Supplementary information

Supplementary Table 1. Main outcomes of intra-operative, epicardial, high resolution mapping studies focused on quantifying features of electropathology.

Study Population	Mapping sites	No.	AF	AF Type	Rhythm	Parameters	Conclusion
WPW CAD MVD AVD	RA RA, LA	25 24	3	PAF LSPAF	AF AF	Focal waves prevalence spatial distribution EGM morphology repetitiveness AFCL irregularities	AF persistence is associated with focal waves Features of focal waves indicate transmural conduction of fibrillation waves (1).
WPW CAD MVD AVD	RA RA, LA	25 24	3	PAF LSPAF	AF AF	Intra-wave CB (%) Inter-wave CB (%) No. of fibrillation waves	Electrical longitudinal dissociation in conduction is a key element in the substrate of human AF (2).
CAD MVD AVD	RA	14	10	PAF:3 PSAF:4 LSPAF: 3	AF	Focal waves (%) Conduction block (%) Endo-Epicardial Asynchrony	First evidence for asynchronous activation of the endo-epicardial wall during AF in humans. Endo-epicardial asynchrony may play a major role in the pathophysiology of AF (3).
CAD	RA BB LA	209	-	N.A.	SR	Conduction delay (%) Conduction block (%)	Intra-atrial and inter-individual variation in conduction delay/block. Predilection site: superior intercaval RA. Extensiveness of CB at the superior intercaval RA or BB does not reflect CB elsewhere (4).

CAD	BB	185	13	PAF:13 PoAF: 56	SR	Activation Patterns Conduction delay (%) Conduction block (%)	Conduction disorders, particularly long lines of longitudinal conduction block, are more pronounced in patients with PoAF episodes (5).
MVD **AVD**	RA BB LA	139	38	PSAF POAF: 63	SR	Conduction delay: prevalence, severity Conduction block: prevalence, severity	MVD patients had a higher maximal degree of CD at the lateral left atrium than AV patients. A history of AF was most strongly correlated to CD/CB at Bachmann's bundle and age (6).
CAD **MVD** **AVD**	RA BB LA	447	75	PAF: 52 PSAF: 21 LSPAF: 2	SR	Conduction delay: prevalence, severity, lengths Conduction block: prevalence, severity, lengths	UHD has no impact on the frequency and severity of conduction disorders (6). AF episodes are associated with more conduction disorders throughout both atria and with more severe conduction disorders at BB.
CAD **MVD** **AVD**	RA BB LA PVA	212	No AF	N.A.	SR	Conduction delay: prevalence, severity, lengths Conduction block: prevalence, severity, lengths	Obese patients have higher incidences of conduction disorders, which are also more extensive and more severe (7).
CHD	RA BB LA	31	5	PAF	SR	Conduction delay: prevalence, severity, lengths Conduction block: prevalence, severity, lengths	Conduction disorders during SR are most pronounced in the RA—particularly the intercaval region—and BB (8).
CHD	RA BB LA	10*	0	N.A.	SR	Conduction delay: prevalence, severity, lengths	Areas of conduction delay and block were present in all patients and were particularly observed at BB and to a lesser degree in the left atrium (9).

						Conduction block: prevalence, severity, lengths	
CAD MVD AVD	PVA	327	62	PAF: 47 PSAF: 14 LSPAF: 1	SR	Activation Patterns	Complex patterns with often multiple entry sites and high interindividual variability. Altered patterns of activation, consisting of multiple opposing wavefronts combined with long lines of conduction slowing, were associated with AF (9).
CAD MVD AVD	PVA	268	49	PAF: 38 PSAF: 11		Conduction delay: prevalence Conduction block: prevalence,	Patients with AF more often present with continuous lines of adjacent areas of CD and CB (10).
CAD MVD AVD	RA BB LA	381	59	PAF: 43 PSAF: 15 LSPAF: 1	SR	Epicardial breakthrough wave: spatial distribution, prevalence, EGM morphology at origin	EBW are: present in 168 patients particularly in thicker parts of the atrial wall most often in ischemic heart disease patients EBW EGM most often consisted of double and fractionated potentials Single potentials: a R wave was observed in 88% of EBW, as opposed to 21% of sinus node breakthrough waves Fractionated EBW potentials were more often observed at the right atrium and Bachmann's bundle (11).
CAD MVD AVD	RA BB LA	253	43	PAF: 33 PSAF: 9 LSPAF: 1	SR	Activation patterns Total atrial activation time	Atrial excitation during SR is affected by underlying heart disease and AF, resulting in alternative routes for BB and left atrioventricular groove

							activation and prolongation of total atrial activation times (10).
CAD MVD AVD	RA (endo-epicardial)	80	8	PAF: 25 PSAF: 4 LSPAF: 2	ST	Conduction block (%) differences between endo- and epicardium Endo-epicardial asynchromy	Conduction disorders: more pronounced at endocardium than the epi- cardium particularly at the superior intercaval region. Length of CB lines longer in patients with presence of cardiovascular risk factors. Conduction disorders and EEA are more present in patients with persistent AF (12)
CAD MVD AVD	RA (endo-epicardial)	20	8	PAF: 8	SR	Conduction block (%) differences between endo- and epicardium Endo-epicardial asynchrony	In patients with AF, sino-atrial node activity occurred more caudally, which indicates changes in preferential sino-atrial node exit pathways (13).
CAD MVD AVD	RA BB LA	164	25	PAF: 19 PSAF:5 LSPAF: 1	SR AES	Activation Patterns Conduction delay (%) Conduction block (%)	Conduction disorders are mainly provoked by prematurely, aberant beats (14).
CAD MVD	RA BB LA	44	23	PAF: 23	SR	RS ratio Unipolar voltages	AF is characterized by decreased amplitudes of single potentials at BB due to loss of S-wave amplitudes and decreased conduction velocity (15).
CAD MVD AVD	RA (endo-epicardial)	21	11	PAF:7 PSAF:2 LSPAF: 2	SR	Endo-epicardial asynchrony EGM fractionation	Local epi–endocardial differences in EGM fractionation occur occasionally during SR but will likely increase during arrhythmias due to increasing endo-epicardial asynchrony and (functional) conduction disorders. EGM fractionation can originate from EEA (16).
CAD	RA	189	0	N.A.	SR	EGM morphology,	The signal fingerprint, consisting of quantified EGM features, including

	BB LA PVA					R/S rations fractionation fractionation delay unipolar voltages	the R/S ratio, the relative frequency distribution of unipolar voltages, the proportion of low-voltage areas, the proportion of the different types of EGMs, and durations of long double and fractionated potentials, may serve as a diagnostic tool to determine the severity and extensiveness of conduction inhomogeneity (17).

AVD: aortic valve disease, BB: Bachmann's bundle, CAD: coronary artery disease, CHD: congenital heart disease EGM: electrograms, EBW: epicardial breakthrough waves, LA: left atrium, LSPAF: longstanding persistent AF, MVD: mitral valve disease, N.A.: not applicable, PAF: paroxysmal AF, PSAF: persistent AF, PVA: pulmonary vein area, RA: right atrium, R/S ratio: amplitude R (positive)-wave/amplitude S (negative)-wave, SR: sinus rhythm.

Inclusion criteria: adult patients undergoing cardiac surgery. Exclusion criteria: presence of an atrial pacing lead, severe liver- or kidney disease, history of ablative therapy in the atria, usage of inotropic agents, impaired left ventricular function (<30%), prior radiation therapy of the chest.

Supplementary Box 1. AF persistence: caused by rotors or endo-epicardial asynchrony?

Proposed mechanisms underlying AF persistence can be divided in either focal (repetitive ectopic activity), re-entrant mechanisms (rotors) or endo-epicardial asynchrony. Though series of elegant experimental studies demonstrated that rotors, identified by dominant frequency analysis, maintained AF (18), multicenter clinical AF trials failed to demonstrate successful outcomes of ablative therapy targeting these rotors (19). In addition, high resolution mapping studies in patients so far failed to demonstrate the presence of such rotors driving AF (1). However, these studies did demonstrate that focal fibrillation waves, defined as fibrillation waves originating in a circumscription area from which they expand into the surrounding tissue, play a key role in AF persistence. These focal waves are caused by transmural conduction of fibrillation waves as a result of endo-epicardial asynchrony. We think it is unlikely that the high-resolution epicardial mapping methodology is too detailed and therefore fails to see rotors is incorrect as linking of consecutive wave fronts combined with stable beat-to-beat-morphology and AF cycle lengths still would reveal the presence of a rotor, irrespective of the mapping resolution.

Supplementary Box 2. Conflicting role of atrial refractoriness in AF pathophysiology.

Although APD shortening was previously recorded in models of atrial tachypacing-induced AF, electrophysiological changes that drive AF include prolongation as well as shortening of action potential duration (APD) (20, 21), reduction in cardiomyocyte excitability and increased APD dispersion (22, 23). APD prolongation was observed in patients with 'lone' paroxysmal AF, in atrial tissue of patients predisposed to AF and in various patient and animal studies for AF with underlying heart failure and structural changes in the atria (21, 24-26). These studies provide compelling evidence that the predominant contributors to the substrate underlying AF are the structural and associated conduction abnormalities i.e. electropathology rather than shortening of APD. Moreover, current pharmacological AF treatments are directed at modulation of atrial refractoriness, and not at molecular root causes of electropathology. This may explain their limited efficacy, while its usage is further limited by pro-arrhythmic effects and noncardiovascular toxicity (27).

References

1. De Groot NM, Houden RP, Smeets JL, Boersma E, Schotten U, Schalij MJ, et al. Electropathological substrate of longstanding persistent atrial fibrillation in patients with structural heart disease: epicardial breakthrough Circulation. 2010;122:1674-82.
2. Allessie MA, de Groot NM, Houben RP, Schotten U, Boersma E, Smeets JL, et al. Electropathological substrate of long-standing persistent atrial fibrillation in patients with structural heart disease: longitudinal dissociation. CirculationArrhythmia and electrophysiology. 2010;3(6):606-15.
3. de Groot N, van der Does L, Yaksh A, Lanters E, Teuwen C, Knops P, et al. Direct Proof of Endo-Epicardial Asynchrony of the Atrial Wall During Atrial Fibrillation in Humans. Circulation Arrhythmia and electrophysiology. 2016;9(5).
4. Lanters EAH, Yaksh A, Teuwen CP, van der Does L, Kik C, Knops P, et al. Spatial distribution of conduction disorders during sinus rhythm. Int J Cardiol. 2017;249:220-5.
5. Teuwen CP, Yaksh A, Lanters EA, Kik C, van der Does LJ, Knops P, et al. Relevance of Conduction Disorders in Bachmann's Bundle During Sinus Rhythm in Humans. Circ Arrhythm Electrophysiol. 2016;9(5):e003972.
6. Heida A, van der Does WFB, van Staveren LN, Taverne Y, Roos-Serote MC, Bogers A, et al. Conduction Heterogeneity: Impact of Underlying Heart Disease and Atrial Fibrillation. JACC Clin Electrophysiol. 2020;6(14):1844-54.
7. Schram-Serban C, Heida A, Roos-Serote MC, Knops P, Kik C, Brundel B, et al. Heterogeneity in Conduction Underlies Obesity-Related Atrial Fibrillation Vulnerability. Circ Arrhythm Electrophysiol. 2020;13(5):e008161.
8. Ramdjan T, Mouws E, Teuwen CP, Sitorus GDS, Houck CA, Bogers A, et al. Progression of late postoperative atrial fibrillation in patients with tetralogy of Fallot. J Cardiovasc Electrophysiol. 2018;29(1):30-7.

9. Kharbanda RK, van Schie MS, Ramdat Misier NL, van Leeuwen WJ, Taverne Y, van de Woestijne PC, et al. First Evidence of Atrial Conduction Disorders in Pediatric Patients With Congenital Heart Disease. JACC Clin Electrophysiol. 2020;6(14):1739-43.
10. Mouws E, van der Does L, Kik C, Lanters EAH, Teuwen CP, Knops P, et al. Impact of the arrhythmogenic potential of long lines of conduction slowing at the pulmonary vein area. Heart Rhythm. 2019;16(4):511-9.
11. Mouws E, Lanters EAH, Teuwen CP, van der Does L, Kik C, Knops P, et al. Epicardial Breakthrough Waves During Sinus Rhythm: Depiction of the Arrhythmogenic Substrate? Circ Arrhythm Electrophysiol. 2017;10(9).
12. Kharbanda RK, Knops P, van der Does L, Kik C, Taverne Y, Roos-Serote MC, et al. Simultaneous Endo-Epicardial Mapping of the Human Right Atrium: Unraveling Atrial Excitation. J Am Heart Assoc. 2020;9(17):e017069.
13. Kharbanda RK, Wesselius FJ, van Schie MS, Taverne Y, Bogers A, de Groot NMS. Endo-Epicardial Mapping of In Vivo Human Sinoatrial Node Activity. JACC Clin Electrophysiol. 2021.
14. Teuwen CP, Kik C, van der Does L, Lanters EAH, Knops P, Mouws E, et al. Quantification of the Arrhythmogenic Effects of Spontaneous Atrial Extrasystole Using High-Resolution Epicardial Mapping. Circ Arrhythm Electrophysiol. 2018;11(1).
15. van Schie MS, Starreveld R, Roos-Serote MC, Taverne Y, van Schaagen FRN, Bogers A, et al. Classification of sinus rhythm single potential morphology in patients with mitral valve disease. Europace. 2020;22(10):1509-19.
16. van der Does L, Knops P, Teuwen CP, Serban C, Starreveld R, Lanters EAH, et al. Unipolar atrial electrogram morphology from an epicardial and endocardial perspective. Heart Rhythm. 2018;15(6):879-87.
17. Ye Z, van Schie MS, de Groot NMS. Signal Fingerprinting as a Novel Diagnostic Tool to Identify Conduction Inhomogeneity. Front Physiol. 2021;12:652128.
18. Jalife J, Berenfeld O, Mansour M. Mother rotors and fibrillatory conduction: a mechanism of atrial fibrillation. Cardiovasc Res. 2002;54(2):204-16.
19. Buch E, Share M, Tung R, Benharash P, Sharma P, Koneru J, et al. Long-term clinical outcomes of focal impulse and rotor modulation for treatment of atrial fibrillation: A multicenter experience. Heart Rhythm. 2016;13(3):636-41.
20. Olson TM, Alekseev AE, Liu XK, Park S, Zingman LV, Bienengraeber M, et al. Kv1.5 channelopathy due to KCNA5 loss-of-function mutation causes human atrial fibrillation. Hum Mol Genet. 2006;15(14):2185-91.
21. Li D, Fareh S, Leung TK, Nattel S. Promotion of atrial fibrillation by heart failure in dogs; atrial remodeling of a different sort. Circulation. 1999;100:87-95.
22. Krogh-Madsen T, Christini DJ. Action potential duration dispersion and alternans in simulated heterogeneous cardiac tissue with a structural barrier. Biophys J. 2007;92(4):1138-49.
23. Bingen BO, Neshati Z, Askar SF, Kazbanov IV, Ypey DL, Panfilov AV, et al. Atrium-specific Kir3.x determines inducibility, dynamics, and termination of fibrillation by regulating restitution-driven alternans. Circulation. 2013;128(25):2732-44.
24. Brundel BJJM, Van Gelder IC, Henning RH, Tuinenburg AE, Tieleman RG, Wietses M, et al. Ion channel remodeling is related to intra-operative atrial refractory periods in patients with paroxysmal and persistent atrial fibrillation. Circulation. 2001;103:684-90.

25. Allessie M. The "second factor": a first step toward diagnosing the substrate of atrial fibrillation? J Am Coll Cardiol. 2009;53(14):1192-3.
26. Stiles MK, John B, Wong CX, Kuklik P, Brooks AG, Lau DH, et al. Paroxysmal lone atrial fibrillation is associated with an abnormal atrial substrate: characterizing the "second factor". J Am Coll Cardiol. 2009;53(14):1182-91.
27. Hindricks G, Potpara T, Dagres N, Arbelo E, Bax JJ, Blomstrom-Lundqvist C, et al. 2020 ESC Guidelines for the diagnosis and management of atrial fibrillation developed in collaboration with the European Association of Cardio-Thoracic Surgery (EACTS). Eur Heart J. 2020.