Hypertension and the roles of the 9p21.3 risk locus: Classic findings and new association data

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ABSTRACT

Background: The band 9p21.3 contains an established genomic risk zone for cardiovascular disease (CVD). Since the initial 2007 Wellcome Trust Case Control Consortium study (WTCCC), the increased CVD risk associated with 9p21.3 has been confirmed by multiple studies in different continents. However, many years later there was still no confirmed report of a corresponding association of 9p21.3 with hypertension, a major CV risk factor, nor with blood pressure (BP).

Theory: In this contribution, we review the bipartite haplotype structure of the 9p21.3 risk locus: one block is devoid of protein-coding genes but contains the lead CVD risk SNPs, while the other block contains the first exon and regulatory DNA of the gene for the cell cycle inhibitor p15. We consider how findings from molecular biology offer possibilities of an involvement of p15 in hypertension etiology, with expression of the p15 gene modulated by genetic variation from within the 9p21.3 risk locus.

Results: We present original results from a Colombian study revealing moderate but persistent association signals for BP and hypertension within the classic 9p21.3 CVD risk locus. These SNPs are mostly confined to a ‘hypertension island’ that spans less than 60 kb and coincides with the p15 haplotype block. We find confirmation in data originating from much larger, recent European BP studies, albeit with opposite effect directions.

Conclusion: Although more work will be needed to elucidate possible mechanisms, previous findings and new data prompt reconsidering the question of how variation in 9p21.3 might influence hypertension components of cardiovascular risk.

Abbreviations: CVD, cardiovascular disease; GWAS, genome wide association studi(es); BP, blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; bp, base pair; kb, kilobase pair; SNP, single nucleotide polymorphism; MAF, minor allele frequency; 1 KG, 1000 Genomes Project; TGF-β, transforming growth factor beta; EGFR, epidermal growth factor receptor; VSMC, vascular smooth muscle cell(s); RAS, renin angiotensin system.

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1. Introduction

It had long been a widely held belief that common genetic variation in the established cardiovascular risk locus of cytogenetic band 9p21.3, as discovered and delimited via corresponding genetic associations in 2007 [1–3], is not reproducibly associated with high blood pressure or hypertension, a prime risk factor for cardiovascular disease [4,5].

The pleiotropic nature of the ~120-kb classic risk locus of 9p21.3, the strongest known single contributor in the human genome to genetic risk of cardiovascular disease [6–8], viewed together with the complexity of the genetic and molecular regulation of hypertension, prompted us to reopen the question if this region might, after all, be associated with a contribution of hypertension to the increased cardiovascular risk that characterizes the locus.

In the Theory section, we briefly depict the classic two-block structure of the region in the light of current knowledge, and review some findings and hypotheses that could admit a step of a hypertension etiology being modulated at this locus.

In the Results we first present results from an original, small Colombian association study focusing on 9p21.3 variation. We then present largely corroborating results from much larger, recently published European studies, which we identified in a second step. Taken together, the evidence accumulating so far suggests that common genetic variation in a well-described block (~60 kb) of the 9p21.3 risk region — and, more specifically, in the regulatory DNA of the p15 gene it harbors — may play a role in promoting hypertension, for example via vascular modifications in resistance arteries (arterioles).

2. Material and methods

2.1. Colombian study

Demographic and clinical information, including selected cardiovascular risk factors, was collected for all participants in a study conducted in Medellín, Colombia (see Supplementary Data). Systolic (S) and diastolic (D) blood pressure (BP) levels were defined by the average of two conventional auscultatory BP measurements through a mercury manometer according to an approach in line with current European guidelines [9]. Presence of hypertension was identified based on physician's diagnosis, prescription of antihypertensive drugs, average office SBP ≥140 and/or DBP ≥90 mmHg, or any combination of these possibilities.

A total of 357 participants were genotyped. In view of this small sample size and in line with traditional case-control methodology [10], [11], Chapter 9), the highest and lowest DBP tertiles of the population were slightly overrepresented in the genotyped individuals.

DNA was extracted from white blood cells following standard salting-out procedures and genotyped by LGC Group, UK with KASP™ technology (https://www.biosearchtech.com). All SNPs genotyped were biallelic in our study sample. SNP rs10738605 is triallelic in some other populations of European ancestry. The minor allele frequency (MAF) plots shown in Fig. 1 depict individual SNP- and haplotype-level variation in the locus. Two of the superpopulations that were sequenced and genotyped by the 1000 Genomes Project (1 KG; [17]) are represented: AMR (populations from the Americas having a Native American ancestry component) and EUR (populations of European ancestry). The ‘left-hand’ block of the risk region, colored green in Fig. 1, is composed of essentially three haplotype classes, giving one low and two higher, similar minor allele frequencies (separated by a green horizontal bar). The ‘right-hand’ block is dominated by two haplotype classes and has fairly constant allele frequencies close to 50%; the lead CVD risk SNPs of the region are at its rightmost fringe, denoted by ochre triangles.

The left-hand block includes the upstream regulatory DNA and start of the protein-coding gene encoding p15 (transcribed leftward). The Figure shows a big difference between AMR and EUR in this block. The Figure also shows a matrix, in 0/1 haplotype (schema) coding, for 16 SNPs that are representative of variation within this block, as obtained by 1000 Genomes’ sampling of 94 individuals (i.e., 188 chromosomes) from CLM (Medellín, Colombia, an AMR population).

Where three or more major haplotype classes dominate a region, individual biallelic SNPs can provide only aggregated resolution of what is happening at the haplotype level [18]. Here, SNP classes s1 (blue identifiers), s2 (red) or s3 provide information on, respectively, the contrast of haplotype class c2, c1 or c3 versus the remaining two haplotype classes of the haplotype block.

2.2. European studies

Methods of European studies were as described in the publications presenting those studies [14–16].

3. Theory

3.1. The bipartite cardiovascular risk locus of 9p21.3

The originally described risk locus of 9p21.3 has a bipartite or two-segment structure, in which each of the two mutually adjacent segments is dominated by a distinct haplotype block. Although the haplotypes (i.e., states) of the two blocks are correlated, via a linkage disequilibrium originally estimated as around 0.6 (for average [D]) [2], their frequencies are different.

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3.2. Antagonistic pleiotropy in 9p21.3

The CVD risk locus in 9p21.3 is a confirmed risk locus not only for cardiovascular disease but also for other diseases, including cancers. The pleiotropies appear to be partly antagonistic, in that the risk allele for one disease can be the protective allele for another disease. For example, the NHGRI-EBI GWAS Catalog (www.ebi.ac.uk/gwas) reports SNPs of the left block where the protective allele for an age-related condition (e.g., CVD, glaucoma) would be the risk allele for a cancer-related condition (e.g., breast cancer, glioma, pediatric brain tumor, endometriosis; see Supplementary Material S2.1). Antagonistic pleiotropies have been noted or proposed before for this locus ([19]; see also [20]), with data suggesting a corresponding positive selection in some populations having Native American ancestry [21].

3.3. Genes in the risk locus

Recent literature on the risk locus of 9p21.3 and its associations has emphasized the enigmatic long noncoding RNA (IncRNA) gene ANRIL, which has regulatory roles that are still only partly understood, but that
can affect expression of protein-coding genes in the risk locus [22].

Earlier molecular biology studies had focused on two protein-coding genes for cyclin-dependent kinase inhibitors, which when activated can arrest or inhibit the cell cycle at the G1 stage: the gene CDKN2A encodes both p14ARF (p14) and p16INK4a (p16), and CDKN2B encodes p15INK4b (p15).

A third protein-coding gene, MTAP, which is located outside the risk locus, is also relevant for understanding the region. MTAP encodes a key enzyme in the biosynthetic pathway of polyamines (which plays an important role in the stabilization of atherosclerotic plaques); there is also evidence that MTAP may be partly regulated from within the 9p21.3 CVD risk locus despite the intervening distance [16,23,24].

Fig. 2 depicts the positions of the risk locus’ genes and other functionally relevant genetic and epigenetic features.

3.4. The p15 gene

Some common genetic variation in the p15 haplotype block is in regulatory DNA, where it could in principle modulate transcription of the p15 gene. Thus, the common SNP rs2069418, in an established promoter of the p15 gene, borders a tetranucleotide that was critical for C/EBPβ binding and transcription of the p15 gene in experiments in vitro (Fig. 2) [25–27]; its common variation might also modulate a predicted stemloop (see Supplementary Material S2.2). The functionally strategic position of SNP rs2069418 was also noted before, but in the apparently antagonistic context of glioma risk [28].

The well-studied promoter of the p15 gene [29] is a ‘battlefield’ in which the proto-oncogene c-Myc and TGFβ/Smad struggle to control the p15 gene. Indeed, control of this gene can be important: where expression of the cell cycle inhibitor and tumor suppressor p15 is suppressed, cancer risk can rise [27]. TGFβ is a main regulator of blood vessel development and maintenance, but it acts through several alternative ‘arms’, or pathways, e.g., via crosstalk. We ask when and where the arm of TGFβ/s activities that utilizes p15, as part of the TGFβ/Smad pathway, might be employed under physiological conditions in a context that would be relevant for the genesis of hypertension.

3.5. Routes to hypertension mediated by TGFβ signaling or crosstalk

Fig. 3 shows a rough sketch of elements of three pathways in which TGFβ can play a role and/or modulate hypertension etiologies: the TGFβ/Smad pathway with target gene p15, in the central lane, and two other pathways that are trans-activatable by “crosstalk” with TGFβ, respectively mediated by the type 1 angiotensin-II receptor (AT1R/classic RAS
pathway, left) and the epidermal growth factor receptor (EGFR, right). The central and rightmost of these three pathways and their crosstalk, for which roles in hypertension etiologies remain to be elucidated in more detail, are examined in this context in Supplementary Material S2.3; refs. [27,30–32] describe experimental results that may be relevant. In addition to such cues from experiments, an independent line of evidence supporting a likely role for the central pathway comes from large-scale whole-genome association studies and metastudies that can now identify whole networks showing evidence of collective association with blood pressure [15,16]. The results indicate that the TGF\(\beta\)/Smad pathway plays an important role in BP regulation, as it is enriched for BP-associated genes [16]. It then seems a plausible hypothesis that p15, as an important TGF\(\beta\)/Smad-responsive target, could be involved in the modulating of blood pressure.
Although the complexity and possible sensitivity of such a network can render difficult the prediction of how a genetic change at a given locus will affect BP or the risk of developing hypertension, already Fig. 3 hints that the p15 context puts us in a locus and a scenario where key players, such as cell cycle inhibitors, are only a step away from etiologically familiar routes to pathogenesis of hypertension or its prevention.

4. Results

4.1. Colombian study

We conducted a local, small study in the city of Medellín, Colombia (n = 357 individuals without overt CVD) to explore possible associations between hypertension and 9p21.3 (67 common SNPs spanning > 300 kb). One of the motivations for the study was that we knew of no 9p21.3 studies of a population living in Latin America. It was immediately clear from the sample size that even for minor allele frequencies (MAF) above 20% we would know of no 9p21.3 studies of a population living in Latin America. It was reviewed as being associated with blood pressure or hypertension in ref. [19, Supplementary Information] (‡; † for pathway) or have been reviewed as being associated with BP or hypertension in ref. [46] (‡). Dashed horizontal arrows indicate experimentally observed or hypothesized crosstalk/transactivation between pathways (not just receptors). Not shown, for simplicity, is another potentially relevant system, descending alongside the classic RAS pathway at left, namely the ‘nonclassical’ RAS, composed primarily of the AngII/Ang III-AT2R pathway and the ACE2-Ang-(1–7)-AT7R axis [47], which generally counteracts the effects of a stimulated classic Ang II-AT1R axis as shown in the Figure, and which is currently of great interest for understanding and possibly treating the Covid-19 disease [48].

We compared the inferred risk and protector alleles for hypertension or blood pressure at our genotyped SNPs with the risk and protector alleles of disease phenotypes that had been previously reported at those same SNPs. Risk alleles were defined as those for which mean difference or beta was positive (continuous variables), for which effect size was greater than one (dichotomous variables), or that were explicitly labeled as ‘risk allele’. Previously published associations listed in the NHGRI-EBI GWAS database suggested two mutually ‘antagonistic’ groups (see section 3.2, Supplementary Material S2.1, or [19, Supplementary Information]), the risk alleles for the CVD- or aging-related conditions (e.g., coronary heart disease, or intracranial aneurism) being the protector alleles for cancer-related conditions (e.g., glioma). In our Colombian study and in the 9p21.3 island where we observed the best association signals, the inferred risk alleles for hypertension or BP (e.g., A, the major allele, for rs7865618) were also the previously reported risk alleles for SBP/DBP increase, with a per-allele Odds Ratio of 2.44 for hypertension and a mean difference (effect size) of 2.94/3.14 mmHg for SBP/DBP levels, after correcting for sex and age. Comparable results were obtained when including smoking and/or BMI as covariates (Supplementary Material S1.4 and Supplementary Data).

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Although our study focused on blood pressure variables and hypertension, we did consider, and subjected to the same basic phenotype-genotype analysis, several dozen (not all independent) alternative candidates for the phenotypic/outcome variable of primary interest, selected on the basis of their hemodynamic or other relevance to the development of hypertension (listed in Supplementary Data). However, the BP and hypertension variables consistently gave by far the best support for an association at the SNPs we genotyped (see Supplementary Data for the results obtained for rs7044859 and rs2069418, representing the two equivalence classes of SNPs s1 and s2, which are characterized by high linkage disequilibrium).
4.2. European studies

A review of the data from recently published large European studies [14–16] with detailed look-ups of results in this region of interest, again showed higher DBP association signals in the same island that we had delimited in the Colombian study than in its flanking DNA, with p-values that almost reached genome-wide significance. Thus, in the studies by Warren et al. [15] and by Evangelou et al. [16] (n > 750,000), the two lowest p-values for DBP, 1.23 × 10⁻⁷ and 9.22 × 10⁻⁷, were found, respectively, at the ‘left’ fringe of the island’s haplotype block (rs3217992) and at a SNP in the interior of the island that we had not genotyped in the Colombian study (rs7874604).

Furthermore, in the European blood pressure studies [15,16] genome-wide significance of DBP associations was attained, outside of the classic 9p21.3 CVD risk locus and its flanking regions, in the next gene MTAP (see Fig. 2 and Theory), with a lowest p-value of 1.3 × 10⁻¹⁰ for the sentinel SNP rs4364717 (red asterisk and red horizontal bar at left in Fig. 2; see also the LocusZoom plot in Supplementary Material S3.2). The BP association with the MTAP gene had not been noted in previous studies, and indeed the region had not been sufficiently covered in earlier imputation panels.

In the earlier study by Ehret et al. ([14], n > 180,000), the best p-values observed were within the island, and again for DBP, although they did not reach values below 0.002 (see Supplementary Material, subsection S3.2). We were even able to detect concordant results as far back as 2011, in a study screening close to 2.5 million SNPs for BP associations [36]. The SNPs in the island again had much lower p-values than those in the lead CVD risk zone and regions flanking the island. Thus, the SNP that had the lowest p-value for hypertension in our Colombian study sample, n564398, had similarly the lowest p-value for DBP in the island in ref. [36], namely 0.0161 as its genome-wide meta-analysis p-value corrected for genomic control.

A notable difference with respect to the Colombian results was the effect direction. Indeed, directions of the BP effects in the European studies [15,16] were consistently opposite to those observed in the Colombian study at the same SNPs. In other words, the BP effects were also opposite to the established CVD effects for those SNPs in the literature. The now established CVD effect directions within the island are the same in European populations, deduced via direct genotyping of island SNPs, as they are in populations with a Latin American or Native American ancestry component (e.g., Refs. [37,38]), where they can be inferred from genotyped SNPs in linkage disequilibrium located close to the island. Thus, in the European study samples, but not in the Colombian study sample, the inferred protector alleles for hypertension (i.e., earlier in disease etiology) are the known, constant risk alleles for cardiovascular disease or events (i.e., later in the etiology).

Three other European reports we found, in Refs. [39] and in the PhenoScanner and Roslin GeneATLAS association databases, had varying significance and independence, but the reported effect directions appear compatible with our findings (Supplementary Material S3.3).

5. Discussion

The results from the trans-ethnic investigations we describe here are compatible with the persisting presence, in a South American and in a European population, of associations between BP levels/hypertension and genetic variation specifically in a previously identified haplotype block or ‘island’ nested within the cardiovascular risk locus of band 9p21.3. In the European population, dense coverage of the association screening also allowed the recognition of an association peak for BP in a neighboring gene, MTAP, which is located at about 140 kb from the risk locus.

According to the data collected so far, the hypertension or BP effect within the CVD risk locus 9p21.3 would have the opposite direction in the South American compared to the European population, whereas the CVD effect appears to be the same in both populations, according to published reports [37,38]. To our knowledge, however, our study is the first screen of the 9p21.3 ‘island’ for hypertension or BP associations in a South American population, and we also know of no dedicated CVD association study with dense coverage in this part of the 9p21.3 risk zone. It is therefore too early to generalize or extrapolate from this one small study, and we must await confirmation from independent genotype-phenotype screening in future, if possible also in South American populations having other ancestry proportions, such as those of Peru or Bolivia (see, for example, [40]). The larger networks in which the p15 gene is embedded (e.g., the pathways of Fig. 3) are likely to be complex and resilient, and not just genetic background but also environment (e.g., diet) and local epidemiology (e.g., disease prevalences) could influence routing or rebalancing of the networks and thus affect associations.

According to theoretical arguments, genotype-phenotype associations that change direction of effect between geographic, population or other environments (e.g., in the form of crossing reaction norms [41,42]) could actually be favored, because a variant that is inferior in all environments would be rendered unstable and would eventually be eliminated from the gene pool [41,43], ([44], section 5). In line with this view, trans-ethnically significant BP associations can exhibit sizable directional inconsistency rates. For example, among 562 SNPs genome-wide that were associated with BP traits (SBP, DBP, or pulse pressure) at a high significance of p < 10⁻¹⁰ in a European sample (n = 757,601) in ref. [16], Supplementary Table 18), 20 of the 32 SNPs having p < 0.01 also in a South Asian population, and 6 of the 22 SNPs having p < 0.01 in an African population, had effect directions that were opposite to those in the European population.

As a final note, a wider view of physical or regulatory gene interactions may help understand potential roles of the CVD risk locus in modulating blood pressure or hypertension. One line of investigation that could be relevant is represented by Hi-C data that capture interactions between different chromosomal regions. Thus, Hi-C data reported in ref. [16], Supplementary Table 8) show, at least in mesenchymal stem cells, an interaction cluster that includes the classic risk region and other regions from almost all of cytotgenetic band 9p21.3 (4.7/5.7 Mb; for details see Supplementary Material S3.4). The clustering regions include (or immediately flank) the 9p21.3 interferon gene group, relevant in inflammation, and two genes (GADD45G and FODAC) that have been considered of interest in cardiovascular as well as cancer contexts. Of particular note, the cluster also includes the MTAP and DMRT1 genes, which have been recently reported to be associated with DBP [15] and SBP [16], respectively, and are located at a distance on either side of the classic risk region. Such findings suggest that a full understanding of the potential role of p15 and the classic CVD risk locus in modulating hypertension and CVD might require a wider view that takes into account the regulatory interactions among genes distributed across, and possibly beyond, the 5.7-Mb cytotgenetic band 9p21.3.

Conflict of interest

M.J.C. is Chief Scientist for Genomics England, a UK Government company. The authors have reported that they have no other relationships relevant to the contents of this paper to disclose.

Other statements

This research has been conducted using the UK Biobank Resource under Application Number 236.
Supervision, Project administration, Funding acquisition. Helen R. Warren: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing - original draft, Writing - review & editing, Visualization, Project administration, Funding acquisition. Elizabeth Misas: Formal analysis, Investigation, Data curation. Monica M. Correa: Investigation, Data curation. Jaime A. Gallo-Villegas: Methodology, Investigation, Data curation, Supervision. Gabriel Bedoya: Methodology, Resources, Visualization, Supervision, Project administration. Dagnývar Árístadóttir: Conceptualization, Methodology, Resources, Supervision, Project administration, Funding acquisition. Juan G. McEwen: Conceptualization, Methodology, Resources, Supervision, Project administration, Funding acquisition. Mark J. Caulfield: Methodology, Investigation, Resources, Data curation, Supervision, Project administration, Funding acquisition. Gianfranco Parati: Conceptualization, Methodology, Writing - original draft, Writing - review & editing, Visualization, Supervision, Funding acquisition. Oliver K. Clay: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijchy.2020.100050.

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