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Original Article

Analysis of Genome-Wide Association Study (GWAS) data looking for replicating signals in Alzheimer's disease (AD)

Hui Shi, Christopher Medway, James Bullock, Kristelle Brown, Noor Kalsheker, Kevin Morgan

Division of Clinical Chemistry, Institute of Genetics, School of Molecular Medical Sciences, Queen's Medical Centre, University of Nottingham, Nottingham, NG7 2UH, UK.

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Abstract: We have performed cross-platform comparisons of output from 4 GWAS in late-onset Alzheimer's disease (LOAD) – Reiman *et al.*, 2007; Li *et al.*, 2008; Beecham *et al.*, 2008 and Carrasquillo *et al.*, 2009 to search for new association signals. The aim was to reveal genes that replicated across studies and hence merit further investigation. All SNPs with p-values ranging between $5x10^{-5}$ - $5x10^{-8}$ from each study were assessed across the other studies (either directly or by using a perfect proxy when comparing data from different chip platforms). This revealed only a single SNP (rs929156 in the tripartite motif-containing protein 15, *TRIM15*, gene) that was replicating across all studies at a level approaching genome-wide significance ($P = 8.77x10^{-8}$) and where meta-analysis of odds ratios showed a significant effect on risk (OR 1.1, 95% CI 1.0-1.2, $P = 0.03$). The vast majority of data analysed failed to replicate across these GWAS. The number of replicating association signals we observed is no higher than would be expected due to chance. However, increasing the power by using additional data from larger studies may enable this approach to identify potential LOAD candidate genes for confirmatory association studies.

Key words: Late-onset Alzheimer's disease (LOAD), meta-analysis, genome-wide association analysis (GWAS), tripartite motif-containing protein 15 (*TRIM15*) gene, replication, single nucleotide polymorphism (SNP)

Introduction

Late-onset Alzheimer's Disease (LOAD) is the most prevalent form of dementia and the most common form of Alzheimer's Disease, representing 95% of AD cases in the population [1]. LOAD affects approximately 10% of individuals aged 65 years and almost half of people aged over 85 years [2]. There were 26.6 million people who suffered from LOAD worldwide in 2006, 700,000 of which were in the UK. This number is estimated to increase to 1 in 85 people - approximately 100 million worldwide by 2050 [3].

LOAD has complex aetiology which includes both genetic and environmental determinants. The disease is characterized at the pathological level by extracellular deposits of β-amyloid (Aβ) plaques in the cerebral cortex, and intracellular neurofibrillary tangles (NFTs) of hyperphosphorylated tau protein. Both observations are understood to be caused by misfolding and gradual conversion of highly soluble proteins into insoluble filamentous polymers [4]. To date hundreds of LOAD candidate genes have been explored, suggesting complex biological pathways exist that might explain disease risk (http://www.alzforum.org/). Susceptibility for LOAD is likely to be governed by an array of common risk alleles across a number of different genes which are involved in variety of biochemical pathways affecting both AD aetiology and pathogenesis [5].

APOE is currently the only gene that has been universally confirmed as a genetic risk factor for LOAD. The APOE protein is involved in the transportation of lipids around the body, and is also found to be responsible for chaperoning cholesterol through the blood stream. APOE has also been found essential for efficient intracellular degradation of soluble Aβ by

microglia [6]. APOE activity in the brain requires lipidation by ATP-binding cassette 1 (ABCA1) [7]. LOAD is found to be associated with the *APOE* e4 allele which greatly increases the risk and reduces the average age at onset of AD. However, the risk polymorphism explains at most 50% of the genetic-risk effect in disease [8]. Therefore, additional genetic components must be involved in the complex aetiology of LOAD.

Genome-wide association studies (GWAS) are one of the most commonly used contemporary approaches to find genetic associations to diseases. These studies make it possible to investigate genetic contributions to LOAD from the entire genome. Genes with $5x10^{-5}$ to $5x10^{-8}$ significance may be genuine AD candidates that due to power constraints, have failed to reach genome-wide significance ($<10^{-8}$). The aim of this paper was to select genes/regions that merit further study by identifying all SNPs with p-values within this range and then comparing their effects across other GWAS, either directly or by using a perfect proxy. The approaches we have employed to identify replicating signals can be applied to other studies to search across GWAS data from different platforms.

Methods

Of the 9 GWAS conducted to date studying Alzheimer's disease (Alzforum: http://www.alzforum.org/res/com/gen/alzgene/largescale.asp), we analysed data from four where the data was readily obtainable; subject-level genotype data from two, Reiman *et al.*, 2007 and Carrasquillo *et al.*, 2009 [9, 10], complete summary data from a third Li *et al.*, 2008 [11] and summary data of top SNP hits ($5x10^{-5}$ to $5x10^{-8}$) in the fourth Beecham *et al.*, 2009 [8]. In each case quality control measures had been applied prior to data release. Further details of each study are listed in **Table 1**.

Generating SNP results from subject-level genotype data (Reiman et al., 2007 and Carrasquillo et al., 2009)

Datasets Reiman *et al.*, 2007 and Carrasquillo *et al.*, 2009 [9, 10] were analyzed using the PLINK analysis toolset version 1.05 [12] (http://pngu.mgh.harvard.edu/~purcell/plink/index.shtml). GWAS data was converted into a file format appropriate for PLINK (.ped and .map) before analysis.

The following protocol was used to generate GWAS output from the genotyping data:

(1) PLINK command: > plink --bfile 'Input file' --assoc --ci 0.95 --mind 1 --geno 1 --out 'Output file' was used for Carrasquillo *et al.*, 2009 data.

(2) PLINK command: > plink –bfile 'Input file' --allow-no-sex --assoc --ci 0.95 --mind 1 --geno 1 --out 'Output file' was used for Reiman *et al.*, 2007 [10] data.

(3) '--bfile' indicates the input data file which PLINK uses is in binary format, '--assoc' is the main method for the allelic association test, which compares the minor allele frequencies between cases and controls, and calculates asymptotic p-values. '--ci' 0.95 generates 95% confidence interval for odds ratios, '--mind 1' indicates that the threshold for missing individuals is equal to 1, '--geno 1' means the threshold for missing genotypes is equal to 1, both '--mind and --geno' commands control the dataset quality in terms of SNP genotyping rate. '--out' specifies the output file name.

(4) To make the Reiman *et al.*, 2007 [10] Affymetrix data comparable with Carrasquillo *et al.*, 2009 Illumina data, the SNP ID was translated from Affymetrix SNP ID format "SNP_A-######" to dbSNP reference ID format "rs######". The translation process utilized a PERL script created by ourselves.

As the sex status of individuals was unspecified in the Reiman *et al.*, 2007 dataset [10], the '--allow-no-sex' command enables PLINK to ignore unspecified sex and include all samples into the calculations.

Only limited information was obtained for the Beecham *et al.*, 2009 and Li *et al.*, 2008 studies [8, 11] (**Table 1**). It was not possible to merge datasets, since the two studies for which we had genotyping data used different chip platforms.

Comparing p-values across different GWAS

For each of the GWAS, all SNPs with p-values between $5x10^{-5}$ to $5x10^{-8}$ were compared across the other studies (where possible) either directly or by using a perfect proxy ($r^2 = 1$). SNAP (SNP Annotation and Proxy Search) (http://www.broad.mit.edu /mpg/snap) was used to look for SNP proxies [13] using the

Table 1. Summary of the four GWAS analyzed in this study

	Number of SNPs (post QC)	CHIP platform	Excluded SNPs (%)	Number of SNPs with LD (r^2 = 1)	Number of LD Clusters (r^2 = 1)	Number of Independent Tests
Li *et al*. (2008)	469,438	Affymetrix 500K	5%	128,139	42,634	383,933
Reiman *et al*. (2007)	312,316	Affymetrix 500K	38%	83,739	29,678	258,255
Beecham *et al*. (2008)	532,000	Illumina 550	4%	-	-	-
Carrasquillo *et al*. (2009)	313,330	Illumina 300	1%	26,284	11,539	298,585

The number of SNPs following QC, the platform utilisied and the percentage of SNPs excluded in each study is listed. Also shown are the number of perfect proxies (r^2 = 1) in the QC'd data together with the number of clusters into which these SNPs fall. The number of independent tests for multiple test correction of combined p-values (listed in the supplementary tables) is shown in the last column and was calculated as described in the methods section.

Table 2. Comparison of odds ratios across GWAS for selected SNPs

Gene	OR (95% CI)			
	Reiman *et al.*, 2007	Carrasquillo *et al.*, 2009	Li *et al.*, 2008	Random effects Meta-analysis of OR's
TRIM15 (rs929156)	1.1 (0.9-1.3) (rs2844775)	1.1 (1.0-1.3)	1.1 (0.9-1.3) (rs2844775)	1.1 (1.0-1.2) *P* = 0.03
TFCP2L1 (rs11682545)	0.8 (0.7-0.9)	-	1.3 (1.1-1.5)	1.0 (0.7-1.6) *P* = 0.95
RBM20 (rs7077757)	0.6 (0.5-0.8)	-	1.3 (1.0-1.5)	0.9 (0.5-1.7) *P* = 0.74

If the SNP was not present in a GWAS a perfect proxy (with r^2 value of 1.0) was used to infer the odds ratio. The proxy SNP ID is shown underneath the corresponding odds ratios. The data shown is for the allelic association model. The 95% confidence interval for odds ratios are shown in brackets. The colours indicate the different GWAS (Reiman *et al.*, 2007 – blue, Carrasquillo *et al.*, 2009 – red and Li *et al.*, 2008 – green). The results from random-effects meta-analysis of these odds ratios is given in the final column.

HapMap Resource CEU population - release 22 (March 2007) as the reference dataset. We have used direct proxies in order to capture as much information as possible across the different chip platforms (each has their own SNP portfolio). Imputation attempts for SNPs in *TRIM15* using PLINK yielded limited information when merging the datasets with the reference dataset. Imputed SNPs generated PLINK INFO (information content metric) scores lower than 0.8, indicating dissatisfied imputed SNPs. This low score is due to the poor LD architecture within this region and the limited availability of data.

We studied SNPs within the significance band $5x10^{-5}$ to $5x10^{-8}$ to search for potential new AD candidates that to date have failed to reach genome-wide significance; we have tested within this band to determine if there are genuine AD candidates that are yet to emerge due to the limited power of the GWAS to date. We appreciate that extending to a lower cut-off (> 10^{-5}) may reveal more substantial information and this could well be a viable approach to use on larger GWAS datasets as they become available. Any SNPs with p-values below $5x10^{-8}$ were not included in our analyses; effectively this resulted in all SNPs in the *APOE* region on chromosome 19 being removed – this region replicated across all the studies. Four tables were created, one table

for each of the GWAS listing the SNPs that were in this significance band together with the corresponding SNP p-values in the three other GWAS. Tables for Li *et al.*, 2008; Beecham *et al.*, 2009; Reiman *et al.*, 2007 and Carrasquillo *et al.*, 2009 [8-11] can be found in the supplementary material (**tables 1-4** respectively).

SNPs were selected for further analysis as described below:

(1) SNPs with p-values $5x10^{-5}$ to $5x10^{-8}$ were selected from each of the GWAS.

(2) p-values for the same SNPs (or proxies r^2 = 1) across the remaining studies were determined.

(3) The Fisher's combined p-value test was used as a summary statistic to give an overall value of association. It must be noted that this test does not correct for disparate effects created by alleles whose direction of association differs between studies – the so-called 'flippers'. For the resultant p-value to be meaningful all effects must be in the same direction.

(4) Combined p-values of 10^{-8} were corrected for the number of independent SNPs on the highest density platform utilised following QC (see section below).

We were only able to access the 'top hits' from Beecham *et al.*, 2009 [8] , which limited our comparison across all 4 studies. Supplementary **table 2** compares GWAS output for all 4 studies, whereas supplementary **tables 1, 3** and **4** compare data from the remaining 3 GWAS.

Calculating the number of independent tests for correcting combined p-values

The protocol outlined below was used to calculate the number of independent tests for each study:

(1) PLINK commands "--extract" and "--make-bed" were used to extract all SNPs (post QC) in each study from HapMap data - CEU population release 22.

(2) The extracted files were used to calculate LD values. All SNPs in perfect LD (r^2 = 1) were calculated using PLINK command: > plink --bfile 'Input file' --r2 --ld-window-kb 1000 --ld-window 99999 --ld-window-r2 1 --out 'Output file.ld'

(3) '--r2' is the command for calculating LD r^2 value. '--ld-window-r2 1' indicates the LD threshold is r^2 = 1. '--ld-window 99999' specifies the maximum number of pair-wise combinations to be calculated for each SNP is 99999.

(4) The number of LD clusters (containing SNPs which share an r^2 = 1) was calculated using a PERL script written 'in-house'. The information from the PLINK result file '.ld' were used for this calculation.

(5) The number of independent tests was calculated using the formula: Number of independent tests = [Number of SNPs (post QC) - Number of SNPs in perfect linkage (r^2 = 1)] + Number of LD Clusters with r^2 = 1. These values are shown in **Table 1**.

The numbers of independent tests were calculated for Li *et al*., 2008, Reiman *et al*., 2007 and Carrasquillo *et al*., 2009 [9-11]. It was not possible to calculate the number of independent tests for the Beecham *et al*., 2008 GWAS [8], since we only had information on the 'top hits'.

Meta-analysis of odds ratios

Any SNPs that showed a corrected combined p-value of less than 0.05 were further analyzed by comparing their corresponding odds ratios. The random-effects method was implemented in the StatsDirect software package. In contrast to Fisher's combined this test accounts for the direction of effect. Significance is only obtained when the effects are all in the same direction. A SNP could therefore be significantly associated using Fisher's combined but fail odds ratio meta-analysis.

Gene-centric analysis for TRIM15

A gene-centric approach was used to conduct an in depth SNP analysis of *TRIM15*. The LD architecture surrounding the gene was identified using LD plots generated in Haploview (version 4.0) using HapMap CEU population data. SNPs flanking the gene (20kb either side) were also analyzed. The base pair co-ordinates were obtained from HapMap. The

study-specific p-values for allelic association for each of the *TRIM15* SNPs were generated in PLINK using the data from the Reiman *et al.*,2007 and Carrasquillo *et al.*, 2009 studies [9, 10]; the values from the summary data are used for Li *et al.*, 2008 study [11].

Results

Analysis of GWAS

The SNP's with p-values 10^{-5} to 10^{-8} were identified for each study and then compared across all datasets. Combined p-values were determined for SNP that occurred in at least 2 studies. SNPs with combined p-values of 10^{-8} were corrected for multiple testing. Using this approach 3 SNPs were identified. rs11682545 (supplementary table 1 – Li *et al.*, 2008 [11] as primary comparator) had a combined p-value of 7.98×10^{-8}, corrected P = 0.0306. This SNP occurs downstream of the *TFCP2L1* gene on chromosome 2. Using Beecham *et al.*, 2008 as the comparator rs929156 (supplementary table 2) gave a combined p-value of 8.77×10^{-8}, corrected P = 0.0467; this occurs in intronic sequence of the *TRIM15* gene on chromosome 6. The third SNP (rs7077757) was identified in supplementary table 3 (Reiman *et al.*, 2007 as primary comparator) with a combined p-value of 6.35×10^{-8}, corrected P = 0.0244. This occurs in intronic sequence of the *RBM20* gene on chromosome 10. No combined p-values of less than 10^{-8} were evident using the Carrasquillo *et al.*, 2009 as the primary comparator (**supplementary table 4**).

Meta-analysis of odds ratio for candidate SNPs

A random-effects meta-analysis of the allelic odds ratios was conducted for the 3 SNPs identified as outlined in section of "*Analysis of GWAS*" above (**Table 2**). The *TRIM15* SNP gave odds ratios in the same direction (causative, Table 2) across 3 studies and on meta-analysis gave an odds ratio of 1.1 (95% CI 1.0-1.2; P = 0.03). The *RBM20* (P = 0.95) and *TFCP2L1* (P = 0.74) SNPs were not significant following meta-analysis.

Gene-centric analysis of TRIM15

A gene-centric analysis of *TRIM15* was undertaken (**Figure 1**) to explore the genetic architecture in more detail. The histogram shows the SNPs present in three different GWAS (Reiman *et al.*, 2007; Carrasquillo *et al.*, 2009 and Li *et al.*, 2008 [9-11]), their associated p-values together with their degree of linkage.

Discussion

LOAD candidate genes

The *APOE* region on chromosome 19 was confirmed as a genetic-risk factor in LOAD by all four GWAS with SNP p-values ranging from 10^{-36} to 10^{-44}. Apart from those linked to the *APOE* locus, there were no other SNPs with p-values less than 10^{-8}. However, it is already known that at least 50% of the genetic-risk effects are independent of the *APOE* gene [8], suggesting that unidentified genes exist which contribute to LOAD pathogenesis. Genes with suggestive significance across different GWAS may infer a genuine Alzheimer's disease candidate.

Currently more than 500 genes and 2000 polymorphisms have been assessed as genetic risk factors in association with AD (http://www.alzforum.org/). Except for the *APOE* gene, most of the genes have conflicting reports regarding their associations with AD. However, each of the studies often uses different populations with varying male and female percentages, as well as differing age ranges and sample sizes. Results are therefore not always directly comparable between different studies [5]. The study approach we have used may help identify potential LOAD candidate genes whose signals replicate across studies.

GWAS association analysis uses very stringent significance levels to avoid the large number of false positives potentially arising from the confounding effects of population substructure and testing of a very large number of SNPs [14]. For example, in a GWAS using 500,000 independent markers, 25000 can be expected to show a nominal p-value $\leq 5 \times 10^{-2}$ by chance alone and five out of this 25000 may be significant with p-values 1×10^{-5}. A widely accepted p-value of $\leq 5 \times 10^{-8}$ is used to indicate genuine disease association in GWAS [15]. However, the SNPs on different chip platforms are often not independent. Many SNPs are in LD with other SNPs, potentially reducing the number of independent markers available for analysis. Secondly, the genotyping rate never reaches 100%, and after quality

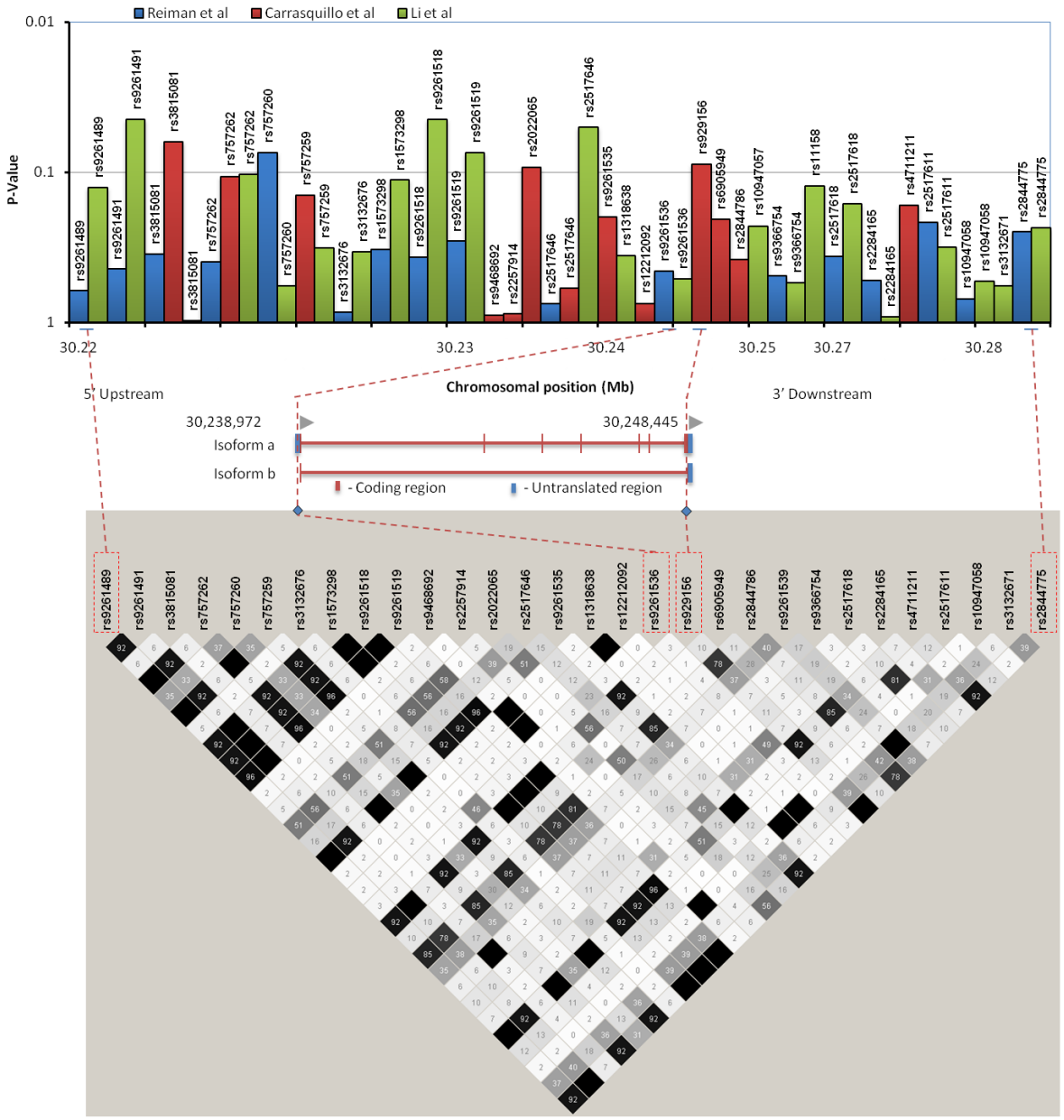

Figure 1. Schematic overview of the *TRIM15* gene and the LD plot for this region. The histogram depicts all GWAS SNPs in *TRIM15*, their p-values and ID's are shown at the top of the figure. These are study colour-coded as indicated at the top of the figure. The two *TRIM15* isoforms and their chromosomal positions are as depicted in HapMap. The LD plot is for the GWAS variants (Haploview 4.0, r^2 values with r^2 colour scheme). The blue diamond shapes and dotted red lines on the LD plot indicate the positions of SNPs with respect to the gene and are boxed in red on the LD plot. The SNPs at the boundaries of this LD block are also boxed in red and their location in the histogram depicted with dotted red lines.

control, significant numbers of SNPs are excluded from study (see **Table 1**). This infers that a p-value of $\leq 5x10^{-8}$ may in some instances be too stringent and SNPs with p-values of 10^{-5} to 10^{-8} might well harbour genuine associations.

The potential role of TRIM15

TRIM15 is a member of the tripartite motif (TRIM) family. The TRIM motif includes three zinc-binding domains, a RING, a B-box type 1 and B-box type 2, and a coiled-coil region. The

protein is localized to the cytoplasm. Two isoforms have been identified and described, however their biological functions have not as yet been identified. *TRIM15* is expressed in various tissues including brain, kidney, prostate, liver, and colon. The biological role of *TRIM15* has not yet been determined [16].

SNP rs929156 in *TRIM15* is located in an exon in one of the two *TRIM15* transcripts. It changes the amino acid from a small, polar Serine to a medium-sized, polar Asparagine. It is located in a B30.2 SPRY like domain (position: 276-465 amino acids). The B30.2-like domain is a conserved domain found in nuclear and cytoplasmic proteins, as well as transmembrane and secreted proteins. The B30.2-like domain may also be associated with a zinc-binding B-box domain in the N-terminal [17]. The SPRY domain is proposed to be a protein interacting module, which recognizes and interacts with specific individual partner proteins [18]. The potential effects of this SNP on protein structure require further investigation. The only other *TRIM15* SNP in these GWAS was rs9261536 which is located in the 5' untranslated region (UTR), a potential regulatory region (i.e. a promoter region or a binding site for an associated transcription factor - **Figure 1**).

Possible linkage has been observed between the *TRIM15* SNP and SNPs in Human leukocyte antigen A-2 alpha chain (*HLA-A*) with r^2 value 0.77 (see supplementary Figure 1). The regions surrounding *TRIM15* have a cluster of *HLA* genes which are associated with the human immune system. The group of *HLA*s encode cell-surface antigen-presenting proteins, which are essential elements in human immune responses. *HLA-A* is essential for immune recognition and apoptosis, and mutations in *HLA-A* have been implicated as a risk factors for various cancers [19]. Ma *et al*., 2008 [20] reported an interaction of *HLA-A* with *APOE* e4, relative risk 2.98 (95% CI = 1.14-8.24, *P* = 0.023) for *HLA-A*24 alleles in AD patients who do not carry *APOE* e4, compared with *APOE* e4 carriers. They also showed mutations in *HLA-A* may be associated with earlier age at onset in AD (by 2.4 years, p-value = 0.03) for those not carrying the *APOE* e4 allele.

The significance of identified SNPs

In this paper we have described an approach to detect replicating signals across different GWAS and platforms in an effort to identify LOAD candidate genes that have failed to reach genome-wide significance previously. Using the data from the four studies listed has generally failed to produce any convincing replicating signals with the possible exception of the *TRIM15* gene which contains the only SNP (rs929156) whose combined p-value (8.77×10^{-8}) survives after multiple testing correction (0.0467) and where the meta-analysis of odds ratios is also significant (OR 1.1, 95% CI 1.0-1.2, *P* = 0.03). The remaining two SNPs that had p-values of 10^{-8} failed to survive the meta-analysis of odds ratios because their effects were discordant between studies (**Table 2**). However, when discussing these observations it must be remembered that in a study of 74 genome-wide SNPs (as selected here) 5% would be expected to appear due to chance: thus we would expect to see in the region of 4 SNPs and we detect 3 signals. Another issue which is evident is the difficulty that exists when trying to compare data across different chip platforms where the SNP complement differs. There are surprisingly few perfect proxies available which results in a significant loss of data and will result in a reduction of power to detect new signals. Collectively these considerations have undoubtedly contributed to the non-replication of data across the GWAS. We propose that approaches such as we describe may prove to be useful when larger data sets are analysed. Any genes thus identified would need confirmation by follow up case/control association studies. They could also be subjected to deep resequencing to determine if they harbour multiple rare variants that may be associated with disease provided, of course, that a large enough resource is available to provide adequate power to be able to detect this. An important argument for GWAS is that the genes in which common variants are found, or genes nearby , may well contain functional rare variants; these may have high enough penetrance to be considered as candidates for possible preventive screening strategies in the future [14].

Note added in proof

Whilst this manuscript was in preparation there were a number of developments that impact on the approaches used in our analyses.

In the current version of PLINK (v1.06), an improved function called '--clump' permits comparison of multiple results from different platforms. The '--clump' utility takes all SNPs that are significant at user defined thresholds and calculates 'clumps' of all other SNPs based on the linkage disequilibrium (LD). However, this function does not perform any meta-analysis. We are aware that the latest version of PLINK (v1.07) (not released yet) will allow meta-analysis by using '--meta-analysis'. This will be a separate function from '--clump' and will not take LD into account.

In the recently published UK LOAD GWAS paper [21] the TRIM15 SNP, rs929156, was shown to be modestly associated with AD (P = 0.049). Adding this data results in a Fisher's combined p-value of 4.30×10^{-9} strengthening the evidence of association for this SNP. The odds ratio from the UK GWAS (OR = 1.07) was also compatible with the odds ratio we observed in the random effect meta-analysis (OR = 1.11).

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Please address correspondences to: Kevin Morgan, PhD, Division of Clinical Chemistry, Institute of Genetics, School of Molecular Medical Sciences, Queen's Medical Centre, University of Nottingham, Nottingham, NG7 2UH, UK. E-mail: Kevin.Morgan@nottingham.ac.uk

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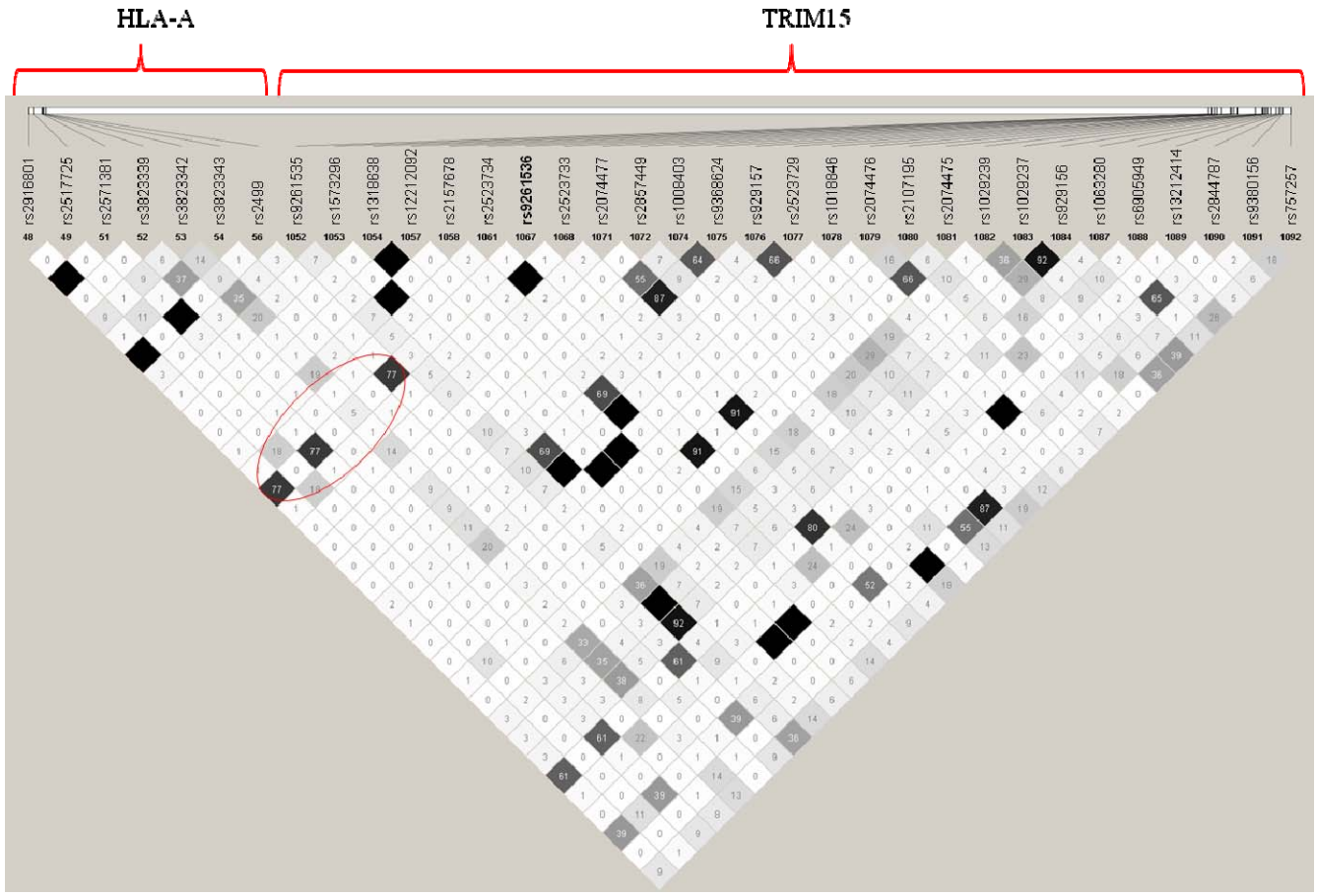

Supplementary Figure 1. Illustration of linkage disequilibrium (LD) between the *TRIM15* and *HLA-A* genes. The red circle highlights the linkage between *TRIM15* SNP rs9261536 (shown in bold) and three other SNPs in *HLA-A* with r^2 value = 0.77. The LD plot was generated using HapMap data (CEU population release 22) and the programme Haploview version 4.0.

Li et al, 2008						Carrasquillo et al, 2009		Reiman et al, 2007		Combined p-value	Corrected p-value
SNP	CHR	BP	Gene	Position	p-value	SNP	p-value	SNP	p-value		
rs4735627	8	00705091	VPS13B	Intron	3.51E-06	rs4735627	8.73E-01	rs4735627	7.66E-01	2.35E-06	
rs7336489	13	59171299	BC041395	Intron	5.38E-06	-		rs7336489	8.78E-01	4.72E-06	
rs370672	5	02501146			9.37E-06	-		rs370672	1.62E-01	1.52E-06	
rs4684083	3	00163865			9.73E-06	-		rs4684083	6.72E-01	6.54E-06	
rs11682545	2	21662295	TFCP2L1	Downstream	1.29E-05	-		rs11682545	6.18E-03	7.98E-08	3.06E-02
rs6805482	3	25435600			1.78E-05	-		rs6805482	9.27E-01	1.65E-05	
rs11166407	1	00410296	LRRC39	Intron	2.00E-05	-		rs11166407	8.62E-02	1.72E-06	
rs8014810	14	35394781	BRMS1L	Intron	2.00E-05	rs2274068	2.33E-01	rs8014810	3.84E-01	1.79E-06	
rs541392	10	30941167			2.76E-05	rs476628	3.66E-01	rs541392	4.19E-01	4.23E-06	
rs13180602	5	60213616	ATP10B	Upstream	2.79E-05	rs4559036	7.00E-02	rs13180602	4.03E-01	7.87E-07	
rs11751998	6	11297073	NEDD9	Intron	3.42E-05	rs10484448	4.86E-01	-		1.66E-05	
rs6571727	14	35210859	GARNL1	Intron	3.49E-05	rs6571727	2.14E-01	rs10132580 #	7.61E-01	5.67E-06	
rs4483549	11	90595620			3.58E-05	rs4483549	3.10E-01	rs4483549	2.12E-01	2.35E-06	
rs1914516	2	15270178			3.61E-05	-		rs1914516	2.21E-01	7.98E-06	
rs4905898	14	99345451	EML1	Intron	3.61E-05	rs10141863	7.74E-01	rs4905897 #	5.44E-01	1.52E-05	
rs4687319	3	93526543	FGF12	Intron	4.60E-05	-		rs4687319	6.18E-01	2.84E-05	
rs16897530	8	00725659	VPS13B	Intron	4.74E-05	-		rs16897530	9.66E-01	4.58E-05	
rs4438299	16	60259838	CDH8	Intron	4.90E-05	rs4438299	9.09E-01	rs4438299	8.81E-01	3.93E-05	

Supplementary Table 1. Li *et al*, 2008 GWAS SNPs ($5x10^{-5} < p < 5x10^{-8}$) compared with Reiman *et al,* 2007 and Carrasquillo *et al*, 2009. The GWAS from which SNPs were initially selected is shown on the left. Each row represents a SNP with a p-value between $5x10^{-5}$ to $5x10^{-8}$. The p-values are highlighted yellow if $p < 0.05$ and they replicated across two or more studies. If a perfect proxy was used the corresponding rs number is listed. The same platform was used in the Reiman et al, 2007 and Li et al, 2008 studies but data from a perfect proxy SNP (shown hatched) was used due to unavailability of data for the initial SNP. The combined p-values across studies are shown if the value approached genome-wide significance of 10^{-8}. The final column shows the corrected p-value adjusted as described in the methods section. SNPs surviving correction for multiple testing are also highlighted in yellow.

Beecham et al, 2008						Reiman et al, 2007		Li et al, 2008		Carrasquillo et al, 2009		Combined p-value	Corrected p-value
SNP	CHR	BP	Gene	Position	p-value	SNP	p-value	SNP	p-value	SNP	p-value		
rs9659092	1	50216176			4.54E-06	rs12022125	4.04E-01	rs12022125	1.48E-01	-		2.71E-07	
rs3807031	6	30141863	PPP1R11	Promoter	1.16E-05	-		-		rs3807031	4.94E-01	5.73E-06	
rs1415985	1	49703336			1.23E-05	rs12022125	4.04E-01	rs12022125	1.48E-01	-		7.35E-07	
rs4926831	1	50062688			1.23E-05	rs4926831	6.32E-01	rs4926831	5.17E-01	-		4.02E-06	
rs929156	6	30247678	TRIM15	Intron	1.69E-05	rs2844775	2.50E-01	rs2844775	2.34E-01	rs929156	8.87E-02	8.77E-08	4.67E-02
rs11583200	1	50332407			1.83E-05	-		-		rs11583200	5.75E-01	1.05E-05	
rs11754661	6	151248771	MTHFD1L	Intron	2.01E-05	-		-		rs11754661	6.27E-01	1.26E-05	
rs3746319	19	49304071	ZNF224	Exon 6	2.96E-05	-		-		rs3746319	9.85E-01	2.92E-05	
rs2180566	20	29482515	DEFB123	Promoter	3.80E-05	-		-		rs2180566	4.75E-01	1.80E-05	
rs2061332	19	49305501	ZNF224	D'stream	3.93E-05	rs2061332	1.49E-02	rs2061332	7.22E-01	rs2061332	8.70E-01	3.68E-07	
rs2681411	3	123268321	CD86	Intron	4.21E-05	-		-		rs2681411	3.09E-01	1.30E-05	
rs2119067	2	165835529			4.38E-05	-		-		rs2119067	1.58E-01	6.92E-06	
rs1402627	18	4123739			4.42E-05	-		-		rs1402627	8.01E-01	3.54E-05	
rs659628	13	76361237	KCTD12	Promoter	4.46E-05	rs659628	4.49E-01	rs659628	1.00E+00	-		2.00E-05	
rs9455973	6	168325855			4.47E-05	rs9455973	9.79E-01	rs9455973	5.99E-01	rs9455973	6.27E-01	1.64E-05	
rs6059244	20	29474144			4.76E-05	-		-		rs6059244	5.43E-01	2.59E-05	
rs11205641	1	49957662			8.41E-05	rs11205641	3.40E-01	rs11205641	4.79E-01	rs11205641	3.85E-01	5.27E-06	

Supplementary Table 2. Beecham *et al*, 2008 GWAS SNPs ($5x10^{-5} < p < 5x10^{-8}$) compared with Reiman *et al*, 2007, Li *et al*, 2008 and Carrasquillo *et al*, 2009. The GWAS from which SNPs were initially selected is shown on the left. Each row represents a SNP with a p-value between $5x10^{-5}$ to $5x10^{-8}$. The p-values are highlighted yellow if $p < 0.05$ and they replicated across two or more studies. If a perfect proxy was used the corresponding rs number is listed. The combined p-values across studies are shown if the value approached genome-wide significance of 10^{-8}. The final column shows the corrected p-value adjusted as described in the methods section. SNPs surviving correction for multiple testing are also highlighted in yellow.

Reiman et al, 2007						Li et al, 2008		Carrasquillo et al, 2009		Combined p-value	Corrected p-value
SNP	CHR	BP	Gene	Position	p-value	SNP	p-value	SNP	p-value		
rs10824310	10	53680643	PRKG1	Intron	6.03E-07	rs10824310	3.06E-01	-		1.84E-07	
rs17330779	7	107663071	NRCAM	Intron	8.80E-07	rs17330779	5.31E-01	-		4.67E-07	
rs6784615	3	52468315	NISCH	Intron	9.89E-07	rs6784615	6.14E-01	-		6.07E-07	
rs12162084	16	26553533			1.30E-06	rs12162084	7.61E-01	-		9.88E-07	
rs2517509	6	31138101			1.35E-06	rs2517509	3.83E-01	-		5.16E-07	
rs7077757	10	112527724	RBM20	Intron	1.52E-06	rs7077757	4.18E-02	-		6.35E-08	2.44E-02
rs249153	12	93837244			2.66E-06	rs249153	8.25E-02	rs249153	7.17E-01	1.58E-07	
rs10747758	12	54287453			3.03E-06	rs10747758	2.45E-01	-		7.42E-07	
rs11958566	5	117719226			4.16E-06	rs11958566	6.16E-01	-		2.56E-06	
rs17505622	13	101759124	FGF14,LOC283480	Intron	5.47E-06	rs17505622	2.55E-01	-		1.39E-06	
rs7079348	10	77742377	C10ORF11	Intron	8.70E-06	rs7079348	3.85E-01	-		3.35E-06	
rs475093	1	43383592	LOC440585	Intron	8.86E-06	rs475093	7.10E-01	-		6.29E-06	
rs11748700	5	15773106	FBXL7	Intron	1.09E-05	rs11748700	2.40E-01	-		2.62E-06	
rs7817227	8	27951747			1.47E-05	rs7817227	4.99E-01	-		7.35E-06	
rs17126808	8	18457737	PSD3	Intron	1.89E-05	rs17126808	7.88E-01	-		1.49E-05	
rs950922	1	21747977	ALPL	Intron	1.96E-05	rs950922	3.45E-01	-		6.74E-06	
rs16842422	1	196346167			1.99E-05	rs16842422	7.48E-01	-		1.49E-05	
rs4759173	12	54262230			1.99E-05	rs4759173	4.52E-01	rs10876820	4.45E-01	4.00E-06	
rs2122339	4	27290902			2.12E-05	rs2122339	5.96E-01	-		1.27E-05	
rs4394475	9	90496717			2.18E-05	rs4394475	5.23E-01	-		1.14E-05	
rs10783760	12	54260896			2.22E-05	rs10783760	3.65E-01	rs10876820	4.45E-01	3.62E-06	
rs13213247	6	81560955			2.29E-05	rs13213247	5.73E-01	rs16892136	4.17E-01	5.46E-06	
rs7097398	10	91782821			2.60E-05	rs7097398	8.02E-01	-		2.08E-05	
rs9982394	21	41191871			2.68E-05	rs9982394	3.06E-01	-		8.19E-06	
rs9934599	16	69220773	IL34	Upstream	2.68E-05	-		rs9934599	4.46E-01	1.20E-05	
rs7031458	9	84704086			2.74E-05	rs7031458	2.16E-02	-		5.91E-07	
rs1923924	9	1581055			2.98E-05	rs1923924	5.00E-01	-		1.49E-05	
rs249154	12	93848520			3.12E-05	rs249154	1.14E-01	rs249153	7.17E-01	2.55E-06	
rs17151710	5	123739233			3.13E-05	rs17151710	7.59E-01	-		2.38E-05	
rs17048904	4	118081372			3.50E-05	rs17048904	1.00E+00	-		3.50E-05	
rs7134292	12	54260239			3.68E-05	rs7134292	3.23E-01	rs10876820	4.45E-01	5.30E-06	
rs7585710	2	10819621	ATP6V1C2	Intron	3.76E-05	rs7585710	1.00E+00	-		3.76E-05	
rs12044355	1	229901524	DISC1*	Intron	3.93E-05	rs12044355	9.07E-01	-		2.92E-07*	
rs6888935	5	117745419			3.93E-05	rs6888935	9.96E-01	-		3.92E-05	
rs17586545	14	51101242	LOC645380,LOC651876	Intron	4.11E-05	rs17586545	8.87E-01	-		3.65E-05	
rs1038891	11	40877959			4.48E-05	rs1038891	4.84E-01	-		2.17E-05	
rs6094514	20	44993488	EYA2	Intron	4.49E-05	rs6094514	3.40E-01	rs11700355	5.60E-01	8.54E-06	
rs10248657	7	112741449			4.56E-05	rs10248657	8.88E-01	-		4.05E-05	

Supplementary Table 3. Reiman *et al*, 2007 GWAS SNPs ($5x10^{-5} < p < 5x10^{-8}$) compared with Li *et al*, 2008 and Carrasquillo *et al*, 2009. The GWAS from which SNPs were initially selected is shown on the left. Each row represents a SNP with a p-value between $5x10^{-5}$ to $5x10^{-8}$. The p-values are highlighted yellow if $p < 0.05$ and they replicated across two or more

studies. If a perfect proxy was used the corresponding rs number is listed. The combined p-values across studies are shown if the value approached genome-wide significance of 10^{-8}. The final column shows the corrected p-value adjusted as described in the methods section. SNPs surviving correction for multiple testing are also highlighted in yellow. *DISC1* is starred to indicate that the combined p-value listed has included the data (p=8.20E-03) from the Beecham et al, 2008 study.

Carrasquillo et al, 2009						Li et al, 2008		Reiman et al, 2007		Combined p-value	Corrected p-value
SNP	CHR	BP	Gene	Position	p-value	SNP	p-value	SNP	p-value		
rs2318144	8	58277297	ncRNA		2.22E-06	rs17194995	2.04E-01	rs17194995	3.13E-01	1.42E-07	
rs1279795	23	123152101			5.02E-06	rs1279795	8.42E-01	-		4.22E-06	
rs3007421	1	6452776	PLEKHG5	Intron	6.54E-06	rs3007421	6.51E-01	rs3007421	4.68E-01	1.99E-06	
rs6546452	2	25834776			8.55E-06	rs17680828	9.00E-01	rs17680828	9.68E-01	7.45E-06	
rs7318037	13	81367146			1.15E-05	rs4456389	9.82E-01	rs4456389	2.39E-01	2.70E-06	
rs2118732	5	79419032			1.32E-05	rs7736549	5.49E-01	-		7.25E-06	
rs8039031	15	34954382	MEIS2	Downstream	2.26E-05	rs8039031	5.04E-01	rs8039031	9.92E-02	1.13E-06	
rs7245160	18	70417826	AK056288/LOC400657	Upstream	2.66E-05	rs7245160	4.60E-01	rs7245160	4.15E-01	5.08E-06	
rs856675	14	84405968			3.83E-05	rs17737309	7.10E-01	rs17737309	2.87E-01	7.81E-06	

Supplementary Table 4. Carrasquillo *et al*, 2009 GWAS SNPs ($5x10^{-5} < p < 5x10^{-8}$) compared with Reiman *et al*, 2007 and Li *et al*, 2008. The GWAS from which SNPs were initially selected is shown on the left. Each row represents a SNP with a p-value between $5x10^{-5}$ to $5x10^{-8}$. If a perfect proxy was used the corresponding rs number is listed. No SNPs replicated across studies using the Carrasquillo et al, 2009 GWAS as the primary dataset.