·论　著·

91 种炎症蛋白与 5 种心血管疾病的因果关系：双向孟德尔随机化研究

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[摘要]　目的　采用孟德尔随机化（MR）和反向 MR 方法评估 91 种炎症蛋白与 5 种心血管疾病（主动脉夹层、动脉瘤、冠心病、非风湿性心瓣膜病和风湿性心瓣膜病）之间的因果关系。方法　使用来自欧洲人群的全基因组关联研究（GWAS）数据，利用 MR 方法和反向 MR 方法对 91 种炎症蛋白与 5 种心血管疾病之间的双向因果关系进行评估分析。MR 分析方法包括逆方差加权法、加权中位数法、MR-Egger 回归、简单模式和加权模式，敏感性分析包括 Cochran's Q 检验、MR-Egger 截距检验、MR-PRESSO 法和留一法。结果　共有 16 种炎症蛋白可能与心血管疾病风险存在相关性，分别为 C-C 趋化因子配体（CCL）20、CD5、CCL28、白细胞介素 20 受体 α（IL-20RA）、潜在转化生长因子 β1 前体（LAP-TGF-β1）、胸腺基质淋巴细胞生成集（TSLP）、胱抑素 D（CST5）、白血病抑制因子（LIF）、翻译起始因子 4E 结合蛋白 1（EIF4EBP1）、CCL4、白细胞介素 22 受体 α1（IL-22RA1）、IL-10、IL-17C、单核细胞趋化蛋白（MCP）-2/CCL8、神经秩蛋白（NRTN）、MCP-3/CCL7。此外，心血管疾病的进展可能会导致 10 种炎症蛋白水平的变化，包括 CCL11、IL-8、TNF-β、成纤维细胞生长因子（FGF）-19、白细胞介素 10 受体 α（IL-10RA）、FGF-21、白细胞介素 10 受体 β（IL-10RB）、β- 神经生长因子（β-NGF）、CD5 和 MCP-1/CCL2。结论　多种炎症蛋白与 5 种心血管疾病之间存在双向因果关系，进一步研究各种炎症蛋白与上述心血管疾病之间的相关性具有潜在临床价值。

[关键词]　炎症；炎症蛋白；心血管疾病；孟德尔随机化；全基因组关联研究

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Causal relationship between 91 inflammatory proteins and 5 cardiovascular diseases: a bidirectional Mendelian randomization

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[Abstract]　Objective　To assess the causal relationships between 91 inflammatory proteins and 5 cardiovascular diseases (including aortic dissection, aneurysm, coronary heart disease, non-rheumatic valve disease, and rheumatic valve disease) by Mendelian randomization (MR) and reverse MR. Methods　MR and reverse MR analyses were used to assess bidirectional causality between 91 inflammatory proteins and 5 cardiovascular diseases based on Genome-Wide Association Studies (GWAS) data from European populations. MR analysis methods involved inverse variance weighted, weighted median, MR-Egger, simple mode and weighted mode approaches. Sensitivity analysis methods included Cochran's Q test, MR-Egger intercept test, MR-PRESSO method, and leave-one-out approach. Results　A total of 16 inflammatory proteins might be associated with the risk of cardiovascular diseases, including C-C motif chemokine ligand (CCL)20, CD5, CCL28, interleukin 20 receptor α (IL-20RA), latency-associated peptide transforming growth factor β1 (LAP-TGF-β1), thymic stromal lymphopoietin (TSLP), cystatin D (CST5), leukemia inhibitory factor (LIF), eukaryotic translation initiation factor 4E binding protein 1 (EIF4EBP1), CCL4, interleukin 22 receptor α1 (IL-22RA1), interleukin (IL)-10, IL-17C, monocyte chemoattractant protein (MCP)-2/CCL8, neurturin (NRTN), and MCP-3/CCL7. Furthermore, the progression of cardiovascular diseases

might potentially lead to changes in the levels of 10 inflammatory proteins, including CCL11, IL-8, tumor necrosis factor β (TNF-β), fibroblast growth factor (FGF)-19, interleukin 10 receptor α (IL-10RA), FGF-21, interleukin 10 receptor β (IL-10RB), β-nerve growth factor (β-NGF), CD5, and MCP-1/CCL2. **Conclusion**　The present study highlights the bidirectional causal relationship between an array of inflammatory proteins and the 5 cardiovascular diseases. Further research on the correlation between various inflammatory proteins and the above cardiovascular diseases has potential clinical value.

炎症是人体正常生理防御机制的一种表现，通常由感染、组织损伤或其他异常情况引起。然而，长期或慢性炎症会对心血管系统产生不利影响，可能会增加罹患心脏疾病的风险。例如，CRP 是一种广泛用于评估全身炎症的标志物，通常与冠心病风险升高有关；一些炎症生物标志物已被证明可以预测心血管疾病，而不受传统风险因素的影响[1-2]。炎症蛋白在炎症中发挥着关键作用，并与心脏疾病产生复杂的相互作用。以往的研究探讨了某些炎症蛋白在心血管疾病中的功能和潜在影响，如 IL-6 和 IL-1 在心血管疾病中起着重要作用，并有望成为某些心血管疾病的治疗靶点[3-4]。尽管这些观察性研究探讨了炎症蛋白在诊断和治疗心血管疾病方面的作用，但其结果可能会受到混杂变量或反向因果关系的影响，从而使因果关系变得更加复杂。因此，一些常见的心脏疾病与大量炎症蛋白之间的因果关系还需要进一步阐明。

孟德尔随机化（Mendelian randomization，MR）分析源于孟德尔遗传定律，通过分析数据中的单核苷酸多态性（single nucleotide polymorphism，SNP）来阐明暴露与结果之间的因果关系[5]。这种分析方法利用了 SNP 的随机分配特点，将其作为工具变量进行分析[6]。由于等位基因在生殖细胞减数分裂过程中会发生随机分配，MR 分析可有效减少混杂因素、测量误差和反向因果关系，从而大大提高研究结果的可靠性[6-7]。本研究利用 MR 分析和反向 MR 分析对 91 种炎症蛋白与 5 种心血管疾病的因果关系进行分析验证。

1　资料和方法

1.1　研究设计

采用 MR 分析和反向 MR 分析来研究 91 种炎症蛋白对 5 种心血管疾病的双向因果效应。要进行 MR 分析，需要提出 3 个重要的假设：相关性假设，即假设工具变量与暴露因素之间存在稳固的相关性；独立性假设，即假设工具变量独立于混杂因素；排他性假设，即假设工具变量只能通过暴露因素对结果产生影响。分析中使用的所有数据都来自公开的和以前发表的数据集，因此无须伦理批准或知情同意。

1.2　数据来源

5 种心血管疾病的全基因组关联研究（Genome-Wide Association Studies，GWAS）数据来自芬兰基因研究项目（FinnGen），主动脉夹层、动脉瘤、冠心病、非风湿性心瓣膜病和风湿性心瓣膜病的数据编号分别为 finngen_R9_I9_AORTDIS、finngen_R9_I9_AORTANEUR、finngen_R9_I9_CHD、finngen_R9_I9_NONRHEVALV、finngen_R9_I9_RHEUVALV。91 种炎症蛋白的 GWAS 数据来自欧洲生物信息研究所 GWAS 目录数据库（EBI GWAS Catalog Database），其数据集来自对 91 种血浆蛋白质的全基因组蛋白质定量性状位点（protein quantitative trait loci，pQTL）分析[8]。该项研究利用了 Olink 精准蛋白质组学技术（Olink Target 96 Inflammation Panels），可以对样本进行系统免疫图谱扫描，检测其中包含的 92 种炎症相关蛋白标志物，但由于检测问题，该研究团队移除了脑源性神经营养性因子（brain-derived neurotrophic factor，BDNF），故而包含 91 种炎症蛋白。该项研究综合了 11 个队列，总计 14 824 名欧洲居民参与者。

1.3　工具变量选择

为确保符合 MR 分析的关联假说，利用 $P < 5 \times 10^{-8}$ 或 $P < 5 \times 10^{-6}$ 的阈值[9]，选择与暴露有强相关性的 SNP。对于每种疾病，采

用 $P<5×10^{-6}$ 的阈值鉴定出 3 个及以上的 SNP,但对于冠心病,采用了 $P<5×10^{-8}$ 这一更为严格的阈值。为了降低连锁不平衡的风险,对这些 SNP 采用了聚类分析(距离设置为 10 000 kb, r^2 设置为 0.001)。在 GWAS 中,等位 SNP 在暴露和结果方面的排列不确定,因此这些 SNP 被排除。同时,利用每个 SNP 的 r^2 值确定暴露变异的比例。工具强度通过 F 值来衡量, $F=r^2(N-2)/(1-r^2)$,其中 N 表示样本量。F 值低于 10 的 SNP 被排除在外,以减少弱工具带来的偏差[10-11]。丢失的 SNP 使用从 LDlink(*https://ldlink.nci.nih.gov/*)获得的 $r^2>0.9$ 的 SNP 进行替代[12]。

1.4　MR 方法　采用多种 MR 分析方法评估 91 种炎症蛋白与 5 种心血管疾病之间的因果关系。通过反向 MR 分析研究潜在的反向因果关系。主要分析采用逆方差加权(inverse variance weighted,IVW)方法,该方法综合了各种 SNP 因果效应的 Wald 比值估计值,对暴露与结果的因果关系进行评估[13]。然而,该方法预设了所有工具变量的有效性,在实际应用中并不总是成立,因此补充了其他允许工具变量存在误差的方法以提高结果的可靠性。加权中位数(weighted median)对无效工具变量的容忍度更高,当有效工具变量权重超过 50% 时,即可提供可靠的估计值[14]。MR-Egger 回归方法侧重于评估无效因果假设并确保因果关系评估的一致性,包括工具变量表现出遗传变异不足的情况[15]。加权模式(weighted mode)通过关注众数的效应值,能够有效地捕捉最具代表性的因果关系,尤其是在涉及多个工具变量的情况下。简单模式(simple mode)也是基于众数效应进行分析的一种方法,它对极端值具有更强的适应能力,不受少量极端数据的影响;同时,在工具变量效应大小分布不均或存在显著异质性的情况下,简单模式可以有效地提供中心倾向的估计值。当 IVW 方法得出 $P<0.05$ 且所有 MR 分析方法的 β 值方向相同时,判定为阳性结果。此外,当 MR-Egger 截距检验和 MR-PRESSO 方法均得出 $P>0.05$ 时,判定为不存在水平多效性。

1.5　敏感性分析　采用 Cochran's Q 检验、MR-Egger 截距检验、MR-PRESSO 方法和留一法(leave-one-out)进行敏感性分析,以提高研究结果的可靠性和稳健性。Cochran's Q 检验用于检测潜在的异质性,旨在评估工具变量的变化是否会导致不同的结果[16]。MR-PRESSO 方法专门用于识别表现出水平多效性的离群 SNP,前提是超过 50% 的工具变量有效[17]。MR-Egger 截距检验用于检测潜在的垂直多效性,其特点是工具变量通过独立于暴露的途径影响结果[15]。留一法逐一地剔除每个 SNP 后重新评估剩余 SNP 的效应值,旨在通过评估每个 SNP 对总体结果的影响来验证结果的可靠性和稳健性。

1.6　统计学处理　使用 R 4.1.2 软件 TwoSampleMR 包和 MR-PRESSO 包进行数据分析。

2　结　果

2.1　9l 种炎症蛋白对 5 种心血管疾病的影响　如图 1 所示,在主动脉夹层中,C-C 趋化因子配体(C-C motif chemokine ligand,CCL)20 和 CD5 为保护因素,CCL28 和白细胞介素 20 受体 α(interleukin 20 receptor α,IL-20RA)为危险因素;在动脉瘤中,潜在转化生长因子 β1 前体(latency-associated peptide transforming growth factor β1,LAP-TGF-β1)和胸腺基质淋巴细胞生成素(thymic stromal lymphopoietin,TSLP)为危险因素;在冠心病中,胱抑素 D(cystatin D,CST5)和白血病抑制因子(leukemia inhibitory factor,LIF)是保护因素,翻译起始因子 4E 结合蛋白 1(eukaryotic translation initiation factor 4E binding protein 1,EIF4EBP1)、CCL4 和白细胞介素 22 受体 α1(interleukin 22 receptor α1,IL-22RA1)是危险因素;在非风湿性心瓣膜病中,IL-10、IL-17C、单核细胞趋化蛋白(monocyte chemoattractant protein,MCP)-2/CCL8 和神经秩蛋白(neurturin,NRTN)是保护因素;在风湿性心瓣膜病中,MCP-3/CCL7 是危险因素。

Cochran's Q 检验结果显示,暴露与结果之间的关系存在相当大的异质性。实施随机效应 IVW 分析可一定程度减轻结果中的异质性。此外,MR-Egger 截距检验和 MR-PRESSO 均未发现偏差,这表明 MR 分析的可靠性及其在异质性中抵御潜在多向性偏差的能力。留一法表明研究结果并非由单个 SNP 驱动。散点图和剔除敏感性分析见图 2 和图 3。

Exposure	Outcome	nSNPs	Method	Beta	P value	OR (95% CI)
			MR Egger	-0.923	0.0935	0.40 (0.14, 1.09)
			Weighted Median	-0.3428	0.2145	0.71 (0.41, 1.22)
CCL20*		17	**Inverse Variance Weighted**	**-0.4227**	**0.0281**	**0.66 (0.45, 0.96)**
			Simple Mode	-0.1882	0.6873	0.83 (0.34, 2.04)
			Weighted Mode	-0.2461	0.55	0.78 (0.35, 1.72)
			MR Egger	-0.2927	0.5156	0.75 (0.32, 1.76)
			Weighted Median	-0.4156	0.0776	0.66 (0.42, 1.05)
CD5*		16	**Inverse Variance Weighted**	**-0.4249**	**0.0121**	**0.65 (0.47, 0.91)**
			Simple Mode	-0.6709	0.1339	0.51 (0.22, 1.17)
	Aorta Dissection		Weighted Mode	-0.2867	0.3718	0.75 (0.41, 1.38)
			MR Egger	0.317	0.4975	1.37 (0.56, 3.36)
			Weighted Median	0.52	0.0661	1.68 (0.97, 2.93)
CCL28		18	**Inverse Variance Weighted**	**0.4643**	**0.0288**	**1.59 (1.05, 2.41)**
			Simple Mode	0.6474	0.1805	1.91 (0.77, 4.74)
			Weighted Mode	0.5888	0.1751	1.80 (0.80, 4.07)
			MR Egger	1.1649	0.1298	3.21 (0.91, 11.31)
			Weighted Median	0.7757	0.0296	2.17 (1.08, 4.37)
IL-20RA		7	**Inverse Variance Weighted**	**0.69**	**0.0356**	**1.99 (1.05, 3.79)**
			Simple Mode	1.2337	0.0881	3.43 (1.05, 11.27)
			Weighted Mode	0.4015	0.4086	1.49 (0.62, 3.62)
			MR Egger	0.4179	0.0219	1.52 (1.10, 2.10)
			Weighted Median	0.3624	0.0062	1.44 (1.11, 1.86)
LAP-TGF-β1		18	**Inverse Variance Weighted**	**0.2344**	**0.0138**	**1.26 (1.05, 1.52)**
			Simple Mode	0.2543	0.346	1.29 (0.77, 2.16)
	Aneurysm		Weighted Mode	0.4027	0.0157	1.50 (1.11, 2.01)
			MR Egger	0.1585	0.6525	1.17 (0.60, 2.28)
			Weighted Median	0.4823	0.0088	1.62 (1.13, 2.32)
TSLP		11	**Inverse Variance Weighted**	**0.35**	**0.0374**	**1.42 (1.02, 1.97)**
			Simple Mode	0.5168	0.0576	1.68 (1.05, 2.69)
			Weighted Mode	0.5168	0.0322	1.68 (1.12, 2.52)
			MR Egger	-0.0194	0.3718	0.98 (0.94, 1.02)
			Weighted Median	-0.0355	0.0337	0.97 (0.93, 1.00)
CST5*		32	**Inverse Variance Weighted**	**-0.036**	**0.0228**	**0.96 (0.94, 0.99)**
			Simple Mode	-0.0986	0.0844	0.91 (0.81, 1.01)
			Weighted Mode	-0.0373	0.0378	0.96 (0.93, 1.00)
			MR Egger	-0.0326	0.8129	0.97 (0.75, 1.25)
			Weighted Median	-0.0846	0.2094	0.92 (0.81, 1.05)
LIF*		7	**Inverse Variance Weighted**	**-0.1325**	**0.011**	**0.88 (0.79, 0.97)**
			Simple Mode	-0.0555	0.5836	0.95 (0.78, 1.14)
			Weighted Mode	-0.0609	0.4697	0.94 (0.81, 1.10)
			MR Egger	0.1377	0.1638	1.15 (0.96, 1.37)
			Weighted Median	0.1204	0.0095	1.13 (1.03, 1.24)
EIF4EBP1	Coronary Artery Disease	10	**Inverse Variance Weighted**	**0.1222**	**0.0005**	**1.13 (1.06, 1.21)**
			Simple Mode	0.1725	0.0358	1.19 (1.04, 1.36)
			Weighted Mode	0.1256	0.0487	1.13 (1.02, 1.26)
			MR Egger	0.0383	0.092	1.04 (1.00, 1.08)
			Weighted Median	0.0417	0.0666	1.04 (1.00, 1.09)
CCL4		15	**Inverse Variance Weighted**	**0.033**	**0.0241**	**1.03 (1.00, 1.06)**
			Simple Mode	0.0037	0.9373	1.00 (0.92, 1.10)
			Weighted Mode	0.0335	0.0578	1.03 (1.00, 1.07)
			MR Egger	0.1235	0.1793	1.13 (0.96, 1.33)
			Weighted Median	0.1224	0.044	1.13 (1.00, 1.27)
IL-22RA1		10	**Inverse Variance Weighted**	**0.0965**	**0.0311**	**1.10 (1.01, 1.20)**
			Simple Mode	0.0656	0.4754	1.07 (0.90, 1.27)
			Weighted Mode	0.1392	0.1583	1.15 (0.96, 1.37)
			MR Egger	-0.1414	0.0779	0.87 (0.75, 1.00)
			Weighted Median	-0.0903	0.0712	0.91 (0.83, 1.01)
IL-10*		15	**Inverse Variance Weighted**	**-0.087**	**0.0166**	**0.92 (0.85, 0.98)**
			Simple Mode	-0.1371	0.1459	0.87 (0.73, 1.04)
			Weighted Mode	-0.0991	0.1687	0.91 (0.79, 1.04)
			MR Egger	-0.2039	0.19	0.82 (0.61, 1.09)
			Weighted Median	-0.0719	0.2645	0.93 (0.82, 1.06)
IL-17C*		13	**Inverse Variance Weighted**	**-0.1166**	**0.0092**	**0.89 (0.82, 0.97)**
			Simple Mode	-0.0035	0.9779	1.00 (0.78, 1.27)
	Non-Rheumatic Valve Disease		Weighted Mode	-0.0094	0.9383	0.99 (0.79, 1.25)
			MR Egger	-0.0393	0.0688	0.96 (0.92, 1.00)
			Weighted Median	-0.0426	0.0098	0.96 (0.93, 0.99)
MCP-2/CCL8*		20	**Inverse Variance Weighted**	**-0.0373**	**0.0245**	**0.96 (0.93, 1.00)**
			Simple Mode	-0.0732	0.2201	0.93 (0.83, 1.04)
			Weighted Mode	-0.0388	0.0254	0.96 (0.93, 0.99)
			MR Egger	-0.0673	0.3868	0.93 (0.81, 1.08)
			Weighted Median	-0.1057	0.0828	0.90 (0.80, 1.01)
NRTN*		11	**Inverse Variance Weighted**	**-0.0857**	**0.0375**	**0.92 (0.85, 1.00)**
			Simple Mode	-0.1928	0.0878	0.82 (0.68, 1.01)
			Weighted Mode	-0.1943	0.0324	0.82 (0.71, 0.96)
			MR Egger	0.6212	0.1149	1.86 (0.91, 3.83)
			Weighted Median	0.404	0.0912	1.50 (0.94, 2.39)
MCP-3/CCL7	Rheumatic Valve Disease	15	**Inverse Variance Weighted**	**0.3656**	**0.0367**	**1.44 (1.02, 2.03)**
			Simple Mode	0.092	0.8379	1.10 (0.46, 2.60)
			Weighted Mode	0.614	0.0369	1.85 (1.10, 3.11)

图 1　91 种炎症蛋白与 5 种心血管疾病之间 MR 分析中的阳性结果

Fig 1　Positive results in MR analysis between 91 inflammatory proteins and 5 cardiovascular diseases

*: Exposure was negatively associated with outcome. *OR* and 95% *CI* indicate change in outcome *OR* per 1 standard deviation increase in exposure. MR: Mendelian randomization; CCL: C-C motif chemokine ligand; IL-20RA: Interleukin 20 receptor α; LAP-TGF-β1: Latency-associated peptide transforming growth factor β1; TSLP: Thymic stromal lymphopoietin; CST5: Cystatin D; LIF: Leukemia inhibitory factor; EIF4EBP1: Eukaryotic translation initiation factor 4E binding protein 1; IL-22RA1: Interleukin 22 receptor α1; IL-10: Interleukin 10; IL-17C: Interleukin 17C; MCP: Monocyte chemoattractant protein; NRTN: Neurturin; nSNPs: Number of single nucleotide polymorphisms; *OR*: Odds ratio; *CI*: Confidence interval.
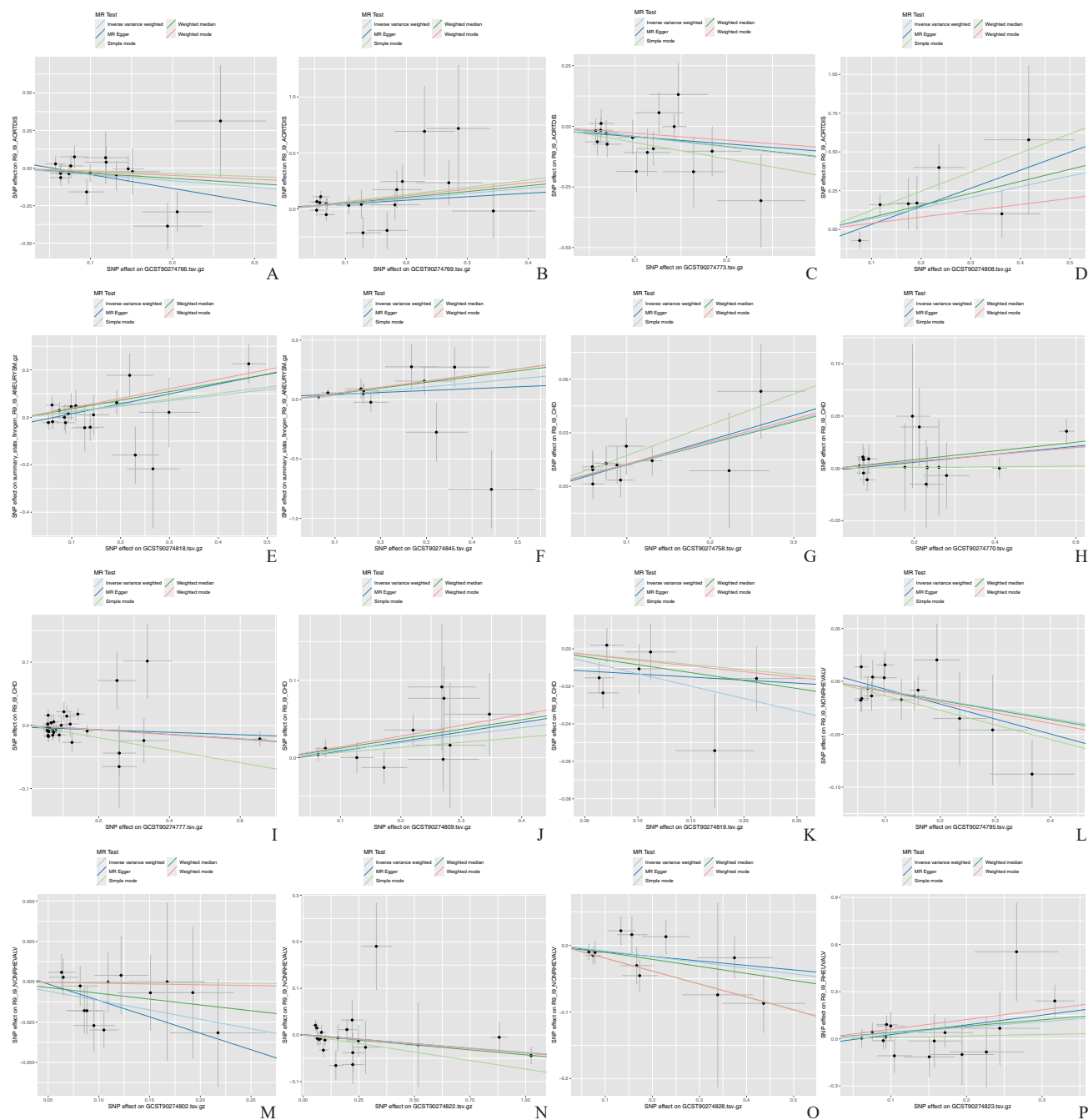
图 2　91 种炎症蛋白与 5 种心血管疾病之间 MR 分析的散点图

Fig 2　Scatter plot of MR analysis between 91 inflammatory proteins and 5 cardiovascular diseases

The single inverse variance (IV) related to exposure risk and the single IV related to outcomes (indicated by black dots) correspond to each other. The 95% confidence intervals of the odds ratios for each IV are represented by vertical and horizontal lines. The slope of these lines indicates the estimated causal effect analyzed by the MR method. A-D: The relationships between aortic dissection (outcome) and inflammatory proteins CCL20, CCL28, CD5, and IL-20RA, respectively; E, F: The relationships between aneurysm (outcome) and inflammatory proteins LAP-TGF-β1 and TSLP, respectively; G-K: The relationships between coronary artery disease (outcome) and inflammatory proteins EIF4EBP1, CCL4, CST5, IL-22RA1, and LIF, respectively; L-O: The relationships between non-rheumatic valve disease (outcome) and inflammatory proteins IL-10, IL-17C, MCP-2/CCL8, and NRTN, respectively; P: The relationship between rheumatic valve disease (outcome) and inflammatory protein MCP-3/CCL7. MR: Mendelian randomization; CCL: C-C motif chemokine ligand; IL-20RA: Interleukin 20 receptor α; LAP-TGF-β1: Latency-associated peptide transforming growth factor β1; TSLP: Thymic stromal lymphopoietin; EIF4EBP1: Eukaryotic translation initiation factor 4E binding protein 1; CST5: Cystatin D; IL-22RA1: Interleukin 22 receptor α1; LIF: Leukemia inhibitory factor; IL-10: Interleukin 10; IL-17C: Interleukin 17C; MCP: Monocyte chemoattractant protein; NRTN: Neurturin; SNP: Single nucleotide polymorphism.

图 3　对 91 种炎症蛋白和 5 种心血管疾病进行 MR 分析的留一图

Fig 3　Leave-one-out analysis for MR studies on 91 inflammatory proteins and 5 cardiovascular diseases

A-D: The relationships between aortic dissection (outcome) and inflammatory proteins CCL20, CCL28, CD5, and IL-20RA, respectively; E, F: The relationships between aneurysm (outcome) and inflammatory proteins LAP-TGF-β1 and TSLP, respectively; G-K: The relationships between coronary artery disease (outcome) and inflammatory proteins EIF4EBP1, CCL4, CST5, IL-22RA1, and LIF, respectively; L-O: The relationships between non-rheumatic valve disease (outcome) and inflammatory proteins IL-10, IL-17C, MCP-2/CCL8, and NRTN, respectively; P: The relationship between rheumatic valve disease (outcome) and inflammatory protein MCP-3/CCL7. MR: Mendelian randomization; CCL: C-C motif chemokine ligand; IL-20RA: Interleukin 20 receptor α; LAP-TGF-β1: Latency-associated peptide transforming growth factor β1; TSLP: Thymic stromal lymphopoietin; EIF4EBP1: Eukaryotic translation initiation factor 4E binding protein 1; CST5: Cystatin D; IL-22RA1: Interleukin 22 receptor α1; LIF: Leukemia inhibitory factor; IL-10: Interleukin 10; IL-17C: Interleukin 17C; MCP: Monocyte chemoattractant protein; NRTN: Neurturin.

2.2　5 种心血管疾病对 91 种炎症蛋白的影响　如图 4 所示,主动脉夹层与 CCL11、IL-8 和 TNF-β 呈负相关;动脉瘤与成纤维细胞生长因子(fibroblast growth factor,FGF)-19 呈正相关,而与白细胞介素 10 受体 α(interleukin 10 receptor α,IL-10RA)呈负相关;冠心病与 FGF-21 和白细胞介素 10 受体 β(interleukin 10 receptor β,IL-10RB)呈负相关;非风湿性心瓣膜病与 β- 神经生长因子(β-nerve growth factor,β-NGF)、CD5 和 MCP-1/CCL2 呈负相关;风湿性心瓣膜病与所研究的 91 种炎症蛋白没有明显的反向 MR 因果关系。

Exposure	Outcome	nSNPs	Method	Beta	P value	OR (95% CI)
Aorta Dissection	CCL11*	15	MR Egger	-0.0291	0.3049	0.97 (0.92, 1.02)
			Weighted Median	-0.0355	0.0525	0.97 (0.93, 1.00)
			Inverse Variance Weighted	**-0.0331**	**0.0122**	**0.97 (0.94, 0.99)**
			Simple Mode	-0.0368	0.3418	0.96 (0.90, 1.04)
			Weighted Mode	-0.0334	0.3034	0.97 (0.91, 1.03)
	IL-8*		MR Egger	-0.0250	0.3323	0.98 (0.93, 1.02)
			Weighted Median	-0.0226	0.1759	0.98 (0.95, 1.01)
			Inverse Variance Weighted	**-0.0269**	**0.0317**	**0.97 (0.95, 1.00)**
			Simple Mode	-0.0207	0.4587	0.98 (0.93, 1.03)
			Weighted Mode	-0.0169	0.4571	0.98 (0.94, 1.03)
	TNF-β*		MR Egger	-0.0025	0.9304	1.00 (0.94, 1.05)
			Weighted Median	-0.0220	0.2686	0.98 (0.94, 1.02)
			Inverse Variance Weighted	**-0.0409**	**0.0036**	**0.96 (0.93, 0.99)**
			Simple Mode	-0.0094	0.7927	0.99 (0.92, 1.06)
			Weighted Mode	-0.0059	0.8437	0.99 (0.94, 1.05)
Aneurysm	FGF-19	24	MR Egger	0.0282	0.2447	1.03 (0.98, 1.08)
			Weighted Median	0.0269	0.1932	1.03 (0.99, 1.07)
			Inverse Variance Weighted	**0.0380**	**0.0135**	**1.04 (1.01, 1.07)**
			Simple Mode	0.0348	0.3353	1.04 (0.97, 1.11)
			Weighted Mode	0.0293	0.1601	1.03 (0.99, 1.07)
	IL-10RA*		MR Egger	-0.0065	0.7869	0.99 (0.95, 1.04)
			Weighted Median	-0.0129	0.5930	0.99 (0.94, 1.03)
			Inverse Variance Weighted	**-0.0318**	**0.0438**	**0.97 (0.94, 1.00)**
			Simple Mode	-0.0563	0.1279	0.95 (0.88, 1.01)
			Weighted Mode	-0.0086	0.7521	0.99 (0.94, 1.04)
Coronary Artery Disease	FGF-21*	53	MR Egger	-0.0568	0.3993	0.94 (0.83, 1.08)
			Weighted Median	-0.0617	0.1219	0.94 (0.87, 1.02)
			Inverse Variance Weighted	**-0.0572**	**0.0402**	**0.94 (0.89, 1.00)**
			Simple Mode	-0.0574	0.5135	0.94 (0.80, 1.12)
			Weighted Mode	-0.0526	0.4324	0.95 (0.83, 1.08)
	IL-10RB*		MR Egger	-0.1596	0.0116	0.85 (0.76, 0.96)
			Weighted Median	-0.0726	0.0512	0.93 (0.86, 1.00)
			Inverse Variance Weighted	**-0.0653**	**0.0112**	**0.94 (0.89, 0.99)**
			Simple Mode	-0.0447	0.5690	0.96 (0.82, 1.11)
			Weighted Mode	-0.0704	0.3032	0.93 (0.82, 1.06)
Non-Rheumatic Valve Disease	β-NGF*	40	MR Egger	-0.0682	0.4176	0.93 (0.79, 1.10)
			Weighted Median	-0.0835	0.0964	0.92 (0.83, 1.02)
			Inverse Variance Weighted	**-0.0703**	**0.0431**	**0.93 (0.87, 1.00)**
			Simple Mode	-0.1800	0.0587	0.84 (0.70, 1.00)
			Weighted Mode	-0.1256	0.0701	0.88 (0.77, 1.01)
	CD5*		MR Egger	-0.1260	0.1854	0.88 (0.73, 1.06)
			Weighted Median	-0.1152	0.0286	0.89 (0.80, 0.99)
			Inverse Variance Weighted	**-0.0782**	**0.0450**	**0.92 (0.86, 1.00)**
			Simple Mode	-0.1093	0.3181	0.90 (0.73, 1.11)
			Weighted Mode	-0.1260	0.0837	0.88 (0.77, 1.01)
	MCP-1/CCL2*		MR Egger	-0.0766	0.4114	0.93 (0.77, 1.11)
			Weighted Median	-0.0427	0.4209	0.96 (0.86, 1.06)
			Inverse Variance Weighted	**-0.0762**	**0.0466**	**0.93 (0.86, 1.00)**
			Simple Mode	-0.0490	0.6620	0.95 (0.77, 1.18)
			Weighted Mode	-0.0490	0.5696	0.95 (0.81, 1.13)

图 4　91 种炎症蛋白与 5 种心血管疾病之间反向 MR 分析中的阳性结果

Fig 4　Positive results in reverse MR analysis between 91 inflammatory proteins and 5 cardiovascular diseases

*: Exposure was negatively associated with outcome. OR and 95% CI indicate change in outcome OR per 1 standard deviation increase in exposure. MR: Mendelian randomization; CCL: C-C motif chemokine ligand; IL-8: Interleukin 8; TNF-β: Tumor necrosis factor β; FGF: Fibroblast growth factor; IL-10RA: Interleukin 10 receptor α; IL-10RB: Interleukin 10 receptor β; β-NGF: β-nerve growth factor; MCP: Monocyte chemoattractant protein; nSNPs: Number of single nucleotide polymorphisms; OR: Odds ratio; CI: Confidence interval.

Cochran's Q 检验表明, 暴露与结果之间存在异质性, 但随机效应 IVW 方法证实了反向 MR 分析的可靠性。这种可靠性归因于随机效应 IVW 方法能够减轻结果中的异质性。此外, MR-Egger 截距测试和 MR-PRESSO 方法进一步验证了 MR 分析结果的可靠性。留一法表明研究结果并不依赖于任何单个 SNP。散点图和剔除敏感性分析见图 5 和图 6。
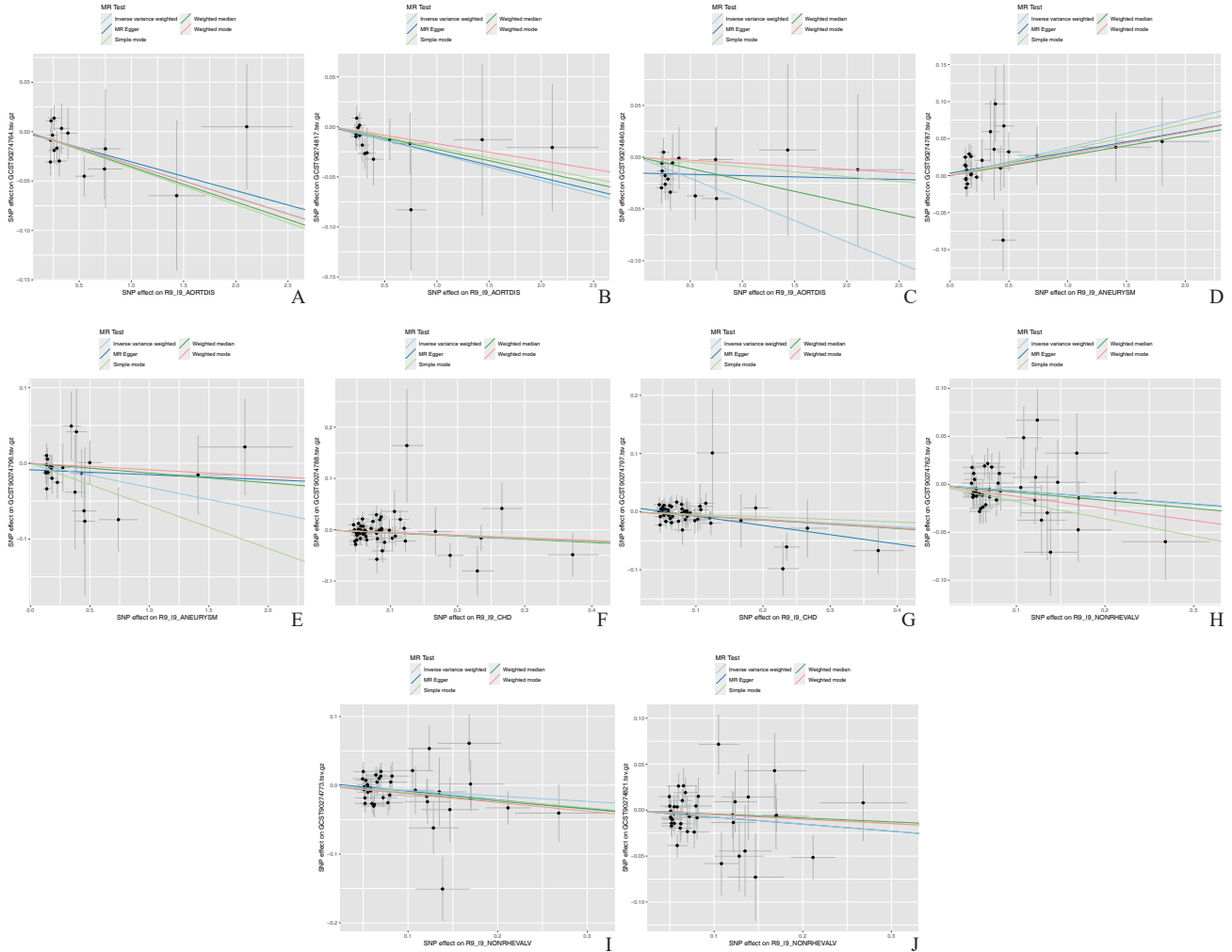

图 5　5 种心血管疾病与 91 种炎症蛋白之间反向 MR 分析的散点图

Fig 5　Scatter plot of inverse MR analysis between 5 cardiovascular diseases and 91 inflammatory proteins

The single inverse variance (IV) related to exposure risk and the single IV related to outcomes (indicated by black dots) correspond to each other. The 95% confidence intervals of the odds ratios for each IV are represented by vertical and horizontal lines. A-C: The relationships between aortic dissection (exposure) and inflammatory proteins CCL11, IL-8, and TNF-β, respectively; D, E: The relationships between aneurysm (exposure) and inflammatory proteins FGF-19 and IL-10RA, respectively; F, G: The relationships between coronary artery disease (exposure) and inflammatory proteins FGF-21 and IL-10RB, respectively; H-J: The relationships between non-rheumatic valve disease (exposure) and inflammatory proteins β-NGF, CD5, and MCP-1/CCL2, respectively. CCL: C-C motif chemokine ligand; IL-8: Interleukin 8; TNF-β: Tumor necrosis factor β; FGF: Fibroblast growth factor; IL-10RA: Interleukin 10 receptor α; IL-10RB: Interleukin 10 receptor β; β-NGF: β-nerve growth factor; MCP: Monocyte chemoattractant protein; SNP: Single nucleotide polymorphism.

3　讨　论

本研究采用 MR 方法对 91 种炎症蛋白与 5 种心血管疾病之间的潜在因果关系进行了初步分析, 随后用反向 MR 方法评估了反向因果关系的可能性。研究结果表明, CCL20 和 CD5 可能是主动脉夹层的上游保护因素, 而 CCL28 和 IL-20RA 可能是主动脉夹层的上游危险因素; 此外, 主动脉夹层的发生可能导致 CCL11、IL-8 和 TNF-β 水平下降。在动脉瘤中, LAP-TGF-β1 和 TSLP 可被视为上游危

险因素, 而疾病进展可能导致 IL-10RA 水平下降和 FGF-19 水平上升。对于冠心病, 上游保护因素包括 CST5 和 LIF, 而上游危险因素包括 EIF4EBP1、CCL4 和 IL-22RA1; 其发病可能会导致 FGF-21 和 IL-10RB 水平降低。在非风湿性心瓣膜病中,

上游保护因素包括 IL-10、IL-17C、MCP-2/CCL8 和 NRTN, 而疾病进展可能会导致下游的 β-NGF、CD5 和 MCP-1/CCL2 水平降低。在风湿性心瓣膜病中, 上游危险因素是 MCP-3/CCL7, 没有发现明显的下游变化。
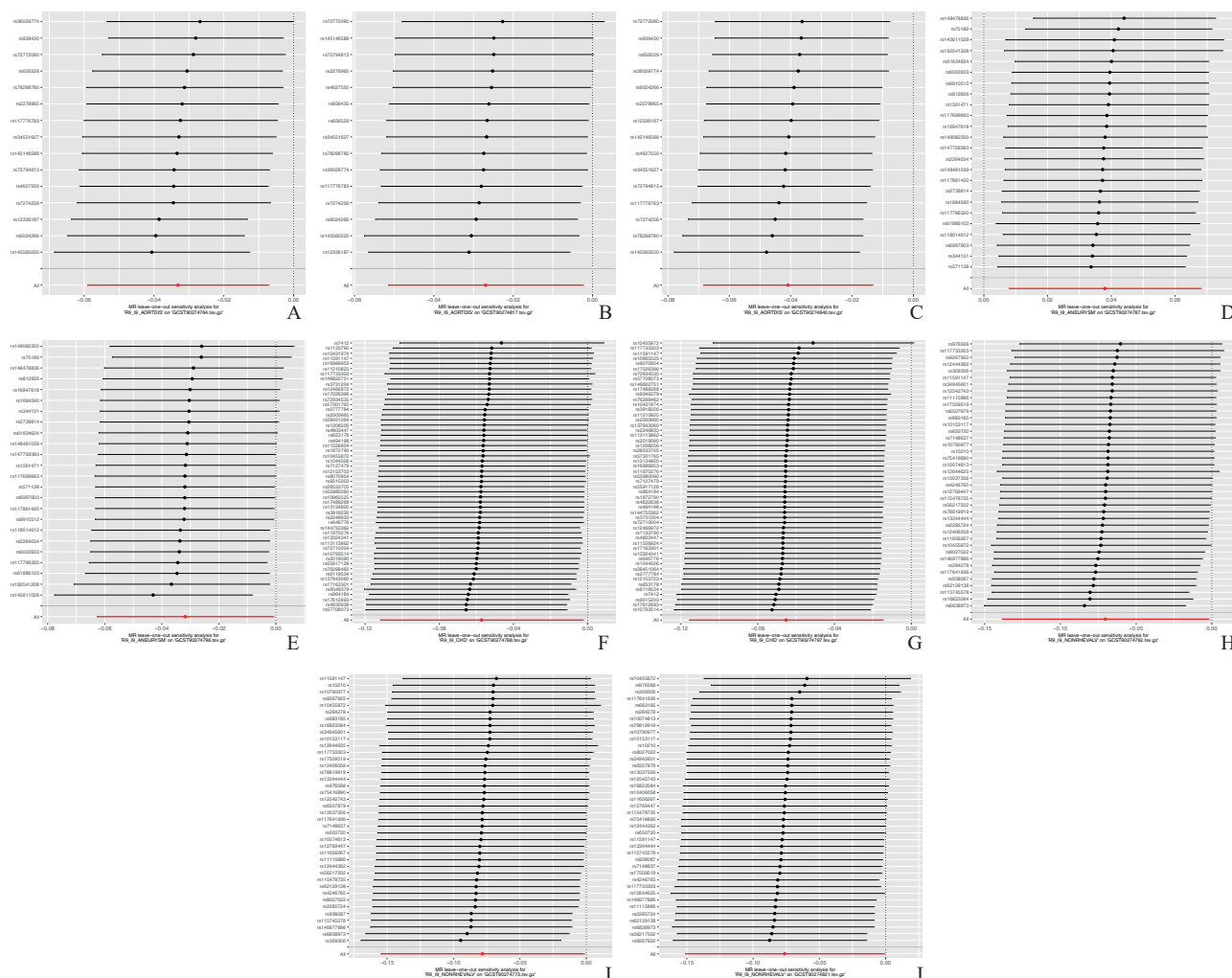

图 6　5 种心血管疾病与 91 种炎症蛋白之间反向 MR 分析的留一图

Fig 6　Leave-one-out plot of inverse MR analysis between 5 cardiovascular diseases and 91 inflammatory proteins

A-C: The relationships between aortic dissection (exposure) and inflammatory proteins CCL11, IL-8, and TNF-β, respectively; D, E: The relationships between aneurysm (exposure) and inflammatory proteins FGF-19 and IL-10RA, respectively; F, G: The relationships between coronary artery disease (exposure) and inflammatory proteins FGF-21 and IL-10RB, respectively; H-J: The relationships between non-rheumatic valve disease (exposure) and inflammatory proteins β-NGF, CD5, and MCP-1/CCL2, respectively. CCL: C-C motif chemokine ligand; IL-8: Interleukin 8; TNF-β: Tumor necrosis factor β; FGF: Fibroblast growth factor; IL-10RA: Interleukin 10 receptor α; IL-10RB: Interleukin 10 receptor β; β-NGF: β-nerve growth factor; MCP: Monocyte chemoattractant protein.

　　值得注意的是, 本研究发现 CD5 水平与非风湿性心瓣膜病和主动脉夹层有显著相关性。基于这一结果, 我们提出了一个假设, 即非风湿性心瓣膜病的进展可能会导致下游 CD5 水平的降低, 鉴于 CD5 被认为是防止主动脉夹层的上游保护因子, CD5 水平的降低可能会增加罹患这种疾病的风险。有趣

的是, 多项研究表明主动脉瓣双瓣疾病有时与主动脉逐渐扩张有关, 有可能导致主动脉夹层风险增加[18-19]。虽然这种现象可能与局部血流动力学变化有关, 但某些研究提出了潜在的细胞原因[20-21]。CD5 是慢性淋巴性白血病中 T 细胞、B1-a 细胞和 B 细胞的生物标志物, 在调节 T 细胞和 B 细胞活化、

促进免疫耐受和减轻过度炎症反应方面起着关键作用[22-23]。非风湿性心瓣膜病经常与慢性炎症和心瓣膜结构改变有关，这些过程涉及免疫系统的激活和调节[24]。这一疾病过程可能会破坏免疫调节的平衡，影响免疫细胞的活性和细胞因子的释放，从而对 CD5 水平产生负面影响。因此，这种破坏可能会引发多米诺骨牌效应，导致严重的免疫反应、炎症和动脉壁损伤，从而损害动脉壁的完整性并增加主动脉夹层的风险。然而，这些推断都是初步的，需要进一步的实验验证，其具体的机制仍未完全阐明。

迄今为止，已有大量研究探讨了炎症蛋白与心血管疾病之间的相关性。随机对照试验结果显示，对于服用他汀类药物的患者来说，高敏 CRP 比低密度脂蛋白胆固醇水平更能预测未来的心血管事件和死亡率[25]。IL-6、IL-18、基质金属蛋白酶 9、可溶性 CD40 配体和 TNF-α 等促炎细胞因子都与冠心病风险的增加独立相关[26]。此外，与安慰剂相比，以 IL-1β 免疫性为重点的抗炎疗法可显著降低心血管事件的复发率[4,27-28]。

之前的研究主要集中于研究炎症与心血管疾病之间的关系，通常是将特定的炎症因素与单一疾病联系起来。已有研究者通过 MR 方法探讨了各类暴露（如肠道菌群[29-30]、血清代谢物[31]）与特定疾病之间的因果关系，但炎症与心血管疾病之间的 MR 分析尚未见报道。本研究通过 MR 和反向 MR 方法初步探讨了 91 种炎症蛋白与 5 种心血管疾病之间的双向因果关系，探索了这几种疾病的潜在上游影响和下游后果。

本研究还存在一些不足：（1）本研究使用的 GWAS 数据集主要来自欧洲居民，缺乏其他种族群体的数据，这可能会导致估计结果有偏差，并影响本研究结果的普适性。（2）本研究收集的数据为横断面数据，考虑到疾病发病率的时间依赖性，缺乏暴露测量的纵向数据可能会给分析结果带来部分偏差。（3）本研究无法全面评估独立性和排他性假说，这有可能导致偏差。未来的研究应采用综合的方法，整合多组学技术[32-33]，以便在复杂的基因－环境相互作用中更深入地了解疾病的发病机制。

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