 2001), although association with lung (Hasadsri et al., 2013), or other cancers (Drlicek et al., 1997; Bruhnding et al., 2017; Valpione et al., 2013; Xia et al., 2003; Matschke et al., 2007; Debes et al., 2007) (Goto et al., 2006; Meglic et al., 2001) is possible.

The pathophysiology of Yo-PCD is poorly understood with two hypothesis that have been advanced, CD8 + T cell killing with help of CD4 cells (Albert et al., 2000, Albert et al., 1998, Albert and Darnell, 2004, Darnell and Albert, 2000, Darnell et al., 2000, Graus et al., 1991, Greenlee et al., 2015, Sutton et al., 2004), and direct pathogenicity of the Yo-Ab on Purkinje cells (Schubert et al., 2014). The cause of this cross-immune reaction is elucidated. Yo proteins are expressed in gynecological cancers (Rojas-Marcos et al., 2012; Totland et al., 2011) while physiologically CDR2 expression is mostly restricted to the testis, ovaries and the cerebellum (Schubert et al., 2014; Corradi et al., 1997). Although the occurrence of Yo-PCD is a rare complication of breast and ovarian cancer (< 10 diagnosed cases per year in France), risk for such complication may increase with exploration of immunotherapy for

Abbreviations: PCD, Paraneoplastic cerebellar degeneration; PNS, Paraneoplastic neurological syndromes; Yo-PCD, Paraneoplastic cerebellar degeneration with anti-Yo antibodies; Yo-Ab, Anti-Yo antibodies; Abs, autoantibodies; HLA, Human Leukocyte Antigens; GWAS, Genome Wide Association Studies

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these malignancies (Spellman and Tang, 2016; Gaillard et al., 2016). For example, autoimmune encephalitis, another type of paraneoplastic disease, has been reported as a side effect of anti-PD1 checkpoint inhibitors (Williams et al., 2016). As some therapies are aiming at increasing T cell tumoral infiltration, a favorable prognostic factor in breast and ovarian cancers (Mesnage et al., 2017; Park et al., 2016), prevalence of such complications may increase. Consistently, the risk to develop PNS after CTLA4 blockade was recently reproduced in a mouse model of PNS that expresses a neo-self antigen both in Purkinje neurons and in implanted breast tumor cells (Yshii et al., 2016). Anti-CTLA4 monoclonal antibody in this mouse model elicited antigen-specific T cell migration into the cerebellum, and significant neuroinflammation and PNS mimicking PCD (Yshii et al., 2016). In this context, identification of predisposing factors, such as genetic factors may be helpful to guide therapy and/or in the selection of tumoral antigen to select in the context of specific Human Leukocyte Antigens (HLA).

In this study, we explored whether anti-Yo immunity is HLA associated, as are naturally occurring autoimmune diseases and previously reported in some (de Graaf et al., 2010; Liu et al., 2008; Martel et al., 2003) but not all paraneoplastic syndromes (Tanaka et al., 1999; Uchuya et al., 1998). We identified HLA associations that were mostly tumor specific, suggesting further heterogeneity in Yo-PCD per tumor type. These findings also suggest a tumor specific neoantigens in the development of autoimmune response that is mediated by specific HLA subtypes.

2. Methods

2.1. Patients and matched controls

All 43 patients were recruited and diagnosed in France by the French reference center on PNS (Pr. Honnorat). To be included in this study, patients had to meet the following criteria: i) high titer of serum or CSF Yo-Abs; ii) histologically proven cancer; iii) and PCD ($n = 40$) or peripheral neuropathy ($n = 3$) diagnosis according to the international guidelines (Graus et al., 2004). Ethnicity was carefully considered, with most patients being of European descent, except for 3 of Maghrebian (North Africa) origin. To build an ethnically and geographically matched control sample, we first recruited and typed 2–3 controls per patient from France thanks to Drs. Honnorat (Lyon), Isabelle Arnulf (Paris) and Yves Dauvillers (Montpellier), ethnicity (European versus North African) being verified using principal component analysis of Genome Wide Association (GWAS) typing results. We next added more controls using the large Genetic Epidemiology Research on Adult Health and Aging (GERA) Kaiser cohort (Banda et al., 2015), with the goal of matching 1 case for approximately 5 controls using the first 2 principal component analysis and identifying nearest neighbors within this cohort. These GERA controls are likely French or North African born first generation immigrants or subjects born from two parents born in France or North Africa, all living in California. As results were identical using the smaller French-only controls versus the extended matched dataset, we elected to only present data of the larger dataset, however comprehensive results for both the larger control set and the French matched controls are displayed in Supplementary Table 1. Supplementary Fig. 1 shows comparable distribution of the first 2 principal components for all cases versus all controls and Supplementary Fig. 2 shares the same approach as Supplementary Fig. 1 but is limited to the French matched controls. Results examining cancer specific associations are displayed in Supplementary Table 2. Clinical and available tumor phenotyping data on all 35 subjects is presented in Supplementary Table 3.

2.2. HLA and GWAS typing

Patients and matched-French controls were high resolution typed at 4-field resolution using HLA sequencing as described in Wang et al. (2012), and results condensed to 2-field (amino acid polymorphism resolution) typing level. The rationale for this is the small sample size and first focus on amino-acid polymorphisms known to alter peptide presentation by HLA. In parallel with this, we genotyped a panel of subjects of European and African descent ($N = 2000$) for HLA imputations using the same HLA genotyping method (Wang et al., 2012). These individuals had been earlier genotyped with Genome-Wide Human SNP Array 6.0 genotyping chip and were used to build European and African descent population specific HLA imputation panels for predicting HLA-DR, DQ and DP. HIBAG (Zheng et al., 2014), a program that performs slightly better than SN2HLA (Kuniholm et al., 2016) was used to infer HLA genotypes on all subjects across various ethnic groups at the same 2-field resolution as reported in Ollila et al. (2015).

Independent testing of a subset of samples using the algorithm and our panel gave the following 2 allele genotype overall accuracy verified by HLA typing for HLA-A: 97.3%, B: 91.6%, C: 97.6%, DRB1: 91.1%, DQA1: 94.9%, DQB1: 95.4%, DPA1: (100%), DPB1 (93.0%). Accuracy for predicting the key alleles found to be associated in this study were: DRB1*04:01 (80%), DRB1*16:01 (77.8%), DRB1*13:01 (93.9%), DQA1*01:02 (97.0%), DQA1*01:03 (91.5%), DQB1*05:02 (100%), DQB1*06:03 (83.3%), DPB1*04:01 (96.1%), DPB1*04:02 (91.3%), DPB1*104:01 (25%), DPB1*17:01 (95.7%). Imputation accuracies are similar to those obtained by other authors in subjects of European descent (Zheng et al., 2014; Karnes et al., 2017). Finally, in cases where alleles well established to be linked between DRB1, DQA1 and DQB1 or DPA1 and DPB1, respectively were increased in parallel in our samples, haplotypes were inferred for ease of discussion.

2.3. Statistics

HLA allele carrier frequencies were compared using χ^2 or fisher exact test depending on the total number of reported alleles present for statistical analysis. $\chi 2$ were used only if > 5 individuals, of either cases or controls, reported the given HLA allele being tested. If 5 or less of the cases or controls reported the given HLA allele, a fisher exact test was used. The p-values reported in results are those observed after using the appropriate test. Our comparisons were performed in cases versus Genome Wide, principal component-matched controls, and verified in the smaller set of fully HLA typed, ethnically matched French subjects for verification. To correct for the analysis of 8 loci, we only considered statistically significant findings with $p < 0.00625$.

3. Results

Table 1 reports on all significant findings.

3.1. Overall sample

Results, biological and clinical data for all 43 cases are reported in Supplementary Table 3. As noted, 40 cases were European French while 3 were of North African ancestry. Most cases ($n = 27$) were with ataxia and secondary to Ovarian cancer. Overall, anti Yo cases were associated with a protective effect of DRB1*04:01 (OR = 0, $p = 0.0008$) and DPB1*04:01 (OR = 0.35, $P = 0.0047$). When only cases with ataxia were considered, only the DRB1*04:01 and its associated allele DQA1*03:03 allele remained significant (OR = 0, $p = 0.0023$).

Detailed comparisons of carrier frequency for cases versus Genome Wide, principal component-matched controls are reported in Supplementary Table 1, and comparisons versus the small subset of French, ethnically matched, controls are also reported in Supplementary Table 1 for verification purposes (findings were similar).